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GALACTOSYLTRANSFERASE FROM COMMERCIAL PREPARATIONS OF FETUIN

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Summary

A galactosyltransferase that transfers galactose from UDPgalactose to asialoagalacto fetuin or *N*-acetylglucosamine was partly purified from two commercial preparations of fetuin and its kinetic properties were characterized. Several other preparations of fetuin were also found to contain galactosyltransferase activity.

Galactosyltransferases from various sources have been characterized and, in some cases, purified [1, 2]. These enzymes catalyze the following reaction:

UDPgalactose + Acceptor → Acceptor galactose + UDP

Fetuin, a glycoprotein from fetal calf serum, is often used as an acceptor to assay for certain galactosyltransferases after many of its ultimate sialic acid and penultimate galactose residues have been removed. When asialoagalacto fetuin was prepared by treating a commercial fetuin preparation with specific glycosidases and used in galactosyltransferase assays, the fetuin preparation itself was found to contain a galactosyltransferase activity. This report describes the separation and kinetic characterization of this enzyme from two commercial fetuin preparations.

Fetuin: Commercial preparations of fetuin were purchased from Sigma Chemical Co. and Grand Island Biologicals Co. (GIBCO). Sigma fetuins are

*Abbreviations: Medium "J", glucose-, phosphate- and bicarbonate-free Hanks' balanced salt solution, pH 7.2, buffered with 10 mM Hepes; Hepes, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; GlcNAc, *N*-acetylglucosamine, 2-acetamido-2-deoxy-D-glucose.

separated from fetal calf serum by the procedure of Deutsch [3] which uses a combination of a sodium trichloroacetate precipitation and ammonium sulfate and ethanol fractionation techniques. Two types of fetuin preparations from GIBCO were used; one was separated from fetal calf serum by the Deutsch procedure, the other by the method of Spiro [4] which utilizes a low temperature ethanol fractionation in the presence of barium and zinc ions. All preparations were in lyophilized form.

Galactosyltransferase assay: UDP[^{14}C]Galactose had a specific activity of 255 Ci/mol, whereas that of the UDP[^{3}H] galactose was 507 Ci/mol. UDP-[^{3}H]Galactose was used in all kinetic experiments. Standard assay incubations included: 110 μM radiolabeled UDPgalactose (20 cpm/pmol), 20 mM GlcNAc, 10 mM $MnCl_2$ in a total volume of 50 μl, and were performed at 37°C for 1 h. The buffers used were either Medium "J" or 25 mM cacodylate, pH 7.2. Assays were begun by the addition of enzyme and stopped by the addition of 10 μl of a 250 mM solution of ice-cold EDTA in 5 mM Tris-HCl, pH 7.2, as well as rapid chilling to 0°C. An aliquot of the assay solution, usually 50 μl, was transferred to Whatman No. 3 chromatography paper. The paper was then saturated with 1% sodium tetraborate and subjected to electrophoresis at 57 V/cm for 40 min [5]. Areas surrounding the origin were cut from the dried electrophoretogram and counted in a toluene-based scintillation system. Radioactivity remaining at the origin corresponded to radioactive galactose moieties incorporated onto acceptors.

Glycosidase-treated fetuin: Asialoagalacto fetuin was prepared by treating fetuin with neuraminidase and β-galactosidase. Neuraminidase (2500 units) and β-galactosidase (1500 units), both from *Diplococcus* and purified by the methods of Hughes and Jeanloz [6], were added to 50 mg of Sigma fetuin in 0.1 M sodium phosphate buffer, pH 6.3, to a final volume of 1 ml. This solution was incubated for 4 h at 37°C, after which time the glycosidases were inactivated by incubating the mixture at 56°C for 30 min. After discovery of the galactosyltransferase, all preparations of asialoagalacto fetuin that were to be used as acceptors in the transferase assay were heated to 80—100°C for 15 min to inactivate the galactosyltransferase. The galactosyltransferase assay described above was used to determine the number of available acceptor sites for galactose. Assuming a molecular weight of 50 000 for the fetuin [4], the number of acceptor sites for galactose per molecule of asialoagalacto fetuin prepared by glycosidase treatment was calculated to be 6.

Affinity chromatography: UDPHexanolamine-Sepharose was synthesized by the method of Barker et al. [7]. Procedures for the application and elution of the enzyme were similar to those described in [8] except that the column size was 0.5 × 6 cm and no protease inhibitor was used in the buffers. 50 mg of Sigma fetuin III were applied and 85% of the activity was recovered with 2% eluting in the void fractions. As shown in Fig. 1, the elution buffer was prepared by replacing $MnCl_2$ with EDTA [8].

DEAE chromatography: A column (25 × 1.2 cm) with a bed volume of 20 ml was equilibrated with 5 mM Tris-HCl, pH 7.2, and 1 mM β-mercaptoethanol. A 52-mg portion of Sigma fetuin II was applied and the column developed in the same buffer with an increasing NaCl gradient at the rate of 1 ml/2 min. Fractions of 5 ml were collected. Recovery of both protein and

activity were over 95%. Protein was determined by a modification of the McGrath assay[9].

Commercial preparations of fetuin are contaminated with a galactosyltransferase that can use either asialoagalacto fetuin or *N*-acetylglucosamine as acceptor. The contamination was discovered when asialoagalacto fetuin was prepared by glycosidase treatments and not boiled and subsequently used in a control assay with radiolabeled UDPgalactose. The endogenous galactosyltransferase transferred galactose to asialoagalacto fetuin in the presence of Mn^{2+}. Controls were done with bovine serum albumin treated with neuraminidase and β-galactosidase to demonstrate that glycosidase treatment of fetuin did not introduce the galactosyltransferase. Fetuin that was not glycosidase-treated demonstrates a small amount of endogenous activity. We then tested the untreated fetuin preparations for activity toward *N*-acetylglucosamine and found significant levels of galactosyltransferase activity for this acceptor. The galactosyltransferase activity is labile when the fetuin preparations are heated to 80—100°C for longer than 10 min.

Several sources of fetuin prepared by different methods were found to have galactosyltransferase activity toward GlcNAc and asialoagalacto fetuin. Sigma fetuins I and II prepared by the Deutsch procedure and Sigma fetuin III, the preparation currently available, all have considerable activity. GIBCO fetuins prepared by the Deutsch or Spiro methods also have this activity, though much lower than the Sigma fetuins. None of these preparations displayed either phosphatase or pyrophosphatase activities. Other glycosyltransferase activities that might be expected also to contaminate fetuin, such as glucosyl-, *N*-acetylglucosaminyl- and *N*-acetylneuraminyltransferases were not detected in these preparations.

The galactosyltransferase has been separated from Sigma fetuin by exchange chromatography on DE-52 (Fig. 1) or affinity chromatography on UDP-Sepharose (Fig. 2). Table I shows the relative purification of the galactosyltransferase by each of these methods. Affinity chromatography yielded the enzyme of greatest purity, 30 nmol/mg per min, which represented a 300-fold purification.

Turco and Heath [10] have purified a galactosyltransferase from fetal calf serum by affinity chromatography on UDP-Sepharose and repeated passages through an α-lactalbumin-Sepharose column. After separation on UDP-Sepharose, and prior to additional purification, the enzyme from fetal calf serum has a specific activity of 210 nmol/mg per min or about 7 times the activity of the enzyme separated from fetuin by UDP-Sepharose chromatography.

The K_m values for the fetuin enzyme purified on DE-52 for UDP-galactose, *N*-acetylglucosamine, and asialoagalacto fetuin are 47 μM, 3.4 mM and 0.1 mM, respectively. All kinetic studies obeyed Michaelis-Menten saturation kinetics. In addition, at α-lactalbumin concentrations of 100 μg per 50 μl assay, 90% of the activity toward the GlcNAc acceptor is inhibited with a concomitant increase in transfer to glucose. This α-lactalbumin effect on galactosyltransferase acceptor specificity has been well characterized [11].

When separated from fetuin, the enzyme rapidly loses activity, whether stored at 4°C or frozen. Solutions of crude fetuin stored frozen retain full

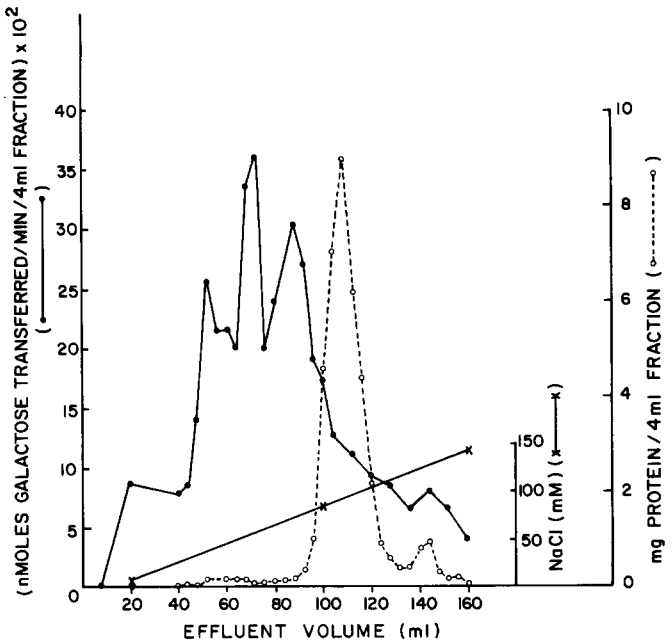

Fig. 1. DEAE chromatography of crude fetuin. The chromatographic procedures and recoveries of protein and galactosyltransferase activity are described in the text. Activity was assayed under standard conditions.

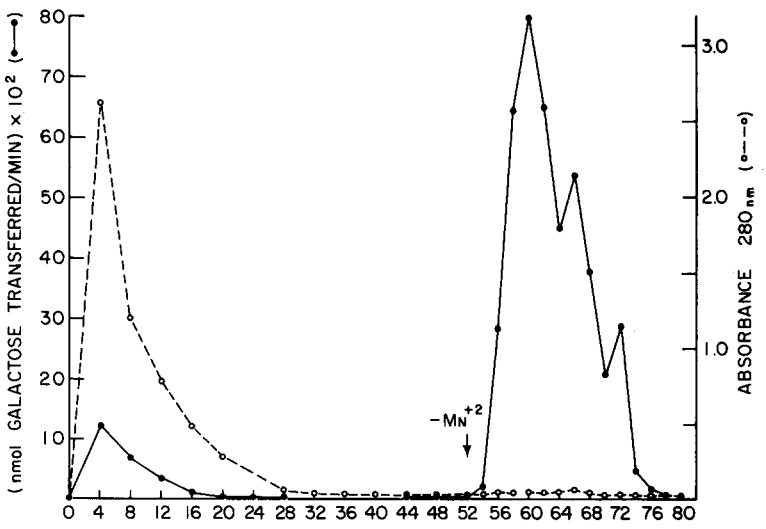

Fig. 2. UDPHexanolamine-Sepharose chromatography of crude fetuin. The chromatographic procedures and recoveries of protein and galactosyltransferase activity are described in the text. Activity was assayed under standard conditions. The numbers on the abscissa refer to effluent volume in ml.

TABLE I

PURIFICATION OF GALACTOSYLTRANSFERASE FROM SIGMA FETUIN BY ION-EXCHANGE OR AFFINITY CHROMATOGRAPHY

Fetuin samples were chromatographed on either DEAE-cellulose or UDP-Sepharose and fractions assayed as described in the text.

Fraction	Protein (mg)	Galactosyltransferase activity nmol Gal transferred to GlcNAc (mg/min)	Relative spec. act.
A. Sigma fetuin II	52	0.08	1.0
DEAE-cellulose	0.31	12.0	150.0
B. Sigma fetuin III	50	0.09	1.0
UDP-Sepharose	0.15	29.5	327.0

activity for up to 3 months. Fetuin stored in lyophilized form retains 100% activity up to at least 1 year. Thus, some commercial fetuin preparations not only contain large amounts of galactosyltransferase, but are a convenient and inexpensive source of the crude enzyme free from phosphatase contamination and can be conveniently used, for example, to screen acceptor preparations for their ability to accept transfer of galactose.

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