BBA 66316

GALACTOSYL TRANSFERASE ACTIVITY IN A VARIETY OF SOURCES

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(Received January 14th, 1971)

SUMMARY

Galactosyl transferase activity was found in a variety of sources including rat and chicken tissues, bovine thyroid particles, rabbit gastric mucosa, L cell membranes, opossum, guinea pig, bat and hamster mammary glands and milk from the bat and horse. Enzymatic activity was measured using glucose and *N*-acetylglucosamine as substrates in the absence and presence of bovine α-lactalbumin.

INTRODUCTION

Lactose synthetase (UDPgalactose:D-glucose 1-galactosyl transferase; EC 2.4.1.22) catalyzes the formation of lactose (Eqn. 1).

$$\text{UDPgalactose} + \text{glucose} \xrightarrow{Mn^{2+}} \text{lactose} + \text{UDP} \qquad (1)$$

This reaction requires two proteins, a galactosyl transferase and α-lactalbumin for significant activity. However, the reaction may be catalyzed by the galactosyl transferase in the absence of α-lactalbumin when the glucose concentration is high ($K_m = 1.4$ M)[2]. α-Lactalbumin lowers the apparent K_m for glucose so that it becomes a good substrate. The galactosyl transferase will transfer galactose to a variety of carbohydrate acceptors such as *N*-acetylglucosamine (Eqn. 2), β-1-4 linked glycosides and the carbohydrate side-chain of glycoproteins such as ovalbumin[3].

$$\text{UDPgalactose} + \textit{N}\text{-acetylglucosamine} \xrightarrow{Mn^{2+}} \textit{N}\text{-acetyllactosamine} + \text{UDP} \qquad (2)$$

α-Lactalbumin markedly inhibits the transfer of galactose to *N*-acetylglucosamine but inhibits the transfer slightly to polymers of *N*-acetylglucosamine and ovalbumin[3]. The principal function of the galactosyl transferase in tissues other than lactating mammary tissue is to transfer galactose to appropriate carbohydrate side chain of glycoproteins and on this basis it would be predicted that the galactosyl transferase would be found in many tissues.

The purpose of this study was to examine a variety of sources for galactosyl transferase activity in order to determine its distribution. The criteria for similarity

of the enzyme from different sources is that α-lactalbumin should stimulate the reaction when glucose is the substrate and inhibit the reaction when *N*-acetylglucosamine is the substrate.

MATERIALS AND METHODS

In general, assays were performed as previously described[4] and exceptions are recorded. The concentration of bovine α-lactalbumin used was 1 mg/ml to stimulate in the presence of glucose and to inhibit in the presence of *N*-acetylglucosamine. No attempt was made to optimize assay conditions for the various tissues. For rat (Holtzman) and chicken (Leghorn) tissues 1 g was homogenized with 2 ml of homogenizing buffer[4] and centrifuged at 500 × *g* for 15 min. The supernatant solution was made 0.2% in Tween 80 and assayed at pH 9.0. The reaction was stopped by boiling for 1 min. Fresh bovine thyroid particles were prepared[5] except that 1 g of tissue was homogenized with 5 ml of homogenizing buffer[4]. The pellet was suspended in a minimum volume of 0.25 M sucrose and assayed at pH 7.5 in the presence of 0.2% Tween 80. L cells[5] were obtained from Dr. Higgins of this department and membranes were prepared by a procedure described for the preparation of membranes from Ehrlich ascites tumor cells[6]. The lyophilized material (Step 2) was dissolved in 0.5 M glycylglycine and assayed at pH 7.5 in the presence of 0.1% Tween 80. The reaction was stopped by boiling and the activity observed represents that from $1.2 \cdot 10^9$ cells. Particles from rabbit, rat and chicken gastric mucosa were prepared by the method of ZIDERMAN *et al.*[7] and the activity assayed at pH 9.0 represents the particles obtained from 1 g of tissue. Homogenates from the mammary glands of the bat (Mexican Free-Tailed), opossum, guinea pig and hamster were prepared as previously described[4]. Horse and bat milk were centrifuged at 15 000 × *g* for 25 min at 4°. The fluid between the fat layer and the precipitate was used as the enzyme source. Activity is expressed as units per ml of milk.

RESULTS AND DISCUSSION

The results of the assays using glucose and *N*-acetylglucosamine as substrates in the absence and presence of α-lactalbumin are presented in Table I. In the highly purified bovine system α-lactalbumin stimulates the reaction when glucose is the substrate and inhibits when *N*-acetylglucosamine is the substrate. Most of the sources examined in the study exhibit this pattern which indicates that this galactosyl transferase is wide-spread and has similar properties. This is compatible with the view that its principal biological function is concerned with the transfer of galactose to a *N*-acetylglucosamine residue in the carbohydrate side-chains of glycoproteins. The mammary gland is unique since it produces α-lactalbumin which can modify this transferase so that it forms lactose readily in the presence of glucose. This study has shown that lactose was synthesized by all tissues in the presence of bovine α-lactalbumin.

SPIRO AND SPIRO[5] have isolated a galactosyl transferase from the thyroid which transfers galactose to thyroglobulin and *N*-acetylglucosamine and have suggested the transfer may be catalyzed by two separate enzymes. They also have reported that the enzyme does not transfer galactose to glucose. The present study shows that

TABLE I

GALACTOSYL TRANSFERASE ACTIVITY IN A VARIETY OF SOURCES*

Source	*Substrate*			
	Glucose		*N-acetylglucosamine*	
	-α-lactalbumin	*+α-lactalbumin*	*-α-lactalbumin*	*+α-lactalbumin*
Rat				
lung	0.0	7.5	5.4	0.0
heart	0.0	1.8	0.9	0.0
spleen	4.9	3.0	9.3	10.9
gastric mucosa	0.7	15.2	9.5	1.1
liver	1.3	1.5	1.0	3.9
Chicken				
liver	0.0	17.3	44.7	21.7
brain	0.0	3.0	0.8	0.0
gastric mucosa	1.9	4.5	7.7	0.0
spleen	0.0	0.1	9.0	0.0
Bovine thyroid particles	1.7	4.2	3.8	13.2
L cell membranes	0.8	1.4	10.1	0.9
Rabbit gastric mucosa	0.0	8.5	16.2	4.7
Opossum mammary gland	76.5	186.3	267.3	176.0
Guinea pig mammary gland	105	558	380	180
Hamster mammary gland	106	758	579	386
Bat mammary gland	50	266	288	73
Bat milk	14.5	27.5	12.5	2.0
Horse milk	67.7	46.9	2.5	0.5

* Activity is expressed as units (nmoles/min at 37°) per g tissue or ml of extract. See text for details.

the thyroid particles transfer galactose to glucose under the proper conditions and this reaction is stimulated by α-lactalbumin. Other studies with the bovine milk galactosyl transferase have shown that the enzyme can transfer galactose to a variety of acceptors under the appropriate conditions[3]. CACCAM AND EYLAR[6] have suggested that it is unlikely that α-lactalbumin would modify the acceptor specificity of a membrane-bound galactosyl transferase prepared from Ascites cells. The results with the L cell membranes (Table I) show that α-lactalbumin does stimulate the formation of lactose from glucose. Recent studies[8] have shown that the galactosyl transferase can be used as an indicator enzyme for Golgi apparatus and hence it is suspected that it would be widely distributed. The results with the mammary gland tissues and milks are similar to those obtained from other species[9] except in horse milk where α-lactalbumin inhibited the transfer of galactose to glucose. High concentrations of α-lactalbumin can inhibit the galactosyl transferase reaction and this may be the reason for the observed inhibition[4]. The galactosyl transferase present in embryonic chick brain[11], rabbit mucosal particles[7], goat colostrum[12], and rat liver Golgi[8] appears to be similar to the one found in other tissues.

ACKNOWLEDGEMENTS

Journal Article No. 2189 of the Agricultural Experiment Station, Oklahoma

State University, Stillwater, Oklahoma. This research was supported by grants from the American Cancer Society P 420 and a Career Development Award IKO4 GM 42396 to K.E.E.

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