THE OCCURRENCE OF A β-GALACTOSYLTRANSFERASE IN NORMAL HUMAN URINE

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1. Introduction

The enzymes of carbohydrate metabolism occurring in urine are the glycosidases [1–4], the properties of which have been studied extensively during the past few years. Glycosyltransferases are widespread in nature and have been found in many mammalian tissues and body fluids [5,6], but their presence in urine has not been reported. Eight samples of human urine were examined for galactosyltransferase activity and an enzyme with the specificity of the A protein of lactose synthetase [7] was detected in each.

2. Materials and methods

Uridine diphosphate-D-[^{14}C]-galactose (300 mCi/mmole) (UDP-[^{14}C]gal) was purchased from the Radiochemical Centre, Amersham, England. Phenyl-β-D-glucoside (ph-β-Glc), phenyl-β-*N*-acetyl-D-glucosaminide (ph-β-GNAc), phenyl-β-D-galactoside (ph-β-Gal), and bovine α-lactalbumin (α-LA) were obtained from Koch-Light Laboratories, Colnbrook, England.

Descending paper chromatography was performed on Whatman No. 1 paper using the following systems: a) ethyl acetate–pyridine–water (10:4:3, by vol); b) ethyl acetate–pyridine–water (2:1:2, by vol); c) ethyl acetate–glacial acetic acid–water (3:1:1, by vol).

Radioactivity was detected using a Packard Radiochromatogram Scanner (Model 7201) and counted on paper using a Nuclear Chicago Liquid Scintillation Counter (Unilux II), with Scintimix II (Koch-Light) in toluene as scintillant. The counting efficiency was approximately 70%.

Urine samples were collected as non-starved samples in mid-afternoon from healthy donors aged between 22 and 36 years. The samples were immediately cooled to 4°C and used within 2 hr.

Serum samples were obtained as described previously [8].

The galactosyltransferase activity was assayed by measuring the transfer of [^{14}C]galactose from UDP-[^{14}C]gal to various low molecular weight acceptors. The reaction conditions are given in table 1. After incubation, the mixture was chromatographed in solvent a) for 24 hr (6 hr using the phenyl glycosides as acceptor).

The anomeric linkage of the transferred [^{14}C]galactose was determined using an α-galactosidase from *Turbo cornutus* and a β-galactosidase from *Charonia lampas* (both supplied by Seikagaku Kogyo Co. Ltd., Tokyo, Japan).

Urinary creatinine was measured using the method of Løken [9].

Galactosidase activity was determined under the conditions of the galactosyltransferase assay be reincubating the [^{14}C]galactoside products (approximately 4000 cpm) as described in table 1, but omitting the UDP-[^{14}C]gal and sugar acceptor.

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Table 1
Transfer of [^{14}C]gal from UDP-[^{14}C]gal to monosaccharides and phenyl glycosides by an enzyme in human urine

Donor	Sex	ABO blood group	Incorporation (cpm/μg creatinine)							
			GNAc		Glc		ph-β-GNAc		ph-β-Glc	
			−α-LA	+α-LA	−α-LA	+α-LA	−α-LA	+α-LA	−α-LA	+α-LA
A.E.	M	A	48	343	9	295	80	658	1	5
P.H.	M	A	243	1121	6	1390	510	1758	6	29
G.P.	F	A	26	160	3	149	91	387	1	2
L.R.*	F	A	244	543	54	1950	567	–	–	25
O.U.	M	B	69	416	0	1339	185	961	0	7
A.C.	M	O	120	571	0	1373	199	1694	0	31
I.K.	F	O	1007	1567	56	1755	1949	2007	35	35
E.W.	F	O	49	217	4	127	150	318	2	5

* Sample obtained at week 31 of pregnancy.
Abbreviations: GNAc, *N*-acetyl-D-glucosamine; Glc, D-glucose.
Reaction mixture: UDP-[^{14}C]gal, 0.17 nmole (77 000 cpm); ATP, 0.5 μmole; Tris–HCl buffer, pH 7.2, 1.25 μmole; $MnCl_2$, 1 μmole; sodium azide, 1 μmole; acceptor, 0.5 μmole; α-lactalbumin (when added), 250 μg; urine, 25 μl. Total volume 150 μl. Incubated at 37°C for 16 hr.

3. Results

Samples of urine from eight donors were tested for galactosyl-transferase activity with GNAc, ph-β-GNAc, Glc, and ph-β-Glc as acceptor (table 1), in the presence and absence of exogenous α-lactalbumin. All the samples tested were capable of transferring [^{14}C]gal to GNAc and ph-β-GNAc and this activity was enhanced by the addition of α-LA under the conditions used. Transfer of [^{14}C]gal to gluclose was very low in the absence of α-LA but was greatly enhanced by the addition of α-LA. Transfer of [^{14}C]-gal to ph-β-Glc was also very low and was increased only slightly by adding α-LA (table 1). Under identical conditions, except using serum as the enzyme source, transfer of [^{14}C]gal in the absence of α-LA was 21 066 cpm to GNAc, 63 867 cpm to ph-β-GNAc, 457 cpm to glucose, and 670 cpm to ph-β-Glc. In the presence of α-LA the transfer of [^{14}C]gal was 37 154 cpm to GNAc and 33 605 cpm to glucose. In all the incubations using serum and urine some unchanged UDP-[^{14}C]gal was detected at the end of the reaction.

The product formed by galactose transfer using GNAc as acceptor and either urine or serum as the enzyme source had a chromatographic mobility relative to lactose (R_L value) of 1.48 in solvent a), 1.3 in solvent b), and 2.1 in solvent c). With glucose as acceptor the products co-chromatographed with lactose in the same three solvent systems. Ph-β-GNAc as acceptor gave a product of R_L = 5.4 in solvent a) and 3.1 in solvent b). Reincubation of the reaction products with urine did not result in the liberation of free [^{14}C]gal, which demonstrated that no galactosidase activity was present in urine under the conditions of galactosyltransferase assay.

Treatment of the reaction products with α- and β-galactosidases suggested that the [^{14}C]gal was β-linked to the acceptor.

Some requirements for galactose transfer are given in table 2. The enzyme shows a requirement for Mn^{2+} which cannot be replaced by Mg^{2+}. Of the compounds tested, only GNAc and ph-β-GNAc were acceptors in the absence of α-LA. In the presence of α-LA, glucose, ph-β-Glc, and possibly ph-β-Gal were also acceptors. D-Galactose, D-mannose, D-xylose, L-fucose, lactose, *N*-acetyl-D-galactosamine, and myo-inositol were not acceptors either with or without α-LA (table 2).

When urine samples were centrifuged at 105 000 *g*

Table 2
Requirements for galactose transfer

Reaction mixture	[^{14}C]gal transfer			
	−α-LA		+α-LA	
	cpm/sample	cpm/μg creatinine	cpm/sample	cpm/μg creatinine
Complete*	1869	120	8915	571
− Mn^{2+}	27	2	–	–
+ 4 μmole Mn^{2+}	3163	203	–	–
− Mn^{2+}, + 1 μmole Mg^{2+}	0	0	–	–
− GNAc	0	0	0	0
− GNAc, + Glc	0	0	21 458	1373
− GNAc, + Glc: − Mn^{2+}	–	–	0	0
− GNAc, + Glc: + 4 μmole Mn^{2+}	–	–	20 939	1342
− GNAc, + Glc: − Mn^{++}, + 1 μmole Mg^{2+}	–	–	204	13
− Enzyme	0	0	0	0
− GNAc, + phenyl-β-GNAc	3104	199	26 469	1694
− GNAc, + phenyl-β-Glc	0	0	484	31
− GNAc, + phenyl-β-Gal	0	0	119	7

* As table 1 with GNAc as acceptor and urine, A.C.

for 1 hr all the enzymic activity remained in the supernatant, which demonstrated that the enzymic activity is not due to cell debris in the urine.

4. Discussion

The ability of urine to transfer galactose from UDP-galactose to GNAc and ph-β-GNAc demonstrates the presence of a UDP-galactose: *N*-acetyl-D-glucosamine galactosyltransferase. The enzyme was also able to transfer small amounts of galactose to glucose and ph-β-Glc. The addition of α-LA to the reaction mixtures resulted in an increase of enzymic activity. This was very pronounced when glucose was the acceptor (table 1): the product being indistinguishable from lactose. This enzyme has a requirement for Mn^{2+} and has properties consistent with those of the A protein of lactose synthetase (EC 2.4.1.22), the presence of which has been demonstrated in human milk [10,11], amniotic fluid [12], and serum [13]; bovine milk [14–17]; bovine and guinea pig mammary gland [18]; porcine serum and liver [19] and a variety of other sources [20].

Although several enzymes have been found in urine, this is the first report of the presence of a glycosyltransferase in this material.

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