Lectin-Tannin Interactions and Their Influence on Pancreatic Amylase Activity and Starch Digestibility

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Interactions of red kidney bean lectin and tannic acid and their individual and combined effects on the digestion of starch by pancreatic α-amylase were investigated. The interaction of tannic acid and lectin resulted in a loss in lectin hemagglutinating activity. Individually lectin and tannic acid could inhibit the activity of pancreatic α-amylase and thus reduce starch digestion. A combination of lectin and tannic acid had no significant inhibitory effect on the α-amylase, indicating that in combination their antinutritional effects toward this enzyme may become abolished. The effects of individual antinutrients may not always relate to the effects observed with consumption of a mixture of antinutrients as commonly encountered in foods.

INTRODUCTION

Different foods possess different nutritional properties, and seeds of certain legumes, including red kidney bean, have been shown to result in some adverse effects following consumption, particularly in the raw state, by man and animals (Public Health Laboratory Service, 1976; Bender and Reaidi, 1982; Ekpenyong and Barchers, 1981; Evans et al., 1973; Furuichi et al., 1988; Greer et al., 1985; Liener, 1986). The effects observed on oral ingestion of such legumes have been attributed, at least in part, to the large amounts of antinutrients, such as lectins, tannins, and enzyme inhibitors, found in these foods (Gupta, 1987). The processing and conventional domestic cooking of antinutrient-containing foods are usually associated with an improvement in the nutritional quality of the food concerned.

Lectins and tannins individually have been shown to have direct inhibitory effects on digestive enzymes such as salivary and pancreatic α-amylases (Thompson and Gabon, 1987; Thompson and Yoon, 1984; Rea et al., 1985) and also to interact with intestinal epithelial cells disrupting absorption of nutrients (Nakata and Kimura, 1985; King et al., 1982; Jaffe, 1980; Welch et al., 1989), explaining some of their effects. Foods containing such antinutrients have recently received much attention as they result in only gradual increases in blood glucose after digestion and appear useful in the management of diabetes (Crapo, 1984; Jenkins et al., 1986; Thompson, 1988) and hyperlipidaemia (Jenkins and Jepson, 1982; Jenkins et al., 1983). Thus, it has been suggested that consumption of low levels of certain antinutrients may produce health benefits while avoiding some of the adverse effects associated with their large intake (Thompson, 1988).

Although lectins and tannins do not exist in isolation, the majority of investigations into their dietary effects have been performed by using the individual antinutrients. Little is known about antinutrient–antinutrient interactions or the effect of a combination of antinutrients on digestive enzyme activity. Antinutrients may come into contact with one another during food processing, cooking, or consumption, and therefore it was of interest to determine the effects of red kidney bean lectin, tannic acid, and combinations of the two on mammalian α-amylase activity and starch digestion. The result of such investigations may extend the understanding of the role that complex mixtures of antinutrients in foods play in nutrition.

MATERIALS AND METHODS

Determination of Hemagglutinating Activity. Fresh rabbit erythrocytes collected into heparin anticoagulant were supplied by the Animal Facilities, the Hospital for Sick Children, Toronto. They were prepared for use according to the procedure of Dacie and Lewis (1984). Cells were washed three times in 0.1 M phosphate buffered saline, pH 7.2 (PBS), and then treated with trypsin. For agglutination tests, cells were used at a concentration of 3% (v/v) in PBS. Solutions, in PBS, containing 1.6 mg mL^{-1} red kidney bean lectin (PHA-P, Sigma Chemical Co., St. Louis, MO) and 1.36 mg mL^{-1} tannic acid (BDH Chemicals Ltd., Toronto, ON) were used to prepare serial twofold dilutions in PBS, each dilution having a final volume of 0.1 mL. An aliquot (0.1 mL) of the 3% suspension of erythrocytes was added to each member of the dilution series. The tubes were gently shaken and left at 18 °C for 3–5 min and then centrifuged at 1000*g* for 2–3 min after which time the degree of macroscopic agglutination was recorded according to the procedure of Boorman et al. (1977) as follows:

4+	one large mass of agglutinated cells, no free cells
3+	between 2 and 4 masses of agglutinated cells, no free cells
2+	between 4 and 20 separate masses of cells, some free cells present
1+	more than 20 separate masses of cells, many free cells present
(+)	agglutination just visible, giving a granular appearance
0	no agglutination, all cells free and evenly distributed

The greatest dilution that could agglutinate the erythrocytes, the hemagglutinating titer, was defined as containing 1 hemagglutinating unit (HU)/mL.

Interaction of Red Kidney Bean Lectin and Tannic Acid. Mixtures were prepared in PBS containing a final concentration of lectin of 50 μg mL^{-1} and either 0.5, 1, 10, 20, 30, 40 or 50 μg mL^{-1} tannic acid. The ratios of antinutrients are close to those encountered in foods, particularly legumes. After incubation for 1 h at 18 °C, the mixtures were centrifuged at 4500*g* for 15 min and the supernatants assayed for hemagglutinating activity as described above.

Pancreatic α-Amylase Activity. The activity of α-amylase was assayed by using, as the substrate, soluble potato starch (Baker Chemical Co., Phillipsburg, NJ), prepared by treatment with sodium borohydride as described by Strumeyer (1967). The

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potato starch substrate was dissolved in 0.05 M phosphate buffer, pH 6.9, containing 6.7 mM NaCl (substrate buffer) to a final concentration of 2% (w/v). Enzyme solution consisted of 2 mg of porcine pancreatic α-amylase (Type VI-A, Sigma) in 90 mL of substrate buffer to which was added 10 mL of 1 mM $CaCl_2$. The assay mixture contained 1 mL of enzyme solution, 2 mL of substrate buffer, and 3 mL of starch substrate solution. This was incubated at 37 °C, and 0.5 mL aliquots were taken at 0, 10, 15, and 20 min after the start of the reaction and analyzed for maltose released according to the method of Bernfeld (1955). The initial velocity of the reaction was recorded as milligram equivalents of maltose released per milliliter of assay mixture per minute at fixed concentration of the enzyme. One unit of α-amylase activity was defined as the amount of enzyme that produced reducing sugar equivalent to 1 μmol of maltose/min under the defined reaction conditions.

Effect of Antinutrients. To determine the effect of antinutrients on α-amylase activity, the same procedure as above was followed but the substrate buffer was replaced in the assay mixture with solutions containing either red kidney bean lectin, tannic acid, or both. In all instances red kidney bean lectin and tannic acid were added to give a final concentration of 125 μg mL^{-1} in the reaction mixture.

Preincubation Effects. The effects of red kidney bean lectin and tannic acid, alone and in combination, on α-amylase activity were investigated by using the following seven incubation systems:

(1) Red kidney bean lectin, tannic acid, or a combination of the two were mixed with the enzyme, and the reaction was started immediately by the addition of starch substrate.

(2) The substrate was incubated with red kidney bean lectin, tannic acid, or a mixture of the two for 15 min before addition of enzyme.

(3) The enzyme was incubated with red kidney bean lectin, tannic acid, or a combination of the two for 15 min before addition of the substrate.

(4) The enzyme was incubated with red kidney bean lectin for 10 min, tannic acid was added, and the mixture was incubated for a further 5 min before addition of substrate.

(5) The enzyme was incubated with tannic acid for 10 min, red kidney bean lectin was added, and the mixture was incubated for a further 5 min before addition of substrate.

(6) Red kidney bean lectin was incubated with tannic acid for 10 min, enzyme was added, and the mixture was incubated for a further 5 min before addition of substrate.

(7) Red kidney bean lectin, tannic acid, or a combination of the two was incubated at 100 °C for 15 min in a boiling water bath and then cooled rapidly in ice to 37 °C. The α-amylase solution was added and the mixture incubated for 5 min before addition of starch substrate.

The concentration of both red kidney bean lectin and tannic acid in the final reaction mixture was 125 μg mL^{-1} in all experiments, and unless specified, all incubations were performed at 37 °C.

RESULTS AND DISCUSSION

Tannic acid at concentrations of 85 μg mL^{-1} and above agglutinated trypsinized rabbit erythrocytes, 85 μg mL^{-1} possessing 1 hemagglutinating unit (HU)/mL (Table I). Red kidney bean lectin at a concentration of 50 μg mL^{-1} demonstrated 128 HU mL^{-1} against the same erythrocytes. However, the hemagglutination produced by 50 μg mL^{-1} red kidney bean lectin was abolished when the lectin was preincubated with subagglutinating doses (20–50 μg mL^{-1}) of tannic acid (Table I). The lowest concentration of tannic acid to completely inhibit the hemagglutinating activity of 50 μg mL^{-1} red kidney bean lectin was 20 μg mL^{-1}. This suggests that tannic acid and red kidney bean lectin can readily combine in aqueous solution, the resulting complex being devoid of hemagglutinating activity. The ability of tannins to bind to proteins in solution is well documented (Neucere et al., 1978; Hagerman and Butler, 1980; Haslam, 1974), and therefore the inactivation of red kidney bean lectin by tannic acid is presumably due to the precipitation of the lectin protein by the polyphenol.

Table I. Hemagglutination of Trypsinized Rabbit Erythrocytes by Red Kidney Bean Lectin, Tannic Acid, and Their Mixtures

RKB lectin, μg mL^{-1}	tannic acid, μg mL^{-1}	hemagglutinating activity (HU)[a]
50.0	0	128.0
50.0	0.5	128.0
50.0	1.0	32.0
50.0	5.0	8.0
50.0	10.0	1.0
50.0	20.0	0
50.0	30.0	0
50.0	40.0	0
50.0	50.0	0
0	80.0	0
0	85.0	1.0
0	170.0	2.0
0	340.0	4.0

[a] The greatest dilution that could agglutinate trypsinized rabbit erythrocytes was defined as containing 1 hemagglutinating unit (HU)/mL.

Table II. Porcine Pancreatic α-Amylase Activity with and without Addition of Red Kidney Bean Lectin and/or Tannic Acid

incubation system[a]	conditions	amylase activity units[b]	amylase activity %[c]
1	B + E + S[d]	94.23[a] ± 5.06	100.0
	L + E + S	98.21[a] ± 3.04	104.22 ± 3.23
	T + E + S	96.42[a] ± 3.71	102.33 ± 3.93
	L + T + E + S	96.36[a] ± 4.48	102.26 ± 4.75
2	(B + S)[e] + E	92.84[a] ± 2.95	100.00
	(L + S) + E	97.51[a] ± 1.59	105.03 ± 1.72
	(T + S) + E	92.61[a] ± 3.24	99.76 ± 3.49
	(L + T + S) + E	89.66[a] ± 1.59	96.58 ± 1.72
3	(B + E)[e] + S	92.95[a] ± 3.48	100.00
	(L + E) + S	10.77[c] ± 4.40	11.58 ± 4.73
	(T + E) + S	53.86[b] ± 6.92	57.96 ± 7.44
	(L + T + E) + S	78.37[a] ± 4.87	84.31 ± 5.23
	[(B + E) + B][f] + S	92.83[a] ± 1.43	100.00
4	[(L + E) + T] + S	9.76[c] ± 1.25	10.52 ± 1.35
5	[(T + E) + L] + S	59.89[b] ± 2.15	64.52 ± 2.32
6	[(L + T) + E] + S	94.18[a] ± 5.26	101.46 ± 5.66
7	[{B} + E][g] + S	92.83[a] ± 1.27	100.00
	[{L + B} + E] + S	104.36[a] ± 1.67	112.43 ± 1.80
	[{T + B} + E] + S	57.65[b] ± 9.14	62.10 ± 9.84
	[{L + T} + E] + S	106.22[a] ± 0.64	114.43 ± 0.69

[a] See Materials and Methods for details. [b] One unit of enzyme activity is defined as the amount of enzyme that produces reducing sugar equivalent to 1 μmol of maltose/min under the defined reaction conditions. Figures represent mean ± SEM ($n = 4$). Activities within one particular group marked with the same letter are not significantly different by Duncan's multiple comparison test ($p \leq 0.05$). [c] Percent relative to the control. [d] B, substrate buffer; E, pancreatic α-amylase; S, potato starch substrate; L, red kidney bean lectin (125 μg mL^{-1}); T, tannic acid (125 μg mL^{-1}). [e] Substances in parentheses were incubated at 37 °C for 15 min. [f] Substances in parentheses were incubated at 37 °C for 10 min, and substances in brackets were incubated for a further 5 min at 37 °C. [g] Substances in braces were heated at 100 °C for 15 min, and substances in brackets were incubated for a further 5 min at 37 °C.

The influence of 125 μg mL^{-1} red kidney bean lectin and 125 μg mL^{-1} tannic acid, alone and in combination, on the activity of α-amylase is shown in Table II and Figures 1–3. Addition of either red kidney bean lectin or tannic acid, without preincubation, to the α-amylase assay mixture resulted in no significant alteration in the rate of maltose production by the enzyme (Table II, incubation system 1). This indicates that for an inhibitory effect to arise, the antinutrients must come into contact with α-amy-

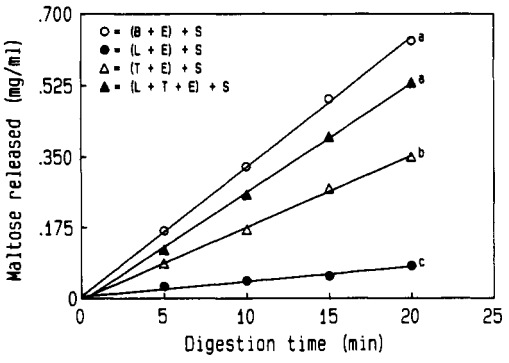

Figure 1. Maltose released from potato starch by α-amylase as affected by addition of red kidney bean lectin and/or tannic acid to the reaction mixture (incubation system 3). The rate of maltose release from lines marked with the same letter was not significantly different by Duncan's multiple comparison test ($p \leq 0.05$). For symbols, see Table II.

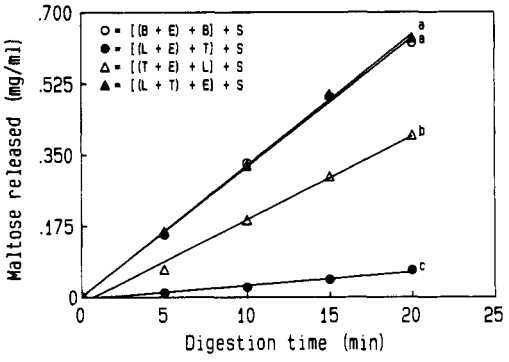

Figure 2. Maltose released from potato starch by α-amylase as affected by addition of red kidney bean lectin and/or tannic acid to the reaction mixture (incubation systems 4–6). The rate of maltose release from lines marked with the same letter was not significantly different by Duncan's multiple comparison test ($p \leq 0.05$). For symbols, see Table II.

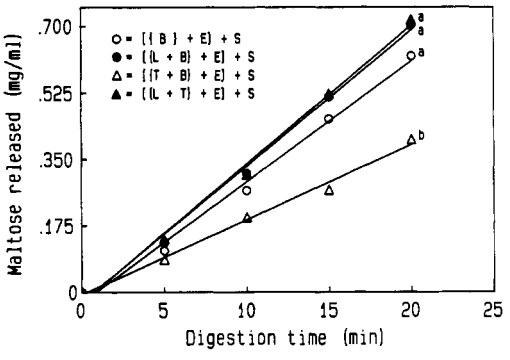

Figure 3. Maltose released from potato starch by α-amylase as affected by addition of red kidney bean lectin and/or tannic acid to the reaction mixture (incubation system 7). The rate of maltose release from lines marked with the same letter was not significantly different by Duncan's multiple comparison test ($p \leq 0.05$). For symbols, see Table II.

lase prior to the enzyme–substrate interaction. Without preincubation the affinity of red kidney bean lectin and tannic acid for α-amylase is not great enough to prevent enzyme–starch combination. Also, the starch substrate is in excess in the reaction mixture, which would favor enzyme–substrate interactions rather than enzyme–antinutrient interactions.

Addition of red kidney bean lectin and/or tannic acid to the starch substrate prior to exposure to α-amylase digestion had no significant effect on the activity of the enzyme (Table II, incubation system 2). Both lectins and polyphenols have been reported to bind to starches, producing a decrease in their in vitro digestibilities (Deshpande and Salunkhe, 1982; Thompson and Gabon, 1987), although this was not observed in this study. Under the conditions defined here, the starch substrate is in excess and thus any starch in combination with lectin or tannic acid (presumably with a lower susceptibility to digestion) would have little, if any, effect on the α-amylase, as the concentration of unaffected starch would be sufficiently high to allow enzyme activity to proceed without inhibition.

Preincubation of α-amylase with red kidney bean lectin or tannic acid prior to the addition of starch substrate resulted in a significant ($p \leq 0.05$) inhibition of enzyme activity (Table II, incubation system 3, and Figure 1). This exposure of α-amylase to antinutrient, prior to substrate addition, presumably facilitates antinutrient–α-amylase interactions accounting for the inhibition. The reduction in α-amylase activity by both red kidney bean lectin and tannic acid is presumably a result of the interaction of the lectin or the polyphenol either with the enzyme itself or with its substrate. Although both red kidney bean lectin and tannic acid produced inhibition of the enzyme at the fixed final concentration of 125 μg mL^{-1}, the lectin was significantly more effective than tannic acid in reducing α-amylase activity (Table II, incubation system 3). This suggests that on a weight basis red kidney bean lectin would have a greater antinutritional effect than tannic acid following consumption. This finding relates to earlier studies on starch digestibility by salivary and pancreatic α-amylases, in which red kidney bean lectin and tannic acid at concentrations approximately equal to that found in legumes produced 64% and 13% reductions in wheat starch digestibility, respectively (Thompson and Yoon, 1984; Thompson and Gabon, 1987). Preincubation of both red kidney bean lectin and tannic acid with α-amylase produced a 15% reduction in enzyme activity (Table II, incubation system 3, and Figure 1), although the enzyme activity under these conditions was not significantly lower than that obtained for the uninhibited enzyme. The inhibition produced as a result of preincubation of α-amylase with either red kidney bean lectin or tannic acid alone was significantly greater than that produced by preincubation of both antinutrients with the enzyme. Thus, red kidney bean lectin and tannic acid neutralize each other's inhibitory effect on α-amylase. This reduced inhibition suggests that the interaction of red kidney bean lectin and tannic acid in solution alters the inhibitory effects of the individual antinutrients.

Preincubation of α-amylase with red kidney bean lectin, followed by additional incubation with tannic acid, prior to substrate addition produced a significant reduction in α-amylase activity (Table II, incubation system 4, and Figure 2). Under these conditions the inhibition produced was similar to that following preincubation of the enzyme with lectin alone (Table II, incubation system 3). Subsequent addition of tannic acid did not reduce lectin-induced α-amylase inhibition as seen when lectin and tannic acid were combined prior to exposure to the enzyme (Table II, incubation system 6). Thus, the inhibitory nature of the lectin was not neutralized by tannic acid once the lectin had combined with the enzyme. It is possible that under these conditions all of the red kidney bean lectin was in combination with the enzyme and was therefore unavailable for interaction with the tannic acid subsequently added.

When tannic acid was preincubated with α-amylase followed by a second preincubation with red kidney bean lectin, the inhibitory effect (Table II, incubation system 5) was similar to that seen with preincubation with tannic

acid alone (Table II, incubation system 3). In this situation subsequent addition of red kidney bean lectin did not reduce the α-amylase inhibition induced by tannic acid. Therefore, in analogy to the lectin-induced inhibition, the inhibitory nature of tannic acid was not neutralized by red kidney bean lectin once tannic acid had complexed with α-amylase. It is possible that after combination of tannic acid with enzyme a proportion of tannic acid remains free in solution. Upon subsequent addition of red kidney bean lectin the tannic acid not in combination with α-amylase interacts with the red kidney bean lectin, thus preventing additional inhibition by the lectin.

Combination and incubation of red kidney bean lectin and tannic acid prior to their exposure to α-amylase resulted in no significant reduction in enzyme activity (Table II, incubation system 6, and Figure 2). Again, it can be seen that in combination red kidney bean lectin and tannic acid can interact so that their individual inhibitory activity is abolished.

Heating the lectin to 100 °C for 15 min, simulating conditions that may be encountered during food processing prior to consumption, abolished the ability of the lectin to inhibit α-amylase (Table II, incubation system 7, and Figure 3). The interaction of lectin and α-amylase is probably dependent on the lectin's biological (carbohydrate binding) activity, as this has been previously reported to be destroyed by heating to 100 °C for as little as 2 min (Bender and Reaidi, 1982). The denatured protein presumably cannot interact with the enzyme and therefore results in no inhibition. Heat treatment of tannic acid did not abolish its inhibitory capacity, α-amylase activity (Table II, incubation system 7, and Figure 3) being significantly reduced after combination of the enzyme with heated tannic acid. The physicochemical properties of tannic acid are not sufficiently altered by the heat treatment to prevent it from interacting with α-amylase, reducing its catalytic activity. Heating red kidney bean lectin and tannic acid together at 100 °C for 15 min abolished the inhibitory capacity of the heated tannic acid (Table II, incubation system 7). Although the lectin is presumably denatured under these conditions, it still is capable of interacting with tannic acid, neutralizing its effect on α-amylase. Cooking has been associated, in some instances, with an improvement in the nutritional quality of foods. This has been attributed, in part, to the inactivation of antinutrients present in the food. This study has shown that even though amylase inhibition by tannic acid was not prevented by heat treatment of the polyphenol, its action could be abolished by the presence of red kidney bean lectin. This indicates that the improved nutritional quality of certain cooked foods may be the result of combination of antinutrients found together in the food as well as to their inactivation by heat.

It is interesting to note that inclusion of lectin without preincubation and inclusion of heat-treated lectin into the reaction mixture resulted in a small enhancement of α-amylase activity over control values, albeit not statistically significant. A slight enhancement of enzyme activity by red kidney bean lectin has also been described for rat small intestine brush border membrane-bound maltase that had been liberated into solution by using the detergent Triton X-100 (Erickson et al., 1985). Maltase enhancement by red kidney bean lectin was greater for the membrane-bound enzyme, indicating that the membrane may also play a role in the changes observed in enzyme activity. Although the exact mechanism of the lectin–enzyme interaction remains unknown, it was suggested by these workers that the lectin may increase the number of enzyme active sites through an alteration in their accessibility to the substrate.

Overall, this study has shown that lectins and tannins can interact with elimination of their hemagglutinating activity; it has also confirmed that, individually, red kidney bean lectin and tannic acid are inhibitors of pancreatic α-amylase and may potentially reduce the rate of starch digestion and the blood glucose response in humans. However, preincubation of lectin and tannic acid was shown to facilitate their interaction so that they have little or no effect on the α-amylase catalyzed digestion of starch. It was also demonstrated that although heat-treated tannic acid could still inhibit α-amylase, heated denatured lectin would abolish this inhibition. In vitro studies may not always predict what may happen in vivo. Nevertheless, the in vitro inhibitory effect of lectin and tannin on starch digestion appears to relate well with their in vivo effect on starch digestion and absorption as indicated by blood glucose response (Rea et al., 1985; Thompson, 1988; Thompson and Gabon, 1987; Thompson and Yoon, 1984; Yoon et al, 1983).

Lectins and tannins have been described in a wide variety of commonly consumed foods, and their presence has been suggested to be responsible for various effects on the digestion of and absorption of nutrients from these foods (Gupta, 1987). Although antinutrients are common in many different legumes frequently consumed by humans, adverse reactions following consumption of such foods are not always encountered. A novel explanation for this lack of ill effect is given by the results of this study. The individual adverse effects of red kidney bean lectin and tannic acid on the activity of α-amylase activity were shown to be markedly reduced when the antinutrients were mixed prior to contact with the enzyme. Several workers have investigated possible methods of removal or inactivation of antinutrients during food processing and cooking in an attempt to improve the nutritional quality of such foods (Liener, 1962; Thompson et al., 1983; Del Valle et al., 1983; Kataria et al., 1988). The results presented here indicate a possibility that, as well as removal and/or inactivation, processing and cooking of foods can facilitate the interaction of antinutrients so that their effects on nutrition and digestion are abolished.

In "real life" situations the composition of foods consumed is normally very complex and may contain a wide variety of antinutrients that may or may not interact prior to digestion. Although the effects of red kidney bean lectin on α-amylase were abolished by heat treatment, the denatured lectin could prevent the inhibitory effect of tannic acid toward the enzyme. Thus, it is important when the effects of individual antinutrients are evaluated to consider how these will relate to the effects encountered upon consumption of a complex mixture of dietary constituents.

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