SOLUBILIZATION OF OLIGOSACCHARIDE TRANSFERASE AND GLUCOSIDASE ACTIVITIES FROM THYROID ROUGH MICROSOMES

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1. Introduction

In previous studies from this laboratory [1,2], an oligosaccharide transferase activity was demonstrated in thyroid rough microsomes: the membranous enzyme catalyzes the transfer of oligosaccharides from exogenously supplied oligosaccharide lipids to the asparagine residue of synthetic Asn-X-Thr containing peptide acceptors. This transferase was also recently described in hen oviduct [3], rat liver [4] and yeast [5] membranes. As a prerequisite for purification, this paper examines the conditions which allow total extraction of the enzyme from membranes while maintaining full activity. Although a few other glycosyltransferases involved in the early lipid-linked pathway of *N*-glycosylation have already been solubilized [6,7], this is the first report dealing with the oligosaccharide transferase.

Several authors have suggested that glucose-containing oligosaccharide lipids constitute the physiological donor of the oligosaccharide transferase [8,9]. Glucose residues might be a signal in the transfer reaction before being removed by specific glucosidases. The present study points out that the oligosaccharide transferase is extracted together with two glucosidases of distinct specificities.

2. Materials and methods

2.1. *Solubilization of oligosaccharide transferase and glucosidase activities*

Thyroid rough microsomes were prepared as previously described [10] and rapidly frozen in liquid nitrogen before storage at −20°C until use. For the detergent extraction, they were resuspended in 20 mM Tris–HCl pH 7.5, 0.14 M sucrose, 1 mM $MnCl_2$ before addition of 1/10 vol. of 5 M NaCl and 1/10 vol. of 2% DOC (final concentration of protein, 5–6 mg/ml). After standing for 30 min at 0°C, the suspension was centrifuged at 100 000 × *g* for 1 h.

Abbreviations: DMSO, dimethylsulfoxide; DOC, sodium deoxycholate

2.2. *Preparation of labeled oligosaccharide lipids and glycopeptides*

(i) Glucose-containing [Man-^{3}H]oligosaccharide lipids were labeled by incubating daily prepared thyroid rough microsomes for 10 min at 37°C in the presence of 10 μM UDP-Glc and 1 μM GDP-[^{3}H]Man [11]. The delipidation was carried out as in [1].

(ii) [Glc-^{3}H]oligosaccharide lipids were similarly prepared by incubating the particles with 10 μM UDP-[^{3}H]Glc [11].

(iii) [Glc-^{3}H]glycopeptides were obtained from the delipidated glycoprotein residue of (ii) by pronase digestion [10]. The proteolytic digest was then heated at 100°C for 5 min and partitioned with chloroform-methanol. The aqueous phase was used as glycopeptides.

2.3. *Oligosaccharide transferase assay*

Assay reactions contained the following components in a final volume of 150 μl: Tris–HCl, 20 mM, pH 7.5; $MnCl_2$, 1 mM; sucrose, 0.14 M; synthetic heptapeptide of porcine ribonuclease (residues 73–79), 50 nmol; glucose-containing [Man-^{3}H]oligosaccharide lipids, 10 000 cpm; DMSO, 10% when mentioned; and 75 μl of the 100 000 × *g* supernatant. After incubating for 2 h at 30°C, the reactions were stopped by adding 10 μl of concentrated formic acid. The reaction mixtures were centrifuged at 11 000 × *g* for 5 min. The [Man-^{3}H]-glycosylated peptides

were isolated by paper high voltage electrophoresis in 1.5 N formic acid [2]. All the assays were in duplicate.

2.4. *Glucosidase assay*

Assay mixtures were as in section 2.3, except that peptide acceptor and DMSO were omitted and that the glucose-labeled substrate (10 000 cpm) was either [Glc-^{3}H]oligosaccharide lipids or [Glc-^{3}H]glycopeptides. After incubating at 30°C for 2 h, the reactions were stopped by adding 2 μl of formic acid, neutralized by NaOH, and submitted to paper high voltage electrophoresis in 1% sodium borate 75 min at 40 V/cm (migration of free glucose, 10 cm). All the assays were in duplicate.

3. Results and comments

3.1. *Effect of detergents on the membranous oligosaccharide transferase*

Fig.1 shows the effect of increasing concentrations of two types of mild detergents on the oligosaccharide transferase activity present in rough microsomes. Either ionic (sodium cholate or deoxycholate) or non-ionic (Triton X-100), they inhibited the enzyme when assayed at 37°C (fig.1A, cholate) but stimulated it to various extents at lower incubation temperature. At 30°C, 0.25% cholate showed the highest stimulation (fig.1A) whereas deoxycholate on Triton produced its maximal effect around 0.125% (fig.1B). The transferase appeared to be sensitive to deoxycholate over a very narrow concentration range.

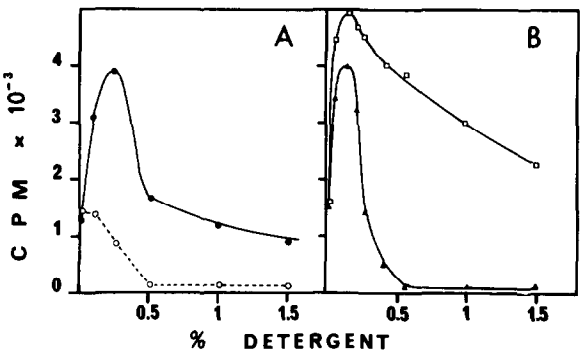

Fig.1. Effects of various detergents on the microsomal oligosaccharide transferase activity. Rough microsomes were incubated for 1 h with the peptide acceptor and [Man-^{3}H]-oligosaccharide lipids dispersed in 0.01% cholate or DOC, without DMSO. All other conditions were as in [1]. (A) sodium cholate at 30°C (●——●) or 37°C (○– – –○). (B) DOC (▲——▲) or Triton X-100 (□——□) at 30°C.

3.2. *Solubilization of the oligosaccharide transferase*

Several extraction procedures were examined for their ability to liberate the transferase from the microsomal membrane. The criterion employed for solubilization was recovery of activity in the supernatant after centrifugation at 100 000 × *g*. As can be seen in table 1, all three detergents tested were found to release significant enzyme activity at a concentration close to that which produced the maximum of stimulation for the membranous enzyme. However, the extraction of the transferase can be enhanced either by repeating freezing and thawing or more efficiently by adding salt in the solubilization buffer. At 0.5 M NaCl–0.2% DOC, essentially all the transferase activity was solubilized (av. 96%; 5 expts) together with 60% of the microsomal proteins; higher concentrations of DOC proved inhibitory. This was not observed

Table 1
Solubilization of oligosaccharide transferase by various detergents

Solubilization conditions		Enzyme activity	
		cpm	% control
pH 7.5	10% DMSO	0	0
With freezing and thawing (4 cycles)			
pH 7.5	0.2% cholate	0	0
	0.5% cholate	220	9
	1 % cholate	50	2
	0.2% cholate – 0.5 M NaCl	755	30
	0.5% cholate – 0.5 M NaCl	750	30
	1 % cholate – 0.5 M NaCl	650	26
pH 7.2	0.2% cholate – 0.5 M NaCl	720	29
pH 8.3	0.2% cholate – 0.5 M NaCl	760	30
Without freezing and thawing			
pH 7.5	0.2% cholate – 0.1 M NaCl	210	8
	0.2% cholate – 0.5 M NaCl	310	12
	0.2% cholate – 0.8 M NaCl	245	10
	0.2% cholate – 0.5 M NaCl	565	22
	0.2% DOC – 0.5 M NaCl	2500	100
	0.5% DOC – 0.5 M NaCl	130	5
	1 % DOC – 0.5 M NaCl	0	0
	0.2% Triton	425	17
	0.2% Triton – 0.5 M NaCl	1240	50
	0.5% Triton	1640	66
	1 % Triton	1930	77

Table 2
Comparative effect of DMSO on the microsomal and solubilized oligosaccharide transferase

Addition	Enzyme activity	
	cpm	% control
Microsomes		
none	1200	100
+ cholate (0.2%)	3930	326
+ cholate (0.2%) + DMSO (10%)	11 528	961
+ cholate (0.5%)	1857	155
+ cholate (0.5%) + DMSO (10%)	6093	508
0.2% cholate – 0.5 M NaCl extract		
none	310	100
+ DMSO (10%)	1470	474
0.2% DOC – 0.5 M NaCl extract		
none	2500	100
+ DMSO (10%)	5161	206
+ Triton X-100 (10%)	2425	97

for Triton which even at 1% final concentration extracted no more than 77% of the control activity. It can be speculated from these data not only that the oligosaccharide transferase is embedded in the membrane by hydrophobic interactions but also that it is tied to some microsomal components by electrostatic interactions since both high salt concentration and ionic detergent are needed to solubilize it entirely.

3.3. *Properties of the solubilized oligosaccharide transferase*

One salient feature already observed for this enzyme [1] is that it appeared stimulated by DMSO. Although this agent itself did not allow solubilization (table 1), it led to increased transferase activity whenever the enzyme was membrane-bound or solubilized (table 2). It could not be replaced by 0.1% Triton. The effect of DMSO was observed over a relatively broad concentration range up to 15% (fig.2). A similar action of this solvent has been reported for an enzyme involved in peptidoglycan synthesis, C_{55}-isoprenylpyrophosphate phosphatase [12].

The oligosaccharide transfer was linear with respect to time of incubation for roughly 30 min under usual assay conditions but had yet utilized 40% of the supplied radioactive lipid donor (fig.3). The presence of 10% DMSO did not significantly modify the time-course of the reaction and it has usually been found to double the yield of oligosaccharide transfer. This might reflect some relative stabilization of the enzyme-active site.

Solubilized enzyme activity was maximal between

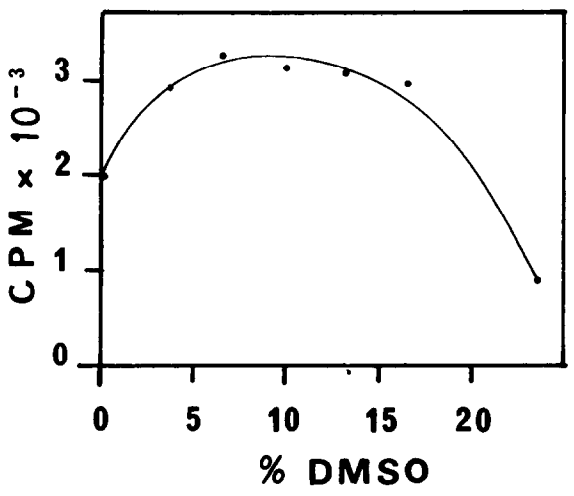

Fig.2. Effect of DMSO on the solubilized oligosaccharide transferase activity. Conditions of standard assay for 30 min.

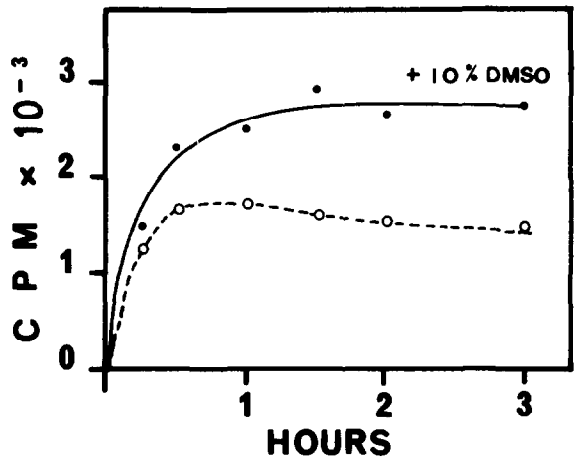

Fig.3. Time-course of oligosaccharide transferase reaction. Conditions of standard assay with the incubation time indicated, in the presence (•——•) or absence (○- - -○) of DMSO.

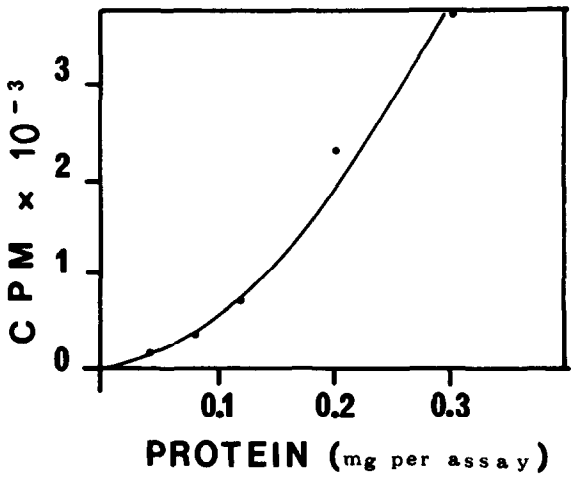

Fig.5. Effect of protein concentration on the solubilized oligosaccharide transferase activity. Conditions of standard assay for 30 min with 10% DMSO. Varying protein concentration was produced by diluting the detergent extract with the reaction buffer.

pH 7.5 and 8.5 and decreased sharply in the acidic range (fig.4) as it was recently reported for the microsomal enzyme from calf thyroid [9].

The dependence of the oligosaccharide transfer upon enzyme concentration is shown in fig.5. The transferase seemed to undergo a loss of activity upon excessive dilution.

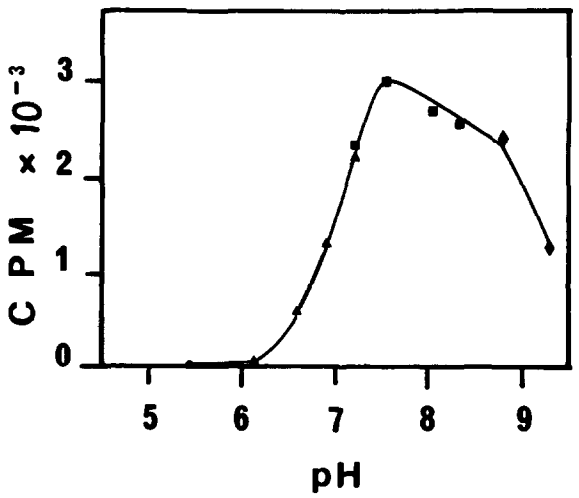

Fig.4. Effect of pH on the solubilized oligosaccharide activity. Conditions of standard assay for 30 min with 10% DMSO. To achieve the desired pH, the following 0.4 M buffers were added at a final concentration of 0.03 M: sodium citrate (•), cacodylate (▲), Tris–HCl (■), sodium glycinate (♦).

The solubilized transferase was stored at −20°C for 2 weeks without loss of more than 25% of activity. Half diluting the DOC extract allowed more prolonged storage. Kept in ice for 1 day, the enzyme lost 50% of its activity. DMSO had no effect on the stability of the transferase.

3.4. *Solubilization of glucosidase activities*

Besides the oligosaccharide transferase, the DOC extract was found to contain enzymatic activities releasing free glucose either from glucose-labeled oligosaccharide lipids or from glucose-labeled glycopeptides (table 3). When using *p*-nitrophenyl-α and β-glucosides as substrates, two distinct glucosidase activities have been distinguished, the most active hydrolyzing the β-anomer. As shown in table 3, the inclusion of these substrates in the standard assay mixture greatly reduced the removal of labeled glucose from oligosaccharides either lipid-linked or protein-linked. Up to 80% of inhibition was observed with the *p*-nitrophenyl-β-glucoside and 30% with the α-anomer when oligosaccharide lipids were substrates. Similar results were obtained when the glucosidases acted on labeled glycopeptides. These observations are suggestive of glucosidase activities of distinct anomeric specificities: the major part of the radioactive glucose being liberated by a β-glucosidase, the minor part by an α-glucosidase.

Structural studies have indicated that the lipid-

Table 3
Substrate specificity of solubilized glucosidase activities

[^{3}H]Glucose substrate	Release of [^{3}H]glucose	
	cpm	% control
Oligosaccharide lipids		
no addition	1601	100
+ 125 mM *p*-nitrophenyl-α-glucoside	1104	69
+ 125 mM *p*-nitrophenyl-β-glucoside	298	18
Glycopeptides		
no addition	36	100
+ 125 mM *p*-nitrophenyl-α-glucoside	274	33
+ 125 mM *p*-nitrophenyl-β-glucoside	327	39

linked oligosaccharide has the composition $(Glc)_3$ $(Man)_9$ $(Glc\,NAc)_2$, the glucose residues being arranged in the linear sequence Glc $\xrightarrow{1,2}$Glc $\xrightarrow{1,3}$Glc $\xrightarrow{1,3}$ Man at the nonreducing end [13,14]. While the present work was in progress, two distinct glucosidase activities have been solubilized and partly characterized [15,16]: one acting on $(Glc)_3$ $(Man)_9$ $(Glc\,NAc)_2$ and the other on (Glc_2) $(Man)_9$ $(Glc\,NAc)_2$. But up to now little information is available on the anomeric specificity of these enzymes. They have been considered as α by some authors [15,17] or β by others [18]. The data presented here favour the idea that one of the glucosidases might be β. They are in agreement with the results in [11] showing that oligosaccharide lipids might be labeled in vitro directly from UDP-glucose and therefore would possess their distal glucose residue in a β-linkage.

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