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Antinutritional Factors Related to Proteins and Amino Acids

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I. PROTEASE INHIBITORS

A. Introduction

Among the many factors that have been implicated as having an adverse effect on the nutritional value of plant proteins is a class of proteins that has the ability to inhibit the proteolytic activity of proteases of diverse origin. Because of the important role soybeans play as a dietary ingredient for animals as well as humans, the protease inhibitor found in this legume has received the most attention since it was first reported by Read and Haas in 1938 (1). The protein fraction responsible for the inhibition of trypsin was partially purified by Bowman (2) and Ham and Sandstedt (3) in 1944 and subsequently isolated in crystalline form by Kunitz one year later (4). The existence of a heat-labile inhibitor of trypsin seemed to offer a reasonable explanation for the observation made many years before by Osborne and Mendel that heat treatment improved the nutritive value of soybean protein (5).

*Retired

The realization that protease inhibitors might be of nutritional significance in soybeans stimulated an intensive search for similar factors in other legumes that provide an important source of protein in the diets of many segments of the world's population. Table 1 provides a partial list of the many plants that are known to contain protease inhibitors as well as their specificity with respect to the proteases they inhibit.

B. Biochemical Properties

The protease inhibitors that have been isolated from soybeans and other legumes fall biochemically into two main categories: those that have a molecular weight of 20,000 to 25,000 with relatively few disulfide bonds and a specificity directed primarily toward trypsin, and those that have a molecular weight of only 6000 to 10,000 with a high proportion of cystine residues and are capable of inhibiting chymotrypsin as well as trypsin at independent binding sites. The most thoroughly characterized examples of these two classes of inhibitors are the so-called Kunitz and Bowman-Birk inhibitors isolated from the soybean.

The complete amino acid sequence of the Kunitz inhibitor is shown in Fig. 1. It consists of 181 amino acid residues with the reactive site (the site directly involved in its interaction with trypsin) located at residues Arg 63 and Ile 64. This molecule combines with trypsin in a stoichiometric fashion, that is, one molecule of the inhibitor inactivates one molecule of trypsin. The complex that forms is analogous to an enzyme–substrate complex that, unlike the usual enzyme–substrate complex that readily dissociates, is bound tightly with a Ki of 10^{-10} M (6). X-ray crystallography has given a closer insight into the detailed nature of the enzyme-inhibitor complex (Fig. 2); the molecular forces involved in this interaction have been reviewed by Laskowski and Kato (7).

Five closely related Bowman–Birk type of inhibitors have been isolated and characterized from soybeans; they are referred to as PI-I through PI-V (8). The amino acid sequence of PI-I is shown in Fig. 3. A unique feature of this molecule is that it has two independent binding sites: a trypsin-reactive site (Lys 16–Ser 17) and a chymotrypsin-reactive site (Leu 44–Ser 45). In contrast to the Kunitz inhibitor, the Bowman–Birk inhibitors are very rich in disulfide bonds, possessing seven. This feature is responsible for the very tight, compact three-dimensional structure revealed by X-ray crystallography of PI-II, a variant of the Bowman–Birk inhibitor that has 65 amino acid residues (9), and by nuclear magnetic resonance spectroscopy of PI-I, the classical variant that is comprised of 71 amino acids (10). The sequences of amino acids surrounding these two reactive sites are remarkably similar to each other, and a high degree of homology has been found

TABLE 1 Distribution of Protease Inhibitors Present in Legumes

Botanical name	Common name	Proteases inhibited
Arachis hypogaea	Peanut, groundnut	T, C, Pl, K
Cajanus cajan	Pigeon pea, red gram	T
Canavalia ensiformis	Jack bean, sword bean	T, C, S
Chamacrista fasiculata	Partridge pea	T
Cicer arietinum	Chick pea, Bengal gram, garbanzo	T, C
Clittoria ternatea	Butterfly pea	T, C, S
Cyamopsis tetragonoloba	Cluster bean	T, C, S
Dolichos biflorus	Horse gram	T
Dolichos lablab	Hyacinth bean, field bean, Hakubenzu bean	T, C, Th
Faba vulgaris	Double bean	T
Glycine max	Soybean	T, C
Lathyrus odoratus	Sweet pea	T
Lathyrus sativus	Chickling vetch	T, C
Lens esculenta (culinaris)	Lentil	T
Lupinus albus	Lupine	T
Mucana deeringianum	Florida velvet bean	T
Phaseolus aconitifolius	Moth bean	T
Phaseolus anguiaris	Adzuki bean	T, C
Phaseolus aureus	Mung bean, green gram	T, endopeptidase
Phaseolus coccineus	Scarlet runner bean	T, C
Phaseolus lunatus	Lima bean, butter bean	T, C
Phaseolus mungo (radiatus)	Black gram	T, C, S
Phaseolus vulgaris	Navy bean, kidney bean, pinto bean, French bean, white bean, wax bean, haricot bean, garden bean	T, C, E, S
Pisum sativum	Field bean, garden pea	T
Psophocarpus tetragonolobus	Winged bean, Gao bean	T
Stizobolium deeringianum	Velvet bean	T
Vicia faba	Broad bean, field bean, faba bean	T, C, Th, Pr, Pa
Vigna unguiculata (sinensis)	Cowpea, black-eyed pea, Southern pea, serido pea	T, C
Voandzeia subterranca	Bambara bean	T

Source: From Ref. 6

C, chymotrypsin; E, elastase; K, kallikrein; Pa, papain; Pl, plasmin; Pr, pronase; S, subtilisin; T, trypsin; Th, thrombin

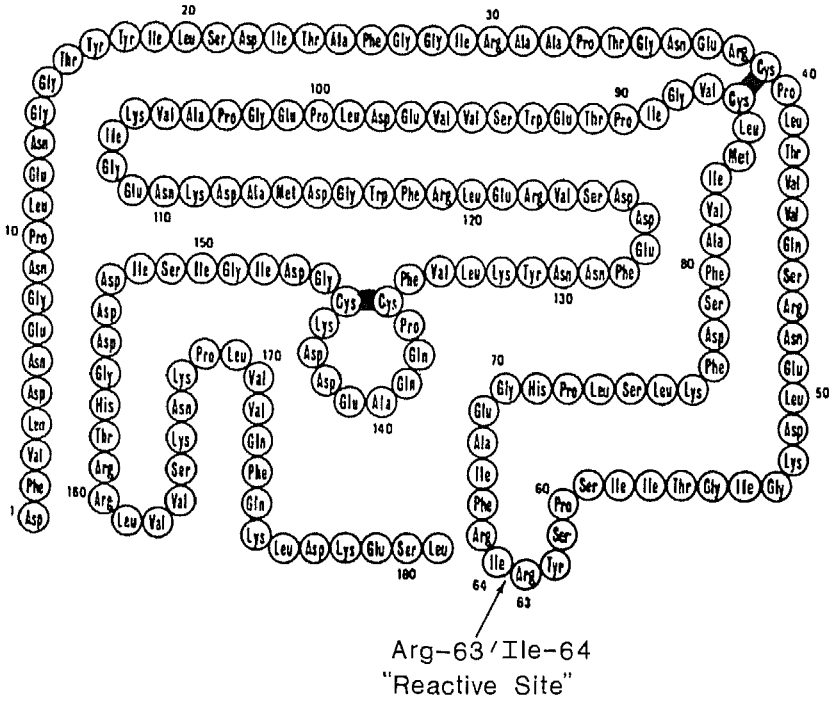


FIGURE 1 Amino acid sequence of the Kunitz soybean trypsin inhibitor. (From T. Koide and T. Ikenaka, Studies on soybean trypsin inhibitors. 3. Amino acid sequence of the carboxyl region and the complete amino acid sequence of soybean trypsin inhibitor (Kunitz). *Eur. J. Biochem.*, 32:417, 1973.)

between the Bowman-Birk inhibitor and a number of other low molecular weight inhibitors that have been isolated from other legumes (Table 2).

C. Nutritional Significance

1. Biological Effects

Not long after soybeans were introduced into the United States, primarily as a source of oil, Osborne and Mendel made the significant observation that soybeans had to be heat treated in order to support the growth of rats (5). With the isolation of a trypsin inhibitor from raw soybeans by Kunitz (4), it generally was assumed that the beneficial effect of heat treatment could be attributed to the destruction of this factor that interfered with

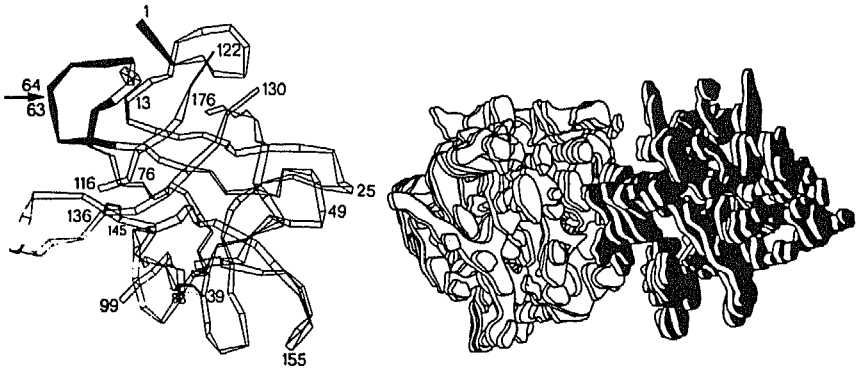


FIGURE 2 Folding of the polypeptide backbone chain of the Kunitz inhibitor is shown on the left. Amino acid residues in intimate contact with trypsin are shown in black. Shown on the right is a model of the Kunitz inhibitor–trypsin complex. The part representing trypsin is shaded less heavily. (From R. M. Sweet, H. T. Wright, J. Janin, C. H. Clothis, and D. M. Blow, Crystal structure of the complex of porcine trypsin and soybean trypsin inhibitor (Kunitz) at 2.6 Å, *Biochemistry*, 13:4212, 1974.)

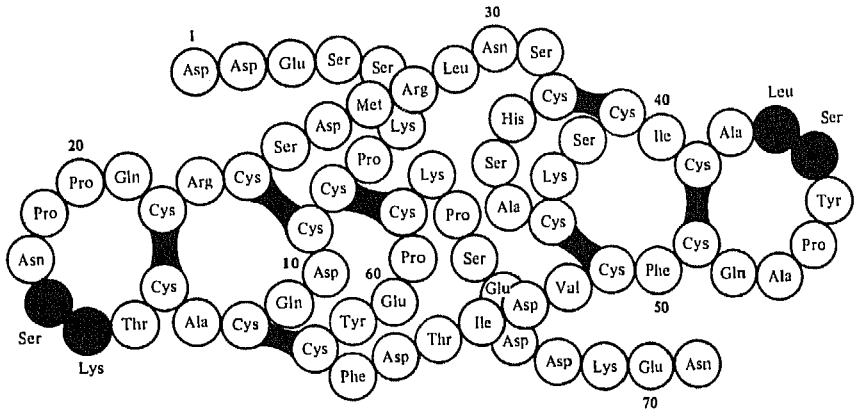


FIGURE 3 Amino acid sequence of the Bowman–Birk inhibitor. The disulfide bonds and reactive sites involved in its interaction with trypsin (Lys 16–ser 17) and chymotrypsin (leu 44–ser 45) are shown in black. (From S. Odani and T. Ikenaka, Studies on the soybean trypsin inhibitors. VII. Disulfide bridges in soybean Bowman–Birk proteinase inhibitor, *J. Biochem. (Tokyo)*, 74:697, 1973.)

TABLE 2 Sequence Homology Around Reactive Sites of Double-Headed Inhibitors in Legumes

	Reactive site									
Soybeans Bowman-Birk	Cys	-Ala	-Cys-Thr	16 -Lys	17 -Ser	-Asn	-Pro	-Pro	-Gln-Cys	Trypsin inhibitors
CII	Cys	-Ala	-Cys-Thr	49 -Arg	50 -Ser	-Met	-Pro	-Gly	-Gln-Cys	
DII	Cys	-Met	-Cys-Thr	24 -Arg	25 -Ser	-Met	-Pro	-Pro	-Gln-Cys	
	Cys	-Met	-Cys-Thr	51 -Arg	52 -Ser	-Gln	-Pro	-Gly	-Gln-Cys	
Lima bean LBI-I, IV	Cys	-Leu -Ala	-Cys-Thr	26 -Lys	27 -Ser	-Ile	-Pro	-Pro	-Gln-Cys	
Garden bean iso-inhibitor II	Cys	-Met	-Cys-Thr	53 -Arg	54 -Ser	-Met	-Pro	-Gly-Lys	-Cys	
Chick pea	Cys	-Val	-Cys-Thr	14 -Lys	15 -Ser	-Ile	-Pro	-Pro	-Gln-Cys	
Runner bean PCI-3			-Ile-Tyr	-Lys	-Ser	-Gln	-Pro			
Soybeans Bowman–Birk	Cys	-Ile	-Cys	43 -Ala-Leu	44 -Ser	-Tyr	-Pro	-Ala	-Gln-Cys	Chymotrypsin inhibitors
Lima bean LBI-I, IV	Cys	-Ile	-Cys-Thr	53 -Leu	54 -Ser	-Ile	-Pro	-Ala	-Gln-Cys	
LBI-IV	Cys	-Ile	-Cys-Thr	53 -Phe	54 -Ser	-Ile	-Pro			
Runner bean PCI-3		-Asp	-Val-Ala	-Leu	-Ser	-Pro				
Soybean CII	Cys	-Met	-Cys-Thr	22 -Ala	23 -Ser	-Met	-Pro	-Pro	-Gln-Cys	Elastase inhibitors
Garden bean iso-inhibitor II			Cys-Thr	26 -Ala	27 -Ser	-Ile	-Pro	-Pro	-Gln-Cys	

Source: From Ref. 6

the digestion of protein in the intestinal tract. Purified fractions from the soybean, which were rich in antitryptic activity, in fact were capable of inhibiting the growth of rats (11), chicks (12), and mice (13), an effect that generally was accompanied by a depression in the digestibility of the protein in the diet. Furthermore, the feeding to rats of a raw soybean extract from which the trypsin inhibitors had been removed by affinity chromatography showed improved growth performance compared to controls from which the trypsin inhibitors had not been removed (14). Despite these observations, it remained unclear why preparations of trypsin inhibitor were capable of inhibiting growth even when incorporated into diets containing predigested protein or free amino acids (15). Such experiments obviously ruled out an inhibition of proteolysis as the sole factor responsible for growth inhibition and thus served to focus attention on some alternate mode of action of the trypsin inhibitors.

Perhaps the most significant observation that ultimately has led to a better understanding of the mode of action of the soybean inhibitors was the finding that raw soybeans or trypsin inhibitor itself could cause hypertrophy and hyperplasia of the pancreas (16–18). This has led to the suggestion that the growth depression caused by trypsin inhibitors might be the consequence of an endogenous loss of amino acids secreted by a hyperactive pancreas (19,20). Since pancreatic enzymes such as trypsin and chymotrypsin are particularly rich in sulfur-containing amino acids, pancreatic hypertrophy and/or hyperplasia diverts these amino acids from the synthesis of body tissue protein to the synthesis of these enzymes. This loss in sulfur-containing amino acids exacerbates an already critical situation with respect to soybean protein, which inherently is deficient in these amino acids.

Related to the cellular proliferation of pancreatic tissue evoked by trypsin inhibitors is the observation that raw soy flour potentiates the carcinogenic effect of azaserine (21) and di(2-hydroxypropyl) nitrosamine (22) on the pancreas of the rat. Even more significant is the fact that the long-term (60 or more weeks) feeding of raw soy flour alone causes the appearance of adenomatous nodules on the pancreas (23). This carcinogenic effect was correlated positively with the level of trypsin inhibitor in the diet when various levels of raw soy flour were incorporated into the diet (24). Similar studies with the mouse (25) and hamster (26), however, revealed no evidence of pancreatic lesions with the long-term feeding of raw soy flour.

2. Mode of Action

Many studies have been conducted in an attempt to elucidate the mechanism by which trypsin inhibitors induce pancreatic hypertrophy. Green and Lyman were the first to postulate that pancreatic secretion is controlled by negative-feedback inhibition that depends on the level of trypsin present at

any given time in the small intestine (27). When the level of this enzyme falls below a certain critical threshold, the pancreas responds in a compensatory fashion by producing more enzyme. Suppression of this negative-feedback mechanism occurs if the trypsin becomes complexed with the inhibitor.

It is believed that the mediating agent between the trypsin and the pancreas is the hormone cholecystokinin (CCK), which is released from the intestinal mucosa when the level of trypsin becomes depleted. This is supported by the observation that elevated levels of plasma CCK accompanied the feeding of raw soy flour to rats (28). These relationships are shown in Fig. 4. Negative-feedback control has been found to be operative in most species of animals, with the exception of the dog (29). In the cases of the pig and calf, however, the existence of the negative-feedback mechanism is not accompanied by pancreatic hypertrophy.

Evidence that such a mechanism occurs in humans comes from experiments involving the infusion of the Bowman–Birk inhibitor into the duodenum that evoked an increase in the level of trypsin, chymotrypsin, and elastase into the small intestine (30). The feeding of a meal containing raw soy flour to human subjects also led to an increase in plasma CCK (31).

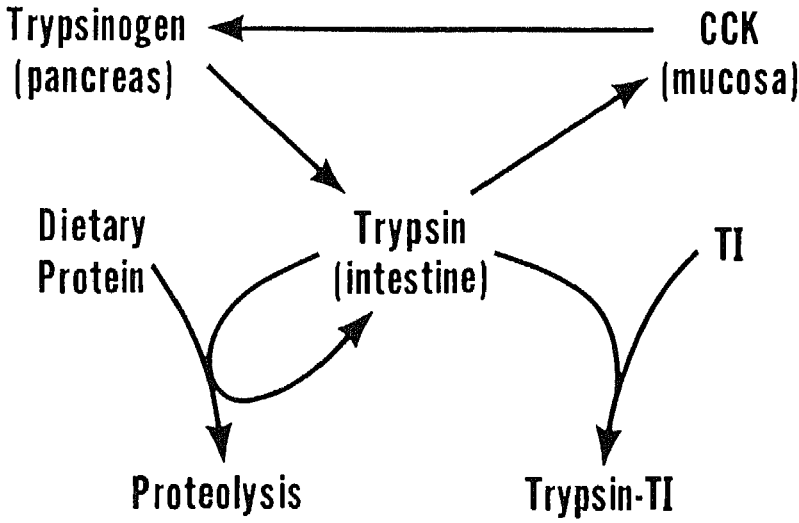


FIGURE 4 Mode of action of soybean trypsin inhibitors on pancreas (CCK = cholecystokinin). (From R. L. Anderson, J. J. Rackis, and W. H. Tallent, Biologically active substances in soy products. In *Soy Protein and Human Nutrition*, edited by H. L. Wilcke, D. T. Hopkins, and D. H. Waggle, Academic Press, New York, 1979, p. 209.)

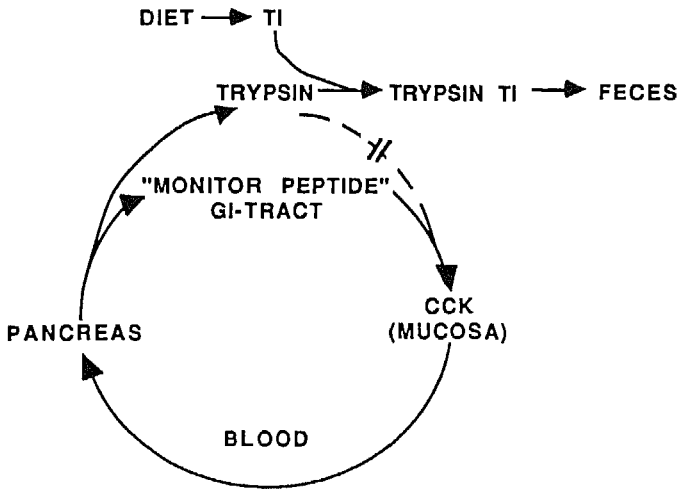


FIGURE 5 Role of monitor peptide in negative-feedback control (TI = trypsin inhibitor; CCK = cholecystokinin). (Based on studies from Ref. 32.)

Until quite recently, it has not been clear how trypsin manages to suppress the secretion of CCK or, conversely, how its inactivation by trypsin causes an increase in CCK production. A trypsin-sensitive peptide ("monitor peptide") with 61 amino acids has been isolated from rat's pancreatic juice that acts as a signal for the release of CCK from the intestinal mucosa (32). This peptide is inactivated by trypsin, thus causing a suppression of CCK release; but, when trypsin is complexed with the inhibitor, this peptide is free to signal the release of CCK, which in turn causes pancreatic hypertrophy and/or hyperplasia with a concomitant increase in the secretion of enzymes. These relationships are shown in the diagram of Fig. 5.

3. Nutritional Significance in Humans

Since trypsin inhibitors are present in a wide variety of foods commonly consumed by humans, including not only legumes but also cereal grains, tubers, fruits, vegetables, nuts, and eggs (33,34), the question may be raised as to whether the presence of trypsin inhibitors in the diet poses any risk to human health. There is a documented instance in which inadequately processed soy protein used as an extender for tuna fish caused an outbreak of gastrointestinal illness (35). Although this does not prove that trypsin inhibitors were responsible for this adverse reaction, it is interesting to note that there is at least one case report of an allergy to the Kunitz trypsin inhibitor (36).

D. Detoxification

1. Effect of Heat Treatment

Because of its economic importance, the soybean has received the most attention with respect to the effect of heat treatment on trypsin-inhibitor activity and the consequences of such treatment on the nutritional quality of the protein. In general, the extent to which the trypsin-inhibitory activity is destroyed by heating is a function of the temperature, duration of heating, particle size, and moisture conditions—variables that are controlled carefully in the commercial processing of soybeans in order to insure a product with maximum nutritional value. An example, using a rat assay, of the relationship that exists between the destruction of the trypsin inhibitor by heat treatment and the concomitant improvement in the nutritional quality of the protein is illustrated in Fig. 6.

Most commercially available soybean products intended for human consumption (e.g., tofu, soy milk, soy protein isolates and concentrates,

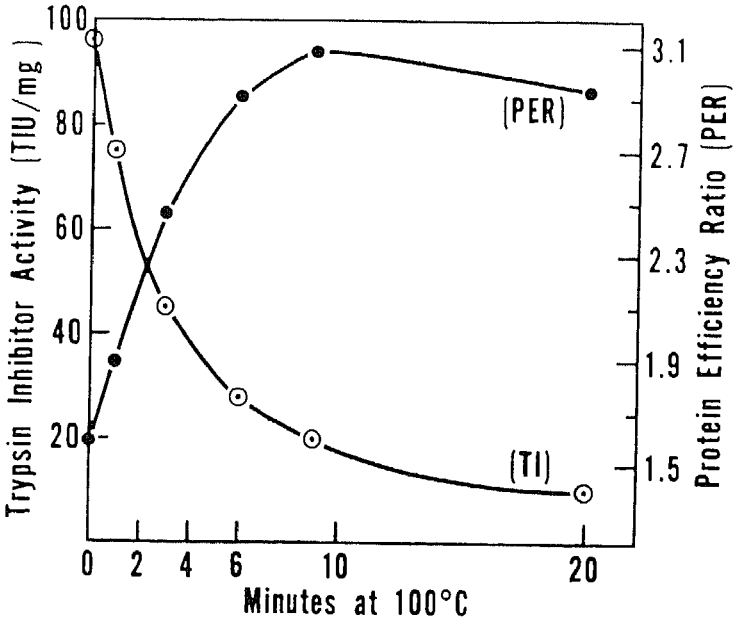


FIGURE 6 Effect of heat treatment on the trypsin-inhibitory activity (TI) expressed as trypsin inhibitor units (TIU) and nutritive value of soybean meal as measured by the protein efficiency ratio (PER). (From J. J. Rackis, Biological and physiological factors in soybeans, *J. Am. Oil Chem. Soc.*, 51:161A, 1974.)

and textured meat analogues) have received sufficient heat treatment so that they contain less than 10% of the trypsin-inhibitor activity originally present in the beans or raw soy flour from which they were derived (37). This a level of activity that is believed to be well below the threshold level necessary to cause pancreatic hypertrophy in rats (38,39).

Although live steam treatment of soybeans (a process referred to as toasting) is the most commonly used method for inactivating the trypsin inhibitor, other modes of heat treatment or processing have proved equally effective. These include boiling soybeans in water (40), dry roasting (41), dielectric heating (42), microwave irradiation (43), extrusion cooking (44), gamma irradiation (45), and infrared radiation (46). The direct infusion of steam into soy milk also inactivates the trypsin inhibitor (47). Because of the compact structure of the Bowman–Birk inhibitor and its stability toward heat in its isolated form (48), it generally has been assumed that the residual trypsin-inhibitor activity found in processed soybean products is due to the Bowman–Birk inhibitor. However, using analytical techniques that serve to differentiate between the Kunitz and Bowman–Birk inhibitors, it has been found the Bowman–Birk inhibitor is destroyed more readily than the Kunitz inhibitor in its natural milieu (e.g., soy flour) (49). The reason for this anomalous situation is not clear, but may be the result of the interaction of the many disulfide groups of the Bowman–Birk inhibitor with other cysteine-rich components comprising the soybean matrix, reactions that may be accelerated by heat.

The popular use of legumes as a staple food item in many parts of the world has prompted numerous studies on the effect of different forms of heat treatment on the inactivation of the trypsin inhibitors present in these beans. The legumes that have received the most attention in this regard include the navy and kidney beans (*Phaseolus vulgaris*), broad beans (*Vicia faba*), peanuts (*Arachis hypogaea*), lima beans (*Phaseolus lunatus*), cowpeas (*Vigna sinensis* or *unguiculata*), winged bean (*Psophocarpus tetragonolobus*), moth bean (*Vigna aconitifolium*), chick pea (*Cicer arietinum*), and pigeon pea (*Cajanus cajan*). In general, the effect of heat treatment on most other legumes follows the same general pattern as that observed with the soybean (see Ref. 6).

2. Germination

Although the germination of soybeans has been reported by some investigators to result in an improvement in the nutritive value of the protein (50,51), the relationship of this to changes in trypsin-inhibitor activity is obscure (52). Changes in net trypsin-inhibitor activity are relatively slight (53–55), although the relative distribution of the Kunitz and Bowman–Birk inhibitors becomes altered. The content of the Bowman–Birk inhibitor in the

cotyledons of soybean gradually disappeared and, by the 13th day, only the Kunitz inhibitor could be detected (56). In this case new forms of the inhibitor were observed that presumably arose as the result of limited proteolysis during germination (57,58).

Gupta (59) and Savelkoul et al. (60) have documented the changes that take place during the germination of many different legume seeds. No consistent pattern could be discerned; some legumes showed little or no change, while others displayed a decrease in trypsin-inhibitor activity. In general, however, there appears to be little or no correlation between changes in trypsin-inhibitor activity and the nutritive value of the germinated seed.

3. Traditional Modes of Preparation

Tofu is comprised mainly of protein that has been precipitated from a hot-water extract of soybeans with calcium-magnesium salts. Soy milk is simply a hot-water extract of whole soybeans that may have undergone clarification. Since the preparation of both tofu and soy milk involves the cooking or steaming of soybeans prior to extraction with water, such products are generally quite low in trypsin-inhibitor activity (61). Fermented preparations of soybeans and such other legumes as tempeh, miso, and natto are virtually devoid of trypsin-inhibitor activity since the beans are subjected to boiling prior to fermentation (62). Fermented legumes have been shown to have superior nutritive value compared to their unfermented counterparts (63–65) but, since both types of preparations were derived from heated beans, this improvement probably has little to do with the trypsin inhibitor.

In only a few instances has an attempt been made to determine the effect of fermentation in the absence of heat. Varying degrees in the reduction of trypsin-inhibitory activity by natural fermentation in the absence of heat treatment have been reported for chick peas (66,67) and cowpeas (67). In the absence of further studies, it can only be assumed that such decreases in trypsin-inhibitory activity were due to proteolytic attack by enzymes secreted by the microorganisms involved in the fermentation.

4. Genetic Studies

The screening of 56 genotypes of grain-type soybean and 17 vegetable-type soybean collections has revealed over a 10-fold variation in trypsin-inhibitor activity (68). Kakade et al. also noticed a wide variation in the trypsin-inhibitor activity of over 100 different varieties and strains of soybeans, but no correlation was obtained between trypsin-inhibitor content and the nutritional value of the protein as measured in rats (69). In a continuing search for soybean genotypes that might be devoid of trypsin

inhibitors, 2944 accessions of soybean germ plasm were screened and only two accessions that lacked the Kunitz inhibitor but still retained about 50% trypsin-inhibitor activity were discovered (70,71), presumably due to the presence of the Bowman–Birk inhibitor (72). Although feeding studies with rats, chicks, and swine have indicated some improvement in the nutritional value of the protein, the strain with only 50% trypsin inhibitor activity still was inferior to that of heat-processed soybean meal (73–75).

E. Physiological Role in Plants

The physiological role of the protease inhibitors in plants sometimes has been presumed to be regulation of protein catabolism during germination or the degradation of storage protein during seed maturation. Attractive as this hypothesis may seem, there is very little experimental support for this concept. For example, the protease inhibitors isolated from specific plants in fact are incapable of inhibiting the endogenous proteases of the same plant (76,77). In Section I.D.2, reference was made to the fact that a diminution in protease-inhibitor activity is not observed consistently during germination.

It is now generally accepted that the protease inhibitors play a key role in the defense mechanism that plants have evolved against insects (78–80) and microbial pathogens (81). Studies of the effect of protease inhibitors artificially introduced into defined diets have shown them to be detrimental to the growth and development of insects from a wide variety of genera (82–85). The mechanism for protection against insects appears to be very similar to what has been observed in animals, namely, the ability of these inhibitors to interfere with the digestive enzymes in the gut of these insects (86–88).

The genes from a number of protease inhibitors from different plants, including soybeans (89–92), potatoes (93,94), tomatoes (95), rice (96), and barley (97) have been cloned, and concerted efforts are being made to transfer these genes into other plants in order to confer resistance to insect attack. Two examples illustrating the successful application of this approach may be cited. The cowpea (*Vigna unguiculata*) trypsin inhibitor, which is a variant of the Bowman–Birk inhibitor, has been introduced into *Agrobacterium tumifasciens* and expressed in the tobacco plant (98). These genetically altered tobacco plants had enhanced resistance to the tobacco budworm (98). The introduction of the gene of potato inhibitor II, an inhibitor of trypsin and chymotrypsin, into tobacco plants using the cauliflower mosaic virus as the vector severely retarded the growth of the larvae of the tobacco horn worm (99).

The development of transgenic plants with protease-inhibitor activity offers exciting possibilities in agriculture. For example, advantage could be

taken of the differences in the specificity of various protease inhibitors so as to provide protection against a wide variety of insects, depending on the type of digestive enzymes that are present in their gut. It also might be possible to alter the specificity of a given protease inhibitor by site-directed mutagenesis of the amino acids comprising its reactive site. In the final analysis, a combination of several different inhibitors may be required to achieve complete protection against insect predation.

F. Analytical Techniques

Rackis et al. have critically evaluated the most commonly employed techniques for assaying for protease-inhibitor activity that generally involve a measurement of the degree to which the activity of a pure sample of a given protease, usually bovine trypsin or chymotrypsin, on casein or a synthetic substrate is inhibited by the test sample (100). The limitation to this type of assay is the fact that it does not serve to distinguish among the different molecular species of inhibitors that might have the same specificity toward a given protease. Furthermore, this type of assay fails to reveal to what extent such nonprotein components as phytates and tannins might contribute to the observed protease-inhibitor activity. These problems can be circumvented by using monoclonal antibodies that distinguish between the Kunitz and Bowman–Birk inhibitors in soybeans (49,101–103), or by a procedure that involves the specific absorption of trypsin inhibitors by affinity chromatography on immobilized trypsin (104,105).

II. AMYLASE INHIBITORS

A. Nutritional Significance

Protein inhibitors of alpha-amylase are distributed widely throughout the plant kingdom and have been purified from wheat, barley, rye, corn, millet, kidney bean, colocasia, and yam. Many of these have been studied extensively with respect to their structure, physicochemical properties, and mechanism of action (see Ref. 106 for details). It is questionable, however, whether these amylase inhibitors really should be considered as true antinutritional factors since biological studies have not been very conclusive regarding their adverse nutritional effects. For example, the alpha-amylase inhibitor isolated from the kidney bean (*Phaseolus vulgaris*) did not affect the growth rate of rats, nor did it affect the availability of energy from dietary starch (107). Although the growth of rats (108) and chickens (109) was not retarded by the feeding of alpha-amylase inhibitor from wheat, there was evidence of pancreatic hypertrophy indicative of degenerative changes in cellular morphology. In summary, therefore, it does not appear that the amylase inhibitors from legumes and cereals are particularly delete-

rious to animals, and perhaps humans, other than producing a possible stimulatory effect on pancreatic function similar to what has been observed with the trypsin inhibitors. It would be desirable, therefore, that foods possessing high levels of amylase inhibitors such as beans and cereals be processed thoroughly by heat treatment to insure their inactivation.

At one time, a number of alpha-amylase inhibitor preparations from kidney beans (so-called starch blockers) were introduced onto the market. These supposedly were capable of inhibiting the digestion of starch in the intestinal tract to produce a reduction in caloric intake and consequent weight loss. Clinical studies, however, did not support these claims (110–112). Moreover, the amylase inhibitor represented only a minor constituent of these commercial formulations, which also contained significant levels of trypsin inhibitor and lectins (113,114). A subsequent study using a more highly purified preparation of amylase inhibitor from white beans showed that such a preparation in fact was capable of inactivating amylase in the human intestinal lumen (115). It remains to be proven, however, whether this effect actually will cause weight loss.

B. Role in Plants

Since most amylase inhibitors of plant origin are active only against animal alpha-amylase, it does not appear likely they serve to regulate carbohydrate metabolism in the plant. A much more likely role for the these amylase inhibitors is that they serve to protect seeds against insect predators. A considerable body of evidence has demonstrated that most of the plant amylase inhibitors are strongly active against amylases from insect species that are known to attack these same plants (106,116). The addition of amylase inhibitors from wheat (117) or kidney beans (118) to synthetic diets fed to the cowpea weevil (*Callosobruchus maculatus*) adversely affected the development and increased the mortality of this pest. Earlier reports on the toxic effect of a bean lectin on the development of the cowpea weevil (119,120) now have been shown to be due to the amylase-inhibitor content of such lectin preparations (121). The successful transfer of the bean amylase-inhibitor gene to the tobacco plant (122) presents the possibility that genetic engineering involving the transfer of the gene for the amylase inhibitor to other plants may be employed to enhance the resistance of such plants to insects toward which they normally would be susceptible.

III. LECTINS

A. Introduction

Paralleling the distribution of protease inhibitors in plants is a class of proteins referred to as lectins. This term was introduced first by Boyd and Shapleigh (123), who pointed out that this class of proteins exhibited a high

degree of specificity toward human blood cells of various blood group types. It was this high degree of specificity that led them to coin the word *lectin* from the Latin word *legere*, meaning to pick or choose, to emphasize the specificity that these proteins exhibit toward blood groups. One obvious manifestation of this property is their ability to agglutinate the red blood cells from various species of animals due to the interaction of multiple binding sites of the lectins with specific glycoconjugate receptors on the cell membrane. In fact, the term *phytohemagglutinin* sometimes is used in referring to lectins of plant origin. Over the ensuing years, it increasingly has become apparent that the lectins exhibit a wide variety of other interesting biological effects that enable them to play a key role as mediators of cell recognition in living systems as well as providing powerful tools for the study of carbohydrates and their derivatives, both in solution and on cell surfaces (124). This brief overview gives primary consideration to their physicochemical properties, nutritional significance, mode of action, and their role in the plant. More specific details on the many varied aspects of lectins may be found in Ref. 125, a book devoted to this subject.

B. Physicochemical Properties

A cursory examination of the physicochemical properties of some representative lectins shown in Table 3 suggests that, although there is a wide diversity in their properties (126), certain broad generalizations can be made. The most-important common feature is that most lectins are comprised of either two or four subunits, each of which contains a specific sugar-binding site. It is this feature of multivalency that accounts for the ability of lectins to agglutinate cells or to precipitate glycoproteins or large polysaccharide polymers. For example, concanavalin A, the lectin from the jack bean, is a tetramer comprised of four identical subunits, each of which has a molecular mass of 26,000 daltons (Fig. 7). A rather more-complex situation is exemplified by the lectins of the kidney bean (*Phaseolus vulgaris*). In this bean, there is a family of five lectins (isolectins), each of which is a tetramer of four subunits designated as L or E (Fig. 8). These two different subunits confer leukoagglutinating (L) activity or erythroagglutinating (E) activity to the parent tetramer. Thus, the isolectins referred to as L_4 and E_4 would have leukoagglutinating and hemagglutinating activity, respectively, exclusively, whereas the other three isolectins (E_3L_1, E_2L_2, and EL_3) would display both activities, depending on the relative proportion of these two subunits.

Not shown in Table 3 is the fact that metal ions (of calcium, manganese, or magnesium) are required for the agglutinating activity by most lectins and also the fact that most lectins are glycoproteins. Concerted

TABLE 3 Physicochemical Properties and Sugar Specificity of Phytolectins

Plant source	Sugar specificity	Molecular weight[a]	Sub-units	Carbo-hydrate content (%)
Abrus precatorius (jequirity bean)	α-D-Gal	65,000[b]	2	
		134,000	4	
Arachis hypogeae (peanut)	α-D-Gal	110,000	4	0
Bandiera simplicifolia	α-D-Gal	114,000	4	
Bauhinia purpurea alba	α-D-GalNAc	195,000		11
Canavalia ensiformis (jack bean)	α-D-Man, α-D-Glc	55,000	2	0
		110,000	4	
Dolichos biflorus (horse gram)	α-D-GalNAc	113,000	4	3.8
		109,000	4	
Glycine max (soybean)	D-Gal, α-D-GalNAc	122,000	4	6
Lens culinaris[c] (lentil)	α-D-Man, α-D-Glc	52,000	2	2
Phaseolus coccineus (scarlet runner bean)	GlcNAc	120,000	4	
Phaseolus lunatus[d] (lima bean)	D-GalNAc	124,000	2	4
		247,000	4	4
Phaseolus vulgaris (black bean)		128,000		5.7
Phaseolus vulgaris (red kidney bean)	D-GalNAc	120,000	4	4.1
Phaseolus vulgaris (wax bean)	D-GalNAc	120,000	4	10.4
Phaseolus vulgaris (navy bean)	D-GalNAc	128,000	4	
Pisum sativum (garden pea)	α-D-Man, α-D-Glc	53,000	4	0.3
Psophocarpus tetragonoiobus (winged bean)	α-L-Fuc	120,000	4	9.4
		58,000	2	4.8
Riclnus communis (castor bean)	D-Gal, D-GalNAc	60,000[b]		
	D-Gal	120,000		
Vicia faba (field bean)	D-Man, D-GlcNAc	50,000	4	

Glc, glucose; GlcN, glucosamine; GlcNAc, N-acetylglucosamine; Gal, galactose; GalN, galactosamine; GalNAc, N-acetylgalactosamine; Fuc, fucose; Man, mannose

[a]If more than one value is given for molecular weight, there is evidence for the existence of multiple forms of the lectins (isolectins)

[b]Nonhemagglutinating toxins

[c]Also known as *Lens esculenta*

[d]Also known as *Phaseolus limensis*

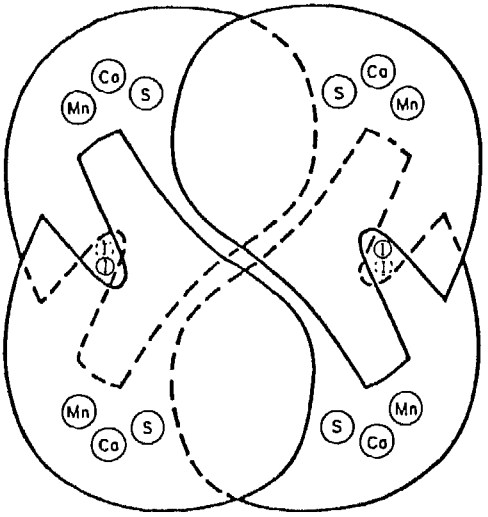


FIGURE 7 Schematic representation of the tetramer of concanavalin A. Each subunit is approximately 42 × 40 × 39 Å. Manganese and calcium sites are indicated by Mn and Ca, respectively. The saccharide-binding site near the metals is indicated by S and the hydrophobic-binding site in the cavity by I. (From J. W. Becker, G. N. Reeke, B. A. Cunningham, and G. M. Edelman, New evidence on the location of the saccharide-binding site of concanavalin A. Reprinted by permission from *Nature* vol. 259, pp. 406–409; Copyright © 1976 Macmillan Magazines Limited.)

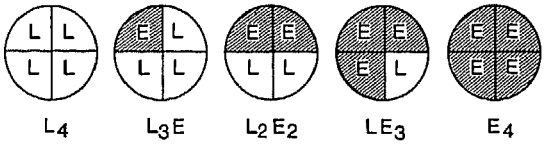


FIGURE 8 Schematic representation of the tetrameric structure of the five isolectins in the red kidney bean in which L and E are the subunits responsible for leukoagglutinating and erythroagglutinating, respectively. (From J. B. Miller, R. Hsu, R. Heinrikson, and S. Yachnin, Extensive homology between the subunits of the phytohemagglutinin mitogenic proteins derived from *Phaseolus vulgaris*, *Proc. Nat. Acad. Sci.*, 72:1388, 1975.)

efforts are being made to determine the primary, secondary, tertiary, and quaternary structure of the various lectins, and such studies have revealed a high degree of homology among the lectins of diverse origin (127). In addition to concanavalin A, the three-dimensional structure based on X-ray crystallography has been reported for the wheat germ agglutinin (128), pea (129), peanut (130), soybean (131), and lentil (132) lectins. Despite the vast amount of information we now have regarding the structural features of many lectins (126), the reason for differences in their sugar specificity remains elusive. A recent X-ray crystallographic study of the lectin from *Erythrina corallodendron* (133) suggests that extensive differences in the topography of the binding pockets of lectins most likely accounts for differences in sugar specificity.

C. Nutritional Significance

The extreme toxicity of the castor bean has been known for a long time, but it remained for Stillmark to demonstrate its hemagglutinating property in 1888 (134). Landsteiner and Raubitschek showed 20 years later that even the seeds of many edible species of legumes contained substances capable of agglutinating the red blood cells of various animals in a very specific fashion (135). Following the pioneering work of Jaffé and co-workers and subsequent research by Liener and Puszta firmly established the fact that the lectins of the common bean (*Phaseolus vulgaris*) mainly are responsible for the toxic effects resulting from the consumption of the raw bean (see Ref. 136 for Liener's review of this). Other legumes from which lectins have been isolated and shown to be toxic upon oral ingestion include the jack bean (*Canavalia ensiformis*), horse gram (*Dolichos biflorus*), hyacinth bean (*Dolichos lablab*), Adzuki bean (*Phaseolus vulgaris*), lima bean (*Phaseolus lunatus*), and the winged bean (*Psophocarpus tetragonolobus*).

The fact that lectins are distributed so widely in food items commonly consumed in the human diet (137) raises the important question of whether they pose any significant risk to human health. Although the lectins of most food items are inactivated by the heat involved in processing or household cooking (137), lectin activity nevertheless has been detected in such food items as dry cereals and peanuts (137), dry-roasted beans (138), and processed wheat germ (139).

There are a number of reports in the literature of human intoxication in which lectins appear to have been the causative agents. For example, in 1948 a severe outbreak of gastroenteritis occurred among the population of West Berlin because of the consumption of partially cooked beans that had been airlifted into the city during its blockade (140). A mixture of beans

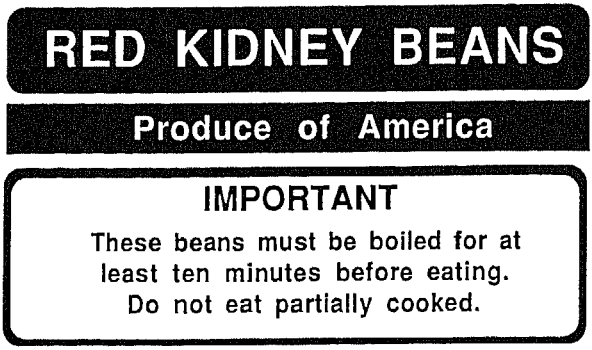


FIGURE 9 Warning label that has been placed on packets of dry beans sold in the retail markets in England. (From Ref. 136.)

and maize prepared by mothers in Tanzania as a porridge for infant food was found still to possess lectin activity because of insufficient cooking (141). Outbreaks of intoxication have been reported in England because of the consumption of raw or partially cooked beans (142–144). Most of these cases have involved individuals who had eaten raw beans as part of a salad or as an ingredient in such dishes as chili con carne prepared in a slow cooker. In the latter case, the conditions of heating were such that the lectin activity was not destroyed completely even though the beans were considered to be acceptable in terms of texture and palatability (145–148). Prompted by these reports, warning labels now may be found on most dry beans sold in retail food stores in England (Fig. 9).

D. Mode of Action

Jaffé and co-workers were the first to propose that the toxicity of bean lectins could be attributed to their ability to bind to specific sites on the surface of cells lining the intestinal tract (149). Subsequent studies by other investigators have fully confirmed the fact that bean lectins bind to the intestinal mucosa (150–154). This is illustrated in Fig. 10, which shows that the lectin ingested by rats in the form of raw kidney beans binds to the brush border region of the small intestine and may be endocytosed in part by cells underlying the brush border membrane. As shown in Fig. 11, the binding of the kidney bean lectin is accompanied by severe disruption of the brush border and abnormal development of the microvilli.

One of the major consequences of lectin-induced damage to the intestinal mucosa is a serious impairment in the absorption of nutrients across

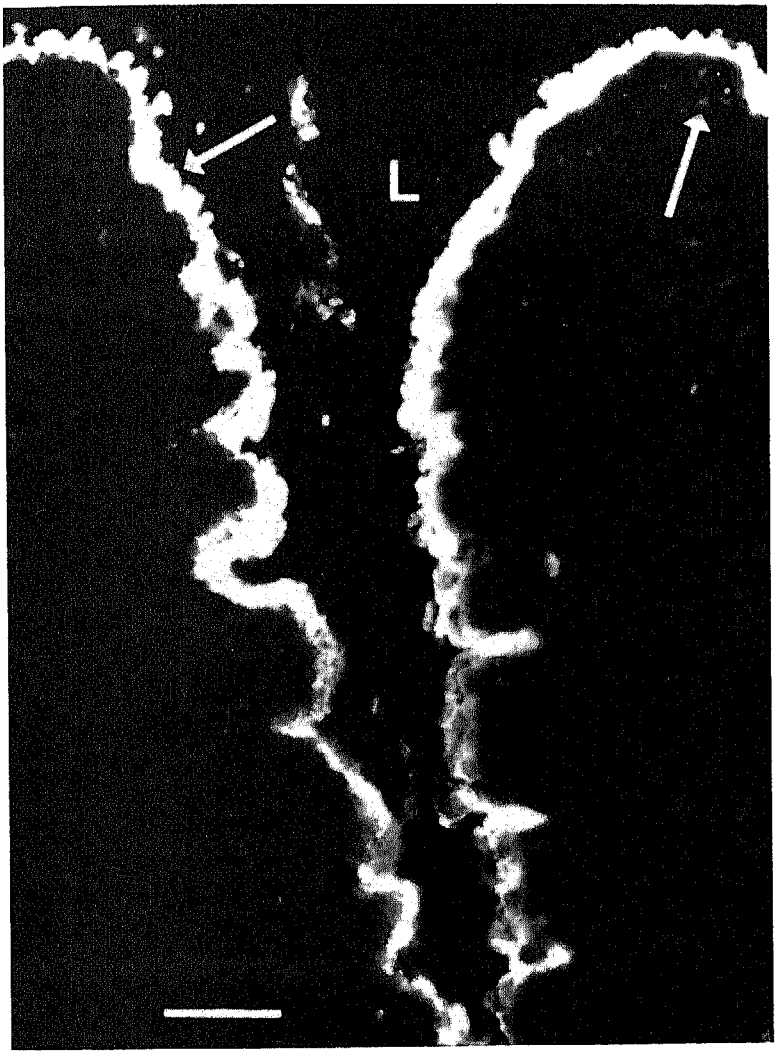


FIGURE 10 Immunofluorescence micrograph of part of a transverse section through the duodenum of a rat fed a diet containing raw kidney beans. Incubation with rabbit antilectin immunoglobulins and fluorescein isothiocyanate–conjugated antirabbit immunoglobulin G, showing immunofluorescence in brush border region and within apical cytoplasm of mature enterocytes (arrows) (L = intestinal lumen; the bar is equal to 50 μm). (From Ref. 151.)

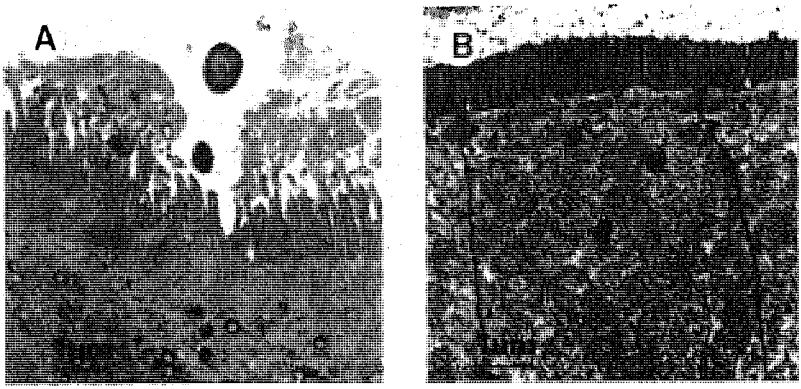


FIGURE 11 Electron micrographs of sections of brush borders from rats fed diets containing (A) 5% raw kidney beans and (B) 10% casein. (From A. Puzstai, E.M.W. Clarke, T. P. King, and J. C. Stewart, Nutritional evaluation of kidney beans (*Phaseolus vulgaris*): Chemical composition, lectin content, and nutritional value of selected cultivars, *J. Sci. Food Agr.*, 30:843, 1979.)

the intestinal barrier. This phenomenon was demonstrated first by Jaffé and Comejo (155) and later was confirmed by Donatucci et al. (156) by the use of radioactive glucose and the technique of vascular intestinal perfusion. This interference with the absorption of nutrients is nonspecific since it also can be demonstrated with amino acids (157,158), lipids (159), and vitamins (160). Superimposed upon this impairment in intestinal absorption is the finding that enterokinase (161) and many of the brush border hydrolases are inhibited by lectins (158,162), a factor that also would contribute to an interference with nutrient availability. The ability of lectins to cause an increase in the weight and number of cells of the small intestine and an increase in the secretion of mucin (163) also could contribute to an endogenous loss of nitrogen and a negative effect on growth.

An alternative explanation for the toxic effects of dietary lectins is suggested by the observation that germ-free animals are better able to tolerate raw beans in their diet compared to conventional animals (164,165). This observation may be related to the fact that rats fed raw beans exhibit an overgrowth or colonization of coliform bacteria in the small intestine (154,159) It has been suggested that, as a result of the altered permeability of the intestinal mucosa, these bacteria, or the endotoxins they produce, gain entrance into the bloodstream and cause toxic systemic effects (166). The exact mechanism by which lectins induce colonization of the small

intestine is not known. A possible explanation may be that lectins, because of their polyvalency, bind to receptor sites on the brush border as well as to the bacterial coat and thus serve to "glue" bacteria to the luminal surface of the small intestines.

Lectins and antibodies to lectins can be detected immunochemically in the blood of rats and pigs fed raw kidney beans (167,168). This would indicate that lectins themselves, either intact or partially digested, may be absorbed and enter the circulatory system. In addition to a direct toxic effect on certain target organs, other systemic effects that may ensue include an increase in protein and fat catabolism, a depletion of muscle glycogen, and an elevation in blood insulin levels (169).

E. Role in Plants

Among the various hypotheses that have been proposed for the role of lectins in plants, at least two have attracted the most attention: that they act as mediators of the symbiotic relationship between N-fixing microorganisms, primarily of the genus *Rhizobium*, and leguminous plants, and that they are part of a defense mechanism against insects and microbial pathogens.

The association between legumes and N-fixing bacteria is highly specific. For example, the rhizobia that infect soybeans cannot nodulate garden peas or white clover and vice versa. That lectins are responsible for this specific interaction is based on the finding that the lectin from a particular legume such as the soybean binds in a sugar-specific manner to the corresponding rhizobial species but not to bacteria that are symbiotic to other legumes. However, a number of exceptions to this general pattern have been reported so that the lectin-recognition hypothesis continues to be a subject of controversy (170). Nevertheless, it is possible to alter the lectin-binding specificity by the transfer of genes essential for nodulation between plants. For example, the pea lectin gene has been introduced into white clover roots using *Agrobacterium rhizogenes* as a vector. The clover roots that resulted then could be nodulated by a *Rhizobium* usually specific for the pea (171). The transfer of the genes coding for the lectin of a N-fixing plant to nonlegumes remains an exciting challenge of obvious agricultural importance.

Various lines of evidence suggest that lectins may be involved in the defense of plants against insects, bacteria, fungi, and viruses (170,172). For example, the lectin from *Phaseolus vulgaris* was found to have a lethal effect on the larvae of the bruchid beetle (119), presumably due to the binding of the lectin to epithelial cells lining the midgut of this insect (173). As pointed out in Section II.B, however, it is believed that it is the amylase

inhibitor that is responsible for the resistance of the common bean to the cowpea weevil (121). A protein isolated from the seeds of a wild variant of *Phaseolus vulgaris* and referred to as arcelin was found to be toxic to the bruchid beetle, an important bean pest (174). It is related closely to the lectin found in most other bean cultivars at both the amino acid and nucleotide levels. The transfer of the arcelin allele to nonresistant bean cultivars by backcrossing or by the addition of purified arcelin to artificial seeds resulted in a high degree of insect resistance. The pea lectin has been expressed in the potato (175) and in the tobacco plant (176). In the latter case, the transgenic tobacco plant was found to have increased resistance to the tobacco bud worm; an additive protective effect was obtained with the introduction of the cowpea trypsin inhibitor (176). In addition to their role as a defensive measure against insects, the lectins from various plants also have been shown to inhibit the growth of phytopathogenic bacteria and fungi (177–179).

All of these studies suggest that plant lectins offer considerable promise for the genetic engineering of disease-resistant plants. Since this strategy would raise the possibility of increasing the toxicity of such plants, careful attention would have to be paid to the elimination of toxins by suitable processing techniques. Although the lectins as well as the protease inhibitors usually can be inactivated by proper heat treatment, the application of such industrial-scale technological methods as air classification, extraction, and texturization in the absence of heat may not be fully effective as a means of detoxification. The best insurance against this possibility is the careful monitoring of all newly introduced transgenic plants for the presence of antinutritional factors.

F. Analytical Techniques

Lectin activity is determined most commonly by measuring the degree to which erythrocytes from the blood of a given animal are agglutinated, the cells sometimes being sensitized by treatment with trypsin or some other protease. The simplest assay is one involving serial dilution in which the end point is determined by visual inspection of the clumped cells. While this method is rapid and simple, it gives only semiquantitative results. A spectrophotometric method has been proposed to increase the precision of such an assay (180). The most-serious limitation of an assay that depends on the agglutination of erythrocytes is the fact that one must choose the blood of an animal for which the lectin is specific. Improper selection of red blood cells may result in very low sensitivity or even negative results. Furthermore, there is no assurance that agglutinating activity *per se* bears any relevance to the *in vivo* effects of the lectin. Assuming that the toxicity

of lectins to a given animal species depends on the lectins' ability to bind to the intestinal mucosa, the most-relevant technique would be one that measures the degree to which a certain lectin binds to the epithelial cells of the target animal. Such a method has been proposed that involves an assay of the enzyme-linked immunosorbent assay (ELISA) type in which one measures the binding of an enzyme-linked lectin to preparations of the brush border membrane of the animal under study (181).

IV. TOXIC AMINO ACIDS

A. Neurotoxins

Lathyrism, as it is known to occur in humans, is a paralytic disease associated with the consumption of *Lathyrus sativus* (more commonly known as chickling or grass pea) or such related species as *L. clymenum* and *L. cicera.* Although this disease has been recognized since ancient times, today the disease is restricted to India, Bangladesh, and Ethiopia. It surfaces during periods of famine resulting from droughts during which field crops become blighted and, as alternative crops, these particular crops are cultivated. As recently as 1975, over 100,000 cases of lathyrism in men between the ages of 15 and 45 years were reported in India (182). The disease is characterized by a nervous paralysis of the lower limbs that forces the victim to walk with short, jerky steps; in extreme cases, death may result (183).

Spencer and Schaumberg have described an outbreak of lathyrism that occurred during World War II among Romanian Jews confined to a forced-labor camp in the Ukraine (184). For a period of 4 months, their daily ration consisted of 400 g of *L. sativus* peas cooked in salt water plus 200 g of bread. The neurological symptoms of this disease persist even today in those survivors who now live in Israel.

Attempts to identify the causative agent of human lathyrism have been complicated by the fact that the sweet pea (*L. odoratus*) produces another form of lathyrism (osteolathyrism) that is characterized in rats by skeletal deformities (185). This is in contrast to what is observed with rats who thrive quite well when they are fed *L. sativus* and do not display the nervous disorder associated with the consumption of this species in humans. Historically, the osteolathyrogen of the sweet pea was the first to be isolated and was identified as 3-aminopropionitrile (I) (186). See Fig. 12 for structures and distribution of the various lathyrogenic neurotoxins.

Several groups of workers in India (187–189) have succeeded in isolating a compound from *L. sativus* that may be the causative factor of human lathyrism. This compound, identified as 3-N-oxalyl-2,3-diaminopropionic acid (II), produced severe neurotoxic symptoms when injected into rats,

	Structure and Name	Occurrence
(I)	$N\equiv C-CH_2-CH_2-NH_2$	*Lathyrus odoratus*
	3-aminopropionitrile[a]	*L. pusillus*
		L. hirsutu
(II)	$HOOC-\overset{\overset{O}{\|\|}}{C}-NH-CH_2-\overset{\overset{NH_2}{\|}}{CH}-COOH$	*L. sativus*
	3-N-oxalyl-2,3-diamino-	*L. cicera*
	propionic acid[b]	*L. clymenum*
(III)	$H_2N-CH_2-CH_2-\overset{\overset{NH_2}{\|}}{CH}-COOH$	*L. latifolius*
	2,4-diaminobutyric acid[b]	*L. sylvestris*
(IV)	$N\equiv C-CH_2-\overset{\overset{NH2}{\|}}{CH}-COOH$	*Vicia sativa*
	3-cyanoalanine[b]	
(V)	$CH_3-NH-CH_2-\overset{\overset{NH_2}{\|}}{CH}-COOH$	*Cycas circinalis*
	3-N-methylaminoalanine[c]	

[a] osteolathyrogen

[b] neurolathyrogen

[c] possible cause of amyolateral sclerosis in man

FIGURE 12 Structures and distribution of lathyrogenic factors.

chicks, and monkeys. This compound, as well as 2,4-diaminobutyric acid (III) and 3-cyanoalanine (IV), also have been isolated from other *Lathyrus* species as well as *Vicia sativa* and have been shown to produce neurotoxic effects when administered by injection into several different species of ani-

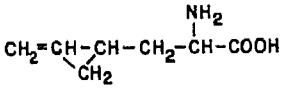
mals. However, attempts to reproduce neurolathyrism in animals by the oral administration of these various neurotoxic amino acids generally have proved unsuccessful (190,191); thus, the true causative agent of human lathyrism has not been established unequivocally.

A high incidence of amyotrophic lateral sclerosis (ALS) is known to occur among the residents of certain islands in the western Pacific (e.g., Guam and Rota). One of the traditional foods consumed by the natives in these islands is the seeds of the false sago palm (*Cycas circinalis*), which has led to the search for the toxic agent(s) in this plant that might be responsible for this neurological disorder. An unusual nonprotein amino acid, 3-N-methylamino-L-alanine (V) was first isolated from the cycad plant by Vega and Bell (192). It subsequently was shown by Spencer et al. that the repeated oral administration of this compound to macaques produced behavioral dysfunction and neuropathological changes that resembled the prominent features of ALS noted in Guam (193). However, since 3-N-methylamino-L-alanine is only one of several potential neurotoxins present in the cycad seed, it is premature to assign a causal role to any single factor until further research is completed (194).

B. Hypoglycin

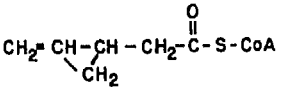
Consumption of the fruit of the plant *Blighia sapida* (known in Jamaica as ackee and in Nigeria as isin) has been linked to a disease of undernourished people, especially in Jamaica, known as vomiting sickness (see review by Kean, Ref. 195). This plant was named after Captain Bligh, who introduced it into the West Indies after he survived the mutiny on the Bounty. The characteristic symptom of violent vomiting that accompanies the consumption of the unripe fruit is followed by convulsions, coma, and even death in some instances. Hypoglycemnia is the principal clinical sign, with sugar levels as low as 20 mg per 100 ml of blood, compared to a normal value of 100 mg per 100 ml. The causative agent has been identified as beta-(methylenecyclopropyl)alanine and is referred to as hypoglycin A (Fig. 13). It also may occur as conjugate as the gamma-glutamyl dipeptide.

Hypoglycin follows essentially the same pattern of metabolism as branched-chain amino acids. Some of these intermediates are shown in Fig. 13. It first is deaminated to β-(methylenecyclopropyl) pyruvate, which then undergoes oxidative deamination to β-(methylenecyclopropyl)acetyl-CoA. Formation of this interferes with the transfer of long-chain fatty acyl CoA to carmitin, thus blocking the process of beta-oxidation. This results in an impairment in gluconeogenesis that is accompanied by depletion of stored glycogen and hypoglycemia. Because of its structural similarity to leucine, hypoglycin also may interfere with the metabolism of this amino acid. This

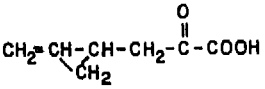


β-(Methylenecyclopropyl)alanine

(hypoglycin A)



β-(Methylenecyclopropyl)acetyl-CoA



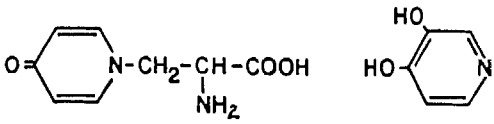
β -(Methylenecyclopropyl) pyruvate

FIGURE 13 Structure of hypoglycin A and some of the intermediates involved in its metabolism.

results in an accumulation of isovaleric acid and alpha-methylbutyric acid, amino acids that could act as depressants of the central nervous system (196). This could explain the syndrome of vomiting that accompanies the ingestion of akee. Hypoglycin also has been reported to produce teratogenic effects in rat and chick embryos (197).

C. Mimosine

The National Academy of Sciences has published a monograph that points out the potential value of the legume *Leucaena leucocephala* (called kao haole in Hawaii) as a forage crop for livestock and human feeding (198). One of the principal factors limiting the use of this plant, however, is the fact that an unusual amino acid, mimosine (Fig. 14), comprises 3%–5% of the dry weight of the protein. This amino acid is believed to be responsible for the poor growth performance of cattle when leucaena makes up more than one-half of the diet. This adverse effect on growth has been attributed to the underproduction of thyroxine, presumably due to the fact that the rumen bacteria convert mimosine to 3,4-dihydroxypyridine (Fig. 14), which acts as a goitrogenic agent (199). In nonruminants such as the horse, pig, and rabbit, the goitrogenic effect is not very marked. These animals nevertheless do very poorly on diets containing leucaena, one of the characteristic



Mimosine 3,4-Dihydroxypyridine

FIGURE 14 Structure of mimosine and its goitrogenic metabolite 3,4-dihydroxypyridine.

features being a loss of hair (200,201). It has been suggested, in fact, that mimosine might be used as a defleecing agent in sheep (202). Certain segments of the human population, particularly in Indonesia, are known to consume portions of the leucaena in their diets, and a loss of hair has been observed frequently among those individuals who have eaten the leaves, pods, and seeds in the form of a soup (203).

Although the goitrogenic effect of mimosine seems to be well established, the precise mechanism of toxicity in other animal species remains obscure. It can act as an inhibitor of pyridoxal-containing transaminases (204), tyrosine decarboxylase (205), and both cystathione synthetase and cystathionase (206). An inhibition of the last two enzymes may be of particular relevance since they play key roles in the conversion of methionine to cysteine, a major component of hair protein, and could account for the hair loss that is so characteristic of mimosine toxicity. Mimosine may exert a more-direct effect on hair growth since it has been reported that leucaena extracts destroyed the matrix of the hair follicles of mice (207).

Matsumoto et al. reported that the mimosine content of the seeds and leaves of leucaena could be decreased by storing the plant in temperatures in excess of 70°C in the presence of moisture (208). Yoshida showed that the addition of ferrous sulfate to the diet of rats fed leucaena leaf meal reduced mimosine toxicity, presumably due to a decrease in the absorption of this amino acid from the gastrointestinal tract (209).

D. Djenkolic Acid

In certain parts of Sumatra, particularly in Java, the djenkol bean is a popular item of consumption (203). The bean is actually the seed of the leguminous tree *Pithecolobium lobatum* and resembles the horse chestnut in size and color. Consumption of this seed sometimes leads to kidney failure, which is accompanied by the appearance of blood and white, needlelike clusters in the urine. These clusters have been identified as a sulfur-

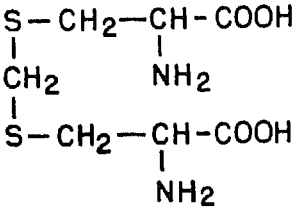


FIGURE 15 Structure of djenkolic acid, an amino acid present in *Pithecolobium lobatum*.

containing amino acid known as djenkolic acid (Fig. 15), which comprises 1%–4% of the seed (210). Despite its structural resemblance to cystine, it cannot replace cystine in the diet of rats, although it apparently can be metabolized by the animal body (211). However, because of its relative insolubility, much of the djenkolic acid escapes metabolic degradation and tends to crystallize out in the urine.

E. Dihydroxyphenylalanine

The amino acid 3,4-dihydroxyphenylalanine or dopa (Fig. 16) is present in fairly high concentrations in the fava bean (*Vicia faba*) (212–215), the velvet bean (*Stizolobium deeringianum*) (216), and wheat and oats (217). Since the consumption of the fava bean frequently is associated with a disease in humans known as favism, the question has been raised as to whether dopa might play a causative role in the etiology of this disease (218). Persons genetically deficient in the enzyme glucose-6-phosphate dehydrogenase appear to be particularly susceptible to this disease, and one of the characteristic clinical features of this disease is believed to be due to a marked lowering of the glutathione content of the erythrocytes (219). In view of these facts, it may be pertinent to note that the *in vitro* addition of dopa to the red blood cells from individuals deficient in glucose-6-phosphate dehydroge-

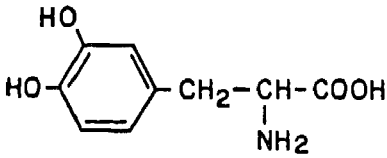


FIGURE 16 Structure of 3,4-dihydroxyphenylalanine (dopa), an amino acid present in the faba bean (*Vicia faba*), the velvet bean (*Stizolobium deeringianum*), and wheat and oats.

nase produced a significant lowering of the glutathione content of such cells (218). Because of its high content of dopa, it has been suggested that *Vicia faba* might be of therapeutic value in the treatment of Parkinson's disease (220).

F. Selenoamino Acids

Selenium poisoning in livestock due to the consumption of selenium-accumulating plants of the genus *Astragalus* has been well documented (221). The selenium of such plants is present in the protein in the form of selenomethionine, selenocysteine, and selenocystine residues (Fig. 17). Digestion of this protein in the digestive tract results in the liberation and absorption of these selenoamino acids and, because of their structural similarity to their natural sulfur analogues, they compete for the synthesis of animal protein. Defective proteins thus formed could account for the loss of hair and sloughing of hoofs, which are characteristic features of selenium poisoning in livestock.

Chronic selenosis in human beings, presumably caused by eating corn grown in seleniferous soil, has been reported in Colombia, South America (222). In certain parts of Venezuela, the ingestion of nuts from a tree known as coco de mono (*Lecythis ollaria*) produces a toxic syndrome in humans characterized by abdominal distress, nausea, vomiting, diarrhea, and loss of scalp and body hair. Using an assay system involving the measurement of the cytotoxicity activity against mouse fibroblasts, the factor responsible for this toxic effect was identified as selenocystathionine (223) (Fig. 17).

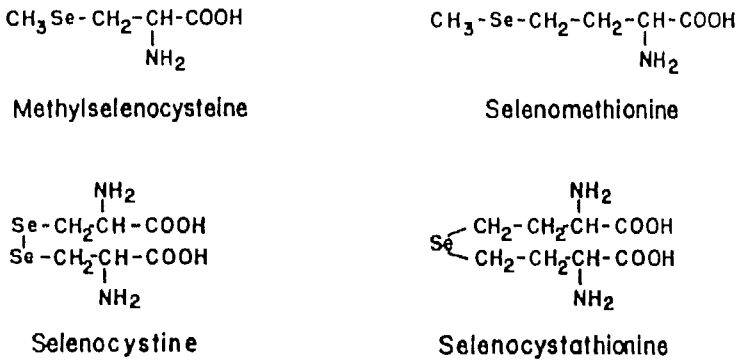


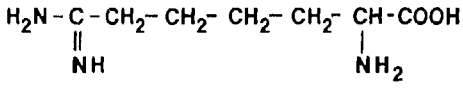
FIGURE 17 Structure of selenoamino acids.

G. Indospicine

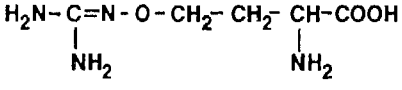
Indigofera spicata, or creeping indigo, is a tropical legume with potential value as a forage and soil-improvement crop. It is known to contain a toxic amino acid, indospicine, which is a structural analogue of arginine (224) (Fig. 18). This compound was shown to cause cirrhosis and other pathological changes of the liver when fed to rats, an effect that was attributed to its role as an antagonist of arginine (225). It also has been reported that indospicine may produce cleft palates in the fetuses of rats given a single oral dose of this compound on the 13th day of gestation (226).

H. Canavanine

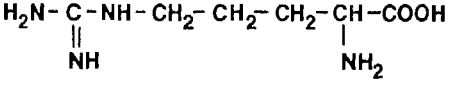
Canavanine, like indospicine, is an analogue of arginine (Fig. 18), and it occurs in high concentrations (up to 5%) in the seeds of the jack bean (*Canavalia ensiformis*), and in a number of other legumes in lesser amounts. Its role as a protective agent against insects has been discussed in detail by Rosenthal (227). Alfalfa sprouts contain about 1.5% of their dry weight of canavanine. A severe lupus erythematosus–like syndrome is produced in monkeys fed alfalfa sprouts, an effect that has been attributed to its canavanine content (228).



Indospicine



Canavanine



Arginine

FIGURE 18 Structures of indospicine and canavanine, antimetabolites of arginine found in creeping indigo (*Indigofera spicata*) and jack bean (*Canavalia ensiformis*), respectively.

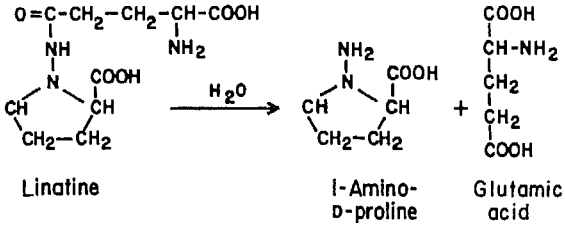


FIGURE 19 Structure of 1-amino-D-proline, the antipyridoxine factor of linseed (*Linium usitatissimum*) and its natural precursor, linatine.

I. Linatine

Flax or linseed (*Linium usitatissimum*) meal is considered a poor source of protein for chicks, but considerable improvement may be effected by extracting the meal with water and autoclaving for 30 min or by supplementation with pyridoxine (229,230). Klosterman and co-workers reported the isolation of a pyridoxine antagonist from flaxseed that was identified as 1-amino-D-proline, which occurs naturally in combination, via a peptide linkage, with glutamic acid (Fig. 19) (231). This peptide was given the name linatine. 1-Amino-D-proline was actually four times as toxic as linatine when injected into chicks, and its toxicity could be counteracted effectively by the simultaneous injection of pyridoxine. Sasaoka et al. carried out similar studies with rats and noted that 1-amino-D-proline caused marked changes in amino acid metabolism (232). These effects most likely are related to its effect on enzyme systems that require pyridoxal phosphate as a cofactor (233).

J. Lysinoalanine

Alkaline extraction of soybeans, which is used frequently in the preparation of soy protein isolates, is known to reduce the nutritive value of the protein, attributable at least in part to the destruction of cystine (234). One of the decomposition products of cystine is dehydroalanine (which also may be derived from the decomposition of serine), which can interact with the epsilon amino group of lysine to form lysinoalanine (Fig. 20). Alkali-treated soybeans produced kidney lesions in rats, an effect that could be reproduced by the administration of lysinoalanine (235,236).

Sternberg et al. have shown lysinoalanine to be widely distributed in cooked foods, commercial food preparations, and food ingredients, many of which had never been subjected to alkaline treatment (237). Many of these foods had levels of lysinoalanine that were considerably higher than

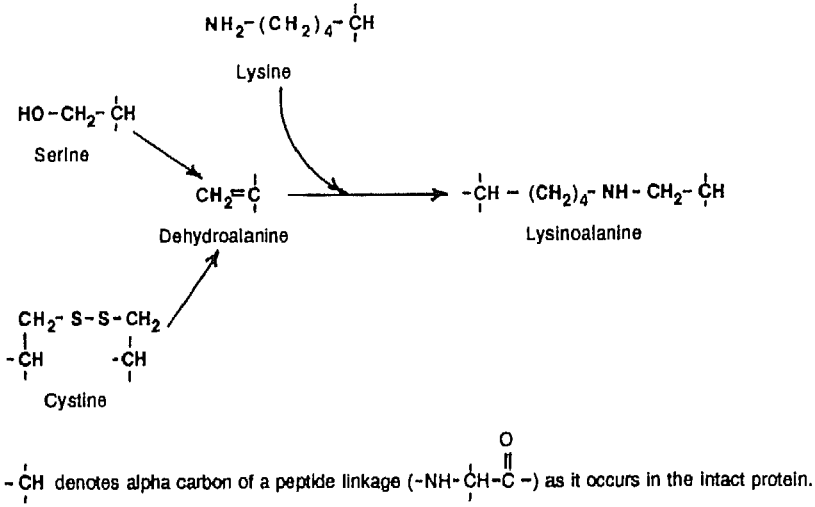


FIGURE 20 Formation of lysinoalanine from cystine (and/or serine) and lysine.

those found in commercial samples of soy protein isolate. The widespread distribution of lysinoalanine among commonly cooked foods would tend to indicate that this is neither a novel or serious problem because humans have been exposed to proteins containing lysinoalanine for a long time with apparent impunity. This conclusion is strengthened by a recent report that the feeding to preterm babies of a heat-processed infant milk formula containing high levels of lysinoalanine had no effect on their renal function (238).

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Volume 3

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