INTESTINAL LACTASE EVALUATION IN VIVO WITH 3-METHYLLACTOSE

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1. Introduction

Intestinal disaccharidases in higher animals are located in the luminal membrane of the intestinal mucosa, where they hydrolyse disaccharides prior to the absorption of sugars as monosaccharides [1]. Lactase deficiency is a rather rare inborn metabolic error [2], but it is also a developmental syndrome normal in adult rats [3–5] and rabbits [6] and very common in adult humans [7]. There seems to be a biological clock with a marked decline of the lactase after the time of weaning, irrespective of the diet, except for people whose ancestors have been long dependent on substantial consumption of milk. Hence, determination of intestinal lactase is important both in pediatrics and in gastroenterology. It can be carried out directly by mucosal sampling or indirectly evaluated from the level of glucose in blood after a lactose load.

Glycosydases are generally tolerant to the aglycon moiety of oligosaccharides. This includes the intestinal lactase [8], although exceptions have been reported [9,10]. It was thought that if 3-methyllactose could be synthesized it would probably be a good substrate for the intestinal lactase, yielding 3-methylglucose which is known to be readily absorbed from the intestine [11] and rapidly eliminated in the urine [12]. This can happen because of a most favorable combination of the specificities of the intestinal glucose carrier [13], for which 3-methylglucose is a good substrate, the hexokinase [14] and glucokinase [15], for both of which it is inert, and the renal glucose carrier [16], for which it is not a substrate.

We report here, 3-methyllactose has been synthesized, found to be readily hydrolysed by the intestinal lactase, and when administered orally to suckling rats led to urinary excretion of 3-methylglucose, that can be estimated simply by reducing power or specifically by gas–liquid chromatography.

2. Materials and methods

As a source of intestinal lactase free of lysosomal β-galactosidase (known to have different specificities [17]), brush border was isolated from rat intestinal mucosa by the method in [18].

Total sugar was estimated by reducing power with the Somogyi-Nelson reagent [19], galactose was determined with galactose dehydrogenase [20] and glucose was determined with glucose oxidase [21].

Auxiliary enzymes and coenzymes were obtained from Sigma.

A Perkin-Elmer 900 gas chromatograph was used with a glass capillary column of 2 mm diam. and 45 m length filled with SE-30, at a constant temperature of 200°C, and with N_2 carrier gas. Trimethylsilyl derivatives of the sugars were prepared as in [22].

3. Results and discussion

3.1. *Synthesis of 3-methyllactose*

To a solution of α-lactose hydrate (20 g) in cooled 20% sodium hydroxide (1 l), benzoyl chloride (100 ml) was added dropwise with stirring. The

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mixture was stirred for 1 h, kept for 1 h at room temperature, filtered and the solid washed with water until neutral and extracted with boiling methanol. The residual syrup crystallized from chloroform–methanol to yield 16 g of 1,2,6,2′,3′,4′,6′-hepta-*O*-benzoyl-lactose, m.p. 195–197°C [23]. This compound (10 g) was methylated with diazomethane and boron trifluoride etherate in dichloromethane to give 1,2,6,2′,3′,4′,6′-hepta-*O*-benzoyl-3-*O*-methyl-lactose (7 g) m.p. 204–205°C (chloroform–methanol) [23]. Treatment of this 3-*O*-methyl derivative (5 g) with 2 M sodium methoxide in methanol (200 ml) for 24 h, gave a clear solution which was neutralized with Amberlite IR-120H$^+$ resin, and evaporated to dryness. The residue was washed with ethyl acetate to give 3-methyllactose as a highly hygroscopic solid (0.32 g) which refused to crystallize. The ^{1}H NMR spectrum of this compound was in good agreement with the assigned structure. Acidic or enzymatic hydrolysis gave galactose and 3-methylglucose, but no glucose.

3.2. *Hydrolysis of 3-methyllactose by intestinal lactase*

The intestinal lactase, isolated as in section 2, hydrolysed 3-methyllactose at pH 6.5 with virtually the same efficiency as lactose: V_{max} 90% and K_m 25 mM, versus 100 mM and 20 mM for lactose.

3.3. *Oral administration to suckling rats*

3-Methyllactose or a mixture of 3-methylglucose and galactose were administered orally to suckling rats, collecting urine and analysing it for sugars as in table 1. The results in table 1 show that 3-methylglucose unaccompanied by 3-methyllactose appears in the urine shortly after the administration of the latter. In this case, the elimination of liberated 3-methylglucose was linear during the first 8 h; the contrast with the rapid elimination in the case of administration of a mixture of 3-methylglucose + galactose indicates that hydrolysis in the intestine was the limiting factor. Nevertheless, the hydrolysis in vivo was slower than expected. The possibility of disturbing impurities could not be ruled out because

Table 1
Elimination of 3-methylglucose (3MG) in urine after oral administration of 3-methyllactose (3ML)

	Oral sugars					Sugar eliminated in urine within 8 h					
							Distribution (%)				
Rats	3MG (mg)	Gal (mg)	3ML (mg)	Volume (ml)	Osmolarity (osm/l)	(mg)	3MG	Glu	Gal	3ML	% 3MG (from 3ML oral)
Controls											
A_1	24	25	–	0.5	0.7 (+Na^+)	24	89	< 3	8	–	–
A_2	24	25	–	0.5	0.7	22	85	< 3	11	–	–
Problems											
B_1	–	–	50	0.5	0.5 (+Na^+)	4	95	< 2	< 2	–	18
B_2	–	–	50	0.5	0.5	5	95	< 2	< 2	–	20
B_3	–	–	50	0.25	0.5						
B_4	–	–	50	0.25	0.5						
B_5	–	–	50	0.25	0.5	3.2 ± 0.5	95	< 3	< 2	–	12 ± 4
B_6	–	–	50	0.25	0.5						

A group of 4 suckling rats of the same litter and age (15 days) (A_1, A_2, B_1, B_2) were fasted for 4 h in metabolic cages at 30°C and orally given sugars in 0.5 ml with NaCl to complete osmolarity as indicated. Another group of 4 similar rats from another litter (B_3–B_6) were treated similarly, but given 3ML in 0.25 ml. Urine was collected by transabdominal bladder pression every 2 h during 8 h. Sugars eliminated during this time are indicated in the table; controls eliminated most of the 3MG within the first 4 h (68% of the 3MG given); 3MG elimination in the problems given 3ML was linear with time during the observed period. No 3ML was detected by paper chromatography in the urine in any of the animals given this sugar

of exhaustion of the lot of 3-methyllactose used in these experiments.

The estimation of 3-methylglucose in urine by reducing power, with enzymatic controls for galactose and glucose, can serve for most purposes. Nevertheless, a much better estimation, both in specificity and sensitivity, can be carried out by gas–liquid chromatography as shown in fig.1. Since of the two peaks of 3-methylglucose, the 2nd overlaps with one of those given by galactose, only the 1st peak should be used for the estimation of 3-methylglucose in urine taking into account that it corresponds to ~50% of the total.

3.4. *Perspectives*

It appears that oral administration of 3-methyllactose followed by estimation of 3-methylglucose in urine could be adapted for the routine non-invasive evaluation of intestinal lactase in vivo. Work is in progress to improve the yield and purity of 3-methyllactose for test in humans, giving some 0.1–0.2 g/kg body wt to fasted subjects and collecting urine for analysis before and during the next 3–5 h. Moreover, the same principle could be considered likely to be applied to other intestinal disaccharidases whose physiological substrates have glucose as aglycon.

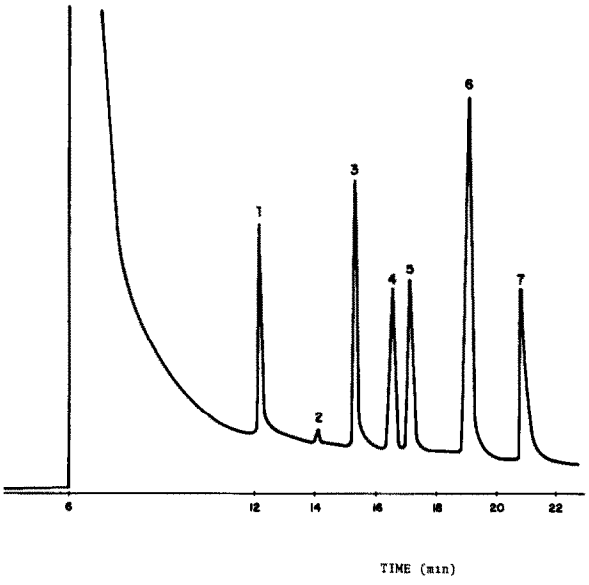

Fig.1. Gas–liquid chromatography of urine of a suckling rat with 3-methylglucose, galactose, glucose and mannitol added. Peak numbers indicated in the figure correspond to: 1, 3MG, 1st peak; 2, gal, 1st peak; 3, 3MG, 2nd peak + gal, 2nd peak; 4, gal, 3rd peak; 5, glu, 1st peak; 6, mannitol; 7, glu, 2nd peak.

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