In vitro Digestibility of eCry3.1Ab Protein under Simulated Mammalian Gastric Conditions

Data Requirement: Not applicable

Author: Shuang Song

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Syngenta Study No.: TK0028111

Performing Laboratory: Syngenta Biotechnology, Inc.
Product Safety
3054 East Cornwallis Road
PO Box 12257
Research Triangle Park, NC 27709-2257, USA

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Company: *Syngenta Seeds, Inc.*

Company Representative:

Demetra Vlachos — October 22, 2010

Demetra Vlachos
Regulatory Affairs Manager

Date

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With the exceptions noted below, this study was conducted in compliance with the relevant provisions of Good Laboratory Practices Standards (40 CFR Part 160, US EPA 1989) pursuant to the Federal Insecticide, Fungicide, and Rodenticide Act.

- The SeeBlue® Plus2 molecular weight standard was characterized by the manufacturer, Invitrogen, Inc. (Carlsbad, CA, USA), prior to use in this study. This characterization was not conducted under Good Laboratory Practices Standards.
- The ImageQuant TL™ software, version 7.0 used during the conduct of this study was not validated according Good Laboratory Practices Standards.

Study Director:

October 22, 2010

Shuang Song
Technical Expert I
Product Safety
Syngenta Biotechnology, Inc.

Date

Submitted by:

October 22, 2010

Demetra Vlachos
Regulatory Affairs Manager
Syngenta Seeds, Inc.
3054 East Cornwallis Road
PO Box 12257
Research Triangle Park, NC 27709-2257, USA

Date

Sponsor:

October 22, 2010

Gerson Graser
Technical Leader I
Product Safety
Syngenta Biotechnology, Inc.

Date

QUALITY ASSURANCE STATEMENT

Study Title: *In vitro* Digestibility of eCry3.1Ab Protein under Simulated Mammalian Gastric Conditions

Study Director: Shuang Song

Study Number: TK0028111

Pursuant to Good Laboratory Practice Regulations (40 CFR Part 160), this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to Management and to the Study Director by the Quality Assurance Unit on the dates listed below.

Inspection/Audit Type	Inspection/Audit Dates	Reporting Dates
Audit Protocol	12-MAY-2010 - 12-MAY-2010	12-MAY-2010
Inspect Analytical	08-JUN-2010 - 08-JUN-2010	08-JUN-2010
Audit Study Data	26-AUG-2010 - 26-AUG-2010	26-AUG-2010
Audit Final Report, 1st audit	19-OCT-2010 - 19-OCT-2010	19-OCT-2010
Audit Final Report, 2nd audit	21-OCT-2010 - 21-OCT-2010	21-OCT-2010

Prepared By: Connie L. Connor Date: October 21, 2010

Connie L. Connor
Senior Quality Assurance Auditor
Syngenta Biotechnology, Inc.

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LIST OF ACRONYMS AND ABBREVIATIONS

Bis-Tris	bis(2-hydroxyethyl)-amino-tris(hydroxymethyl)methane
E. coli	*Escherichia coli*
EDTA	ethylenediaminetetraacetic acid
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
kDa	kilodalton
LDS	lithium dodecyl sulfate
LOD	limit of detection
mCry3A	modified Cry3A
MES	2-(*N*-morpholino)ethanesulfonic acid
mg	milligram
ml	milliliter
mM	millimolar
Mol. Wt	Molecular Weight
MW	molecular weight
ng	nanogram
PAGE	polyacrylamide gel electrophoresis
PVDF	polyvinylidene difluoride
SDS	sodium dodecylsulfate
SGF	simulated mammalian gastric fluid
US EPA	United States Environmental Protection Agency
µg	microgram

SUMMARY

The purpose of this study was to assess the *in vitro* digestibility of eCry3.1Ab in simulated mammalian gastric fluid (SGF). Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), Western blot and densitometry analyses were used to evaluate the *in vitro* digestibility of eCry3.1Ab in SGF over a 15 minute time course at 37°C.

The eCry3.1Ab protein degraded rapidly upon exposure to the pepsin enzyme in SGF. Intact eCry3.1Ab (molecular weight 74.8 kDa) was readily digested in less than 30 seconds, as assessed by SDS-PAGE analysis. Further analysis of the eCry3.1Ab protein bands in the SDS-PAGE gel by densitometry revealed that 3% and 0% of the eCry3.1Ab remained after incubation in SGF for 15 and 30 seconds, respectively. Following incubation in SGF for 30 seconds, no intact eCry3.1Ab or eCry3.1Ab-derived fragments were observed by Western blot analysis.

The results of this study support the conclusion that eCry3.1Ab is readily digested by the mammalian gastric enzyme, pepsin.

INTRODUCTION

The purpose of this study was to assess the *in vitro* digestibility of eCry3.1Ab in simulated mammalian gastric fluid (SGF). Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), Western blot and densitometry analyses were used to evaluate the *in vitro* digestibility of eCry3.1Ab in SGF over a 15 minute time course at 37ºC.

Syngenta has transformed maize (*Zea mays*) to produce Event 5307 maize, a new cultivar that has insecticidal activity against certain corn rootworm (*Diabrotica*) species. Maize plants derived from transformation Event 5307 contain the gene *ecry3.1Ab*, encoding the eCry3.1Ab protein. The eCry3.1Ab protein is an engineered chimera of the modified Cry3A (mCry3A) and Cry1Ab proteins.

MATERIALS AND METHODS

eCry3.1Ab Test Substance

The eCry3.1Ab test substance was prepared from a microbial expression system. A modified gene *ecry3.1Ab* was introduced into a vector derived from pET24a and transformed into *Escherichia coli* (*E. coli*) strain DH5α.

In June 2008, eCry3.1Ab was prepared from *E. coli* cell paste by Syngenta Protein Science, Jealott's Hill International Research Centre (Bracknell, UK). Briefly, *E. coli* cells were ruptured and the cell debris was removed by centrifugation. The eCry3.1Ab protein was further purified using immobilized metal affinity chromatography and anion exchange chromatography. The purified protein was pooled, concentrated, aliquoted and lyophilized. The resulting lyophilized powder was designated test substance ECRY3.1AB-0208. The test substance was shipped on dry ice to Syngenta Biotechnology, Inc. (Research Triangle Park, NC, USA), where it was stored at –20ºC ± 8°C.

Test substance ECRY3.1AB-0208 was the source of eCry3.1Ab in this study. Prior to this study, ECRY3.1AB-0208 was characterized in detail and determined to contain 89.6% eCry3.1Ab by weight; the molecular weight of eCry3.1Ab was determined to be 74.8 kDa (Nelson 2008). Additionally, eCry3.1Ab as contained in test substance ECRY3.1AB-0208 was previously shown to be biochemically and functionally equivalent to, and a suitable surrogate for, eCry3.1Ab as produced in Event 5307 maize plants (Nelson 2009). For use in this study, ECRY3.1AB-0208 was solubilized and diluted in 10 mM ammonium bicarbonate (pH 10.0).

In vitro Digestibility of eCry3.1Ab under Simulated Mammalian Gastric Conditions

The SGF digestibility assay was performed at 37°C ± 2°C over a 15 minute time course. Samples of the digestion reaction mixture were taken at 0, 15, 30 and 45 seconds, 1 minute, 1 minute 15 seconds, 1 minute 30 seconds, 1 minute 45 seconds, and 2, 3, 5, 10 and 15 minutes for analysis.

The SGF (USP 2000) was prepared the day of use and consisted of 2 mg/ml sodium chloride (pH 1.2) and 2,600 units/ml pepsin (Sigma-Aldrich Cat. No. P6887). The digestion reaction was initiated by the addition of eCry3.1Ab to SGF at a ratio of 1 ug eCry3.1Ab to 10 pepsin activity units (Thomas *et al.* 2004). The reaction mixture was incubated at 37°C ± 2°C. At each time point, an aliquot of the reaction mixture was removed and mixed with stop solution (described below) to terminate the reaction. The samples were then incubated for 10 minutes at 95°C ± 5°C in preparation for SDS-PAGE analysis.

The time zero sample was prepared by adding an aliquot of eCry3.1Ab to a mixture of SGF and stop solution, after which it was prepared for SDS-PAGE analysis as described above. Mixing SGF with stop solution to inactivate pepsin prior to the addition of eCry3.1Ab ensured an accurate time zero sample. This time zero sample served as an undigested control to which all samples were compared, allowing the digestion of eCry3.1Ab in SGF to be assessed over the time course.

The stop solution was a mixture of 200 mM sodium bicarbonate (pH 11.0) and the NuPAGE® LDS (lithium dodecyl sulfate) Sample Buffer. Upon the addition of the stop solution, pepsin was inactivated by the shift in the sample pH, which terminated the reaction. All samples were stored at -20°C ± 8°C until SDS-PAGE and Western blot analyses were conducted.

Table 1 details the composition of the SGF digestibility assay samples.

Assay Control Samples

Two controls were utilized in this study, an eCry3.1Ab control and an SGF control. The eCry3.1Ab control contained eCry3.1Ab and SGF without pepsin. This control examined the potential hydrolysis of eCry3.1Ab in SGF without pepsin over the 15 minute time course. The SGF control contained SGF and 10 mM ammonium bicarbonate (pH 10.0), the solvent used to solubilize and dilute the eCry3.1Ab. This control was examined to evaluate the potential for self-hydrolysis of pepsin over the 15 minute time course.

Both controls had the same concentration or amount of eCry3.1Ab or pepsin, respectively, as initially contained in the SGF digestibility assay samples. Both eCry3.1Ab and SGF controls were incubated at 37°C ± 2°C and sampled at zero and 15 minutes. Each control was combined with the stop solution and prepared for SDS-PAGE analysis in the same manner as described above for the SGF digestibility assay samples. All assay control samples were stored at -20°C ± 8°C until SDS-PAGE and Western blot analyses were conducted.

Table 1 details the composition of the assay control samples.

Table 1. SGF *in vitro* digestibility time course and control samples

Name	Composition	Time points examined	Purpose
SGF digestibility assay samples	eCry3.1Ab, SGF	Time zero (undigested control), 15, 30 and 45 seconds, 1 minute, 1 minute 15 seconds, 1 minute 30 seconds, 1 minute 45 seconds, and 2, 3, 5, 10 and 15 minutes	Examined the *in vitro* digestibility of eCry3.1Ab in SGF
eCry3.1Ab control	eCry3.1Ab, SGF without pepsin	Time zero and 15 minutes	Examined the potential hydrolysis of eCry3.1Ab in SGF without pepsin
SGF control	10 mM ammonium bicarbonate (pH 10.0)[1], SGF	Time zero and 15 minutes	Examined the potential for self-hydrolysis of pepsin over the 15 minute time course

[1]10 mM ammonium bicarbonate (pH 10.0) was the solvent in which eCry3.1Ab was solubilized and diluted

Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis Analysis

Equivalent volumes of each SGF digestibility assay sample were subjected to SDS-PAGE under reducing conditions using a 4-12% Bis-Tris gel and 2-(*N*-morpholino)ethanesulfonic acid (MES) running buffer. Based on the concentration of eCry3.1Ab and the amount of pepsin at the initiation of the digestion reaction, these volumes were equivalent to 0.5 µg eCry3.1Ab and 5 units of pepsin (a ratio of 1 ug eCry3.1Ab to 10 pepsin activity units). Volumes of the control samples, containing either 0.5 µg eCry3.1Ab or 5 units of pepsin, were also subjected to SDS-PAGE. The molecular weight standard was the SeeBlue® Plus2 pre-stained standard. The gel was stained with Coomassie® blue and examined for the presence of bands consistent with the molecular weight of eCry3.1Ab (74.8 kDa).

The limit of detection (LOD) is defined as the lowest amount of analyte in a sample that can be detected. The LOD for the SDS-PAGE was determined by subjecting serial dilutions of eCry3.1Ab to SDS-PAGE. Amounts tested were 0.1, 0.025, 0.0063, 0.0016, and 0.0004 µg of eCry3.1Ab. The gel was stained with a Coomassie® stain, and the lowest amount of eCry3.1Ab visible on the gel was designated the LOD of eCry3.1Ab for SDS-PAGE.

Densitometry Analysis

The densitometry analysis was performed to quantitatively analyze the remaining eCry3.1Ab at each time point during the time course. For the densitometry analysis, the Coomassie® blue stained gel obtained from the SDS-PAGE analysis was scanned on a laser densitometer and the resulting image was analyzed using the ImageQuant TL™ software, version 7.0. The eCry3.1Ab band of each SGF digestibility assay sample was

selected and quantified through measuring the densitometric volume[1] of the band. The percent of remaining eCry3.1Ab relative to the amount of eCry3.1Ab at time zero was then calculated for each time point of the time course.

Western Blot Analysis

Equivalent volumes of each SGF digestibility assay sample were subjected to SDS-PAGE. Based on the the concentration of eCry3.1Ab and the amount of pepsin at the initiation of the digestion reaction, these volumes were equivalent to 10 ng eCry3.1Ab and 0.1 units of pepsin (a ratio of 1 ug eCry3.1Ab to 10 pepsin activity units). Volumes of the control samples, containing either 10 ng eCry3.1Ab or 0.1 units of pepsin, were also subjected to SDS-PAGE. The molecular weight standard was SeeBlue® Plus 2 pre-stained standard. Proteins were transferred to a polyvinylidene difluoride (PVDF) membrane *via* electroblotting. The membrane was probed with a polyclonal goat antibody capable of detecting eCry3.1Ab. An alkaline phosphatase conjugated donkey anti-goat antibody was used to bind to the primary antibody. The protein was visualized by developing the blot with an alkaline phosphatase substrate solution. The Western blot was visually examined for the presence of intact immunoreactive eCry3.1Ab or other immunoreactive eCry3.1Ab-derived fragments.

The LOD for the Western blot analysis was determined by subjecting serial dilutions of eCry3.1Ab to SDS-PAGE. Amounts tested were 1.6, 0.4, 0.1, 0.024, and 0.006 ng eCry3.1Ab. The protein was transferred to a PVDF membrane and then probed with the same antibodies used to monitor the SGF assay. The lowest amount of eCry3.1Ab visible on the membrane was designated the LOD of eCry3.1Ab for the Western blot analysis.

Statistical Analysis

No statistical analysis was required for any parameter evaluated in this study.

[1] Densitometric volume is the integrated intensity of all the pixels in the band, excluding the background.

RESULTS AND DISCUSSION

Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis Analysis

The SDS-PAGE analysis results (Figure 1) demonstrate that eCry3.1Ab is readily digested when incubated in SGF. A very faint band corresponding to intact eCry3.1Ab (74.8 kDa) was visible following incubation in SGF for 15 seconds (Lane 8). However, this band was no longer visible in the digestibility assay sample taken following incubation in SGF for 30 seconds (Lane 9), indicating that eCry3.1Ab is readily digested in SGF in less than 30 seconds.

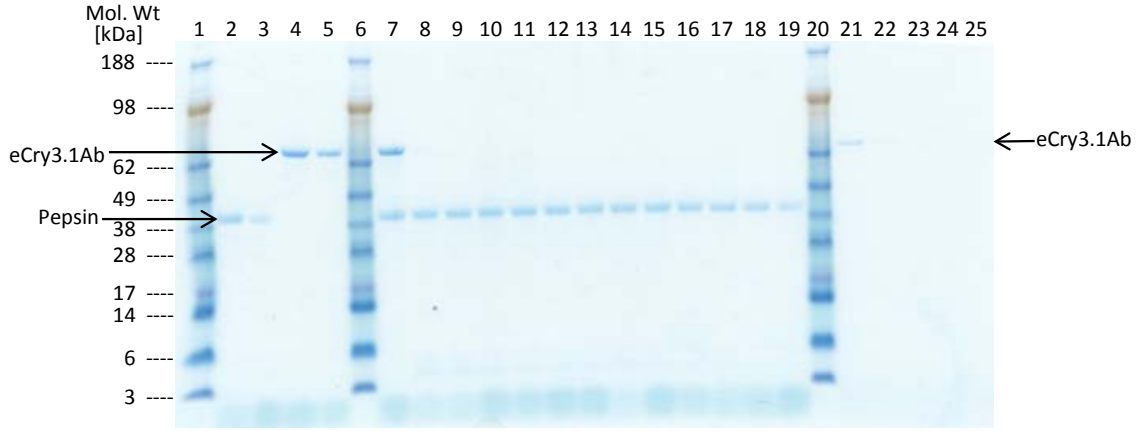
Two very faint, diffuse bands with molecular weights of approximately 4 kDa and 5 kDa, respectively, were visible after incubation in SGF for 15 seconds (Lane 8). These two bands diminished in intensity over the time course and were no longer detectable after incubation in SGF for 10 minutes (Lane 18).

The intensity of the pepsin band (34.6 kDa) slightly decreased after incubation of the SGF solution alone (SGF control) for 15 minutes (Lanes 2 and 3), indicating minor degradation of pepsin. The eCry3.1Ab protein incubated in SGF without pepsin (eCry3.1Ab control) showed no significant degradation over 15 minutes (Lanes 4 and 5), which indicates that the hydrolysis of eCry3.1Ab, seen in the SGF samples (Lanes 7 through 19), can be attributed to pepsin.

Similar band intensities were visualized for the time zero digestibility assay sample (Lane 7) and the eCry3.1Ab time zero control (Lane 4), confirming that equal amounts of eCry3.1Ab were applied to the SDS-PAGE.

The lowest amount of eCry3.1Ab visible on the gel (Lanes 21 through 25) was 0.025 µg (Lane 22). Therefore, the LOD of eCry3.1Ab for the SDS-PAGE used in this study was determined to be 0.025 µg.

Figure 1. SDS-PAGE analysis of eCry3.1Ab following digestion in SGF



Lane 1: molecular weight standard
Lane 2: SGF control – time zero
Lane 3: SGF control – 15 minutes
Lane 4: eCry3.1Ab control (eCry3.1Ab in SGF without pepsin) – time zero
Lane 5: eCry3.1Ab control (eCry3.1Ab in SGF without pepsin) – 15 minutes
Lane 6: molecular weight standard
Lane 7: *in vitro* digestibility assay - time zero[2]
Lane 8: *in vitro* digestibility assay - 15 seconds[2, 3, 4]
Lane 9: *in vitro* digestibility assay - 30 seconds[2]
Lane 10: *in vitro* digestibility assay - 45 seconds[2]
Lane 11: *in vitro* digestibility assay - 1 minute[2]
Lane 12: *in vitro* digestibility assay - 1 minute 15 seconds[2]
Lane 13: *in vitro* digestibility assay - 1 minute 30 seconds[2]
Lane 14: *in vitro* digestibility assay - 1 minute 45 seconds[2]
Lane 15: *in vitro* digestibility assay – 2 minutes[2]
Lane 16: *in vitro* digestibility assay – 3 minutes[2]
Lane 17: *in vitro* digestibility assay – 5 minutes[2]
Lane 18: *in vitro* digestibility assay – 10 minutes[2]
Lane 19: *in vitro* digestibility assay – 15 minutes[2]
Lane 20: molecular weight standard
Lane 21: 0.1 µg eCry3.1Ab for LOD determination
Lane 22: 0.025 µg eCry3.1Ab for LOD determination[4]
Lane 23: 0.0063 µg eCry3.1Ab for LOD determination
Lane 24: 0.0016 µg eCry3.1Ab for LOD determination
Lane 25: 0.0004 µg eCry3.1Ab for LOD determination

The molecular weight of eCry3.1Ab is 74.8 kDa. The molecular weight of pepsin is 34.6 kDa[5].

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[2] The lanes selected for the eCry3.1Ab band detection and quantification by densitometry analysis (Table 2).
[3] Due to the resolution limits of the scanner and the printer, the very faint bands (approximately 4 kDa and 5 kDa) visible on the actual gel might not be visible on the printed image that appears here.
[4] Due to the resolution limits of the scanner and the printer, the very faint band (74.8 kDa) visible on the actual gel might not be visible on the printed image that appears here.
[5] The 34.6 kDa pepsin band showed slightly lower mobility and therefore appeared to have a higher apparent molecular weight (MW) when compared to the MW standards on the gel. The difference between the expected and observed MWs can be explained by the limitations of SDS-PAGE for accurate MW determination. Dube and Flynn (1988) reviewed the reliability of SDS-PAGE for MW determinations and concluded that the apparent MW of a protein by this method is typically within 10% of its true MW. This depends greatly on the similarity between the properties of the protein of interest and the proteins in the standard set (Sadeghi *et al*., 2003).

Densitometry Analysis

The densitometry analysis of the Coomassie® blue stained gel (Figure 1) confirms that eCry3.1Ab is readily digested in SGF in less than 30 seconds. After incubation of eCry3.1Ab in SGF for 15 and 30 seconds, 3% and 0% of the eCry3.1Ab remained, respectively (Table 2).

Table 2. The eCry3.1Ab remaining at each time point as determined by densitometry analysis

Lane (in Figure 1)	Time point	Densitometric volume	Percent (%) of eCry3.1Ab remaining[1]	Amount (µg) of eCry3.1Ab remaining[2]
7	Time zero	373.9	100	0.50
8	15 seconds	12.3	3	0.02
9	30 seconds	0.7	0	0
10	45 seconds	1.0	0	0
11	1 minute	0.6	0	0
12	1 minute 15 seconds	1.1	0	0
13	1 minute 30 seconds	0.7	0	0
14	1 minute 45 seconds	1.2	0	0
15	2 minutes	0.7	0	0
16	3 minutes	0.8	0	0
17	5 minutes	0.6	0	0
18	10 minutes	1.4	0	0
19	15 minutes	0.9	0	0

[1] Percent (%) of eCry3.1Ab remaining relative to the amount of eCry3.1Ab at time zero as determined by densitometry.
[2] The amount of eCry3.1Ab at time zero was equivalent to 0.5 µg (Materials and Methods).
The amount of remaining eCry3.1Ab at each time point was calculated as follows:
amount of eCry3.1Ab remaining = amount of eCry3.1Ab at time zero (0.5 µg) × percent (%) of eCry3.1Ab remaining
This calculation was based on the fact that equivalent volumes of each SGF digestibility assay sample were subjected to SDS-PAGE (Materials and Methods).

Western Blot Analysis

The Western blot analysis results (Figure 2) confirm that eCry3.1Ab is readily digested in SGF in less than 30 seconds. After incubation of eCry3.1Ab in SGF for 30 seconds (Lane 9), no protein bands representing either intact eCry3.1Ab or eCry3.1Ab-derived fragments were visible.

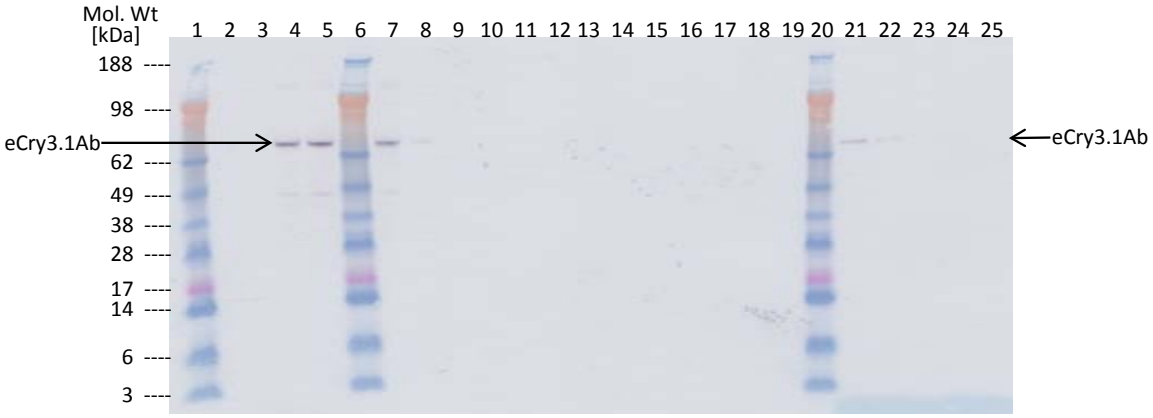
A very faint band with an approximate molecular weight of 150 kDa was visible in the eCry3.1Ab control samples (Lanes 4 and 5) and the time zero digestibility assay sample (Lane 7). This protein cross-reacted with the antibody capable of detecting eCry3.1Ab and displayed a mobility consistent with the molecular weight of two eCry3.1Ab molecules (150 kDa). Therefore, it most likely represents a dimer of eCry3.1Ab. An additional faint band with an approximate molecular weight of 47 kDa was also visible in the time zero digestibility assay sample (Lane 7). This band cross-reacted with the antibody capable of detecting eCry3.1Ab and was also present in the eCry3.1Ab control (Lanes 4 and 5). This suggests that the 47 kDa band most likely corresponds to a minor eCry3.1Ab hydrolysis product derived from the sample and is not related to pepsin digestion. Both bands (150 kDa and 47 kDa) were no longer detectable following exposure to SGF for 15 seconds (Lane 8).

The eCry3.1Ab protein incubated in SGF without pepsin (eCry3.1Ab control) showed no significant degradation over 15 minutes (Lanes 4 and 5), which indicates that the hydrolysis of eCry3.1Ab, seen in the SGF samples (Lanes 7 through 19), can be attributed to pepsin.

Similar band intensities were visualized for the time zero digestibility assay sample (Lane 7) and the eCry3.1Ab time zero control (Lane 4), confirming that equal amounts of eCry3.1Ab were applied to the SDS-PAGE and electroblotted.

The lowest amount of eCry3.1Ab visible on the blot (Lanes 21 through 25) was 0.4 ng (Lane 22). Therefore, the LOD of eCry3.1Ab for the Western blot used in this study was determined to be 0.4 ng.

Figure 2. Western blot analysis of eCry3.1Ab following digestion in SGF



Lane 1: molecular weight standard
Lane 2: SGF control – time zero
Lane 3: SGF control – 15 minutes
Lane 4: eCry3.1Ab control (eCry3.1Ab in SGF without pepsin) – time zero[6]
Lane 5: eCry3.1Ab control (eCry3.1Ab in SGF without pepsin) – 15 minutes[6]
Lane 6: molecular weight standard
Lane 7: *in vitro* digestibility assay - time zero[6]
Lane 8: *in vitro* digestibility assay - 15 seconds[7]
Lane 9: *in vitro* digestibility assay - 30 seconds
Lane 10: *in vitro* digestibility assay - 45 seconds
Lane 11: *in vitro* digestibility assay - 1 minute
Lane 12: *in vitro* digestibility assay - 1 minute 15 seconds
Lane 13: *in vitro* digestibility assay - 1 minute 30 seconds
Lane 14: *in vitro* digestibility assay - 1 minute 45 seconds
Lane 15: *in vitro* digestibility assay – 2 minutes
Lane 16: *in vitro* digestibility assay – 3 minutes
Lane 17: *in vitro* digestibility assay – 5 minutes
Lane 18: *in vitro* digestibility assay – 10 minutes
Lane 19: *in vitro* digestibility assay – 15 minutes
Lane 20: molecular weight standard
Lane 21: 1.6 ng eCry3.1Ab for LOD determination
Lane 22: 0.4 ng eCry3.1Ab for LOD determination[7]
Lane 23: 0.1 ng eCry3.1Ab for LOD determination
Lane 24: 0.024 ng eCry3.1Ab for LOD determination
Lane 25: 0.006 ng eCry3.1Ab for LOD determination

The molecular weight of eCry3.1Ab is 74.8 kDa.

[6] Due to the resolution limits of the scanner and the printer, the very faint bands (47 kDa and 150 kDa) visible on the actual blot might not be visible on the printed image that appears here.
[7] Due to the resolution limits of the scanner and the printer, the very faint band (74.8 kDa) visible on the actual blot might not be visible on the printed image that appears here.

Data Quality and Integrity

No circumstances occurred during the conduct of this study that would have adversely affected the quality or integrity of the data generated.

CONCLUSIONS

The eCry3.1Ab protein degraded rapidly upon exposure to SGF. Intact eCry3.1Ab was digested in SGF in less than 30 seconds as assessed by SDS-PAGE analysis. Further analysis of the eCry3.1Ab protein bands in the SDS-PAGE gel by densitometry revealed that 3% and 0% of the eCry3.1Ab remained after incubation in SGF for 15 and 30 seconds, respectively. Following incubation in SGF for 30 seconds, no intact eCry3.1Ab or eCry3.1Ab-derived fragments were observed by Western blot analysis. The results of this study support the conclusion that eCry3.1Ab is readily digested by the mammalian gastric enzyme, pepsin.

RECORDS RETENTION

Raw data, the original copy of this report, and other relevant records are archived at Syngenta Biotechnology, Inc., 3054 East Cornwallis Road, Research Triangle Park, NC 27709-2257, USA.

CONTRIBUTING SCIENTISTS

The analytical work reported herein was conducted by Ian Kietzman, B.S. and Shuang Song, M.S. at Syngenta Biotechnology, Inc., 3054 East Cornwallis Road, Research Triangle Park, NC 27709-2257, USA.

CRITICAL DATES

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Experimental end date: August 31, 2010

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