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Mechanism of Action, Toxicity and Nutritional Significance of Heat-Labile Antinutritional Factors in Some Legumes: A Review

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Abstract: Legumes which are widely consumed all over the world have constituent that depress their utilization. Some of these constituents called antinutritional factor, trypsin inhibitor and haemagglutinin, are heat-labile. Trypsin inhibitor inhibits growth by interfering with the digestion of protein in the intestine of animals thereby causing hypertrophy of the pancreas. Unless legumes are heated before consumption, methionine would be slowly liberated by the proteolytic enzymes and its absorption delayed. Because iectins are capable of agglutinating red blood cells, intake of or improperly cooked legumes is deleterious. Phytohaemagglutinins are known to exert a non-selective adverse effect on the absorption of nutrients form the intestinal tract rather than a direct effect on the digestive process.

Key words: Mechanism of action, toxicity and nutritional significance, antinutritional factors, Nigeria

INTRODUCTION

Most edible Iegumes have constituents referred to as antinutritional factors (Leiner and Kakade, 1969; Oke *et al*., 1990, 1996). While some of these factors are susceptible to heat treatment, some are heat-resistant at varying degrees (Oke *et al*., 1996). Heat-labile ones have been identified to be trypsin inhibitors and lectins.

It has been recognized for many years that the nutritive value and protein digestibility of legumes are very poor unless subjected to cooking or some other form of heat treatment (Leiner, 1962). This depression in protein value and digestibility has been generally attributed to the presence of protease inhibitor (Leiner and Kakade, 1969). For the fact that legumes are widely consumed all over the world and also fed to livestock, it becomes imperative to have some vital information about them. This study would therefore, address pertinent information on mechanism of action, toxicity and nutrition significance of trypsin inhibitor and phytohaemagglutinin which are heat-labile. Mechanism of action of trypsin inhibitor.

The ubiquity of Proteinase Inhibitor (Pl) in the plant kingdom led the discovery of two types of PI known as the Kunitz and Bowman-Birk inhibitors (Ramshaw, 1992). It has been revealed (Reiner and Kakade, 1982) unlike the Kunitz inhibitor that the structural rigidity provided by so many disulphide bridges most likely accounts for the resistance of Bowman-Birk inhibitor to the rather severe treatment with heat, acid, alkali, as well as action of pepsin and papain. However, when treated with 8M urea mixed with marcaptoethanol, the activity of the inhibitor was completely abolished. The ability of the Bowman- Birk inhibitors to inhibit both trypsin and chymotrypsin simultaneously led to their description (Norton *et al*., 1985) as double-headed or polyvalent. Information abound that Kunitz inhibitor from soybean seeds has a single polypeptide chain composed of about 200 amino acid residues devoid of methionine. This confirms the presence of very few disulphide bridges in Kunitz inhibitor.

The most logical explanation for the growth inhibition produced by PI would be that they interfere with the digestion of proteins in the intestinal tract of animals. It has been found (Leiner, 1980) that if the soybean inhibitor was added to pre-digested protein, it led to an inhibition of intestinal proteolysis, which caused hypertrophy of the pancreas due to exogenous loss of protein as a result of repeated intake of inhibitors. This represents one of the primary physiological factors responsible for the poor growth response on a diet that contains raw legume seeds. It was further observed (Nowacki and Walker, 1973) that removal of trypsin inhibitor by affinity chromatography on sepharose-bound trypsin effected a 38% increase in protein efficiency ratio and a corresponding decrease of 40% in the size of the pancreas as compared to the unheated soy extract. It was found that trypsin inhibitor itself accounted for only about 40% of the growth inhibition and pancreatic effect of raw soybean seeds.

TOXICITY OF TRYPSIN INHIBITOR

The manner in which purified proteinase inhibitors combine with certain enzymes has been the object of considerable study because it provides an excellent model system for studying protein-protein interaction. Early studies (Kunitz, 1947) had shown that combination of the inhibitor with trypsin was accompanied by a decrease in the sum of the free-amino group, thus suggesting that the interaction occurred through ionic groups.

It has been observed (Finkenstadt and Laskowski, 1965, 1967) that the first stage in the interaction of trypsin and Kunitz inhibitor involved the specific cleavage of arginly-isoleucine bond that lies within a disulphide loop of the Kunitz inhibitor. This modified inhibitor was still active but removal of the newly formed C-terminal residue by treatment with carboxypeptidase B produced an inactivated derivative. The reduction of the modified inhibitor and its subsequent carboxymethylation led to two inactive fragments, one having 64 (Hayward and Hafner, 1941; Evans and McGinnins, 1948; Leiner *et al*., 1949; Barners *et al*., 1962) have been undertaken to determine whether supplementing the raw protein with various amino acids would achieve the same effect as heating. Such experiments showed that addition of methionine or cystine to unheated soybean meal improved protein untilisation to essentially the same extent as proper heating but it is important to note that additional mechionine will not raise the nutritive value of raw soy to the level of heated soybean similarly supplemented with methionine. This is an indication that the anti-tryptic factor is solely localized in the soybean protein. On the basis of experiments involving the *in vitro* release of amino acids from soybean protein by pancreatin, it was suggested (Melnick *et al*., 1946) that methionine of raw soybean was liberated more slowly by the proteolytic enzymes of the intestines than the other essential amino acids. As a result, the absorption of methionine was delayed and it was not available for mutual supplementation of the remainder of the other amino acids. This concept was supported by Kunitz's (1947) discovery of a heat-labile trypsin inhibitor in raw soybeans and that active anti-tryptic fractions from raw soybeans were capable of inhibiting the growth of rats (Leiner *et al*., 1949) and chicks (Ham *et al*., 1945).

MECHANISM OF ACTION AND TOXICITY OF LECTINS

Another heat-labile substance that appears to be distributed universally among legumes is a glycoprotein with the unique property of being able to agglutinate red blood cells. It is called phytohaemagglutinin, although originally coined to denote those plant agglutinins which showed blood group-specificity, the term lectin is now applied to blood group-specific and blood group non-specific agglutinins of both plant and invertebrate origin (Jeffe *et al*., 1947, 1980a). Lectins have been found to elicit impairment of the Kreb's cycle by interfering with the synthesis of citric acid through the inhibition of citric synthase activity; intensive inflammation of the lymphatic tissues characterized by destruction of the epithelial cells, hyperemia and haemorrhage; necrosis of the liver with fatty degeneration; the myocard may show degenerative lesions while capillaries of all organs may be extended and filled with blood clots and a rise in blood values of urea, glucose, bilirubin, transaminases and lactic dehydrogenases in experimental animals injected with ricin (Jeffe, 1980b; Dirheimer *et al*., 1946). Albumin and haematuria were also detected leading to the conclusion that a hepatonephritis with hepatic cytolysis as an early manufestation of toxicity. As a result of a catalogue of toxic physiological effects of lectin, it could be advanced that the reaction between the agglutinin and cell membrane could result in an alteration of the cell function. The reduced intestinal absorption caused by orally ingested haemagglutinins could have resulted from the combination of lectin with the carbohydrate moiety of substances present in the cell lining the intestinal wall thus causing non-specific interference with absorption. Following the ingestion of raw kidney beans, acute gastroenteritis with nausea, vomiting, diarrhoea and abdominal pain were observed in both infants and adults because lectins can combine with the carbohydrate moiety of substances present in the cells lining the intestinal wall thus causing non-specific interference with absorption attesting to the inability of humans to tolerate lectin-rich foods that have not been adequately heated (Kushawa and Tawar, 1973; Noah, 1980).

NUTRITIONAL SIGNIFANCE OF LECTINS

Digestibility measurements performed in rats fed a diet containing small amounts of isolated black bean lectin showed low food absorption and nitrogen retention in these animals (Jaffe, 1950) due to reduced glucose absorption and inhibition to protein synthesis in a cell-free system by inactivating some components essential for the elongation of peptides. Nutritional significance of lectins would be appreciated if method of inactivation are appraised. However, practical application of detoxification is limited to thermal treatment that has been known since the discovery of this antinutritional factor lending weight to the fact that it is heat-labile.

Infact, the pioneering research (Stillmark, 1989) in which the poisonous action of ricin was destroyed by heat stimulated the interest of investigators. The nutritive value of many legumes is enhanced by autoclaving but preliminary soaking before autoclaving is also effective (Jehkins, 1963; Gardener *et al*., 1960; Kakade and Evans, 1965; Bender, 1983; Oke *et al*., 1996).

Having confirmed the effectiveness of autoclaving in the removal of trypsin inhibitor and haemagglutinin in cowpeas (Oke *et al*., 1996) what is perhaps of importance is the question of cowpea protein digestibility and hence amino acid availability.

CONCLUSION

Though cowpea is widely consumed in the developing countries, its nutritional potential is yet to be elucidated implying that isolation of protein and subsequent determination of its digestibility seem neglected. This challenge would serve as an outlook for the future and also pave the way for cowpea inclusion in livestock diets especially monogastric animals.

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