NOTE ON MODIFICATION OF THE KUNITZ SOYBEAN TRYPSIN INHIBITOR DURING SEED GERMINATION

R. C. FREED and D. S. RYAN, Department of Food Science, University of Wisconsin, Madison, WI 53706

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A number of workers (1–3) have examined the changes in legume seed proteins that occur during germination. Changes in proteinase inhibitors during germination have been of particular interest, since these proteins might be involved in regulating storage protein mobilization during germination or might simply be storage proteins that are degraded during germination (4). Several reports of the appearance or disappearance of electrophoretically distinct proteinase inhibitors have appeared (5,6). Pusztai (5) has reported that a number of the proteinase inhibitory proteins of kidney bean change independently of each other during germination. Some proteinase inhibitors disappear completely during germination, while others increase; some new bands with inhibitory activity that are not present in the dry seed appear. Orf et al (6) showed the appearance of a new band with proteinase inhibitory activity during germination of soybean seeds and suggested that it may be a modified form of the Kunitz inhibitor. Our research confirms the observations of Orf et al (6), and establishes the immunochemical identity of the newly formed proteinase inhibitor with the Kunitz soybean trypsin inhibitor.

MATERIALS AND METHODS

Glycine max (L.) var. Steele soybeans (lot LA 1-4) were obtained from Olds Seed Co., Madison, WI. The Harosoy, T-245, and PI 246.367 varieties were obtained from the U.S. Regional Soybean Laboratory, Urbana, IL.

Antibody production and rocket immunoelectrophoresis were as previously described (7). Discontinuous polyacrylamide gel electrophoresis was done using the buffer system that Davis (8) described and a 10% slab gel in an apparatus obtained from Hoefer Scientific Instruments, San Francisco. Gels were stained with coomassie blue, destained by diffusion, and dried onto filter paper before photography. Seeds were washed, rinsed with 10% Clorox® (The Proctor & Gamble Co., Cincinnati), and soaked for 5 hr in deionized water at 30° C before germination at 25° C in the dark. Seeds were sprayed twice daily with deionized water to prevent dehydration.

RESULTS AND DISCUSSION

Discontinuous gel electrophoresis of crude 0.01*N* NaOH extracts of soybean seeds after various periods of germination (Fig. 1) shows that a number of different changes take place in the seed proteins. Some bands decrease, others increase, and some show little change. The decrease in intensity of the band, which is known to be the Kunitz soybean trypsin inhibitor, is somewhat surprising, since rocket immunoelectrophoresis assay has shown that the Kunitz

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inhibitor content of seeds does not decrease dramatically during germination (7). The appearance of a new band with electrophoretic mobility slightly lower than that of the Kunitz inhibitor, which increases in intensity as the Kunitz inhibitor decreases in intensity, suggests that this new band may be related to the Kunitz inhibitor.

Figure 2 shows rocket immunoelectrophoresis assays of individual slices from disc gels of one-day and nine-day germinated Steele soybean seeds. This assay is specific for the Kunitz soybean trypsin inhibitor; other proteinase inhibitors in the seed do not cross-react. The one-day germinated seed (like the dry seed) contains only one band that is recognized by antibodies to the Kunitz inhibitor. The nine-day germinated seed, however, contains two bands that are both recognized in the rocket immunoelectrophoresis system. Crossed immunoelectrophoresis of nine-day germinated samples (results not shown) showed two precipitin bands that completely fused together, suggesting that the two are immunochemically identical.

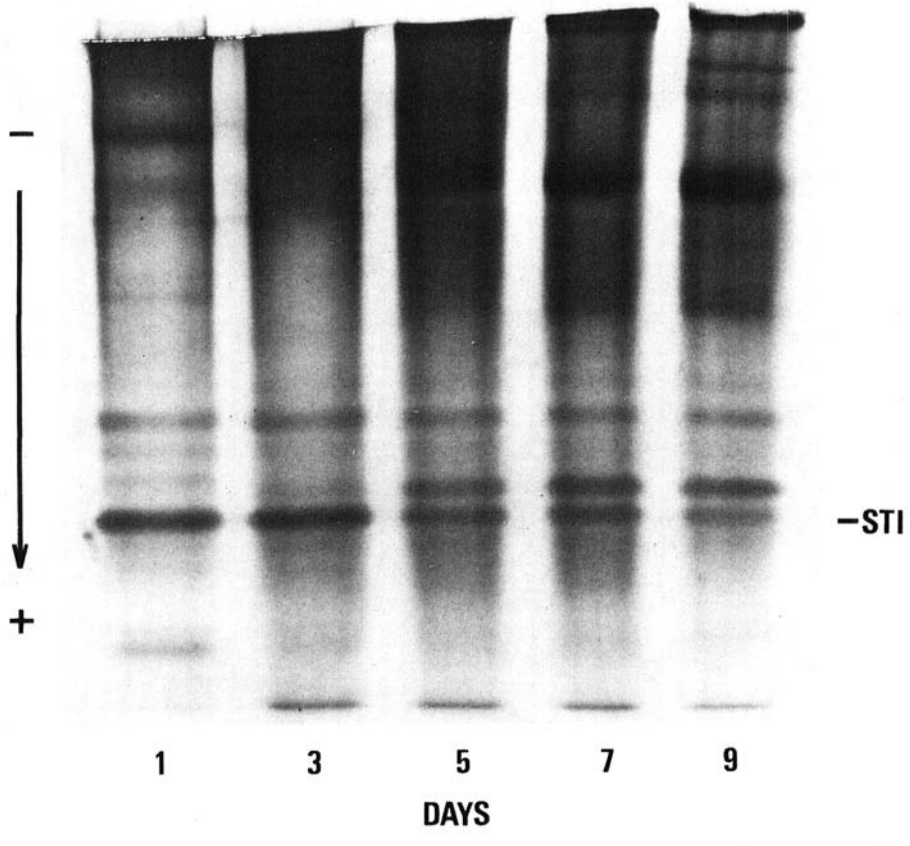

Fig. 1. Discontinuous gel electrophoresis of 0.01*N* NaOH extracts of *Glycine max* (L.) var. Steele after various periods of germination. Line at right indicates location of Kunitz soybean trypsin inhibitor.

Some workers have used discontinous gel electrophoresis or similar tools either to determine the number of inhibitors present in the seed or to examine the changes in inhibitors during germination (5,6). Our results suggest that caution should be exercised in evaluating results from experiments of this kind. The results of enzyme inhibitor assays of slices from disc gels of germinating soybean seeds, for example, suggest that there are two inhibitors with relative mobility near the Kunitz inhibitor, one that decreases and one that increases in intensity during germination. Our results suggest, however, that only a single inhibitor migrates in this region of the gel, that it occurs in two electrophoretically distinct but immunochemically identical forms, and that its content (the sum of both forms) does not change dramatically during germination.

The nature of the new form of Kunitz trypsin inhibitor that appears during germination is not known. Its electrophoretic mobility is similar to that of a naturally occurring genetic variant of the Kunitz inhibitor (9,10), and it seemed possible at least that the new form and the genetic variant could be structurally identical. As shown in Fig. 3, however, soybean varieties that have Kunitz trypsin inhibitors with different electrophoretic mobilities also show the appearance of a

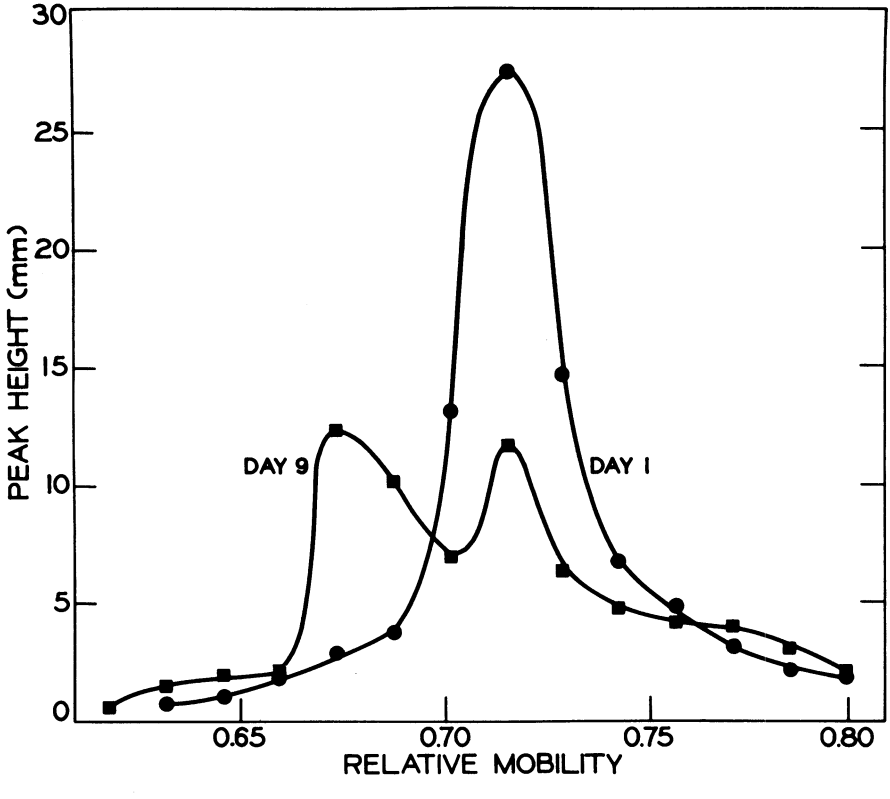

Fig. 2. Rocket immunoelectrophoresis measurement of Kunitz trypsin inhibitor content of slices from disc gels of one-day and nine-day germinated Steele soybean seeds.

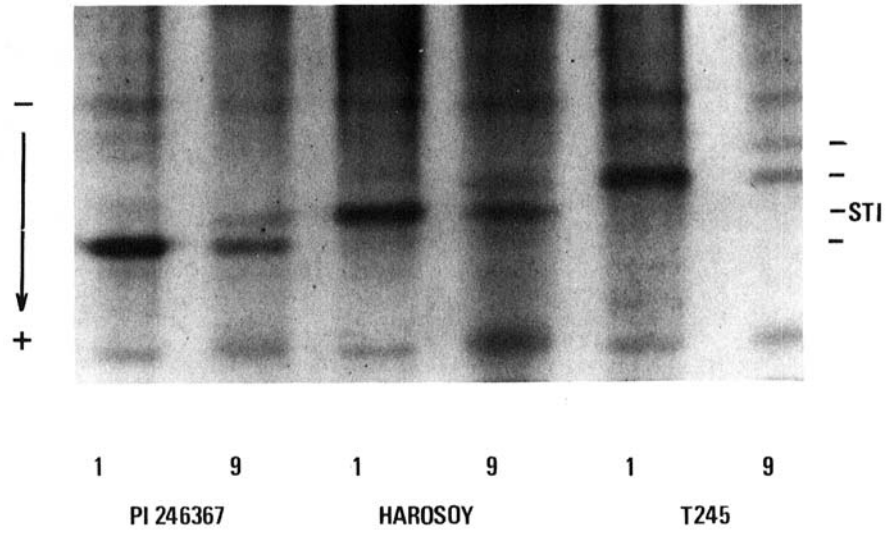

Fig. 3. Discontinuous gel electrophoresis of 0.01*N* NaOH extracts of one-day germinated and nine-day germinated soybeans of different varieties. Unmodified Harosoy Kunitz inhibitor has a mobility identical to that of unmodified Steele Kunitz inhibitor (Fig. 1) and is labeled as STI on right. Other lines at right indicate either genetic variants of STI or modified forms of STI appearing after germination.

new band of slightly lower mobility during germination. The mobility of Harosoy Kunitz inhibitor is the same as that of the Kunitz inhibitor from Steele seeds (Fig. 1).

Orf et al (6) has suggested that the new form of the Kunitz inhibitor may be a proteinase-modified molecule. While this is possible, it seems improbable since a trypsin-modified Kunitz inhibitor has an electrophoretic mobility higher than that of the unmodified molecule (11). This likely would be the case for any proteinase modification, since peptide bond hydrolysis results in the production of a new carboxyl group that would be unprotonated at the alkaline pH of the usual discontinuous gel electrophoresis system. It also seems unlikely that the new form is a complex with an endogenous proteinase, since the band retains inhibitory activity (6) and since any proteinase-proteinase inhibitor complex would probably have a molecular weight at least twice that of the free inhibitor and therefore a significantly altered mobility. Whatever the difference between the two forms of the inhibitor, the antigenic determinants on the molecule do not appear to be affected.

Acknowledgments

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