Food Chemistry **16** (1985) 37–47

Effect of Maillard Reaction Products on Carbohydrate Utilization—Studies *in vitro* and *in vivo*

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(Received: 18 June, 1984)

ABSTRACT

The effect of Maillard reaction products on the digestion of carbohydrates was studied. Low molecular weight compounds from a glucose–lysine model reaction mixture were weak competitive inhibitors of lactase, invertase, maltase and trehalase in vitro, *when tested at concentrations of 5–10 mg/ml. High molecular weight compounds from the reaction mixture were strong inhibitors of the same disaccharidases at considerably lower concentrations (1·4–2·8 mg/ml). However, when fed to growing rats in amounts up to 40 mg, the high molecular weight compounds failed to reduce the uptake of intubated lactose or sucrose. α-Amylase was not at all affected.*

INTRODUCTION

The handling of foodstuffs prior to consumption may include treatments leading to the formation of new molecules, i.e. compounds that are not naturally present in the fresh food. One of the most important mechanisms behind these effects is the Maillard reaction between amino compounds and reducing carbohydrates. This reaction, leading to the formation of strongly coloured products that give the characteristic colour and flavour to fried or baked food, has been the subject of studies from chemical, technological, nutritional and physiological points of view (Eriksson, 1981; Waller & Feather, 1983). However, in spite of its

Food Chemistry 0308-8146/85/$03·30

wide occurrence in prepared foods, knowledge of its health implications is still limited.

In a recent study (Öste & Sjödin, in press; and results to be published) on the effects of Maillard reaction products on the digestion and uptake of dietary proteins, it was observed that some compounds formed in a glucose–lysine model reaction mixture inhibited the action of intestinal peptidases. The present study was performed in order to examine whether Maillard reaction products had any effect on the activity of enzymes involved in the digestion of dietary carbohydrates (for a review of carbohydrate digestion see Dahlqvist (1978)). Since such an effect was observed *in vitro*, absorption tests of carbohydrates on rats were conducted as well. These tests, however, did not reveal any effect on the uptake of dietary carbohydrates *in vivo*.

EXPERIMENTAL

Model Maillard reaction mixtures

A low molecular weight (LMW) fraction and a high molecular weight (HMW) fraction from a glucose–lysine reaction mixture were prepared as described previously (Öste & Sjödin, in press).

Enzyme assays

α-Amylase (EC 3.2.1.1)

A stock solution of salivary amylase was prepared by diluting 1 ml of saliva with 40 ml 50 % glycerol. Before collecting the saliva the mouth was thoroughly rinsed with water. The amylase activity was measured with soluble starch nach Zulkowsky (from Merck) as the substrate. A 0·05 M K–Na–phosphate buffer, pH 6·9, with 0·04 % NaCl was used to get optimal conditions for the enzyme (Dahlqvist, 1962*a*). The stock solution was adjusted to contain 12 IU/ml (1 IU = 1 μmol maltose liberated/min). The rate of starch digestion was measured by following liberated reducing groups with 3,5-dinitrosalicylate (DNS) reagent (Hostettler *et al.*, 1951). Maltose was used as the standard. Enzyme solution (1 ml) was incubated with 1 ml substrate (1–9 mg starch) at 37 °C. The incubation time was kept short to get a low degree of hydrolysis and thus avoid product inhibition. After 6 min 2 ml of DNS reagent were added to the incubation mixture,

which immediately inactivated the amylase. A substrate/enzyme blank was obtained by mixing the substrate with the DNS reagent before adding the enzyme.

Disaccharidase

Activities of lactase (EC 3.2.1.23), maltase (EC 3.2.1.20), isomaltase (EC 3.2.1.10), sucrase (EC 3.2.1.48) and trehalase (EC 3.2.1.28) were measured on perorally obtained biopsy specimens of human small intestine mucosa as described by Dahlqvist (1968) with the following modification (Dahlqvist, 1984): the IGO reagent was obtained by dissolving 5·6 g Glox (Kabi Diagnostica AB, Stockholm, Sweden) in 100 ml 0·5 M tris buffer, pH 7·0.

Animal experiments

General performance

Absorption tests were performed on growing male Sprague–Dawley rats weighing 150–180 g. The uptake of sucrose and of lactose was studied by orally intubating the lightly anaesthetized (diethyl ether) fasting (overnight) rats with a 4 ml solution of 760 mg sucrose or 400 mg lactose. After the time period allowed for absorption (0·5, 1, 2, 3, 4 or 6 (for lactose) h) the rats were narcotized with diethyl ether, the abdomen was opened and the stomach, upper, middle and lower third of the small intestine and the colon and caecum were removed and collected as segments. Each segment was put into a test-tube with a small amount of distilled water and immediately placed in a boiling water-bath for 2 min before freezing.

The sum of sucrose, fructose and glucose was determined in the samples from the rats given sucrose by assay of reducing power after enzymatic hydrolysis of the sucrose. Total anthrone-reactive carbohydrates were determined in the samples obtained from the rats given lactose.

The rat experiments were performed with and without the addition of the HMW fraction to the carbohydrate solution. In some experiments the HMW fraction (1 ml water solution) was intubated separately prior to intubation of the lactose solution.

Handling of intestinal segments

Each segment was homogenized (Ultra-Turrax homogenizer) at low temperature (ice-bath) and diluted to 100 ml with distilled water. The homogenate (4 ml) was deproteinized by adding 1 ml 0·3 N $Ba(OH)_2$ and

1 ml 5% $ZnSO_4$ (Somogyi, 1945). After centrifugation (1000 *g*, 10 min) the supernatant was recovered and subjected to carbohydrate analysis as described.

Analysis of total carbohydrates after invertase treatment
Part of the deproteinized supernatant (0·5–1·0 ml) was diluted to 1·0 ml with distilled water and 1·0 ml of a solution of sucrase (2·5 mg/ml β-fructosidase from yeast, purchased as powder from Boehringer Mannheim GmbH, W. Germany, in 0·2 M Na–acetate buffer, ph 4·75) was added. After incubation at 30 °C for 60 min, 2·0 ml of the DNS reagent were added and the solution was brought to 100 °C for 10 min, 20 ml distilled water were added and the colour development was measured (room temperature) at 530 nm against a reagent blank. Total reducing carbohydrates in the samples were expressed as glucose equivalents.

Analysis of total carbohydrates with anthrone
The deproteinized homogenate (0·05–0·5 ml) was diluted to 2·0 ml and placed in an ice-bath. Anthrone reagent (4 ml) (300 mg anthrone per 100 ml conc. H_2SO_4, see Scott & Melvin (1953)), chilled in an ice-bath, was slowly added. The mixture was boiled for 7·5 min in a water-bath and the colour development was measured at 625 nm against a reagent blank. The total amount of anthrone-reactive carbohydrates was calculated after analysis of standard solutions of lactose.

RESULTS AND DISCUSSION

Table 1 shows the effect of the glucose–lysine reaction mixtures on the kinetics of the carbohydrate-hydrolysing enzymes. The values of Michaelis' constant (K_m) and maximum reaction velocity (V_{max}) were obtained from Lineweaver–Burk plots (Dixon & Webb, 1964). Amounts of 5–10 mg/ml of the LMW fraction in the assay mixture inhibited the disaccharidases, the inhibition being competitive or of the mixed type. This concentration range was of the same magnitude as that of the substrate and, since the inhibition was relatively weak, the effect is probably of little practical significance. It is known that polyols may competitively inhibit disaccharidases, the strength of inhibition being dependent on the configuration of the polyol (Condue & Lewy, 1957;

TABLE 1

Effects of the Glucose–Lysine Reaction Mixture on the Activities of Human Carbohydrate-Hydrolysing Enzymes *in vitro*

Enzyme	*No addition*	*Addition of the LMW fraction*		*Addition of the HMW fraction*		
		5 mg/ml	*10 mg/ml*	*0·8 mg/ml*	*1·4 mg/ml*	*2·8 mg/ml*
α-Amylase						
K_m[a] (g/litre)	$9{\cdot}2 \times 10^{-4}$	$8{\cdot}5 \times 10^{-4}$	—	$11{\cdot}5 \times 10^{-4}$	—	—
$V_{max}(app)/V_{max}$	—	1·0	—	1·0	—	—
Lactase						
K_m[a] [M]	$1{\cdot}5 \times 10^{-2}$	$3{\cdot}6 \times 10^{-2}$	$6{\cdot}7 \times 10^{-2}$	—	$2{\cdot}0 \times 10^{-1}$	—
$V_{max}(app)/V_{max}$	—	1·1	0·7	—	0·3	—
Invertase						
K_m[a] [M]	$2{\cdot}5 \times 10^{-2}$	—	$3{\cdot}1 \times 10^{-2}$	—	—	$2{\cdot}1 \times 10^{-1}$
$V_{max}(app)/V_{max}$	—	—	1·1	—	—	0·2
Maltase						
K_m[a] [M]	$4{\cdot}3 \times 10^{-3}$	$6{\cdot}3 \times 10^{-3}$	11×10^{-3}	—	—	$14{\cdot}7 \times 10^{-3}$
$V_{max}(app)/V_{max}$	—	1·0	0·9	—	—	0·8
Trehalase						
K_m[a] [M]	$2{\cdot}7 \times 10^{-3}$	—	$3{\cdot}7 \times 10^{-3}$	—	—	[b]
$V_{max}(app)/V_{max}$	—	—	0·9	—	—	—

[a] Apparent K_m.

[b] Trehalase was inhibited by the HMW fraction but the obtained substrate concentration/velocity values did not fit to a straight line in the Lineweaver–Burk plot and K_m or V_{max} could not be calculated.

Kelemen & Whelan, 1966). During the initial stages of the Maillard reaction, various polyols are formed as the result of condensation of the carbohydrate moiety with the amino compound and a subsequent partial dehydration. During later stages, further dehydration removes more hydroxy groups and gives rise to various aromatic compounds. However, if present, the polyols formed, at least initially during the Maillard reaction, may very well be responsible for the observed competitive inhibition.

The intense colour of the HMW fraction limited the concentration that could be tested with reasonable accuracy in the assay. In spite of this, the presence of 1·4–2·8 mg/ml of this fraction in the assay strongly inhibited all the disaccharidases. The inhibition was of the mixed type with a pronounced competitive element. Sucrase and lactase were most affected. Trehalase was inhibited as well but the substrate concentration/velocity values obtained did not fit a straight line in the Lineweaver–Burk plot.

The complete structure of the polymeric compounds (melanoidins) formed during the Maillard reaction is unknown. However, various functional groups and partial structures have been detected, including hydroxy groups as well as more dehydrated, aromatic parts (Pernemalm, 1978; Kato & Tsuchida, 1981). It is thus not unreasonable to assume that these polymers have both polyolic and more lipophilic regions. Indeed, a repeating unit in the polymer involving polyolic structures has been proposed (Kato & Tsuchida, 1981). In addition, the melanoidins are known to adsorb to proteins (Horikoshi & Gomyo, 1976). With both polyolic structures and adsorptive capacities, the compounds of the HMW fraction may in fact possess such inhibitory properties as we observed with the disaccharidases.

Salivary α-amylase was not inhibited by either the LMW or the HMW fraction (the relatively low concentrations of them in the assays were due to the interfering colour). Since the properties of salivary amylase are close to those of pancreatic amylase (Meyer *et al.*, 1948), the enzymes are probably identical and it is reasonable to extrapolate the results and assume that the intestinal digestion of starch in general should not be affected by the presence of Maillard reaction compounds.

The intestinal uptake of sucrose and of lactose in the rat with (1 %w/w) and without the HMV fraction added is shown in Fig. 1. No effect on the time curve of the absorption or the total amount absorbed could be observed. The concentrations of the HMW fractions in the in-

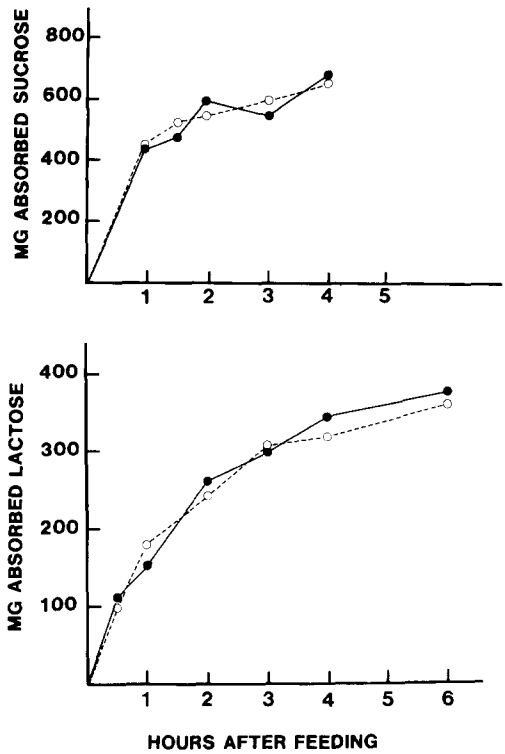

Fig. 1. Rats (150–180 g) were fed (orally intubated) a sucrose (760 mg) or a lactose (400 mg) solution with (filled symbols) or without (open symbols) the addition of 1 % high molecular weight Maillard reaction compounds (HMW fraction). After the time allowed for absorption, the total carbohydrate remaining in the gastro-intestinal tract was analysed. Each point represents the average of two or three rats.

tubated solutions were low compared with the carbohydrates but still high enough (a total amount per rat of 7·6 mg in the sucrose study and 4 mg in the lactose study) to most likely bring about an inhibition *in vivo*, since the observed *in vitro* effect was very strong. However, in rat as well as in man the hydrolytic capacity of sucrose exceeds the transport capacity for the monosaccharides formed (Dahlqvist & Thomson, 1963; Herman, 1974). Thus a partial inhibition might not be detected with the present methodology and may in that case also be of little practical significance. On the other hand, the intestinal lactase activity is rate-limiting for the utilization of dietary lactose, both in most adult humans (Dahlqvist, 1962*b*) and in the rat (Dahlqvist & Thomson, 1964). This means that if the

lactase activity was affected, this should be reflected in the amount of remaining lactose in the intestine. No such effect was observed in our study. Possible explanations for this may include too low a concentration of the inhibiting compounds of the HMW fraction at the site of hydrolysis. However, if the volume of the intestinal content was between 5–10 ml, the concentration of the HMW fraction should have been about 0·4–0·8 mg/ml, or enough to get a notable inhibition. In addition, rats receiving considerably more of the HMW fraction (up to 40 mg) were still capable of absorbing lactose to the same extent as control rats, as judged from the amount of lactose remaining in the gastro-intestinal tract 2 h after feeding (Table 2).

It thus seems evident that the lack of an *in vivo* effect is not the result of too low concentrations of these compounds in the rat intestine. However, it is still possible that the inhibitor concentrations at the actual moment of lactose hydrolysis were not sufficient to bring about an effect. Horikoshi *et al.* (1981) reported that the polymers of a glucose–glycine reaction mixture were strongly adsorbed to the mucus membrane of the small intestine in the rat. If such an adsorption occurred in our experiments, the polymers might have been excluded from an action of competitive inhibition of the enzymes. The same authors also reported that the brown polymers after feeding by intubation formed a hard gel in the stomach. Such an effect is also in concordance with our observation that radiolabelled high molecular weight compounds from a glucose–lysine reaction mixture to a large extent still remained in the stomach 8 h after

TABLE 2

Effect of the Intubated Amount of High Molecular Weight Maillard Reaction Products (HMW Fraction) on 2 h Lactose Absorption in Rats

HMW fraction (*% of administered lactose (400 mg)*)	*Remaining lactose* (*% of administered dose*)	
	Stomach	*Intestine*
0 (10)	0·78 ± 0·44	9·9 ± 2·9
1 (10)	0·57 ± 0·50	9·6 ± 2·3
3 (10)	0·77 ± 0·60	7·3 ± 3·6
10 (8)	0·97 ± 0·49	7·8 ± 2·0

All values are mean ± SD.
(Within parentheses: number of rats.)
Mean rat weight: 133 g.

intubation (Nair *et al.*, 1981). If so, the polymers and the disaccharide solution might have left the stomach at different times and the absorption of lactose would then proceed unaffected. To test if such an effect was present in our experiments, we fed rats the HMW fraction 2, 4, 6 or 16 h in advance of the lactose solution. However, as seen in Table 3, the lactose absorption was still unaffected.

The results suggest that there is no effect *in vivo* on the lactase activity in the rats. The HMW fraction may mediate its effect *in vitro* through properties of the free enzyme that are not present when the enzyme is membrane-bound in the intestine. Another, possibly more likely, explanation could be the presence of a diffusion barrier close to the epithelial cell membrane in the intestine, through which the substrate molecules have to penetrate before reaching the enzyme. A barrier in the form of an 'unstirred layer of water' has been observed to greatly influence the rate of diffusion of molecules to the microvillus membrane and into epithelial cells *in vitro* (Wilson & Dietschy, 1974). The effect was more pronounced with larger molecules than with smaller ones. Molecules of the HMW fraction have a size exceeding 6000–8000 daltons (Öste & Sjödin, in press) and may thus exhibit a considerable restraint to diffusion through this layer, compared to the lactose molecule.

In conclusion, the presented results showed that low molecular weight compounds from a glucose–lysine reaction mixture are weak inhibitors of

TABLE 3

Effect of a Time-lag Between Intake of High Molecular Weight Maillard Reaction Products (HMW Fraction) and Intake of Lactose on 2 h Lactose Absorption in Rats

Time-lag from intake of HMW fraction (20 mg) to lactose intake (h)	*Remaining lactose (% of administered dose (400 mg))*	
	Stomach	*Intestine*
0 (5)	2·4 ± 1·9	30·1 ± 6·4
2 (4)	3·7 ± 2·8	25·9 ± 9·0
4 (5)	3·4 ± 2·6	29·3 ± 9·9
6 (5)	1·9 ± 1·2	22·9 ± 6·8
16 (5)	6·3 ± 2·5	23·8 ± 6·6

All values are mean ± SD.
(Within parentheses: number of rats.)
Mean rat weight: 136 g.

the disaccharidases of the gastro-intestinal tract. High molecular weight compounds, on the other hand, are potent inhibitors *in vitro*, especially of lactase and invertase. During *in vivo* conditions, the same compounds failed to affect the uptake of dietary disaccharides.

REFERENCES

Condue, I. & Lewy, G. A. (1957). Inhibition of glycosidases by aldonolactones of corresponding configuration. *Biochem. J.*, **65**, 389–95.

Dahlqvist, A. (1962*a*). A method for the determination of amylase in intestinal content. *Scand. J. Clin. Lab. Invest.*, **14**, 145–51.

Dahlqvist, A. (1962*b*). Specificity of the human intestinal disaccharidases and implications for hereditary disaccharide intolerance. *J. Clin. Invest.*, **41**, 463–70.

Dahlqvist, A. (1968). Assay of intestinal disaccharidases. *Anal. Biochem.*, **22**, 99–107.

Dahlqvist, A. (1978). Disturbances of the digestion and absorption of carbohydrates. *Biochemistry of Carbohydrates II*, Vol. 16, Manners, D. J. (ed.), University Park Press, Baltimore, pp. 179–207.

Dahlqvist, A. (1984). β-Glucosidases (disaccharidases) In: *Methods of Enzymatic Analysis*, Vol. IV, Bergmeyer, H. U. (ed.), Verlag Chemie, Weinheim (in press).

Dahlqvist, A. & Thomson, D. L. (1963). The digestion and absorption of sucrose by the intact rat. *J. Physiol.*, **167**, 193–209.

Dahlqvist, A. & Thomson, D. L. (1964). The digestion and absorption of lactose by the intact rat. *Acta Physiol. Scand.*, **61**, 20–33.

Dixon, M. & Webb, E. C. (1964). *The Enzymes*, Longmans, Green and Co. Ltd, London.

Eriksson, C. (ed.) (1981). Maillard reactions in food. *Prog. Fd. Nutr. Sci.*, **5**, 1–6.

Herman, R. M. (1974). Hydrolysis and absorption of carbohydrate, and adaptive responses of the jejunum. In: *Sugars in Nutrition*, Sipple, H. C. & McNutt, K. W. (eds.), Academic Press, New York.

Horikoshi, M. & Gomyo, T. (1976). Precipitation of protein in the presence of melanoidin. *Agr. Biol. Chem.*, **40**(8), 1531–7.

Horikoshi, M., Ohmena, H., Gomyo, T., Kuwabara, Y. & Ueda, S. (1981). Effects of browning products on the intestinal microflora of the rat. *Prog. Fd. Nutr. Sci.*, **5**, 223–8.

Hostettler, F., Borel, E. & Deuel, H. (1951). Über die Reduktion der 3,5-Dinitrosalicylsäure durch Zucker. *Helv. Chim. Acta*, **34**, 2132–9.

Kato, H. & Tsuchida, H. (1981). Estimation of melanoidin structure by pyrolysis and oxidation. *Prog. Fd. Nutr. Sci.*, **5**, 147–56.

Kelemen, N. V. & Whelan, W. J. (1966). The inhibition of glucosidases and galactosidases by polyols. *Biochem. J.*, **100**, 5.

Meyer, K. H., Fischer, E. H., Bernfeld, P. & Duckert, F. (1948). Purification and crystallization of human pancreatic amylase. *Arch. Biochem.*, **18**, 203–5.

Nair, B. N., Öste, R., Asp, N-G. and Pernemalm, P-Å. (1981). Absorption and distribution of a ^{14}C-glucose lysine reaction mixture in the rat. *Prog. Fd. Nutr. Sci.*, **5**, 217–22.

Öste, R. & Sjödin, P. Effect of Maillard reaction compounds on protein digestion, *in vivo* studies on rats. *J. Nutrition* (in press).

Pernemalm, P-Å. (1978). Studies on the Maillard reaction, PhD thesis, Swedish University of Agricultural Sciences, Uppsala.

Scott, T. A. & Melvin, E. H. (1953). Determination of dextran with anthrone. *Anal. Chem.*, **25**, 1656–61.

Somogyi, M. (1945). Determination of blood sugar. *J. Biol. Chem.*, **160**, 69–73.

Waller, G. R. & Feather, M. S. (eds) (1983). The Maillard reaction in foods and nutrition, *ACS Symposium Series 215*, ACS, Washington.

Wilson, F. A. & Dietschy, J. M. (1974). The intestinal unstirred layer. Its surface area and effect on active transport kinetics. *Biochem. Biophys. Acta*, **363**, 112–26.