Inhibition of Enzymic Digestion of Amylose by Free Fatty Acids In Vitro Contributes to Resistant Starch Formation

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Timothy C. Crowe,*† Sophie A. Seligman† and Les Copeland†1

Human Nutrition Unit and †Department of Agricultural Chemistry and Soil Science, University of Sydney, NSW, Australia 2006

ABSTRACT The effect of lipids on the enzymic breakdown of starch was investigated using an in vitro assay system. Mixtures of potato amylose, amylopectin and starch and various lipids were incubated at 37°C for 10 min and subjected to digestion by α-amylase (EC 3.2.1.1) and amyloglucosidase (EC 3.2.1.33). Lauric, myristic, palmitic and oleic acids and lysolecithin inhibited enzymic hydrolysis of amylose by ~35% ($P < 0.05$). Stearic acid and cholesterol had no effect on the enzymic breakdown of amylose. Retrograded amylose was hydrolyzed less readily ($P < 0.05$) than solubilized amylose, but the breakdown was not further inhibited in the presence of lauric acid. Fatty acids had no effect on the enzymic hydrolysis of amylopectin, whereas inhibition by fatty acids of the breakdown of whole starch was consistent with only the amylose fraction being affected. The possibility that interactions between starch and fatty acids in the digestive tract could contribute to the formation of resistant starch is considered. J. Nutr. 130: 2006–2008, 2000.

KEY WORDS: • resistant starch • fatty acids • amylose • amylolysis of starch

Starch is a homopolymer of glucose that contains two fractions: amylose, which is an essentially unbranched α[1→4]-linked glucan, and amylopectin, which contains glucose units in α[1→4] and α[1→6] links. Unbranched α[1→4] glucan chains such as amylose have a helical conformation and can form inclusion complexes with a variety of small hydrophobic molecules, including certain types of lipids. The formation of such complexes may result in significant changes in the properties of the glucan, including decreased solubility, increased gelatinization temperature and retarded retrogradation during storage (Eliasson et al. 1981, Krog 1971).

Ingested starch was previously considered to be completely hydrolyzed to glucose in the upper gut by digestive enzymes. However, it is now known that a starch fraction, termed resistant starch, makes an important contribution to dietary fiber because it passes through the small intestine and is fermented in the hind gut by resident microflora yielding short-chain fatty acids (SCFA), which can have beneficial actions on the colon (Annison and Topping 1994, Baghurst et al. 1996, Brown 1996, Englyst and Cummings 1982, Muir et al. 1994). The amount and form of resistant starch in foods vary depending on the botanical source of the starch, the molecular structure of the starch and interactions that may occur between the starch and other food constituents during processing, cooking and eating (Englyst and Cummings 1982, Englyst et al. 1996, Muir and O'Dea 1992). One such interaction that can change the behavior of starch is the formation of complexes with lipids. Complex formation has been suggested to occur in situ in the digestive tract (Holm et al. 1983) and is thought to decrease the digestibility of starch and modulate the glycemic response to ingested carbohydrate (Murray et al. 1998). In this study, we demonstrate that certain fatty acids inhibit the enzymic hydrolysis of the amylose component of starch. This inhibition results in the increased availability of α[1→4] glucan substrates for fermentation in the hind gut.

MATERIALS AND METHODS

Materials. Potato starch, potato amylose, potato amylopectin, fatty acids, lysolecithin (egg yolk; predominantly palmitic and stearic acids) and cholesterol were from Sigma (St Louis, MO). Porcine pancreatic α-amylase (EC 3.2.1.1) was from Boehringer Mannheim (Mannheim, Germany) and amyloglucosidase (EC 3.2.1.33) was from Megazyme International (Bray, Ireland).

Enzymic digestions. Amylose, amylopectin and starch (25 mg) were dissolved in 5 mL of 0.1 mol/L NaOH by heating for 10 min in a boiling water bath with mixing. The resulting solution was cooled to room temperature and freed of NaOH with an Econo-Pac 10 DG column (Bio Rad, Hercules, CA) according to the supplier's instructions. Neutralized glucan solution (100 μL containing 0.36 mg of glucan), 400 μL of 0.2 mol/L sodium acetate (pH 4.5) and 10 μL of a methanolic solution of the lipid were incubated in microcentifuge tubes for 10 min at 37°C before initiating enzymic digestions by the addition of α-amylase (1.4 U) and amyloglucosidase (0.03 U). The final volume of the reaction mixtures was 1 mL. Reactions were stopped by heating in a boiling water bath for 10 min, and free glucose was determined colorimetrically using the glucose oxidase–peroxidase method as described by Blakeney and Matheson (1984). The amylose and amylopectin content of the starch were determined by iodine binding according to the method of Chang (1979). Significant differences between two measurements were determined using paired *t* tests.

RESULTS

When amylose was subjected to enzymic digestion as described, 60% was converted to glucose after 1 h and after 2 h the extent of hydrolysis was ~80% (**Fig. 1**). More than 90% of the amylose was converted to glucose in 6 h. In the presence of lauric acid, 45% of the amylose was hydrolyzed after 1 h, 50% after 2 h and ~60% after 6 h (Fig. 1). In a control experiment, the addition of 10 μL of methanol to the reaction mixture had no effect on the enzymic breakdown of amylose (data not shown). Based on results shown in Figure 1 and

[1] To whom correspondence should be addressed.

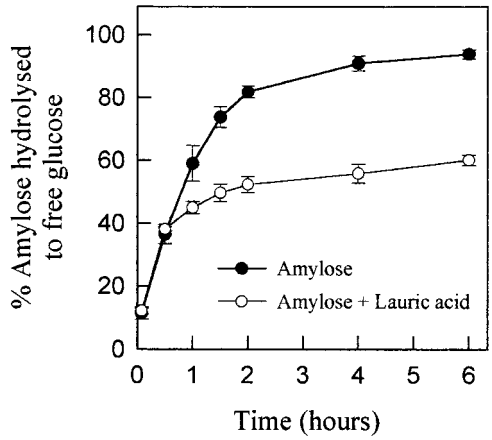

FIGURE 1 Effect of lauric acid on the enzymic hydrolysis of amylose. Amylose (0.36 mg) and amylose (0.36 mg) with added lauric acid (0.1 mg) were hydrolyzed to free glucose in the presence of α-amylase and amyloglucosidase at 37°C as described. Data are means ± SD of at least three independent experiments in which each measurement was made with triplicate samples. Differences between samples with and without lauric acid were significant ($P < 0.05$) for incubation times of ≥1 h.

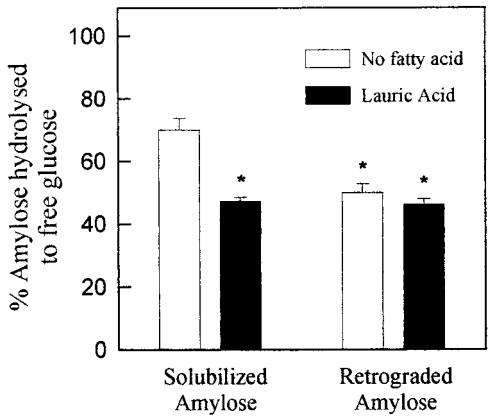

FIGURE 2 Enzymic hydrolysis of solubilized and retrograded amylose to free glucose. Amylose (0.36 mg) was solubilized and stored overnight at 4°C before mixing with lauric acid (0.1 mg) as described. Data are means ± SD of four replicate experiments. Significant differences ($P < 0.05$) from the control, which contained only solubilized amylose, are indicated with an asterisk.

taking into account the definition of resistant starch as being that portion of starch not hydrolyzed after 2 h in an in vitro assay (Englyst et al. 1996), we chose an incubation time of 2 h in subsequent experiments to monitor the formation of enzymically resistant starch.

Myristic, palmitic and oleic acids and lysolecithin had similar effects to that of lauric acid, reducing the extent of amylose hydrolysis to ~50% in 2 h (**Table 1**). However, neither stearic acid nor cholesterol inhibited the enzymic breakdown of amylose (Table 1), and there was no effect of stearic acid when the temperature of the enzymic digestion was increased to 50°C (data not shown).

Amylose was less readily hydrolyzed after it had been solubilized and stored at 4°C overnight to promote retrogradation (**Fig. 2**). The extent of hydrolysis of retrograded amylose was ~50% after 2 h, and the addition of lauric acid had no further inhibitory effect (Fig. 2). Lauric acid had no effect on the enzymic breakdown of amylopectin, whereas the breakdown of whole starch was inhibited 12% by lauric acid (results not shown). This value was consistent with lauric acid affecting the hydrolysis of the amylose fraction only, which was determined by iodine binding to be 31% of the starch.

TABLE 1

Effect of lipids on the enzymic hydrolysis of amylose[1]

Addition to amylose[2]	Conversion to glucose
	%
None	77 ± 7
Lauric acid	51 ± 6*
Myristic acid	48 ± 6*
Palmitic acid	54 ± 6*
Stearic acid	71 ± 7
Lysolecithin (palmitic acid)	56 ± 6*
Oleic acid	53 ± 5*
Cholesterol	78 ± 10

[1] The data are the means ± SD of at least three independent experiments in which each measurement was made with triplicate samples. * Significantly different ($P < 0.05$) from the value for amylose alone as determined by a paired t-test.

[2] Mixtures of amylose (0.36 mg) and fatty acids (0.1 mg), lysolecithin (0.1 mg) and cholesterol (5 μg) were prepared and incubated with α-amylase and amyloglucosidase for 2 hours at 37°C as described.

DISCUSSION

The ability of amylose to form complexes with various types of lipids has long been recognized (Eliasson and Krog 1985, Guraya et al. 1997, Hanna and Lelievre 1975, Hoover and Hadziyev 1981, Krog 1971). Complex formation with monoglycerides has been shown to inhibit digestion of amylose (Carlsson et al. 1979, Guraya et al. 1997), but information on the effect of free fatty acids is limited. In this study, complexes were formed by incubating mixtures of glucans (2.2 μmol glucose units) and fatty acids (0.04–0.05 μmol) for 10 min at 37°C. Previous studies on interactions between the saturated 12-carbon lauric acid and amylose have shown that complexes can form rapidly at 37°C under conditions resembling those that occur physiologically (Seligman et al. 1998). Amylose binds one lauric acid molecule per ~20 glucose units in the glucan chain, but in contrast, very little lauric acid binds under the same conditions to amylopectin and other branched glucans (Seligman et al. 1998). The experiments were performed at a pH of 4.5 to reflect the moderately acidic pH of partially digested food as it enters the duodenum and because of the acidic pH optima of the enzymes used.

The initial rate of glucose release was rapid from amylose alone and from amylose that was complexed with lauric acid. However after 1 h, hydrolysis of the complexed amylose had slowed down significantly compared with amylose alone. After 6 h, only 60% of the amylose complexed with lauric acid had converted to glucose, whereas in the same time >90% of the uncomplexed amylose had completely hydrolyzed. Myristic, palmitic and oleic acids and lysolecithin were similar to lauric acid in their inhibitory effect on the enzymic breakdown of amylose. However, stearic acid did not inhibit amylose hydrolysis, which may be because it did not form a complex under the conditions of our experiments. The hydrolysis of amylopectin was not inhibited by fatty acids, which is consistent with fatty acids binding poorly to amylopectin (Guraya et al. 1997, Seligman et al. 1998). Our observations on the retardation of amylose breakdown by free fatty acids may have phys-

iological relevance. The duration of our experiments was comparable to the time frame for the transit of food through the small intestine, which is considered to be ~6 h (Holgate and Read 1983).

Starch-containing foods that digest slowly and release glucose for absorption along the length of the small intestine result in reduced postprandial glucose and insulin responses (Jenkins et al. 1982), which could be beneficial for glycemic control particularly for type 2 diabetics. The amylose content is an important factor that determines the digestibility of starch, and therefore, foods that contain starches with a high amylose content are considered to be effective in producing a lower glycemic response (Björck et al. 1994, Goddard et al. 1984). The susceptibility of amylose and amylopectin to enzymic attack will be determined by the structural characteristics of these molecules and may be influenced by retrogradation and complex formation with small molecules. Amylose has a greater tendency to retrograde than amylopectin and thereby to regain a semicrystalline structure that is more resistant to enzyme attack (Berry 1986, Biliaderis 1991). Our results suggest that retrogradation also reduced the capacity of amylose to bind fatty acids. Further, amylose forms inclusion complexes more readily than amylopectin, which can also reduce digestibility. Foods may contain small amounts of free fatty acids that could complex with amylose during eating. Complexes could also form in the small intestine by the interaction of linear $\alpha[1\rightarrow4]$ glucan fragments from partially digested amylose and amylopectin with free fatty acids released from triglycerides by the action of lipases. The apparent digestibility of linear glucose oligomers, produced by debranching of amylopection, is decreased in ileal-canulated dogs by mixing with monoglycerides of stearate and palmitate (Murray et al. 1998). We suggest that glucan-fatty acid complexes with increased resistance to enzymic hydrolysis can form rapidly at 37°C and the breakdown of glucan in these complexes could be retarded sufficiently for it to contribute to the resistant starch component of dietary fiber.

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