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A Comparative Study of Histamine Release from Rat Mast Cells by Cry1Aa, Cry1Ab and Cry1Ac Fragmented with Simulated Gastric Fluid (SGF).

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Activated Cry1A were digested for 15 min with simulated gastric fluid (SGF) in different pH. SGF digestive activity differed among Cry1A. Only Cry1Aa was completely fragmented, but the fragmentation pattern was similar on Cry1Aa and Cry1Ac. The effect of Cry1A fragments on histamine release from the cultured rat mast cells (RBL2H3) also varied among Cry1A. Activated Cry1A did not induce histamine release whereas Cry1Aa and Cry1Ab fragments did. These results suggest that a few different amino acid residues exposed on those fragments may affect histamine release. However the final destination of those fragments in intestinal juice should be assessed to obtain a comprehensive conclusion.

Introduction

Cry toxins produced by *Bacillus thuringiensis* have been widely used as highly specific insecticides and as source of insect resistance in transgenic plants. In the future we can expect the reduction of chemical pesticide usage by *B. thuringiensis* formulations. However, in 2000, the so called "StarLink" problem occurred and the transgenic corn expressing Cry9Ca was doubted to have potential to cause allergies (1). As it is now clear that the symptoms were caused by corn proteins and not by Cry toxins, the patients might have had allergy against corn proteins. But the consumers started to avoided genetically-modified crops. For public acceptance, safety assessment of Cry toxins is very important. It is known that histamine, which is a major cause of the allergic symptoms, was released by stimulation of mast cells through IgE antibody and not through IgE (2)(3). We hypothesized that histamine release may be caused by pore formation on mast cells by Cry toxin, based on analogy of the mode of action of Cry toxin against insects. Therefore, we focused our research on whether Cry1A induces histamine release by stimulation without throughing IgE.

Materials and methods

Activated Cry1A digestion with SGF at various pH. Each 20 µg of trypsin activated Cry1Aa, Cry1Ab and Cry1Ac were digested in 100 µl of the pre-warmed digestion mixture containing 40 µl of simulated gastric fluid (SGF; 0.32% (w/v) pepsin, 0.2% (w/v) NaCl, 0.7% (v/v) HCl) for 15 min. Digestion mixture was adjusted to various pH by 1M buffer to mimic pH of gastric juice which often change from 2 to 6 after ingestion of food (4). The reaction was stopped by adding Na_2CO_3 up to a final pH of approximately 7. Digests of Cry1A were separated by SDS-PAGE with 15 % polyacrylamide gel, and detected by western blot analysis using six antisera raised against various parts of Cry1Aa such as α-2,3, α-4,5, α-6,7, β-1-5, β-6-11 and domain III.

Reaction of activated Cry1A and digests of Cry1A with the cultured rat mast cell (RBL2H3) and quantification of histamine. About 1 x 10^6 of RBL2H3 cells (5) were reacted with 200 µg of Cry1A or digests of Cry1A in a ml of Tyrode buffer for 60 min at 37 ◦C. Reaction mixture was centrifuged at 270 × g for 5 min and supernatant was recovered. Histamine released into the supernatant was extracted and quantified as described previously (6). Amount of histamine released was expressed as % of whole histamine amount in RBL2H3 cells which was obtained when they were totally destroyed by sonication.

Results

Western blot analysis of Cry1A digests. Activated Cry1Aa was completely fragmented in SGF digestion for 15 min at pH 2.0, but undigested Cry1Ab and Cry1Ac remained (Fig.1). The fragmentation pattern of Cry1Aa and Cry1Ac was shown to be similar each other and 12 and 15 kDa peptides were dominant. Very few fragments, however, were not detected by anti α-6,7, β-1-5,

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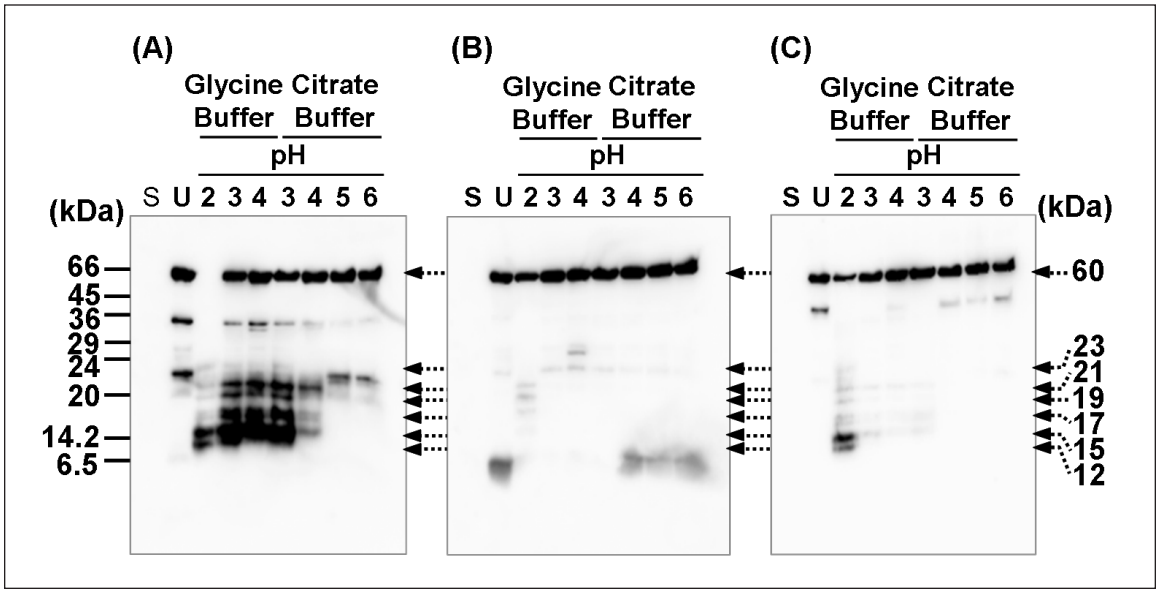

FIG. 1. Cry1A digestion with SGF at various pH. (A) Cry1Aa, (B) Cry1Ab and (C) Cry1Ac of digests were detected with western blot analysis using anti α-4,5 helices antiserum. LaneS, SGF only; LaneU, Untreated Cry1A; another numbers, pH of digestion mixture.

β-6-11 and domain III antisera. Both the 12 and 15 kDa peptides should be proteolytic resistant ones containing α-2-5 helices. On the other hand, in Cry1Ab, 12 and 15kDa peptides were hardly detected, suggesting that both peptides were digested further. These results indicated that the digestive activity of SGF against Cry1A differed among them and domain I may be more resistant than the other domains. Thin 39 kDa peptide shown in LaneS must be pepsin.

The evaluation of histamine release activity of digests of Cry1A resulted by SGF on RBL2H3 cells. Activated Cry1Aa, Cry1Ab andCry1Ac did not induce histamine release as well as BSA (Fig.2). However, when RBL2H3 cells were reacted with Cry1Aa digests obtained by SGF digestion at pH2 and pH4, the amount of histamine release significantly increased. Cry1Ab digests also induced histamine release but did not seem to depend on the digestion pH.

Discussion

In the initial phase of our research, we hypothesized that there were two mechanisms for histamine release. One is direct pore formation by Cry1A fragments. The other is interaction between mast cells and Cry1A fragments without pore formation. Interestingly, three results were revealed in histamine release assay: 1) All activated Cry1A did not induce histamine release, 2) Histamine release activities differed between Cry1Aa and Cry1Ac, in spite of the similar digestion patterns at pH 2, and 3) Cry1Ab digested at pH 6 induced histamine release. These results suggested that histamine was released by stimulation of Cry1A fragments without pore formation. We would like to propose the mechanism of histamine release as follows. Cry1A fragments obtained by the digestion are almost the same in size. These fragments, however, have micro-heterogeneity in N and C -terminal amino acid residues and some fragments among digests may induce histamine release by interaction with mast cells such as Ca^{2+} movement, but others may not.

In our study, it was established that activated Cry1A did not induce histamine release from the cultured rat mast cells. This suggests that activated Cry1A must be safe even if Cry1A acts directly on mast cells in various mucous membranes of human bodies through insecticide sprays, pollen derived from transgenic plants and so on. However, Cry1A digests obtained by SGF digestion could induce histamine release. Generally, a substance which induces 20% or more histamine release is judged to be an allergen, thus, Cry1Aa fragments were thought to trigger allergy-like symptoms. In actual digestive tract, after gastric juice digestion, these Cry1A fragments are attacked further by intestinal juices. Further comprehensive investigations should warrant a comprehensive conclusion.

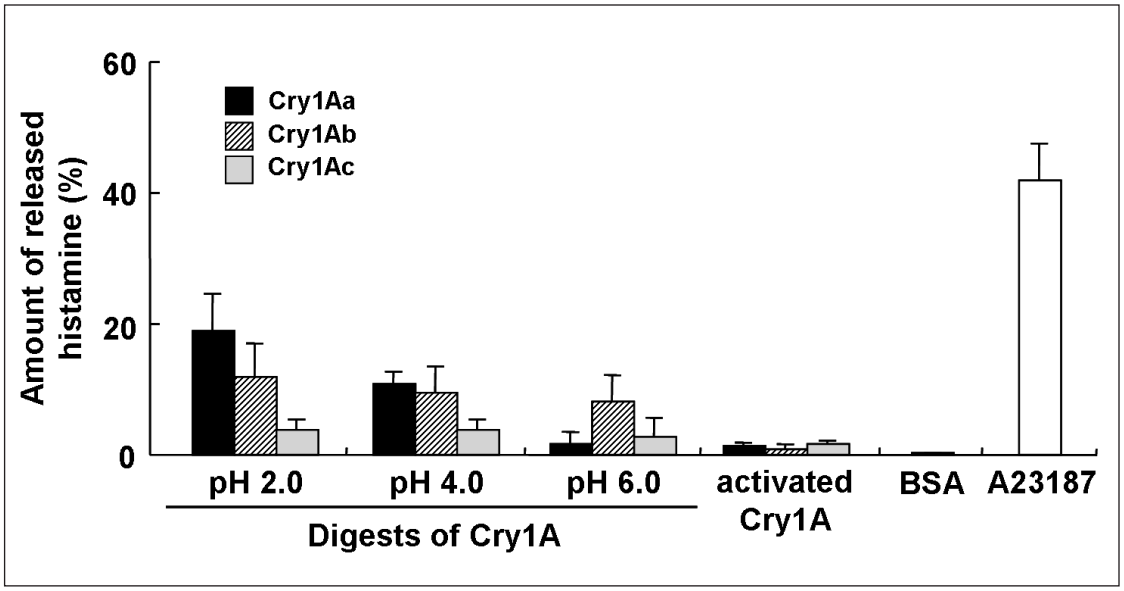

FIG. 2. Histamine release assay. The amount of released histamine was calculated in ratio of whole histamine.

References

1. **SAFER • HEALTHIER • PEOPLE.** 2001. Investigation of Human Health Effects Associated with Potential Exposure to Genetically Modified Corn. A Report to the U.S. Food and Drug Administration from the Centers for Control and Prevention.
2. **Sampson, H. A.** 2003. Food allergy. J. Allergy Clin. Immunol. **111:** S540-547.
3. **Aas, K.** 1978. What makes an allergen an allergen. Allergy **33:**3-14.
4. **James, A. H., and G. W. Pickering.** 1949. The role of gastric acidity in the pathogenesis of peptic ulcer. Clin. Sci. **8:**181.
5. **Mizuno, K., N. Nakahata, and Y. Ohizumi.** 1998. Characterization of mastoparan-induced histamine release from RBL-2H3 cells. Toxicon. **36:**447-456.
6. **Shore, P. A., A. Burkhalter, and V. H. Cohn.** 1959. A method for the fluorometric assay of histamine in tissues. J. Pharmacol. Exp. Therapeut. **127:**182-186.