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SOLUBILIZATION AND PARTIAL CHARACTERIZATION OF UDP-*N*-ACETYLGALACTOSAMINE:GLOBOSIDE α-*N*-ACETYLGALACTOSAMINYLTRANSFERASE FROM DOG SPLEEN MICROSOMES

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Summary

UDP-*N*-acetylgalactosamine:globoside α-*N*-acetylgalactosaminyltransferase (EC 2.4.1.—) synthesizing Forssman hapten was solubilized from dog spleen microsomes by a combination of Triton X-100 treatment and sonication. The solubilized enzyme was partially purified by calcium phosphate gel, ammonium sulfate fractionation and then DEAE-cellulose column chromatography. The enzymatic activity of the purified preparation was stimulated by exogenously added phosphatidylserine, as found in the particulate enzyme.

When the properties of the purified enzyme were examined in the presence of exogenous phosphatidylserine, the enzyme had an absolute requirement for Mn^{2+}; this was not substituted by Ca^{2+} or Mg^{2+}. Apparent K_m values for UDP-*N*-acetylgalactosamine and globoside were $1 \cdot 10^{-5}$ and $5 \cdot 10^{-4}$ M, respectively. It had a pH optimum of 6.55 regardless of the presence or absence of exogenous lipids.

Since the partially purified enzyme was completely free of uridine diphosphatase which was found in the particulate preparation, the effect of UDP on the transferase activity could be studied. Thus, UDP inhibited 85% of the activity at a concentration of 1.5 mM. *p*-Chloromercuribenzoate inhibited over 90% of the activity at 2 mM, indicating the transferase to be SH-enzyme.

Introduction

Forssman hapten, one of the most potent haptenic sphingoglycolipids [1,2], has been shown to possess in its molecule one more additional α-linked *N*-ace-

Abbreviation used: UDP-GalNAc, uridine 5'-diphosphate *N*-acetylgalactosamine.

tylgalactosamine at the non-reducing end of globoside [3,4].

The biosynthesis of the carbohydrate chains of sphingoglycolipids has been demonstrated to proceed by the sequential addition of one glycosyl unit from sugar-nucleotide donors to precursor by specific glycosyltransferases [5,6]. Investigations of the properties and structure of these glycosyltransferases have been hampered by the fact that these enzymes are bound tightly to membranes and not readily obtained in soluble form. In the past, nevertheless, attempts to solubilize and separate membrane-bound glycolipid glycosyltransferases have met increasing success [7—11].

In previous studies, the biosynthesis of aminoglycolipids, globoside and Forssman hapten, by *N*-acetylgalactosaminyltransferases have been demonstrated using enzymes bound to microsomes from guinea pig tissues [12,13]. To elucidate in detail the reaction mechanism catalyzed by *N*-acetylgalactosaminyltransferase, we have attempted the solubilization and purification of these enzymes. In the present studies, exogenous phospholipids were found not only to stimulate microsomal UDP-GalNAc:globoside α-*N*-acetylgalactosaminyltransferase (EC 2.4.1.—) activity, but to prevent partially a profound loss of the activity in the solubilized enzyme preparation which appears to encounter necessarily during the purification process of membrane-bound enzymes. The present communication also describes partial purification of this enzyme from dog spleen microsomes and some properties of the purified enzymes.

Materials and Methods

The following chemicals were obtained from commercial sources: UDP-*N*-acetyl[1-^{14}C]galactosamine (51.5 Ci/mol, New England Nuclear Co.); non-labeled nucleotides (Yamasa Shoyu Co.); DEAE-cellulose (type DE 32, Whatman Co.); Triton X-100 (Wako Chem. Co.); phospholipids (Serdary Res. Lab. Inc.). Total phospholipids mixture (named Lipid L) from lymphoma tissue was prepared as described previously [14]. $Ca_3(PO_4)_2$ gel was prepared by the method of Keilin and Hartree [15]. Glycosphingolipids were isolated from pig spleen or equine kidney by previously published methods [2,16]. All other chemicals were reagent grade.

Preparation of microsomes

The method obtaining microsomal fraction was the same as described before [12]. Briefly, spleen from adult mongrel dog was homogenized in 4 vols. of ice-cold 0.32 M sucrose/14 mM β-mercaptoethanol/1 mM EDTA, and cell debris and nuclei were removed by centrifugation at 450 × *g* for 8 min. After the mitochondrial fraction had been sedimented at 10000 × *g* for 12 min, the supernatant was centrifuged at 105000 × *g* for 1 h. The pellet thus obtained was suspended in 0.25 M sucrose and used as microsomal or membrane-bound enzyme preparation.

Removal of lipids

This was carried out by extraction of microsomes with 90% acetone according to the method of Lester and Fleisher [17]. This extraction removes at least 80% of phospholipids [18].

Solubilization of microsomes by Triton X-100

The subsequent fractionation procedures were carried out at 0—4°C. To 36 ml of a microsomal suspension (30—40 mg of protein per ml) in 0.25 M sucrose was added 10% (w/v) Triton X-100 to give a final concentration of 1%. The mixture was sonicated for 5 × 1-min periods. The mixture was stirred gently for 30 min, diluted with 40 ml of 0.25 M sucrose to lower the concentration of detergent to 0.5% and centrifuged at 105000 × *g* for 1 h.

$Ca_3(PO_4)_2$ gel fractionation

To the 105000 × *g* supernatant (Fraction I, 79.5 ml) was added $Ca_3(PO_4)_2$ gel in a ratio of 35.5 ml/g powder. After stirring for 20 min, the mixture was centrifuged at 10000 × *g* for 10 min, and the supernatant was obtained. To remove completely the enzyme weakly bound to the gel, the gel was suspended in 0.125 M potassium phosphate buffer (pH 5.4) in a ratio of 58.4 ml/g powder, stirred for 20 min, and centrifuged at 10000 × *g* for 10 min. The combined supernatants were pooled (Fraction II).

$(NH_4)_2SO_4$ fractionation

As a preliminary experiment, the Triton-solubilized enzyme preparation (Fraction I) was fractionated by adding solid $(NH_4)_2SO_4$ and neutralized with 0.2 M NH_4OH. Fractions were taken at 0—30, 30—50, and 50—70% saturation. The precipitates obtained after centrifugation at 10000 × *g* for 20 min were suspended in a minimal volume of 0.25 M sucrose and then dialyzed against 10 mM sodium cacodylate/HCl buffer (pH 6.7) overnight. The dialyzates were assayed for α-*N*-acetylgalactosaminyltransferase activity. Since the enzymatic activity was detected in the precipitates of 0—30 and 30—50% saturated fractions, the precipitate with 0—50% saturation was used for subsequent experiment. The activity recovered in 0—50% saturated fraction was 95.7% and the specific activity was approximately doubled as compared to that of the initial solubilized enzyme preparation. On the basis of these results, to Fraction II (249 ml) was added solid $(NH_4)_2SO_4$ to 50% saturation. The precipitate obtained was dissolved in a minimal volume of 10 mM sodium cacodylate/HCl buffer (pH 6.7) and then dialyzed overnight against this buffer containing 0.5% Triton X-100 (Fraction III).

DEAE-cellulose column chromatography

The dialyzate was applied to a column of DEAE-cellulose (OH^-, 1.8 × 10 cm) equilibrated with 10 mM sodium cacodylate/HCl buffer, pH 6.7, containing 0.5% Triton X-100. The column was eluted with a stepwise gradient of 0, 0.1, 0.2, and 0.3 M KCl in the buffer used for the equilibration. A typical chromatographic profile is shown in Fig. 1. The tubes of No. 5 and 6 containing the α-*N*-acetylgalactosaminyltransferase were combined (Fraction IV). This enzyme fraction (8.6 ml) was used for subsequent enzymatic studies.

Enzyme assays

The activity of UDP-GalNAc:globoside α-*N*-acetylgalactosaminyltransferase was assayed according to the modification of the methods described previously [13]. The incubation mixture contained 50 μg of globoside, various amounts of

phospholipids if added, 300 μg of Triton X-100, 10 mM $MnCl_2$, 0.1 M sodium cacodylate/HCl buffer (pH 6.7), 10 μM UDP-*N*-acetyl[1-^{14}C]galactosamine and enzyme preparation in a final volume of 0.1 ml. Detergent, globoside and phospholipids were transferred to the incubation tubes as solutions in chloroform/methanol (2 : 1, v/v) and the solvent was evaporated under nitrogen stream before addition of other components. After incubation for 2 h at 37°C with continuous shaking, the reaction was stopped by adding 0.5 ml of chloroform/methanol (2 : 1, v/v). After shaking and centrifugation, the lower phase was sucked up carefully with capillary pipette and washed two times with 0.2 ml of "theoretical upper phase" according to the method of Folch et al. [19]. The resultant lower phase was evaporated and measured for radioactivity by liquid scintillation counter (Method I) or after thin layer chromatography (Method II) [13]. To ascertain propriety of this assay Method I, the reaction products in the washed lipids extract were pooled from five incubation tubes and chromatographed on a thin layer plate (silica gel H) developing with chloroform/methanol/water (58 : 35 : 8, by volume). The radioactive peak was localized only in the position corresponding to authentic Forssman hapten. The non-lipid radioactive degradation products from UDP-*N*-acetyl[1-^{14}C]galactosamine [20] were not detected.

Uridine diphosphatase activity was measured as follows. Reaction mixture containing 3 mM UDP, 50 mM Tris · HCl buffer (pH 7.5), 4 mM $MgCl_2$ and enzyme preparation in a final volume of 0.25 ml was incubated for 15 min at 37°C. The reaction was terminated by adding trichloroacetic acid in 5% of final concentration, centrifuged, and the amount of inorganic phosphate released was determined with an aliquot of the supernatant by the method of Fiske and Subbarow [21].

Analytical procedures

Protein was determined by the method of Lowry et al. [22], with crystalline bovine serum albumin as a standard. Phospholipid was quantitated by determining phosphorous content [21].

Results

I. Requirement for phospholipids on UDP-GalNAc:globoside α-N-acetylgalactosaminyltransferase activity

Effect of total phospholipids added exogenously in the intact microsomes. In previous report [14], it was found that phospholipids mixture (Lipid L), which was mainly composed of phosphatidylethanolamine, phosphatidylcholine, and sphingomyelin, stimulated strikingly the activity of UDP-galactose: lactosylceramide α-galactosyltransferase. Similary, Lipid L approximately doubled the activity of UDP-GalNAc:globoside α-*N*-acetylgalactosaminyltransferase in the intact microsomes as compared with the control (Table I). The enhancing effect was also observed by addition of commercial phospholipids mixture of the composition similar to Lipid L. The maximum enhancing effect of phospholipids on the enzymatic activity was observed at 90—120 min of incubation time. A lower activating effect observed less than 20 min may indicate the time required for binding of phospholipids with the enzyme.

TABLE I

EFFECT OF EXOGENOUS PHOSPHOLIPIDS ON UDP-GalNAc:GLOBOSIDE α-*N*-ACETYLGALACTOSAMINYLTRANSFERASE OF INTACT MICROSOMES

Enzymatic activities were determined by assay Method I as described in the text with varying species and amounts of exogenous phospholipids as indicated. Each incubation tube contained 365 μg of protein. Specific activity was corrected for endogenous activity.

Phospholipids added (μg)	μg	Specific activity (cpm/2 h per mg protein)	Activation factor
None	—	1230	1
Phosphatidylcholine	250	2926	2.4
	500	2381	1.9
Phosphatidylethanolamine	250	2403	2.0
	500	2096	1.7
Phosphatidylserine	250	2658	2.2
	500	2526	2.1
Diphosphatidylglycerol	250	2493	2.0
	500	1764	1.4
Phosphatidic acid	250	2345	1.9
	500	1855	1.5
Phosphatidylinositol	250	3093	2.5
	500	3016	2.5
Sphingomyelin	250	1449	1.2
	500	1277	1.0
Lysophosphatidylethanolamine	250	926	0.8
	500	1175	1.0
	750	1096	0.9
Lysophosphatidylcholine	250	1129	0.9
	500	858	0.7
	750	789	0.6
Phosphatidylethanolamine + phosphatidylcholine	250 each	2630	2.1
Phosphatidylethanolamine + phosphatidylcholine + sphingomyelin	250 each	2493	2.0
"Lipid L"	475	2556	2.1

Effect of individual phospholipids on the transferase of intact microsomes. Among the nine individual phospholipids examined, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, diphosphatidylglycerol, phosphatidic acid and phosphatidylinositol showed moderate effect but sphingomyelin, lysophosphatidylethanolamine and lysophosphatidylcholine had no effects (Table I). The stimulation was more effective at the lower concentration of 250 μg of phospholipid than at that of 500 μg per 365 μg protein. The greatest activation observed was 2.5-fold with phosphatidylinositol.

Decrease of the enzymatic activity by delipidization and reactivation by adding phospholipids. The removal of phospholipids from microsomes with 90% acetone extraction resulted in 72% decrease of the transferase activity (Table II). Restoration experiments of the enzymatic activity were carried out on the delipidized microsomes by introducing phospholipids. Among the phospholipids tested phosphatidylserine at a concentration of 250 μg per 200 μg protein restored the decreased activity of the acetone-extracted microsomes almost completely to the level of intact microsomes. Lipid L at a concentration

TABLE II

EFFECT OF EXOGENOUS PHOSPHOLIPIDS ON UDP-GalNAc:GLOBOSIDE α-*N*-ACETYLGALACTOSAMINYLTRANSFERASE OF INTACT AND ACETONE-EXTRACTED MICROSOMES

A fixed amount of phospholipids (250 μg, except for Lipid L) was added in each incubation tube containing 200 μg of enzyme protein, because this concentration of phospholipids was more effective for reactivation of the enzymatic activity than that of 500 μg of phospholipids per tube (cf. Table I).

Microsomes	Phospholipids added	μg	Specific activity (cpm/2 h per mg protein)	Activation factor	Relative activity (%)
Intact	None	—	1230		100
Acetone-extracted	None	—	345	1	28.0
	Phosphatidylcholine	250	735	2.1	59.8
	Phosphatidylethanolamine	250	540	1.6	43.9
	Phosphatidylserine	250	1165	3.4	94.7
	Diphosphatidylglycerol	250	440	1.3	35.8
	Phosphatidic acid	250	920	2.7	74.8
	Phosphatidylinositol	250	860	2.5	69.9
	Sphingomyelin	250	405	1.2	32.9
	Lysophosphatidylethanolamine	250	295	0.9	24.0
	Phosphatidylethanolamine + phosphatidylcholine	250 each	804	2.3	65.4
	Phosphatidylethanolamine + phosphatidylcholine + sphingomyelin	250 each	765	2.2	62.2
	"Lipid L"	475	1283	3.7	104.3

of 475 μg also reactivated completely the decreased activity to the original level. The other phospholipids and their mixture restored only partially the decreased activity. A complete restoration of the enzymatic activity to the level of intact microsomes was obtained at 150 μg phosphatidylserine versus 200 μg protein, of which amount was also optimal when the intact microsomes were used.

II. Solubilization and partial characterization of UDP-GalNAc:globoside α-N-acetylgalactosaminyltransferase.

Effect of Triton X-100 on microsomal enzyme activity. In previous paper using guinea pig microsomes, the highest activity was observed with Triton X-100 among the detergents tested, Triton X-100, Triton CF 54, Tween 80, sodium deoxycholate and sodium taurocholate, although an appreciable activity (70—80% of that with Triton X-100) was found with crude sodium taurocholate [13]. Therefore, Triton X-100 was chosen as solubilizing agent for dog spleen microsomal enzyme, and its optimal concentration was determined. Triton X-100 stimulated the transferase and gave a maximum activation only over a narrow range of 0.3—0.6% (w/v) in final concentration. Consequently, the concentration of 0.5% of Triton X-100 was used for subsequent experiments.

Solubilization and purification. The results of purification process of α-*N*-acetylgalactosaminyltransferase from dog spleen microsomes are summarized in Table III. After solubilization of microsomes with 1% (w/v) Triton X-100 and

TABLE III

PURIFICATION OF UDP-GalNAc:GLOBOSIDE α-*N*-ACETYLGALACTOSAMINYLTRANSFERASE FROM DOG SPLEEN MICROSOMES

Procedures for preparation of Fractions and for the enzyme assay are described in Materials and Methods.

Fraction	Volume (ml)	Protein (mg)	Total activity (cpm × 10^{-3})		Specific activity (cpm/2 h per mg protein)		Purification (-fold)		Recovery (%)	
			— PS *	+ PS	— PS	+ PS	— PS	+ PS	— PS	+ PS
Microsomes	36	1020	555	1181	544	1158	1	1	100	100
Triton-treated microsomes	80	1020	610	1148	598	1125	1.1	1.0	109.9	97.2
I. 105 000 × *g* supernatant	79.5	775	525	888	677	1146	1.2	1.0	94.6	75.2
II. $Ca_3(PO_4)_2$ gel	249	195	158	295	810	1514	1.5	1.3	28.5	25.0
III. $(NH_4)_2SO_4$ (0—50%)	15.4	158	157	185	996	1174	1.8	1.0	28.4	15.7
IV. DEAE-cellulose	8.6	8.6	19	37	2229	4329	4.1	3.7	3.5	3.2

* Phosphatidylserine.

sonication the recovery of the total transferase activity in the 105000 × *g* supernatant was 94.6% with a specific activity 1.2 times that of original microsomes, in the absence of exogenous phosphatidylserine. Compared to original microsomes, the specific activity of final preparation showed approximately 4-fold purification and the final recovery was 3.5% of the total activity in the starting material.

Although steps to prepare Fractions II and III contributed apparently little to purification, they would be necessary for obtaining an active and stable solubilized enzyme preparation. Although the yield and degree of purification of the α-*N*-acetylgalactosaminyltransferase obtained by these procedures were not high, the other microsomal components such as nucleoside diphosphatase could be eliminated. That is, the majority of α-*N*-acetylgalactosaminyltransferase was eluted in unabsorbed fraction with no uridine diphosphatase activity on DEAE-cellulose column chromatography, while the bulk of uridine diphosphatase was eluted with 0.1 M KCl in the buffer as shown in Fig. 1.

Some properties of partially purified α-*N*-acetylgalactosaminyltransferase (Fraction IV) were studied.

Effect of addition of phospholipid on the α-N-acetylgalactosaminyltransferase activity. Since present observation showed an absolute requirement of phospholipids for full activity of UDP-GalNAc:globoside α-*N*-acetylgalactosaminyltransferase bound to microsomal membranes, the effect of the phosphatidylserine, which restored completely the decreased activity of delipidized microsomes, was examined for the purified enzyme preparation. An exogenous phosphatidylserine also stimulated the reaction rate catalyzed by the soluble enzyme preparation. The optimal effect was obtained with 150 μg of this lipid per tube (70 μg of protein). Therefore, the transferase activity in subsequent

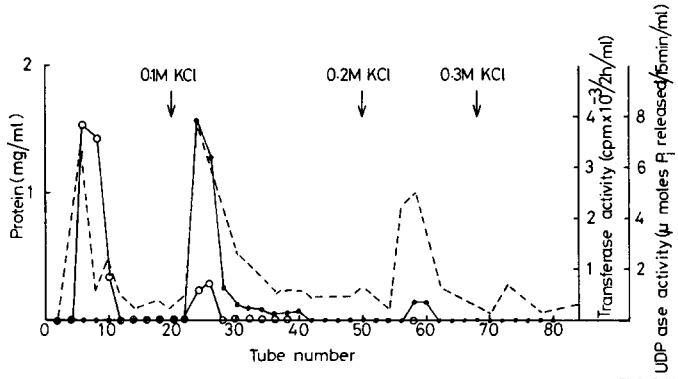

Fig. 1. Purification of the α-*N*-acetylgalactosaminyltransferase by DEAE-cellulose column chromatography. Fraction III of the enzyme preparation (158 mg of protein in 15.4 ml) was applied to a column (1.8 × 10 cm) of DEAE-cellulose equilibrated in 10 mM sodium cacodylate/HCl buffer (pH 6.7) containing 0.5% Triton X-100, and eluted with the same buffer containing stepwise 0—0.3 M KCl at a flow rate of 30 ml/h. The eluate (4.3 ml/tube) was analyzed for protein (- - - - -), the transferase activity (assay Method I) (○) in the presence of exogenous phosphatidylserine (150 μg/tube) and for uridine diphosphatase activity (●), as described in Materials and Methods. Arrows indicate KCl concentration in the column buffer.

experiments was determined under the addition of phosphatidylserine at 150 μg unless otherwise stated. Higher concentration of exogenous phosphatidylserine inhibited the reaction catalyzed by both the membrane-bound and solubilized transferase.

Relationships to protein concentration and to reaction time. The rate of incorporation of radioactive *N*-acetylgalactosamine into Forssman hapten was linear with the amounts of enzyme protein ranging from 20 to 70 μg (Fig. 2) and with incubation time up to 2 h.

Effect of different substrate concentrations. The effects of varying the concentration of globoside and of UDP-GalNAc on the rate of the reaction were

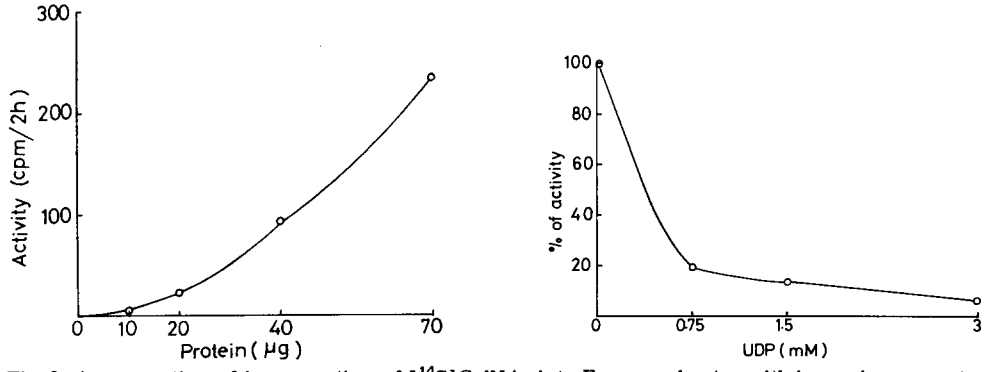

Fig. 2. Augmentation of incorporation of [^{14}C]GalNAc into Forssman hapten with increasing amounts of purified enzyme. Enzyme assay was carried out by Method I, in the presence of 150 μg of phosphatidylserine per tube.

Fig. 3. Effect of UDP on the activity of the purified transferase (50 μg of protein). Enzymatic activity was assayed in the presence of exogenous phosphatidylserine (150 μg/tube) by Method I, except that UDP at various concentrations was added.

TABLE IV

EFFECT OF VARIOUS CHEMICALS ON UDP-GalNAc:GLOBOSIDE α-*N*-ACETYLGALACTOSAMINYLTRANSFERASE ACTIVITY

Enzymatic activity was determined by assay Method I in the presence of phosphatidylserine (150 μg/tube), except that different chemicals were added to the incubation mixture.

Chemicals added	mM	Relative activity (%)
None	—	100
ATP	2	45.1
NaF	2	102.3
NaN_3	2	91.0
p-Chloromercuribenzoate	2	8.3
Reduced glutathione	2	116.9
EDTA	25	2.3

examined. The calculated values of K_m for globoside and UDP-GalNAc are $5 \cdot 10^{-4}$ and $1 \cdot 10^{-5}$ M, respectively.

Effect of pH. A pH optimum of 6.55 in 0.1 M sodium cacodylate/HCl buffer was found irrespective of the presence or absence of exogenous phosphatidylserine.

Effect of divalent cations. A requirement of Mn^{2+} for the α-transferase reaction was absolutely essential with an optimal concentration of 10 mM. The other divalent cations, Mg^{2+} and Ca^{2+} were not at all substituted for Mn^{2+}.

Effect of various chemicals. EDTA had strong inhibitory effect for the transferase activity (Table IV). Sulfhydryl-binding agents such as *p*-chloromercuribenzoate inhibited the reaction over 90% at 2 mM. On the other hand, reduced glutathione slightly stimulated the reaction rate. NaF had no effect and NaN_3 inhibited a little. In contrast to the stimulatory effect of ATP on the particulate-bound transferase from guinea pig [13], the nucleotide inhibited by 55% at 2 mM the activity of the purified enzyme preparation.

Effect of various nucleotides. It was demonstrated in the previous paper that UDP inhibited markedly the α-*N*-acetylgalactosaminyltransferase activity of the particulate enzyme preparation [20]. However, since high level of uridine diphosphatase activity was detected in the dog spleen microsomes, the effect of UDP on the α-transferase reaction might be obscured by this phosphatase

TABLE V

EFFECT OF VARIOUS NUCLEOTIDES AND URIDINE ON UDP-GalNAc:GLOBOSIDE α-*N*-ACETYLGALACTOSAMINYLTRANSFERASE ACTIVITY

Enzymatic activity was assayed by Method I in the presence of exogenous phosphatidylserine (150 μg/tube), except that different nucleotides and uridine were added to the assay system.

Materials added	mM	Relative activity (%)
None	—	100
UDP	1.5	13.4
GDP	1.5	36.1
IDP	1.5	11.3
ADP	1.5	54.1
CDP	1.5	48.9
Uridine	2.0	78.2

activity. In the present study, the uridine diphosphatase-free enzyme preparation obtained was examined for the nucleotide effects. UDP also inhibited markedly the activity of the purified transferase preparation (Fig. 3). Other nucleoside diphosphates examined inhibited more or less the transferase (Table V). Among them, UDP and IDP were the most powerful inhibitors (85—90% inhibition at 1.5 mM). Uridine showed only a weak inhibition on the transferase activity as compared to the nucleoside diphosphates.

Stability. The purified α-*N*-acetylgalactosaminyltransferase preparation did not show any appreciable loss of the activity after storage for 2 weeks at −20° C.

Discussion

Mammalian tissues contain approximately 20 different glycosyltransferases involved in the biosynthesis of the complex carbohydrate moieties of glycolipids. Studies leading to a clear understanding of properties and functions of these enzymes and their relationship to membrane environment have been limited by the lack of purified enzyme preparation. Solubilization and purification of the membrane-bound glycosyltransferases have been exceedingly difficult because of loss of activities during procedures used for solubilization and purification of enzymes.

The procedure for solubilization has remained without rational basis. As shown in the text, Triton X-100 is not only effective in solubilizing UDP-GalNAc:globoside α-*N*-acetylgalactosaminyltransferase from microsomes (Table III) but is also essential for the enzyme activity [13]. Resolution of microsomal membranes was accomplished with a combination of Triton X-100 (final concentration, 1%, w/v) and sonication without appreciable loss of the transferase. We were able to extract virtually 96.4% of the enzyme activity remaining in the supernatant following centrifugation at 105000 × *g* for 1 h. The degree of protein release brought about by this treatment was 76%. After the final purification steps, however, the overall 4-fold increase in the specific activity of the transferase was also lower than expected for the recovery of the enzyme activity. The further purification is likely to be subject to the difficulties encountered with membrane enzymes isolated by detergent treatment, which depend upon whether the enzyme is isolated in a true water-soluble form or present as a multimolecular complex in the solution by the presence of detergent.

The properties of the α-*N*-acetylgalactosaminyltransferase solubilized from dog spleen microsomes were virtually the same as those of the particulate enzyme from guinea pig tissue; i.e. optimum pH, K_m for acceptor globoside, requirement for Mn^{2+} [13] and inhibition by UDP [20]. The solubilized enzyme, however, had 1/10 the K_m for UDP-GalNAc of that with particulate enzyme and its activity was significantly inhibited by ATP which was rather stimulative for the particulate enzyme [13]. ATP requirement in the crude microsomal system remains unclear. One possibility may be that ATP has no direct relationship to the enzyme to be studied, but is utilized to prevent a degradation of sugar-nucleotide donor. This degradative system, however, is possibly not present in the purified enzyme used in this study.

The transferase preparation in the present studies catalyzed also transfer

of *N*-acetylgalactosamine into trihexosylceramide, forming globoside. This fact would indicate poor separation of α- and β-*N*-acetylgalactosaminyltransferase, or co-purification of the transferases which may exist as a single multiglycosyltransferase [5] consisted of respective enzymes responsible for the synthesis of all the glycolipid members present in the tissue. To clarify questions cited here, further investigations will be necessary.

Previously published results showing the activation of membrane-bound [14, 23—25] and solubilized [10,11] glycolipid glycosyltransferases by exogenously added phospholipids suggested phospholipid dependence of the α-*N*-acetylgalactosaminyltransferase. In fact, a requirement of phospholipids for this transferase was clearly demonstrated by delipidization experiments of microsomal membranes. The solubilized and partially purified enzyme preparation was also stimulated by the addition of exogenous phosphatidylserine, as shown in this paper. Although the role of phospholipids, presumably enzyme-phospholipid interaction or formation of phospholipid-acceptor-detergent complex [26] to manifest catalytic activity, is not known, it seems likely that phospholipids participate in maintenance of the active structure of the transferase, as has been postulated for other membrane enzymes [27,28].

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