BIOSYNTHESIS OF FORSSMAN HAPTEN FROM GLOBOSIDE BY α-N-ACETYLGALACTOSAMINYLTRANSFERASE OF GUINEA PIG TISSUES

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SUMMARY UDP-N-acetylgalactosamine:globoside α-N-acetylgalactosaminyltransferase activity was demonstrated in the microsomes from guinea pig tissues. The product was identified as Forssman hapten by thin layer chromatography, enzymatic hydrolysis, and immunoprecipitin reaction. Apparent Km values of the enzyme at optimal pH 6.7 were 4×10^{-4} M and 1×10^{-4} M for globoside and UDP-N-acetylgalactosamine, respectively. In the presence of UDP-glucose, the activity of the enzyme was strongly inhibited, in contrast with an activating effect of UDP-glucose on the β-N-acetylgalactosaminyltransferase described previously (1).

Forssman hapten known as a heterophile antigen (2) is one of the most potent haptenic sphingoglycolipids (3-4). The hapten (6,7) has been shown to possess in its molecule one more additional α-linked N-acetylgalactosamine (GalNAc) at the non-reducing terminal of globoside whose structure is β-N-acetylgalactosaminyl-galactosyl-galactosyl-glucosyl-ceramide.

Very recently, we reported biosynthesis of globoside and Forssman hapten from trihexosylceramide as an acceptor by guinea pig enzyme (1). Biosynthesis of "globoside-type glycolipid" has been shown using chick brain enzyme (8). Present communication describes that guinea pig enzyme catalyzes transfer of GalNAc from UDP-GalNAc to globoside to form Forssman hapten. Some properties of the α-N-acetylgalactosaminyltransferase are also described.

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MATERIALS AND METHODS

Glycolipids, anti-Forssman and -globoside antisera, α- and β-N-acetylgalactosaminidases and UDP-N-acetyl-[1-^{14}C]-galactosamine (51.5 Ci/mole) were obtained as described previously (1). The enzyme assay method was shown in Table 1, and essentially identical with that in previous report (1).

Table I. UDP-GalNAc: Globoside α-N-acetylgalactosaminyltransferase activity in various conditions

Incubation mixture	dpm/mg protein	Addition	Relative activity(%)
Complete	720[a], 2060[b]	None	100
Minus Globoside	41	ATP(2mM)	120
Minus Mn^{2+}	19	UDP-Glc(2mM)	19
Plus EDTA (25mM)	16	" (0.5mM)	27
Minus Mn^{2+}, plus Mg^{2+}	57-111	NaF(2mM)	100
Minus Mn^{2+}, plus Ca^{2+}	93	NaN_3(2mM)	48

(a) Complete incubation mixture (100 μl) contained 50 μg (375μM) of human kidney globoside, 300 μg of Triton X-100, 0.1 M of sodium cacodylate-HCl buffer, pH 6.7, 10 mM of $MnCl_2$, 560 μg of microsomal protein from new-born guinea pig spleen and 50 nCi of UDP-[^{14}C]-GalNAc. This mixture was employed throughout the experiments in this study unless otherwise stated.

(b) UDP-[^{14}C]-GalNAc was increased three times (150 nCi) as much as (a), and 40 μg (300 μM) of globoside was used.

After the reaction mixture was incubated at 37°C for 10 min, the reaction was stopped by adding 10 μl each of 0.5 M KCl, 0.25 M EDTA, and 0.6 ml of chloroform-methanol (2:1). After shaking and centrifugation, resultant lower phase was applied to thin layer chromatography developing with chloroform-methanol-water (65:25:4). The radioactivity corresponding to the spot of Forssman hapten was determined by liquid scintillation technique.

RESULTS

α-N-Acetylgalactosaminyltrasnferase activity under the various conditions. As shown in Table 1, exogenous globoside markedly stimulates synthesis of Forssman hapten. UDP-Glc strongly inhibited the α-N-acetylgalactosaminyltransferase activity.

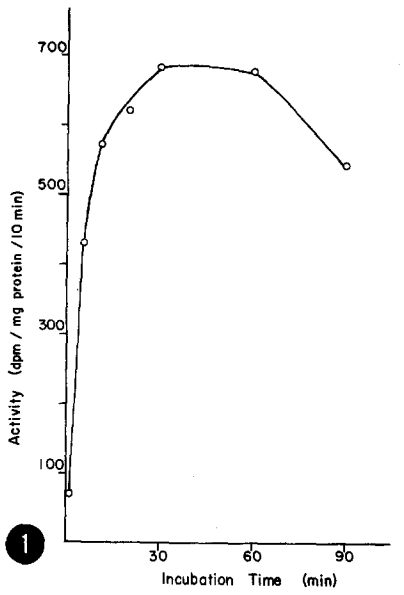
Following agents showed no effect on the α-transferase: p-chloromercury benzoic acid at 45 μM, glutathion, GalNAc, GlcNAc and glucuronolactone at 2 mM, similar with the reaction by the β-transferase (1). Optimal concentration of Mn^{2+} was 10 mM, and this cation was hardly substituted by Mg^{2+} or Ca^{2+}. As to detergents, the highest activity was observed with Triton X-100. Although an appreciable activity (70-80 % of the case with Triton X-100) was found with crude sodium taurocholate, the others (Triton CF-54, sodium deoxycholate, and Tween 80 and 20) were rather inhibitory as compared with the case in the absence of detergents. The optimal pH of the transferase was 6.7 with sodium cacodylate-HCl buffer.

The enzyme activity correlated with the time of incubation and the amounts of enzyme protein (Fig. 1 and 2, respectively). Substrate specificity was the same as that in the previous paper (1)

The transferase activity versus the concentrations of globoside and UDP-GalNAc were shown in Fig 3. The apparent Km of the enzyme for globoside and UDP-GalNAc were 4×10^{-4} M and 1×10^{-4} M, respectively.

Distribution of the enzyme activity. Of a number of tissues tested, the transferase activity was much higher in spleen and kidney than in others (Table II). The maximum specific activity was localized in the microsomal fractions. Brain and liver tissues exhibited little activity. In small intestine the exogenous globoside did not stimulate the transfer activity, probably because of a high concentration of the endogenous acceptor in the tissue.

Identification of the radioactive product. The radioactive product which was prepared as shown in Table I but with large scale and purified by chromatography, was subjected to identification as follows:



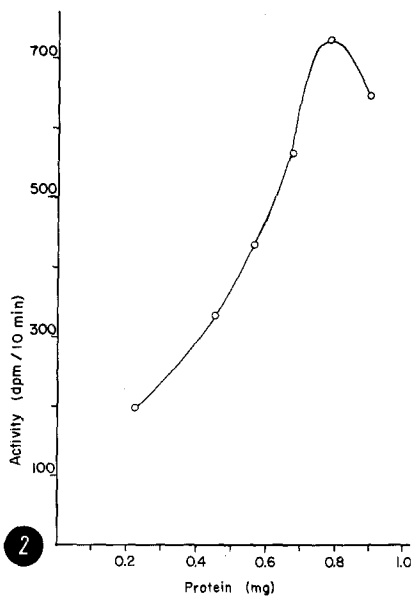


Figure 1. Time course of incorporation of GalNAc into Forssman hapten. The incubation conditions were as given in Table I (a).

Figure 2. Dependence of Forssman hapten formation on **increasing** amounts of microsomal protein.

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1) Thin layer chromatography. The enzymatic product had the same mobility with authentic Forssman hapten on a thin layer chromatography (Fig. 4).

2) Enzymatic hydrolysis. The radioactive product (1700 dpm) was hydrolyzed (843 dpm) by α-N-acetylgalactosaminidase, but not (40 dpm) by β-N-acetylgalactosaminidase under the conditions described previously (1).

3) Immunoprecipitin reaction. The radioactive product reacted most strongly with anti-sheep erythrocyte (anti-Forssman) serum, though some cross reaction of the product with anti-glogoside serum was observed (Fig. 5).

On the basis of the evidence described above, the radioactive product catalyzed by guinea pig enzyme was identified as Forssman hapten.

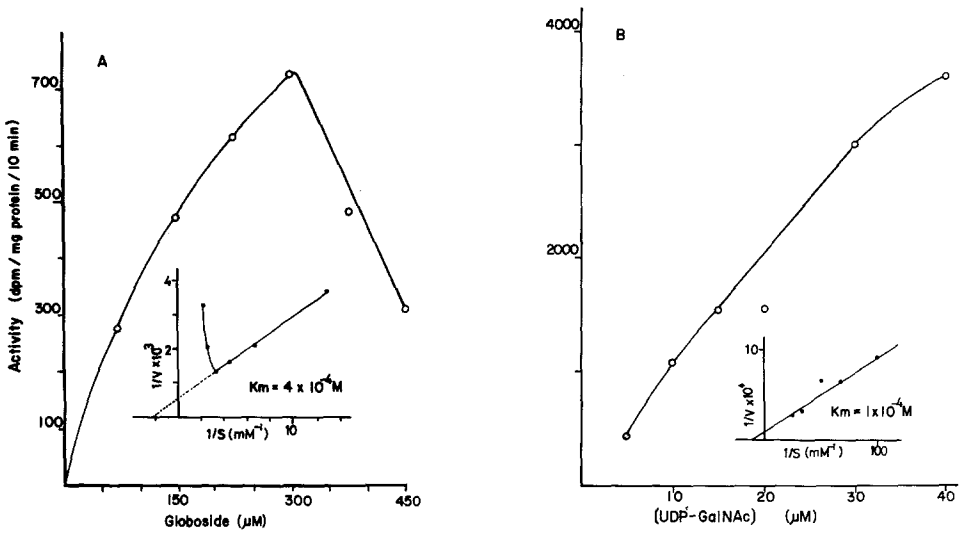

Figure 3. A; The effect of globoside concentration on the rate of formation of Forssman hapten. The conditions were the same as in Table I (a), except that varied concentrations of globoside were used. The inset shows a Lineweaver-Burk plot of the same data. B; The effect of UDP-[^{14}C]-GalNAc concentration on the rate of formation of Forssman hapten. The conditions were the same as in Table I except for the concentrations of globoside (300 μM) and UDP-^{14}C-GalNAc.

Table II. Distribution of the enzyme activity in different tissues and the subcellular fractions

Tissues	Subcellular fractions	dpm/mg protein
Spleen	Whole homogenate	97
	Nuclear fraction	78
	Mitochondrial fraction(Mt)	207(45)
	Microsomal fraction(Ms)	520(44)
	105,000 x g supernatant	20
Kidney	Mt	250(25)
	Ms	410(57)
Brain	Mt	55(10)
	Ms	96(23)
Liver	Mt	27
	Ms	31
Small intestine	Mt	157(141)
	Ms	335(326)

Numbers in parenthesis indicate endogenous values of the activity.
The activity was determined as given in Table I, (a).

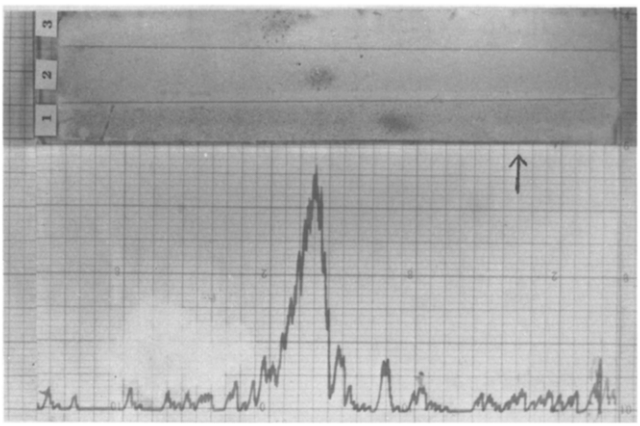

Figure 4. Thin layer chromatography of radioactive product. The product was chromatographed on a thin layer plate (Silica gel G) using chloroform-methanol-water (58:35:8) and scanned for radioactivity. The authentic standards were coloured by spraying orcinol reagent for glycolipids and naphthoresorcine reagent for GalNAc. The arrow shows origin.
Lane 1, GalNAc; lane 2, Forssman hapten; lane 3, globoside.

DISCUSSION

This article shows the presence of α-N-acetylgalactosaminyltransferase in guinea pig tissues. The distribution of the enzyme accords with that of Forssman hapten in various tissues of guinea pig (9), indicating in situ synthesis of the hapten.

N-Acetylgalactosaminyltransferase(s) (10-12) from rat brain tissue has been demonstrated for ganglioside synthesis and did not catalyze transfer of GalNAc to trihexosylceramide and globoside. The anomeric nature of N-acetylgalactosaminidic linkage in brain gangliosides has been shown to be β-configuration (13, 14). Therefore, the brain enzyme(s) is most probably β-N-acetylgalactosaminyltransferase. On the other hand, blood group-A dependent α-N-acetylgalactosaminyltransferases have been demonstrated in milk (15), submaxillary gland (16), stomach (17), serum (18, 19), erythrocyte membranes (18) and ovarian cyst (20) in soluble or

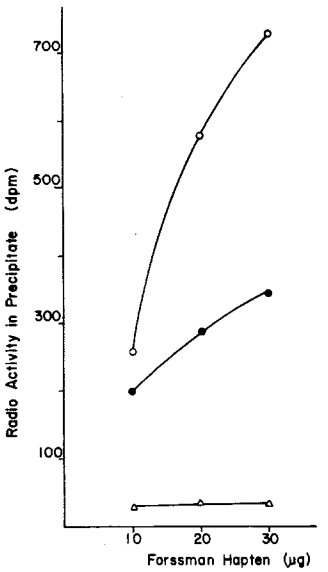

Figure 5. Immunoprecipitin reaction of the radioactive product. The details of the method were described previously (1). To the radioactive product was added the authentic Forssman hapten to make a solution of 770 dpm/10 μg of the hapten in 10 μl. Ten to thirty μl of the solution and 0.1 ml each of various sera were incubated. Precipitates were washed and counted the radioactivity. Reactions with anti-Forssman serum, o——o; anti-globoside serum, ●——●; and normal rabbit serum, △——△.

particulate-bound forms using specific oligosaccharides and glycoprotein as acceptors of GalNAc. Though Forssman hapten has been shown serologically to possess also blood group-A activity (5), the enzymes which catalyze the synthesis of Forssman hapten and blood group-A substance are probably different. α-N-acetylgalactosaminyltransferase reported here, therefore, is the first enzyme toward glycolipid.

The effects of UDP-Glc upon the α- and β-N-acetylgalactosaminyltransferases from guinea pig were quite different from each other. UDP-Glc had some activating effect on the β-transferase (1), while the nucleotide sugar inhibited the activity of the α-transferase.

Some activating effect of ATP observed in both enzymes may

be due to prevent the degradation of UDP-GalNAc by pyrophosphatase as discussed previously (18).

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