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The nutritional significance of plant protease inhibitors

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It has been recognized for many years that the nutritive value and protein digestibility of many plant proteins, particularly those derived from legumes, are very poor unless subjected to cooking or some other form of heat treatment (Liener & Kakade, 1969). This beneficial effect of heat has been generally attributed, at least in part, to the destruction of a unique class of proteins which have the ability to combine in a very specific fashion with the enzymes (trypsin and chymotrypsin) which play a key role in the digestion of proteins in the intestinal tract of animals. Elucidation of the precise manner in which these so-called protease inhibitors lead to growth inhibition, however, has proved to be more elusive than might be suggested by this simple concept. The situation is further complicated by the fact that factors other than the protease inhibitors may affect the digestibility of dietary proteins. Interest in the nutritional role of the protease inhibitors has continued to mount largely as a consequence of the recent introduction of texturized vegetable proteins as a possible substitute for meat protein in the human diet. This paper will address itself to a consideration of the mode of action of the protease inhibitors and the implications this may have with respect to the consumption of plant proteins by humans.

Mode of action

Because of the important role which the soya bean has assumed in the feeding of animals and its potential contribution to the human diet, it is understandable why the protease inhibitors from that plant have received the most attention. It was not long after soya beans were first introduced into the United States, primarily as a source of oil, that Osborne & Mendel (1917) made the significant observation that soya beans had to be heated in order to support the growth of rats. With the discovery of a heat-labile inhibitor of trypsin (Ham & Sandstedt, 1944; Kunitz,

1945) and the demonstration of its ability to inhibit the growth of animals (Liener *et al.* 1949), it was generally assumed that the trypsin inhibitor was largely responsible for the poor nutritive value of raw soya beans.

Although the most logical explanation for the growth inhibition evoked by the trypsin inhibitor would be the fact that it interfered with the normal digestive reactions in the intestinal tract, the true explanation has not proved to be that simple. For example, adding the soya-bean trypsin inhibitor to a diet containing predigested protein still led to an inhibition of growth (Liener *et al.* 1949), thus ruling out an inhibition of intestinal proteolysis as being directly responsible for growth inhibition. Perhaps the most significant observation which has ultimately led to a better understanding of the mode of action of the trypsin inhibitor was the finding that rats and chicks fed raw soya beans or purified preparations of the inhibitor developed an enlarged pancreas (Chernick *et al.* 1948) resulting in an increased secretion of pancreatic enzymes (Gertler *et al.* 1967). Lyman & Lepkovsky (1957) were the first to suggest that the growth depression caused by the trypsin inhibitor might be the consequence of an endogenous loss of essential amino acids produced by the hypersecretory activity of the pancreas. Since pancreatic enzymes are particularly rich in the sulphur-containing amino acids, pancreatic hypertrophy serves to divert the supply of these amino acids from the synthesis of body tissue to the synthesis of pancreatic enzymes which are irretrievably lost by excretion. This loss in the S-containing amino acids accentuates an already critical situation with respect to soya-bean protein which is inherently deficient in these amino acids. It is not surprising, therefore, that methionine supplementation will effectively counteract much of the growth depression caused by raw soya beans despite the persistence of pancreatic hypertrophy (Booth *et al.* 1960).

The mechanism whereby the trypsin inhibitor actually causes pancreatic hypertrophy is still not fully understood. Green & Lyman (1972) have suggested that the degree of pancreatic secretion is determined by the level of free trypsin present at any given time in the intestine. As the level of trypsin drops below a certain threshold level, the pancreas is induced to produce more enzyme, and conversely, when the level of trypsin is restored to normal levels, the secretory activity of the pancreas is inhibited. The agent directly responsible for these effects is believed to be the pancreas-stimulating hormone, cholecystokinin (CCK), whose release from the intestinal mucosa is inhibited by free trypsin (Wilson *et al.* 1978). It is obvious from these considerations that any set of circumstances that leads to a reduction of free trypsin in the intestines, such as complexation with an inhibitor (or with dietary protein, see p. 111), will serve to release CCK resulting in a hyperactive pancreas.

Other factors affecting the digestibility of protein

If the trypsin inhibitor is indeed the major factor responsible for the poor growth of animals fed on raw soya beans, then it should be possible to reduce the nutritive value of heated soya beans to that of raw soya beans by adding the same level of

antitryptic activity to heated soya beans as is present in the raw product. That this is not the case was demonstrated a number of years ago (Liener *et al.* 1949). Furthermore, examination of over 100 varieties of soya beans revealed the absence of any correlation between trypsin inhibitor activity and PER, although PER and the size of the pancreas were significantly related in an inverse fashion (Kakade *et al.* 1972). It would appear, therefore, that there must be present in raw soya beans some other factor, totally unrelated to the trypsin inhibitor, which is also causing pancreatic hypertrophy as well as an inhibition of growth. This situation was clarified when it was found that removal of protease inhibitors from unheated soya-bean extracts by affinity chromatography on Sepharose-bound trypsin produced only a 40% improvement in growth and reduction in the size of the pancreas compared to heat treatment (Kakade *et al.* 1973).

The above findings raise the question as to what is responsible for the remaining 60% of the growth-retarding and pancreatic hypertrophic effects of raw soya beans. A comparison of the in vitro digestibility of raw soya-bean protein from which the protease inhibitors had been removed by affinity chromatography with a heat treated control revealed that the latter was more readily digested by trypsin (Kakade *et al.* 1973). This observation suggests that native soya-bean protein is in itself resistant to digestion by trypsin unless denatured by heat. A related observation is the fact that the isolated globular proteins of *Phaseolus vulgaris* are also very resistant to attack by proteolytic enzymes (Seidl *et al.* 1969; Thompson & Liener, 1978). If undenatured protein is in fact capable of binding trypsin by forming an enzyme-substrate complex, as suggested by Green *et al.* (1973), this could also serve to remove the feed-back inhibition of pancreatic secretion by trypsin and thus cause hypertrophy of the pancreas.

Another factor which may influence the digestibility of the proteins of legumes are the lectins (Liener, 1974). Jaffé & Camejo (1961) have shown the lectin of black bean can reduce the digestibility of dietary protein presumably by interfering with the ability of the intestinal mucosal cells to absorb nutrients.

Physiological significance in humans

It should be appreciated that most of the experiments dealing with the nutritional effects of the protease inhibitors have involved the use of the rat or the chick. What can be said about the relevance of such experiments to the human diet which may contain plant proteins as a potential carrier of these inhibitors?

Many of the soya-bean products intended for human consumption are manufactured from protein isolates which, depending on their mode of preparation, may contain as much as 30% of the inhibitor activity of the original raw bean. An examination of the trypsin inhibitor activity of several textured meat analogs reveals that, although the protein isolate from which they were made may be rich in antitryptic activity, the final products generally contain less than 10% of the activity of raw soya-bean flour (Liener, 1975). Churella *et al.* (1976) have likewise shown that the heat treatment involved in the processing and sterilization of infant soya-bean formulas reduced the trypsin inhibitor activity to less than

10% of the activity of the original isolate. This residual activity did not produce any weight reduction or pancreatic hypertrophy in rats. These observations are consistent with the findings of Rackis *et al.* (1975) who found no pancreatic hypertrophy in rats fed soya-bean flour in which only 54% of the trypsin inhibitory activity had been destroyed. Although a further enhancement in growth is produced when more of the inhibitor was destroyed, this can be attributed to an increase in protein digestibility *per se* rather than to a further destruction of the inhibitor.

Assuming for the moment that processing conditions may have been inadequate to reduce the level of trypsin inhibitor activity below that of the threshold level established for rats, would this activity still pose a risk to human health? Human trypsin is known to exist in two forms, a cationic species, which is the major component of human pancreatic juice, and an anionic species, which comprises about 10 to 20% of the total trypsin activity (Figarella *et al.* 1975). While the latter is fully inactivated by the soya-bean inhibitor, the predominant cationic species is only weakly inhibited (Figarella *et al.* 1974).

In further support of the probability that the soya-bean inhibitor is relatively ineffective against human trypsin is the rather interesting relationship that appears to exist between the size of the pancreas of various species of animals and their sensitivity to pancreatic hypertrophy induced by raw soya beans or the inhibitor (Liener, 1977). The pancreas of those species of animals whose weight exceeds 0.3% of their body-weight become hypertrophic when fed raw soya beans, whereas those whose weights are below this value are insensitive to this effect. Since man has a pancreas which is 0.09 to 0.12% of his body-weight (Long, 1961), one would predict that the human pancreas would be insensitive to the effects of the soya-bean trypsin inhibitor.

Role of trypsin inhibitors in other legumes

To what extent the protease inhibitors account for the poor nutritive value of plants other than soya beans is difficult to assess. Inhibitors which have been purified from the lima bean (Klose *et al.* 1949) and peanut are capable of inhibiting the growth of rats whereas those isolated from *Dolichos lablab* (Phadke & Sohonie, 1962) and maize (Mitchell *et al.* 1976) do not. Nevertheless, it may be significant to note that the trypsin inhibitors of many legumes are quite rich in cystine, and, may in fact account for about 30 to 40% of the total cystine content of some bean proteins (Kakade *et al.* 1969). It is conceivable, therefore, that a dietary loss of cystine from the inhibitor itself could contribute in a significant fashion to the poor nutritive value of these legumes in their native unheated state. Kakade *et al.* (1969) have indeed shown that the cystine of the unheated navy-bean protease inhibitor is only approximately 45% available to the chick compared to 76% availability for the heat-inactivated inhibitor. Thus the protease inhibitors of some legumes may be a double-edged sword; they not only reduce the digestibility of the protein and cause pancreatic hypertrophy, but may also 'lock-in' a significant fraction of the

total cystine content of the protein which is already limiting in the S-containing amino acids.

REFERENCES

Booth, A. N., Robbins, D. J., Ribelin, W. E. & De Eds, F. (1960). *Proc. Soc. exp. Biol. Med.* **104**, 681.
Chernick, S. S., Lepkovsky, S. & Chaikoff, I. L. (1948). *Am. J. Physiol.* **155**, 33.
Churella, H. R., Yao, B. C. & Thompson, W. A. B. (1976). *J. agric. Fd Chem.* **24**, 393.
Figarella, C., Negri, G. A. & Guy, O. (1974). In *Bayer Symposium on Proteinase Inhibitors*, p. 213 [H. Fritz, H. Tschesche, L. J. Greene and E. Truscheit, editors]. Berlin: Springer-Verlag.
Figarella, C., Negri, G. A. & Guy, O. (1975). *Eur. J. Biochem.* **53**, 457.
Gertler, A., Birk, Y. & Bondi, A. (1967). *J. Nutr.* **91**, 358.
Green, G. M. & Lyman, R. L. (1972). *Proc. Soc. exp. Biol. Med.* **140**, 291.
Green, G. M., Olds, B. A., Mathews, G. & Lyman, R. L. (1973). *Proc. Soc. exp. Biol. Med.* **142**, 1162.
Ham, W. E. & Sandstedt, R. M. (1944). *J. biol. Chem.* **154**, 505.
Jaffé, W. G. & Camejo, G. (1961). *Acta cient. venez.* **12**, 59.
Kakade, M. L., Arnold, R. L., Liener, I. E. & Waibel, P. E. (1969). *J. Nutr.* **99**, 34.
Kakade, M. L., Hoffa, D. E. & Liener, I. E. (1973). *J. Nutr.* **103**, 1772.
Kakade, M. L., Simons, N. R., Liener, I. E. & Lambert, J. (1972). *J. agric. Fd Chem.* **20**, 87.
Klose, A. A., Hill, B., Greaves, J. D. & Fevold, H. L. (1949). *Archs Biochem.* **23**, 215.
Kunitz, M. (1945). *Science* **101**, 668.
Kwaan, H. C., Kok, P. & Astrup, T. (1968). *Experientia* **24**, 1125.
Liener, I. E. (1974). *J. agric. Fd Chem.* **22**, 17.
Liener, I. E. (1975). In *Protein Nutritional Quality of Foods and Feeds*, p. 528 [M. Friedman, editor]. New York, NY: Dekker.
Liener, I. E. (1976). *J. Fd Sci.* **41**, 1076.
Liener, I. E. (1977). In *Biochemical Aspects of New Protein Food*, p. 129 [J. Alder-Nissen, editor]. New York, NY: Pergamon Press.
Liener, I. E., Deuel, H. J. Jr. & Fevold, H. L. (1949). *J. Nutr.* **39**, 325.
Liener, I. E. & Kakade, M. L. (1969). In *Toxic Constituents of Plant Foodstuffs*, p. 7 [I. E. Liener, editor]. New York, NY: Academic Press.
Long, C. (1961). *Biochemists Handbook*, p. 675. Princeton, N. J.: D. Van Nostrand.
Lyman, R. L. & Lepkovsky, S. (1957). *J. Nutr.* **62**, 269.
Mitchell, H. L., Parrish, D. B., Corney, M. & Wassom, C. E. (1976). *J. agric. Fd Chem.* **24**, 1254.
Osborne, T. B. & Mendel, L. B. (1917). *J. biol. Chem.* **32**, 369.
Phadke, K. & Sohonie, K. (1962). *J. scient. Res., Djakarta* **21C**, 272.
Rackis, J. J., McGhee, J. E. & Booth, A. N. (1975). *Cereal Chem.* **52**, 85.
Seidl, D., Jaffé, M. & Jaffé, W. G. (1969). *J. agric. Fd Chem.* **17**, 1318.
Thompson, R. M. & Liener, I. E. (1978). *Fedn Proc. Fedn Am. Socs exp. Biol.* **37**, 278.
Wilson, P. A., Melmed, R. N., Hampe, M. V. & Holt, S. J. (1978). *Gut* **19**, 260.