Effect of Purified Lectins on Pancreatic α-Amylase Activities†

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The effect of purified native and heated lectins [commercially purified red kidney bean lectin (9017), laboratory-purified navy and red kidney bean lectins] on the digestion of starch (potato starch and wheat starch) by two commercial preparations of pancreatic α-amylase (types I-A and VI-B) was studied and compared to the effect of a commercial semipurified red kidney bean lectin (L-8754). Among all lectins tested, only native semipurified L-8754 lectin significantly ($p < 0.05$) reduced the activity of α-amylase. Native commercially purified L-9017 lectin and all laboratory-purified lectins, including affinity-chromatography-repurified L-8754 lectin, significantly ($p < 0.05$) increased α-amylase activity. All heat-treated lectins, including the heated semipurified L-8754 lectin, significantly ($p < 0.05$) increased α-amylase activity. The inhibition effect of the native semipurified L-8754 lectin was probably due to contamination with α-amylase inhibitors.

INTRODUCTION

The fact that legume starches are digested at a slower rate and are richer in antinutrients than cereal products and root vegetables has led to the findings that some antinutrients, including lectins, are able to lower the rate of starch digestion and decrease blood glucose response (Thompson, 1988). An excellent review on the nutritional effect of lectins has been written by Liener (1986). When large amounts of lectins are ingested in the free form or in raw beans, lectins can inhibit growth and/or cause death of animals. Several hypotheses of the reaction mechanism of lectins have been proposed. Lectins may interfere with the absorption of nutrients by binding to the specific receptor sites on the surfaces of the intestinal cells, thereby causing malfunction, disruption, or lesion of the small intestine. Lectins may disrupt intestinal brush border cells and make intestinal surface susceptible to bacteria growth. Lectins, intact or partially digested, may themselves enter the circulation system to exert toxic reaction. Some studies showed that lectins also may directly interact with digestive enzymes.

Kim et al. (1976) found that lectins from *Phaseolus* species could inhibit brush border cell membrane peptidase in the intestine of rats. Rouanet et al. (1983) found no effect of lectins on pancreatic proteases. However, the enterokinase, a glycoprotein which activates zymogens by limited hydrolysis, was inhibited by affinity-chromatography-purified lectins from legume seeds. The source of lectin also affected the rate of enterokinase inhibition. Lectins from *Phaseolus vulgaris* were more potent than concanavalin A and soybean lectin (Rouanet et al., 1983). Lectins (hemagglutinins) may be responsible for approximately half of the differences in both digestibility and blood glucose responses between wheat starch and red kidney bean starch (Rea et al., 1985). Thompson et al. (1986) found that pepsin and pancreatin also were inhibited by the heated bean extract and concanavalin A and suggested this inhibition was due to the lectins in beans. Thompson and Gabon (1987) studied the effect of lectins on salivary and pancreatic α-amylase and concluded both enzymes were significantly inhibited.

However, most reported lectin–enzyme experiments were conducted using commercial lectins of unreported purity. Therefore, to ascertain the effect of lectin, their activities should be tested with purified lectins. Lectins from different sources differ in their reactivities (Rouanet et al., 1983) and may affect starch digestion and glycemic response in some foods. The most studied lectin from a *P. vulgaris* species is from red kidney bean (Liener, 1986). The effect of lectins from navy beans (*P. vulgaris*) on digestive enzyme activities has not been studied.

The objectives of this study are (1) to purify lectins from navy and red kidney beans and from a commercial semipurified lectin and (2) to investigate the effect of native and heated purified and semipurified lectin on the digestion of starch by pancreatic α-amylase.

MATERIALS AND METHODS

Materials. The Agri-1 and C-20 cultivars of navy beans (*P. vulgaris*) and red kidney beans (*P. vulgaris*) were obtained from a bean handling company (Agri Sale, Casselton, ND). Commercial preparations of lectins from red kidney bean (semipurified PHA-P L-8754 and chromatographically purified PHA-P L-9017) and porcine pancreatic α-amylase (type I-A with 1260 units of α-amylase activity/mg of protein, and type VI-B with 25 units of α-amylase activity and 7.4 units of β-amylase activity/mg of solid) were obtained from Sigma Chemical Co. (St. Louis, MO). Wheat starch (AYTX-P, food grade) was purchased from Henkel Corp. (Minneapolis, MN). Soluble potato starch was obtained from Baxter Scientific Co. (Minneapolis, MN). G-1 protein, which was isolated from navy C-20 bean according to the procedure of Hall et al. (1977), was used as a molecular mass marker.

Isolation and Purification of Lectins. Beans were finely ground with a Tekmar A-10 analytical mill (Tekmar Co., Cincinnati, OH). Lectin was isolated and purified by affinity chromatography with porcine thyroglobulin–Sepharose using the procedure of Felsted et al. (1975). A portion of the commercial lectin (type L-8754) also was subjected to the same affinity chromatographic purification in our laboratory. The chromatographically purified type L-8754 lectin was labeled as repurified L-8754 lectin.

The purity and subunit molecular mass (M_r) of the isolated lectins were determined using the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) method of Laem-

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mli (1970). A modified Lowry's method (Hartree, 1972) was used to measure protein concentration in the lectin samples. The protein concentration in each sample was adjusted with SDS sample buffer to 1 μg/μL prior to loading to the polyacrylamide gel. After SDS electrophoresis, the gels were stained in 0.25% Coomassie Brilliant Blue 250-R in 50% methanol and 10% acetic acid solution. The density of protein bands was determined using a gel scanner (ISCO, Lincoln, NE), and percentage of purity was estimated by dividing the area of the lectin subunit peak by the total area of all peaks.

Lectin activity was measured by a microtiter method using cow red blood cells according to the procedure of Felsted et al. (1975). The red blood cells were trypsinized and treated with glutaraldehyde according to the procedures of Turner and Liener (1975). The lectin activity was expressed as hemagglutinating unit (HU) per milligram of protein.

Interaction of Lectins with Pancreatic α-Amylase. The commercial semipurified L-8754 and purified L-9017 red kidney lectins and laboratory-purified lectins, including the repurified L-8754 lectin, were used for the enzyme interaction experiments. α-Amylase activity was assayed by measuring the amount of maltose release from a starch solution according to the procedures described in the *Worthington Manual* (Worthington, 1988). The lectin concentration and general procedures used for studying lectin-enzyme interactions were similar to that reported by Thompson and Gabon (1987). Three milliliters of lectin solution (0.1 mg/mL, native or heat treated at 100 °C for 30 min) was added to 3 mL of type I-A (0.08 unit) or VI-B (0.16 unit) pancreatic α-amylase solution. The mixture was preincubated at 37 °C for 30 min, and the hydrolytic reaction was initiated by adding 6 mL of 2% potato starch or wheat starch solution (prepared in 0.02 M, pH 6.9, phosphate-buffered solution). After 25, 50, 75, and 100 min, a 1-mL aliquot was taken from the reaction mixture and analyzed for maltose. Three milliliters of phosphate-buffered saline instead of the lectin solution was used as an enzyme control.

The same procedures were used to determine the effect of different concentrations (0.1, 0.2, and 0.3 mg/mL) of three purified lectins (native Agri-1, C-20, or red kidney bean lectins) except that only type I-A α-amylase and potato starch were used.

Statistical Analysis. Significant differences among maltose releasing rates (the slopes of the curves) were analyzed by Duncan's multiple range test ($p < 0.05$) using the SAS (Statistical Analysis System) program (SAS, 1986).

RESULTS AND DISCUSSION

Purity of the Lectins. Figure 1 shows the photograph of the SDS-PAGE gel of the lectins from various sources. The subunit molecular mass of lectins from red kidney bean is approximately 34 kDa (Miller et al., 1973, 1975). Lectins in the albumin fraction of mavy beans are composed of two types of subunits with 30 and 35 kDa in the ratio of 3 to 1 (Pusztai et al., 1975). The major subunit molecular mass of lectin from red kidney and navy beans in this study was approximately 32 kDa. Differences in subunit molecular mass among all sources are small.

The navy beans (lanes 5 and 6, Figure 1) also contained a small band at 35 kDa, which may be an isolectin (Pusztai et al., 1975). The purity of the laboratory-purified lectins, including lectins from Agri-1 (lane 5), C-20 (lane 6), and red kidney beans (lane 7), were 93.8%, 92.4%, and 90.4%, respectively. The commercially available purified red kidney bean lectin L-9017 (lane 4) had a purity of 92.5%, while commercial L-8754 lectin (lane 2) had only 54.8% purity. Major contaminants (bands other than 32 kDa) included bands at approximately 42 kDa, which corresponded to the molecular mass of the G-1 protein (lane 8) and bands in the region of 12-20 kDa (lane 2). The purity of the repurified L-8754 lectin (lane 3) was increased by approximately 2-folds from 54.8% to 97.7%.

The hemagglutinating activities of the purified lectins from navy Agri-1 and C-20 and red kidney beans were 2^{10}, 2^9, and 2^{13} HU/mg, respectively. The hemagglutinating activity of the commercial L-8754 lectin was 2^{12} HU/mg and was increased to 2^{13} HU/mg after laboratory repurification. The 2-fold increase in the hemagglutinating activity of the repurified lectin corresponded with an approximately 2-fold increase in purity as determined by SDS-PAGE. This result indicates the purification procedure has not altered the structure of the lectin. The hemagglutinating activity of the commercially purified L-9017 lectin also was 2^{13} HU/mg.

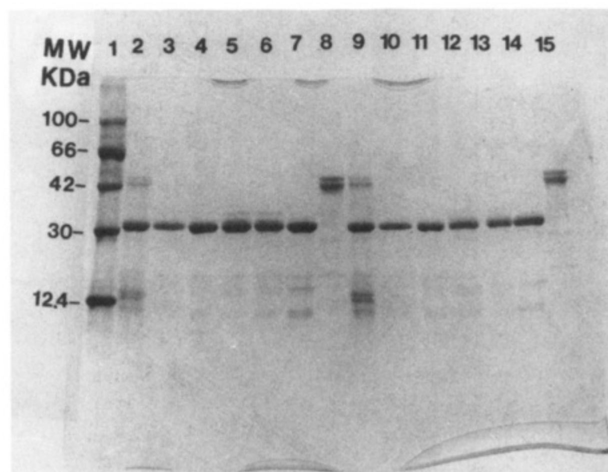

Figure 1. SDS-PAGE patterns of lectins from various sources. The gel slab was a 7.5-20% gradient gel. Proteins in lane 1 are molecular mass markers representing α-actinin (100 kDa), bovine serum albumin (66 kDa), actin (42 kDa), carbonic anhydrase (30 kDa), and cytochrome *c* (12.4 kDa). Protein load in lanes 2-15 was 20 μg each lane. Lanes 2-8 represent native L-8754 lectin, laboratory-repurified L-8754 lectin, L-9017 lectin, laboratory-purified navy Agri-1 lectin, laboratory purified navy C-20 lectin, laboratory purified red kidney bean lectin, and G-1 protein (Phaseolin) from navy C-20 bean, respectively. Lanes 9-15 represent heat-treated L-8754 lectin, laboratory-repurified L-8754 lectin, L-9017 lectin, laboratory purified navy Agri-1 lectin, laboratory-purified navy C-20 lectin, laboratory-purified red kidney bean lectin, and G-1 protein, respectively.

Interaction of Lectins with Pancreatic α-Amylase. After incubation of the native lectins with type I-A pancreatic α-amylase, the digestion of both potato starch and wheat starch was significantly ($p < 0.05$) reduced by commercial semipurified L-8754 lectin (15.42% and 12.84% inhibition, respectively, Figure 2A,B). This result agrees with the earlier paper of Thompson and Gabon (1987). However, all laboratory-purified lectins, including lectins purified from navy Agri-1, C-20 beans, and red kidney beans, significantly ($p > 0.05$) increased the digestion of potato starch from 28.14% to 47.15% and wheat starch from 19.83% to 26.11% (Figure 2A,B).

Both commercially purified L-9017 red kidney bean lectin and the laboratory-repurified L-8754 red kidney bean lectin also significantly ($p < 0.05$) increased the digestion of potato starch and wheat starch (Figure 2A,B). This result indicates that the inhibition effect of the commercial semipurified lectins may be due to contaminants, which might include α-amylase inhibitor. Purified wheat germ lectin and phytohemagglutinin dramatically increased the activity of maltase on small intestine brush border cell membranes of rats (Erickson et al., 1985). The mechanism of the lectin effect on hydrolytic enzyme activity is not clearly understood. However, it has been proposed that lectin may affect the enzyme activities through binding to these enzymes at places other than their substrate binding sites (Kim et al., 1976). Lectins might increase the number of enzyme active sites by altering their accessibility to the substrate (Erickson et al., 1985).

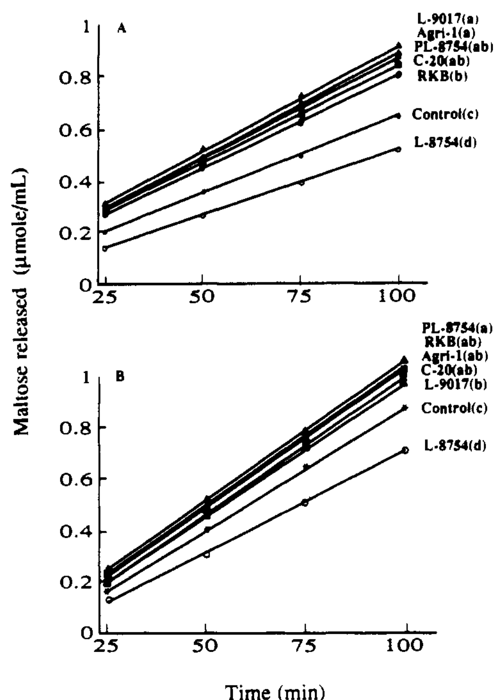

Figure 2. Effect of native lectins from various sources on the digestion of starch by type I-A α-amylase. (A) Potato starch; (B) wheat starch. Lectin concentration was 0.1 mg/mL. Control, enzyme without any lectin; L-9017, purified red kidney bean lectin obtained from Sigma; L-8754, semipurified red kidney bean lectin obtained from Sigma; PL-8754, lectin repurified from L-8754 in our laboratory; Agri-1, lectin purified from Agri-1 cultivar of navy bean; C-20, lectin purified from C-20 cultivar of navy bean; RKB, lectin purified from red kidney bean. Agri-1, C-20, and RKB were purified in our laboratory.

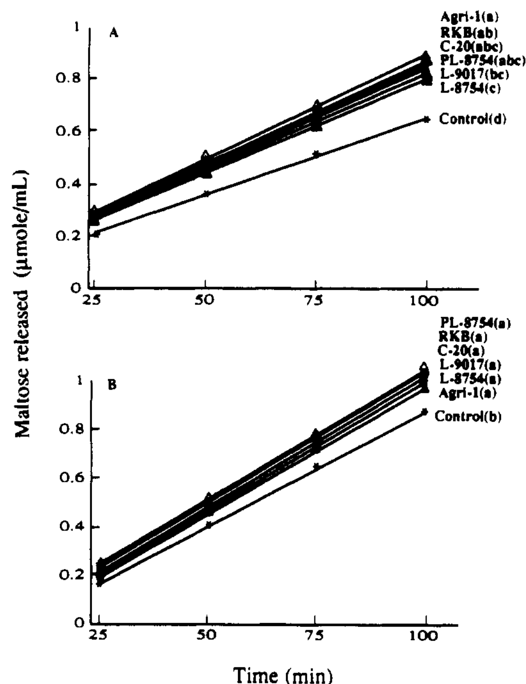

Figure 3. Effect of heated lectins from various sources on the digestion of starch by type I-A α-amylase. Lectins were heated at 100 °C for 30 min. (A) Potato starch; (B) wheat starch. The representations of various lectins are the same as in Figure 2.

The enhancement effect of lectins on the digestion of wheat starch by α-amylase was small compared to the effect on the digestion of potato starch by the same enzyme (28.14% to 47.15% increase for potato starch and 19.83% to 26.11% increase for wheat starch, parts A and B of Figure 2, respectively). This implies that the type of starch also affects lectin–enzyme interaction. Since lectins also could bind to the substrates, they might increase the enzyme activity through binding to both enzymes and their substrates and might increase the affinity of the enzymes to their substrates.

Fish and Thompson (1991) found that preincubation of lectin with the enzyme significantly ($p < 0.05$) reduced the enzyme activity. However, in our study, all purified lectins were preincubated with α-amylase for 30 min and enzyme enhancement effect was observed (Figure 2A,B). The discrepancy in the effect of lectins on α-amylase activity between this and other studies (Rea et al., 1985; Thompson and Gabon, 1987; Fish and Thompson, 1991) might be due to the differences in the purity of lectin. Crude saline extract or unreported purity of lectin were used in other studies (Rea et al., 1985; Thompson and Gabon, 1987; Fish and Thompson, 1991). The lectin used by Rea et al. (1985) was the same as the semipurified L-8574 lectin (private communication with Dr. R. Larson, Sigma Chemical Co.).

The lectins treated at 100 °C for 30 min lost all of the hemagglutinating activities. Unexpectedly, all heat-treated lectins, including laboratory-purified lectins and the commercial lectin L-8754, increased the digestion of both potato starch and wheat starch by pancreatic α-amylase (Figure 3A,B). The native semipurified L-8754 lectin inhibited the α-amylase activity, while repurified and heated L-8754 lectin increased α-amylase activity. These results suggest that semipurified L-8754 lectin might be contaminated with proteins such as α-amylase inhibitors, which were heat sensitive and could be destroyed by boiling for 8 min (Jaffe et al., 1973). The results of this study coincide with the recent findings of Huesing et al. (1991) that α-amylase inhibitor, not lectin, is responsible for the resistance of common bean seeds to cowpea weevil.

Compared to the effect on type I-A α-amylase, similar results also were obtained with type VI-B porcine pancreatic α-amylase (figures not shown). However, the increasing effect of all heated lectins and purified native lectins on type VI-B α-amylase activity (10.71% to 27.91% increase) was smaller than the effect on type I-A α-amylase (20.15% to 47.15% increase; Figures 2 and 3). However, the native commercial L-8754 lectin had relatively greater inhibition effect on type VI-B α-amylase than on type I-A α-amylase. The rates of inhibition on type VI-B α-amylase by native L-8754 lectin were 34.66% and 31.00% for potato starch and wheat starch, respectively, and the rates of inhibition on type I-A α-amylase by native L-8754 lectin were 15.42% and 12.84% for potato starch and wheat starch, respectively (Figure 2A,B). Type I-A α-amylase was commercially treated with phenylmethanesulfonyl fluoride (PMFS), while type VI-B α-amylase was not. Thus, the interaction between the native L-8754 lectin and α-amylase also may be influenced by the pretreatment of the enzyme with PMFS, which is commonly used to inhibit serine proteinases such as trypsin and chymotrypsin during isolation and purification of pancreated α-amylase.

In most situations (Figures 2 and 3), the differences in the effect of purified native and heated lectins on α-amylase activities due to bean variety were minimum with a nonsystematic pattern among the three bean varieties studied. This study suggests that the protein subunit differences between the protein structures of the lectins from navy and red kidney beans (Miller et al., 1973, 1975; Pusztai et al., 1975) have not caused significant difference in their capability to interact with α-amylase.

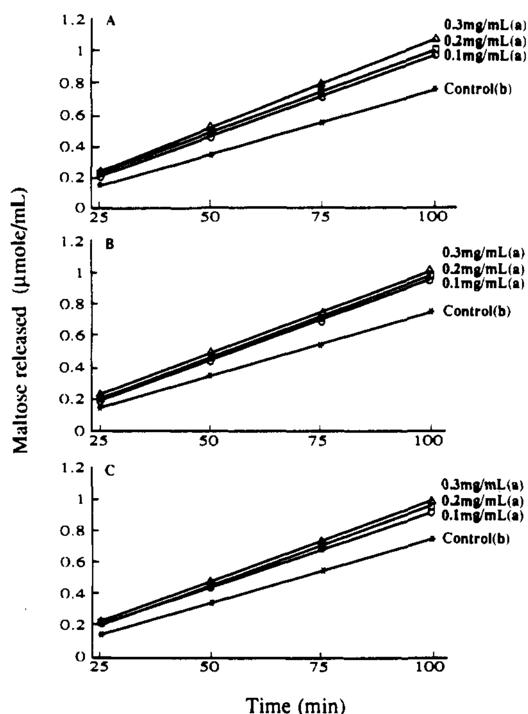

Figure 4. Effect of concentration of laboratory-purified bean lectin on the digestion of potato starch by type I-A α-amylase. (A) Agri-1 navy; (B) C-20 navy; (C) red kidney bean.

There is a systematic trend to increase the activities of α-amylase with increasing concentrations of lectins purified from the three bean varieties (Figure 4). However, the increases in α-amylase activity with increased lectin concentrations above 0.1 mg/mL were not significant ($p > 0.05$). The enzyme molecules were saturated with lectins since all lectin concentrations used in this study were much higher than the α-amylase concentration.

Conclusion. This study demonstrated that purified lectins increased the activity of pancreatic α-amylase. The inhibition effect of commercial L-8754 lectin was probably due to contamination since repurified or heated L-8754 lectin increased α-amylase activity. The slow rate of starch digestion in bean flour compared to that in wheat flour and other cereal products, both in vivo and in vitro, might be due to factors other than direct interactions of lectins with pancreatic α-amylase. Further studies to understand the mechanism responsible for the interactions of lectins and hydrolytic enzymes are recommended.

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