BIOSYNTHESIS OF CHONDROITIN SULFATE: INTERACTION BETWEEN XYLOSYLTRANSFERASE AND GALACTOSYLTRANSFERASE

Nancy B. Schwartz*, Lennart Rodén† and Albert Dorfman*

* Departments of Pediatrics and Biochemistry, Joseph P. Kennedy, Jr. Mental Retardation Research Center, Pritzker School of Medicine, University of Chicago, Chicago, Illinois 60637. † Institute of Dental Research and Departments of Medicine and Biochemistry, University of Alabama in Birmingham, Birmingham, Alabama 35294.

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SUMMARY

An affinity matrix consisting of the core protein of cartilage proteoglycan coupled to Sepharose was used to study the interaction between the glycosyltransferases which catalyze the first two reactions in the biosynthesis of chondroitin sulfate. Xylosyltransferase, for which the core protein is a substrate, is quantitatively adsorbed to the matrix. In contrast, UDP-galactose:xylose galactosyltransferase is not significantly adsorbed, but does bind to matrix which has been previously equilibrated with xylosyltransferase. By virtue of this enzyme-enzyme interaction, a 7-fold purification of galactosyltransferase can be obtained.

The chondroitin sulfate chains of cartilage proteoglycan are synthesized by the concerted action of six distinct glycosyltransferases (1). It has been suggested that enzymes which catalyze consecutive glycosyltransfer steps are located in adjacent positions on the membranes of the endoplasmic reticulum, perhaps as a result of specific enzyme-enzyme interactions (2). Experiments reported here demonstrate that this type of interaction occurs between the glycosyltransferases catalyzing the first two steps of chondroitin sulfate biosynthesis, i.e., xylosyltransferase[1] and galactosyltransferase[1].

MATERIALS AND METHODS

UDP-[^{14}C]xylose (252 mCi per mmole) and UDP-[^{14}C]galactose (270 mCi

[1] Xylosyltransferase refers to UDP-D-xylose:core protein xylosyltransferase and galactosyltransferase to UDP-D-galactose:D-xylose galactosyltransferase.

per mmole) were purchased from New England Nuclear. Core protein from bovine cartilage proteoglycan (PGSD)[2] was prepared by Smith degradation as described by Baker et al (3).

Xylosyltransferase[1] - The enzyme used in these studies was purified 30-fold from an embryonic chick cartilage homogenate by differential centrifugation, ammonium sulfate precipitation and Sephadex gel filtration, as described previously (2). Xylosyltransferase was assayed by procedures which have been described in detail (4). Briefly, incubation mixtures contained 0.4 μmole of $MnCl_2$, 0.75 μmole of KCl, 1.5 nmoles of UDP-[^{14}C]xylose (specific activity approximately 30 μCi per μmole), varying amounts of enzyme protein, and 0.2 mg of PGSD in a total volume of 0.075 ml. After incubating the reaction mixture for 60 min at 37°, 0.25 mg of bovine serum albumin and 0.20 ml of 10% trichloracetic acid-4% phosphotungstic acid were added. Precipitated protein was recovered by centrifugation, washed twice with 5% trichloroacetic acid and redissolved in 0.1 ml of 1.0 M NaOH for liquid scintillation counting.

Galactosyltransferase[1] - This enzyme was obtained from a particulate fraction of an embryonic chick cartilage homogenate, sedimenting between 10,000 x g and 100,000 x g. The enzyme was solubilized by treatment with 1% Nonidet P-40 and 0.50 M KCl in 0.05 M Tris-acetate buffer, pH 5.5 (4), yielding a preparation with a protein concentration of 10-15 mg per ml. It was partially purified by gel filtration on a column (2.5 x 143 cm) of Sephadex G-200 which was eluted with 0.05 M Tris-acetate, pH 5.5, containing 0.25 M KCl and 0.001 M EDTA. A minor portion of the galactosyltransferase activity emerged with the void volume and a major portion in an included position with an effluent volume of 125-160 ml. The enzyme in the included fraction had been

[2] The abbreviations used are: MES, (2-N-morpholino) ethane sulfonic acid; PGSD, Smith degraded chondroitin sulfate proteoglycan.

purified approximately 40-fold and was well separated from the peak of xylosyltransferase and the second chondroitin sulfate galactosyltransferase (UDP-galactose: 4-O-β-D-galactosyl-D-xylose galactosyltransferase). The pooled included fractions were concentrated approximately 10-fold by ultrafiltration through an Amicon XM-50 membrane and then diluted to a protein concentration of 0.2-0.3 mg per ml with 0.05 M MES-0.05 M KCl, pH 6.5. The galactosyltransferase was assayed by incubating 2 μmoles of xylose, 1.5 nmoles of UDP-[^{14}C]galactose (34.5 μCi per μmole), 1 μmole of $MnCl_2$, and varying amounts of enzyme protein in a total volume of 0.075 ml. After 60 min at 37° the reaction was terminated by heating the tubes at 100° for 2 min. The product (4-O-β-D-galactosyl-D-xylose) was separated and quantitated as previously described (4).

PGSD-Sepharose - Sepharose was activated by treatment with cyanogen bromide as described by Cuatrecasas (5). PGSD was coupled to the activated Sepharose in 0.2 M borate buffer, pH 9.5, for 18-20 hrs at 4°. The PGSD-Sepharose was washed well with water and finally equilibrated with 0.05 M MES-0.05 M KCl, pH 6.5. Further details of the coupling procedure and the use of the PGSD-Sepharose to purify xylosyltransferase will be described elsewhere.

Protein determination - Protein was determined by the method of Lowry et al (6), except for the galactosyltransferase eluted from affinity columns, which was estimated by amino acid analysis following hydrolysis in 6 M HCl at 100° for 20 hrs.

RESULTS

Evidence for a specific interaction between xylosyltransferase and galactosyltransferase is presented in Fig. 1 which shows that 88% of a

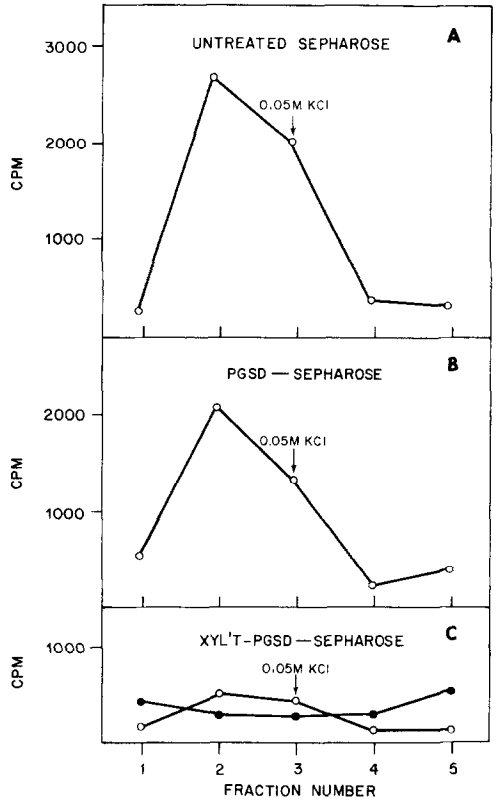

Fig. 1. Interaction of galactosyltransferase with xylosyltransferase on PGSD-Sepharose. Three columns (0.6 x 1.5 cm) containing Sepharose 4B (A) and PGSD-Sepharose (B and C) were equilibrated with 0.05 M MES, pH 6.5, containing 0.05 M KCl, and xylosyltransferase (1.0 ml; 18,000 cpm; spec. act. 5.5 x 10^4 cpm per mg protein) was applied to column C followed by a thorough buffer rinse. (Amounts of enzyme used in these experiments are expressed as radioactivity of the products in cpm under standard assay conditions.) Galactosyltransferase (1.0 ml; 84,000 cpm; spec. act. 5.2 x 10^5 cpm per mg protein) was then applied to each column in two 0.5-ml portions and, after elution with 0.05 M KCl in buffer (1.0-ml fractions), galactosyltransferase (o ——o) and xylosyltransferase (● ——●;column C only) in the effluents were assayed as described in Methods. Of the galactosyltransferase activity applied, 80% (67,000 cpm) was recovered from column A, 66% (55,000 cpm) from column B, and 12% (10,000 cpm) from column C.

galactosyltransferase preparation was retained by a column of PGSD-Sepharose to which xylosyltransferase had been adsorbed (Fig. 1C). In contrast, only 20% and 34%, respectively, of the galactosyltransferase activity was bound to untreated Sepharose (Fig. 1A) or PGSD-Sepharose in the absence of xylosyl-

transferase (Fig. 1B). In subsequent experiments, as little as 10% of the galactosyltransferase was non-specifically adsorbed to Sepharose and PGSD-Sepharose which had been preconditioned with albumin (unpublished results).

As illustrated in Fig. 2, a large proportion (65%) of the galactosyltrans-

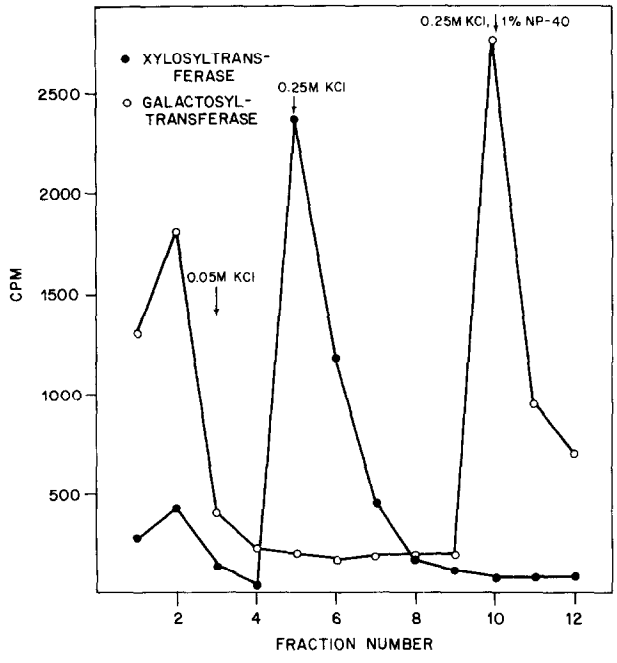

Fig. 2. Elution of xylosyltransferase and galactosyltransferase from an affinity column. A column (0.6 x 5.0 cm) of PGSD-Sepharose was saturated with xylosyltransferase (50 ml; 384,000 cpm; spec. act. 4.6 x 10^4 cpm per mg protein) as described in Fig. 1. A solution of galactosyltransferase (4.0 ml; 360,000 cpm; spec. act. 5.2 x 10^5 cpm per mg protein) was applied to the column, resulting in binding of 63% (228,000 cpm) of the total activity. Elution was then carried out (2.0-ml fractions) with 0.05 M MES, pH 6.5, containing 0.05 M KCl (fractions 3 and 4), 0.25 M KCl (fractions 5 to 9), and 0.25 M KCl-1% NP-40 (fractions 10 to 12).

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ferase activity adsorbed to a xylosyltransferase-saturated column could be eluted with buffer containing 1% Nonidet P-40 and 0.25 M KCl, whereas no activity was eluted by buffer containing only 0.25 M KCl. Approximately 1/3 of the xylosyltransferase activity was recovered in the buffer-salt eluate and no additional activity was eluted with detergent and salt.

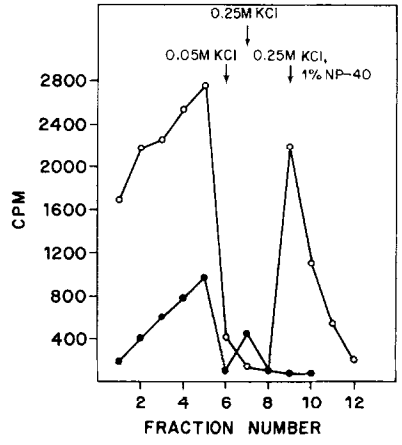

Fig. 3. Chromatography of a solution containing both xylosyltransferase and galactosyltransferase. A solution (25 ml) of xylosyltransferase (615,000 cpm; spec. act. 5.9×10^4 cpm per mg protein) and galactosyltransferase (1,579,000 cpm; spec. act. 5.2×10^5 cpm per mg protein) was applied to a column (0.6 x 5.0 cm) of PGSD-Sepharose prepared as previously described. The column was eluted consecutively with 0.05 M MES, pH 6.5, containing 0.05 M KCl, 0.25 M KCl, and 0.25 M KCl-1% NP-40. ● ——●, xylosyltransferase; o ——o, galactosyltransferase.

In a similar experiment, xylosyltransferase and galactosyltransferase were mixed and applied as a single solution to PGSD-Sepharose in excess of the column capacity (Fig. 3). Under these conditions, 36% of the xylosyltransferase activity and 28% of the galactosyltransferase activity of the mixture were retained by the affinity matrix. When this column was eluted with buffer containing only salt, a small fraction of the bound xylosyltransferase activity was recovered (15%), and no galactosyltransferase activity was detected. Elution with detergent yielded no xylosyltransferase activity but nearly complete recovery of the bound galactosyltransferase (88%).

The interaction between xylosyltransferase and galactosyltransferase is at least partially specific, since the galactosyltransferase eluted from the affinity matrix has been purified approximately 7-fold, resulting in a total purification of 310-fold over the crude cartilage homogenate.

DISCUSSION

It is apparent from these experiments that Sepharose-bound core protein from cartilage proteoglycan, previously used to purify the chain-initiating xylosyltransferase to homogeneity (7), may be used as well to study the interactions between various enzymes. The results described are consistent with three types of interactions: 1) the enzyme-substrate interaction between xylosyltransferase and the core protein which can be dissociated only by the substrate itself (7); 2) an interaction between individual xylosyltransferase molecules which can be dissociated with salt and which correlates well with the tendency of this enzyme to aggregate at low ionic strength (2); and 3) an interaction between xylosyltransferase and galactosyltransferase which can only be dissociated by detergent at elevated ionic strength and which most likely involves binding between hydrophobic regions of the two enzyme molecules.

Further evidence for the interaction between these two enzymes has been obtained by immunochemical procedures (manuscript in preparation). When a mixture of galactosyltransferase and xylosyltransferase was incubated with an antiserum to purified xylosyltransferase, both enzyme activities were found in the resulting precipitate. Under identical conditions, other enzymes (catalase and lactate dehydrogenase) did not precipitate along with the antigen-antibody complex, nor did the antiserum precipitate galactosyltransferase in the absence of xylosyltransferase. It remains to be established whether the enzyme-enzyme interaction demonstrated here extends to other chondroitin sulfate glycosyltransferases and is specific enough to regulate the positioning of the enzymes within the membranes of the endoplasmic reticulum.

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