GLYCOPROTEIN METABOLISM: A UDP-GALACTOSE:GLYCOPROTEIN GALACTOSYLTRANSFERASE OF RAT SERUM[1]

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SUMMARY

Rat serum was found to contain an enzymatic activity capable of catalyzing the transfer of galactose from UDP-galactose to ovalbumin, a glycoprotein whose carbohydrate complement does not include galactose. Other proteins examined could not serve as galactose acceptors, nor would ovalbumin accept the glycosyl groups of UDP-N-acetylglucosamine or GDP-mannose. The enzyme displayed an absolute requirement for Mn^{++}, which could not be replaced by Mg^{++} or Ca^{++}. The pH optimum for the galactosyltransferase is approximately 6 in Tris-maleate buffer. The K_m for UDP-galactose is 2.95×10^{-5} M. The activity is present in soluble form, and is stable to storage at -20° for 3 weeks. Acid hydrolysis releases all of the radioactivity of the labeled product as ^{14}C-galactose. Mild alkaline hydrolysis releases less than 4% of the incorporated radioactivity, demonstrating that galactose is not attached to serine or threonine residues via O-glycosidic linkages.

Most plasma glycoproteins, with the exception of the γ-globulins, are synthesized in the liver (1,2), and in some cases, the subcellular sites of attachment of the carbohydrate moieties to the polypeptide chains have been defined to a significant extent (3-7). Recently Mookerjea et al. (8) have described an N-acetylglucosaminyltransferase in human and rat sera, and Den et al. (9) and Roseman (10) have reported the presence of soluble glucosaminyltransferase and galactosyltransferase in the fluid surrounding embryonic chick brain. This latter activity was also detected in chicken serum, amniotic fluid, vitreous humour, and in human spinal fluid. These activities, in contrast to the

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liver enzymes, which appear to be membrane bound, are found in soluble form. The present report describes some properties of an enzyme obtained from rat serum which transfers galactose from UDP-galactose to ovalbumin, a glycoprotein whose carbohydrate moiety contains only N-acetylglucosamine and mannose.

MATERIALS AND METHODS

UDP-^{14}C-galactose was purchased from New England Nuclear Corp. Ovalbumin (Grade V) was obtained from Sigma. Serum was prepared by allowing fresh whole rat blood to clot, followed by centrifugation at 7000_g for 15 min.

The standard incubation mixture contained, in a total volume of 100 µl: 20 - 60 µl of serum (1.5 - 4 mg protein), 2.5 nmoles (26,000 cpm) of UDP-^{14}C-galactose, 1.5 mg of ovalbumin, 1.0 µmole of $MnCl_2$, 0.2 µmoles of EDTA, and 5.0 µmoles of Tris-maleate buffer, pH 6.0. Reactions were terminated by the addition of 3 ml of cold 5% trichloracetic acid - 1% phosphotungstic acid. Transfer of ^{14}C-galactose to ovalbumin was measured as radioactivity precipitated with trichloracetic acid - phosphotungstic acid. Precipitates were washed 5 times with 3 ml of trichloracetic acid - phosphotungstic acid and once with 3 ml of ethanol/ether (3:1). The washed precipitates were heated with 1 ml of Protosol at 50° until dissolved. The digested samples were added to 10 ml of Liquifluor, and radioactivity was measured by liquid scintillation spectrometry.

Protein was determined by a modification of the method of Lowry et al. (11). Sugars were detected on paper following chromatography with a triphenyl tetrazolium chloride reagent (12).

RESULTS AND DISCUSSION

The requirements for the transfer of galactose to ovalbumin are summarized in Table I. Transfer to endogenous acceptors accounted for less than 1% of the total activity. The galactosyltransferase displayed an absolute

TABLE I

REQUIREMENTS FOR GALACTOSE TRANSFER

Reaction mixture	Galactose-^{14}C transfer	
	cpm/sample	(pmoles)
Complete*	3014	289.8
Zero time	23	2.2
Minus serum	3	0.3
Minus ovalbumin	39	3.8
Minus Mn^{++}	18	1.7
Plus 12 mM EDTA	9	0.9
Minus Mn^{++}, plus 10 mM Mg^{++}	14	1.4
Minus Mn^{++}, plus 10 mM Ca^{++}	13	1.3

*Complete incubation mixture as described in the text, but with the omission of EDTA. Incubation time was 20 min. Each sample received 30 µl of serum (2.2 mg protein).

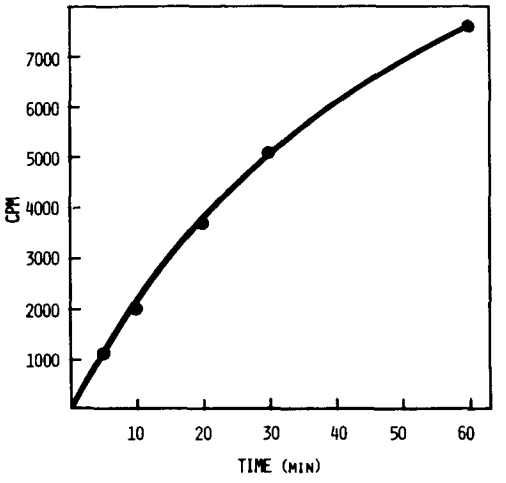

Fig. 1. Time course of transfer of ^{14}C-galactose to ovalbumin. Incubation conditions were as described in Materials and Methods. Each sample received 40 µl of serum (3.0 mg protein).

requirement for Mn^{++}, which could not be replaced by either Mg^{++} or Ca^{++}. The enzyme is fairly specific in that ribonuclease A, ribonuclease B, or Cohn plasma fraction VI (consisting largely of orosomucoid) could not function as acceptors of galactose. In addition, ovalbumin would not accept mannose from GDP-mannose, nor glucosamine from UDP-N-acetylglucosamine.

Fig. 1 illustrates the transfer of galactose to ovalbumin as a function of time. (Unless otherwise stated, results are expressed as cpm/sample and are corrected for zero time values). Fig. 2 shows the linear relationship

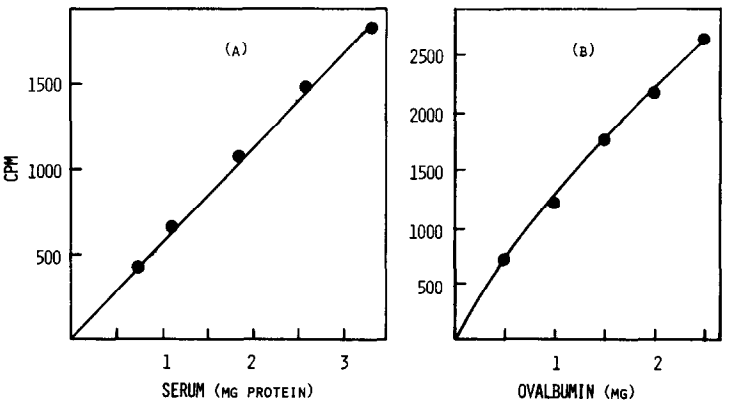

Fig. 2. Relationship of galactose transfer to (A) enzyme, and (B) ovalbumin concentration. Incubation time was 10 min under conditons described in the text. Samples illustrated in (B) received 30 μl of serum (2.5 mg protein).

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of the activity to serum and ovalbumin concentrations. The pH optimum in Tris-maleate buffer is approximately 6. The K_m for UDP-galactose is 2.95×10^{-5} M (Fig. 3). The transferase retained full activity after storage at -20° for a period of 3 weeks.

This galactosyltransferase differs from the liver enzyme(s) referred to (7) and from other glycosyltransferases (7,13,14) not only in that it appears to be present in soluble form, rather than being membrane-bound, but also in its susceptibility to inhibition by nucleoside monophosphates. Thus, galactose transfer to endogenous acceptors in a Golgi-rich fraction from liver is inhibited 44% by 4×10^{-4} M UMP (7). In contrast, 6×10^{-4} M UMP causes only 13% in-

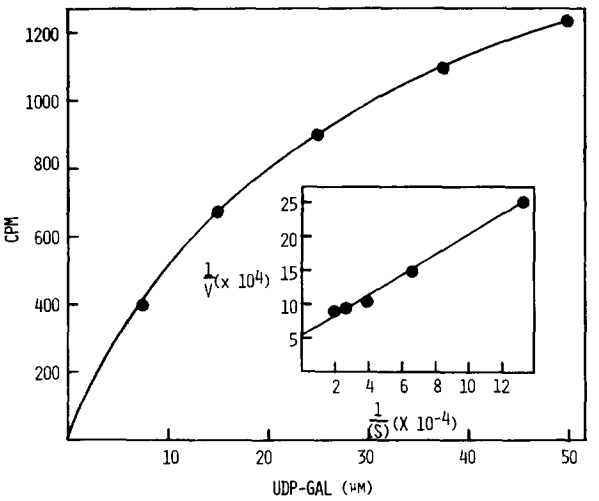

Fig. 3. Effect of UDP-galactose on galactose transfer. Assay conditions as described. Incubation time was 10 min. Each sample received 40 μl of serum (3.0 mg protein).

hibition of the serum enzyme, while 2 x 10^{-3} M UMP is necessary to produce 43% inhibition.

Galactose-^{14}C-labeled ovalbumin was subjected to hydrolysis with 2 N HCl at 100°, for 4 hrs. HCl was removed by evaporation at 50°, and aliquots of the hydrolysate were subjected to descending chromatography in a solvent system consisting of ethyl acetate/pyridine/water (12:5:4) for 24 hrs. The sole radioactive spot migrated with standard galactose, and was well separated from both glucose and mannose.

Since the carbohydrate moiety of ovalbumin does not normally contain galactose (15), the possibility of formation of O-glycosidic linkages to serine or threonine was considered. This possibility was excluded however, since treatment of galactose-^{14}C-labeled ovalbumin with mild alkali under conditions which would split this linkage by β-elimination (0.5 N NaOH, 24°, 90 min) resulted in the release of less than 4% of the incorporated radioactivity.

The specific site(s) of attachment of the galactose residues to the ovalbumin molecule are of considerable interest. It has not yet been possible to determine whether galactose is added directly to the polypeptide via N-gly-

cosidic linkage to asparagine residues, or incorporated into the carbohydrate chain of ovalbumin.

The chicken galactosyltransferase (9) catalyzes the transfer of galactose to α_1-acid glycoprotein pretreated with sialidase and β-galactosidase. Similarly, the serum glucosaminyltransferase (8) exhibited maximal activity with this glycoprotein after sequential treatment with sialidase, β-galactosidase, and hexosaminidase, although it did display partial activity with chick albumin and ribonuclease B. Thus, the significance of serum-catalyzed transfer of galactose to ovalbumin is of great interest, in so far as this glycoprotein, unlike the α_1-acid glycoprotein, is not normally present in blood.

The tissue of origin, function and physiological significance of this enzyme, as well as other serum glycosyltransferases, are currently under investigation. The presence of a similar galactosyltransferase has also been detected in human serum*.

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*I. Neufeld, unpublished observations.