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AFFINITY CHROMATOGRAPHY OF GLYCOSYLTRANSFERASES

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SUMMARY

This review summarizes the use of biospecific chromatography techniques in the purification of mammalian glycosyltransferases. Ligands that are analogues of donor or acceptor substrates have been linked to cyanogen bromide-activated agarose for use as affinity adsorbents. Immobilized lectins have been employed to recognize the carbohydrate moieties of glycosyltransferase and remove them from complex mixtures. The application of these methods has permitted extensive purification of many membrane-bound glycosyltransferases, some to homogeneity.

INTRODUCTION

One of the earliest examples of biospecific adsorption that foreshadowed the development of affinity chromatography was the observation[1] that amylase specifically adsorbed to starch, its physiological substrate (for a historical review, see ref. 2). The development of affinity chromatography as a means for the purification of biological macromolecules was impeded for many years, however, by the lack of methods for synthesizing adsorbents that would specifically bind the desired macromolecule. The key to designing specific adsorbents was discovered only in 1967 when Porath and coworkers[3,4] reported that insoluble polysaccharides such as cellulose reacted with cyanogen bromide in alkali to give derivatives that covalently combined with primary and secondary amines. By this means a wide variety of ligands could be linked to cyanogen bromide-activated polysaccharides to give the insoluble, specific adsorbents needed for affinity chromatography of macromolecules, especially enzymes. In the years since its discovery, cyanogen bromide-activated agarose has been used to prepare hundreds of adsorbents, many of which have been useful for the purification of specific macromolecules, including some that could not be purified by other methods.

The purpose of this report is to review the application of affinity chromatography for the purification of glycosyltransferases, the group of enzymes that participate in the biosynthesis of oligosaccharides in glycoconjugates, each catalyzing a reaction of the following general form:

$$\text{Glycosylnucleotide} + \text{acceptor} \leftarrow \text{glycosyl–acceptor} + \text{nucleotide} \qquad (1)$$

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Glycogen synthase[5] was the only glycosyltransferase extensively purified prior to the development of affinity chromatography, but several kinds of specific adsorbents, most of which contain ligands structurally related to the acceptor or donor substrates for a glycosyltransferase (reaction 1) are now available, and have been used to purify many transferases, some in homogeneous form[6].

The properties of the glycosyltransferases reflect the difficulties to be encountered in their purification. Most are present in small amounts in tissues, bound to the membranes of the endoplasmic reticulum or the Golgi apparatus. Prior to purification they require solubilization, usually with aqueous solutions of a neutral detergent such as Triton X-100. Many require detergent solutions for expression of enzymic activity, and detergents must be present throughout their purification. After partial purification they may be easily inactivated by adsorption to surfaces, especially glass. Many transferases are structurally heterogeneous, since most are glycoproteins exhibiting microheterogeneity in their oligosaccharide groups. Moreover, they may also differ in size, possibly as the result of proteolysis during isolation, and active species with the same substrate specificities and turnover numbers but with different molecular weights may be encountered.

The foregoing properties of the glycosyltransferases preclude their purification by methods that separate molecules on the basis of size, shape, charge, or a combination of these properties, but affinity chromatography under conditions specially chosen to accomodate the unusual properties of a given transferase, can effect their purification. Large volumes of detergent-containing tissue extracts are often required to obtain a reasonable amount of enzyme at the first step in a purification. Enzyme can be concentrated from such extracts by adsorption to and elution from a biospecific matrix, often with an increase in stability. If the enzyme requires detergent for activity, the detergent may be present during any affinity chromatographic step. If, however, it is desirable to remove detergent, this is easily accomplished by elution of enzyme from an affinity adsorbent with detergent-free buffer. Those transferases susceptible to inactivation on dilution, or by adsorption to surfaces can be purified in plasticware or in siliconized glassware. Since only active enzyme species are adsorbed to a specific adsorbent, structural heterogeneity presents a minor problem during purification. The large number of glycosyltransferases that have been purified by biospecific chromatography (Table I), some to homogeneity, bears witness to the utility of the technique for the isolation of these enzymes.

It is a pleasure to dedicate this report to Professor Jerker Porath, whose contributions to affinity chromatography form the foundation on which the methods for purification of glycosyltransferases are based, and without which many recent advances in oligosaccharide structure, function and biosynthesis would have been impossible.

ACCEPTOR SUBSTRATE ANALOGUES AS BIOSPECIFIC LIGANDS

In principle, biospecific chromatography of glycosyltransferases using analogues of acceptor substrates might have very high specificity, since acceptors for these enzymes frequently have no net electrical charge that would contribute to nonspecific interactions. Experience has shown that the binding affinity of glycosyltransferases for acceptor analogues is often poor[6], nevertheless, several enzymes have been highly purified with the aid of such adsorbents.

TABLE I

GLYCOSYLTRANSFERASES PURIFIED BY BIOSPECIFIC CHROMATOGRAPHY

Enzyme	*Ligand*	*Reference*
Chondroitin sulfate β-xylosyltransferase	Chondroitin sulfate core protein	9, 10
β-Xyloside β1 → 4 galactosyltransferase	Xylosyl-core protein	11
	β-Xylosyltransferase	11
Collagen hydroxylysyl β-galactosyltransferase	Denatured collagen	13
	UDP–galacturonic acid	44
	Concanavalin A	44
Galactosylhydroxylysine α1 → 2 glucosyltransferase	Denatured collagen	13
	UDP–glucuronic acid	14
	Concanavalin A	15
Mannoside β1 → 2 N-acetylglucosaminyltransferase	Ovomucoid	18
	UDP–hexanolamine	19, 21
Dolichyl-pyrophosphoryl-oligosaccharide: protein oligosaccharyltransferase	α-Lactalbumin	27
A-blood group N-acetylgalactosaminyltransferase	Agarose	30, 31, 32
	UDP–hexanolamine	33
N-Acetylglucosamine β1 → 4 galactosyltransferase (lactose synthase A protein)	Aminohexyl-β-N-acetylglucosamine	34
	p-Aminophenyl-β-N-acetylglucosamine	35
	UDP–hexanolamine	34
	α-Lactalbumin	45
	Ovalbumin	25, 53
β-Galactoside α2 → 6 sialyltransferase	CDP–ethanolamine	38
	CDP–hexanolamine	38
β-Galactoside α2 → 3 sialyltransferase	CDP–hexanolamine	39
α-N-Acetylgalactosaminide α2 → 6 sialyltransferase	CDP–hexanolamine	40
Glucuronyltransferase	UDP–hexanolamine	42, 43
β-Galactoside α1 → 2 fucosyltransferase	GDP–hexanolamine	47
	Concanavalin A	49
	Wheatgerm agglutinin	50
N-Acetylglucosaminide α1 → 3 and α1 → 4 fucosyltransferase(s)	GDP–hexanolamine	48
Glycogen synthase	UDP–hexanolamine	34

Derivatives of chondroitin sulfate core-protein

The chondroitin sulfates consist of linear polysaccharides attached to some of the serine residues of a polypeptide backbone, or "core-protein", which constitutes up to 7–8% of the weight of the intact proteoglycan[7,8]. Chondroitin 4-sulfate contains six distinct glycosidic linkages: [GlcUAβ1 → 3GalNAc-4Sβ1 → 4]$_n$-GlcUAβ1 → 3Galβ1 → 3Galβ1 → 4xylβ1 → O-Ser. Chondroitin 6-sulfate differs only in the site of sulfation of the N-acetylgalactosamine residues.

The core-protein can be freed of carbohydrate by Smith degradation (periodate oxidation followed by reductive β-elimination), and then attached to cyanogen bromide-activated agarose. This matrix has been useful in the purification to homogeneity of the chondroitin sulfate core-protein β-xylosyltransferase from chick embryo cartilage[9] and from a rat chondrosarcoma[10]. In the presence of manganous ion, the enzyme adsorbs to core-protein–agarose, from which it can be eluted quantitatively by buffers containing the soluble Smith-degraded proteoglycan. However, Smith-degraded proteoglycan contaminates the purified enzyme, and non-specific

elution with sodium chloride was found to be adequate for purification of the transferase, although its yield was decreased.

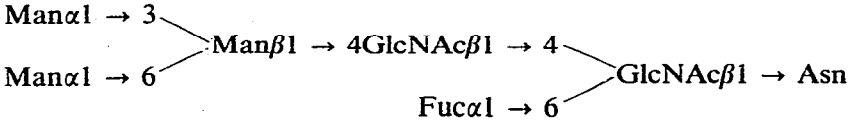
The xylosylated core-protein is an effective substrate and biospecific adsorbent for the galactosyltransferase that forms the Galβ1 → 4Xyl-O-Ser linkage region in chondroitin sulfate. Thus, β-xyloside β1 → 4 galactosyltransferase from embryonic chick cartilage has been partially purified by adsorption onto xylosylated core-protein–agarose, followed by elution with buffers containing either 0.2 *M* xylose, 10 mg/ml xylosylated core-protein, or 0.25 *M* KCl plus 1% Nonidet P-40 (ref. 11).

Derivatives of collagen

Mature collagen contains varying amounts of carbohydrate, predominantly in the structures Gluα1 → 2Galβ1 → O-Hyl and Galβ1 → O-Hyl (ref. 12). Denatured collagen peptides are acceptor substrates for both the collagen β1 → O-hydroxylysine galactosyltransferase and galactosylhydroxylysine α1 → 2 glucosyltransferase, and serve as specific adsorbents for these enzymes when coupled to agarose. Both enzymes were solubilized from whole chick embryos with Triton X-100 and concentrated by ammonium sulfate precipitation, then adsorbed to citrate-soluble rat skin collagen linked to agarose. Binding to collagen–agarose was enhanced by manganous ion. The enzymes were eluted together by buffers containing small collagenase-peptides prepared from rat skin or bovine Achilles tendon collagen. The glucosyltransferase and galactosyltransferase were resolved and separated from the eluting peptides by gel filtration chromatography on Sephadex G-150, giving an overall purification of 4600-fold for the glucosyltransferase, and 1000-fold for the galactosyltransferase[13]. The glucosyltransferase was subsequently purified 39,000-fold to homogeneity by a combination of this step with biospecific chromatography on a uridine 5′-diphosphate (UDP)–glucuronic acid–agarose matrix (see *Derivatives of uridine*)[14,15] and on concanavalin A-agarose (see the section Biospecific ligands that are not substrate analogues)[16,17].

Ovomucoid

Hen egg white ovomucoid contains a variety of branched oligosaccharide structures terminating in mannose residues, and serves as an acceptor substrate for N-acetylglucosaminyltransferase activities in extracts of hog tracheal mucosa. Glycosyltransferases have been identified in this tissue which apparently form the linkage, GlcNAcβ1 → 2Man, with either terminal mannose residue of the structure:



These activities require a divalent metal ion for catalysis, and have been purified 60,000-fold by a method which includes adsorption in the presence of 10 m*M* manganous ion onto ovomucoid–agarose, followed by elution with buffer containing EDTA[18]. This highly purified preparation retains the ability to form the GlcNAcβ1 → 2Man linkage with either mannose residue in the above structure, although the suggestion that a single enzyme might catalyze both reactions was not established.

Indeed, two different enzymes are likely to be present in this preparation since they have been resolved from rabbit and porcine liver[19,20] and bovine colostrum[21] by chromatography on UDP–hexanolamine–agarose (see *Derivatives of uridine*).

Ovalbumin

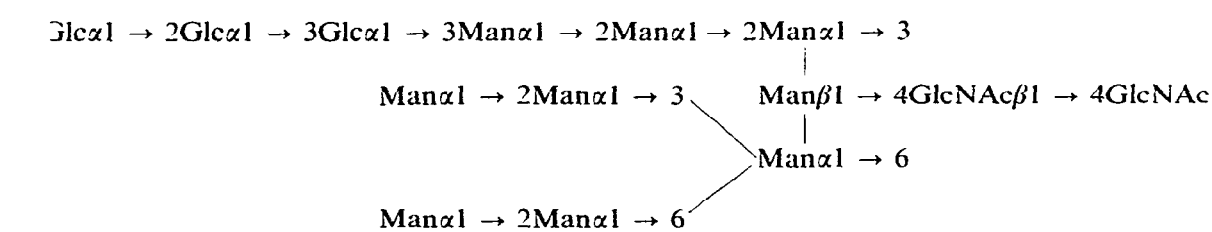
The polypeptide moiety of hen egg white ovalbumin contains a mixture of branched mannose-containing oligosaccharides, some of which terminate in N-acetylglucosamine[22], and ovalbumin serves as an acceptor for an N-acetylglucosamine $\beta 1 \rightarrow 4$ galactosyltransferase that is found in many mammalian tissues. In mammary gland and milk, this enzyme serves as the A-protein component of lactose synthase[23] while elsewhere it forms the Galβ1 → 4GlcNAc linkage of complex-type asparagine-linked glycoprotein oligosaccharides[24]. The galactosyltransferase of fetal calf serum has been purified by chromatography on ovalbumin-agarose, to which the enzyme was adsorbed in the presence of 10 m*M* manganous ion and 1 m*M* uridine 5′-monophosphate (UMP). Elution was achieved by omitting manganous ion and UMP from the column buffer, giving approximately 30-fold purification of the galactosyltransferase in 83% yield. Additional chromatography on UDP–hexanolamine–agarose (see *Derivatives of uridine*) and α-lactalbumin–agarose (see the section Biospecific ligands that are not substrate analogues) yielded homogeneous enzyme[25].

α-Lactalbumin

α-Lactalbumin is known to modify the acceptor substrate for the galactosyltransferase found in bovine mammary gland permitting the formation of lactose in milk[23]. However, fragments of denatured α-lactalbumin fortuitously serve as acceptor substrates for an oligosaccharyltransferase of hen oviduct that catalyzes reactions of the form:

$$\begin{aligned}&\text{Oligosaccharyl-pyrophosphoryl-dolichol} + \text{Asn}\rightarrow\text{X-Thr/Ser-Peptide} \rightarrow \\ &\text{Oligosaccharyl-Asn-X-Thr/Ser-(peptide)} + \text{pyrophosphoryl-dolichol} \qquad (2)\end{aligned}$$

The oligosaccharide portion of one such donor substrate has been determined to be:



This reaction is central to the construction of most, if not all, of the asparagine-linked oligosaccharides found in mammalian glycoproteins, as reviewed by Struck and Lennarz[26]. The hen oviduct enzyme has been solubilized from microsomes with Nonidet P-40 and adsorbed to α-lactalbumin-agarose in the presence of manganous ion. The enzyme has an absolute requirement for divalent metal ion, and is eluted with 2000-fold purification by removing manganous ion from the column buffer[27].

Agarose beads

Agarose is a linear polymer of the disaccharide unit, D-galactopyranose ($\beta 1 \rightarrow 4$)3 $\rightarrow$ 6-anhydro-L-galactopyranose($\alpha 1 \rightarrow 3$)[28,29]. Thus, commercially available agarose gel beads clearly have potential as biospecific matrices for the adsorption of enzymes that utilize substrates containing β-D-galactopyranose residues. In fact, the A-blood group specific N-acetylgalactosylaminyltransferase of human serum which catalyzes reaction 3 will adsorb

$$\text{UDP-GalNAc} + \text{Fuc}\alpha 1 \rightarrow 2\text{Gal}\beta 1 \rightarrow \text{R} \rightarrow \text{Fuc}\alpha 1 \rightarrow \begin{matrix} 2 \\ 3 \end{matrix}\!\!\diagdown\!\!\diagup \text{Gal}\beta 1 \rightarrow \text{R} + \text{UDP} \quad (3)$$
$$\text{GalNAc}\alpha 1 \rightarrow 3$$

to 4% agarose gel beads (Sepharose 4B), from which it can be eluted with 1000-fold purification by UDP[30]. The human milk enzyme has been similarly purified[31]. In a refinement of this method, the human serum N-acetylgalactosaminyltransferase was purified over 135,000-fold to apparent homogeneity, achieving a 77,000-fold purification in the biospecific chromatography step alone[32]. Interestingly, the similar enzyme from porcine submaxillary gland does not adsorb well to agarose, although it too, has been purified to homogeneity on a different adsorbent, UDP–hexanolamine–agarose[33] (see *Derivatives of uridine*).

N-Acetylglucosamine

β-N-Acetylglucosamine containing an aminohexyl arm attached to the anomeric carbon was synthesized by condensation of N-trifluoroacetyl-6-amino-1-hexanol with 2,3,6-tri-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl chloride in dimethylformamide, in the presence of mercuric chloride. The product was a mixture of α- and β-anomers which were separated by fractional crystallization in ethyl acetate–hexane, and the desired β-anomer was deacetylated with barium methylate in methanol[34]. The final product was linked to cyanogen bromide-activated agarose, and the galactosyltransferase of bovine milk was found to utilize this matrix as an acceptor substrate. Binding of the enzyme to N-acetylglucosamine–agarose was enhanced by manganous ion, UDP or UMP, but decreased by EDTA, borate, N-acetylglucosamine, or urea. An effective method for biospecific chromatography was devised employing adsorption to N-acetylglucosamine–agarose in the presence of 25 m*M* manganous ion and 0.5 m*M* UMP, followed by elution with buffer containing 25 m*M* EDTA and 5 m*M* N-acetylglucosamine. In combination with chromatography on UDP–hexanolamine–agarose (see *Derivatives of uridine*), essentially homogeneous galactosyltransferase was obtained[34].

The galactosyltransferase from hog lymph nodes was found to adsorb to aminophenyl-N-acetylglucosamine covalently attached to agarose[35]. As for the bovine milk enzyme, adsorption was performed in the presence of manganous ion and UMP. Elution with 50% yield and 630-fold purification in the step was achieved with buffer containing EDTA.

DONOR SUBSTRATE ANALOGUES AS BIOSPECIFIC LIGANDS

Except for certain enzymes using dolichol–phosphosugars, the glycosyltransferases utilize nucleotide–sugars as donor substrates. In general, the sugar moiety alone

has not proved to be a useful biospecific ligand, correlating with the failure of most free saccharides to inhibit glycosyltransferase reactions[6]. Nucleosides also are not effective inhibitors of most glycosyltransferase reactions, but nucleotides having one or more phosphates at the 5′-position frequently are potent inhibitors, with K_I (inhibitory constant) values often much lower than the K_m (michaelis constant) or K_D (dissociation constant) for the corresponding nucleotide–sugar substrate. Thus, efforts to construct chromatographic matrices using analogues of the donor substrates have concentrated on the nucleotide moiety.

As reviewed recently[36], the methods that have been developed for coupling nucleotides to chromatography supports can be divided into three classes depending upon whether the covalent linkage to the spacer arm is via (1) the base, (2) the ribose ring, or (3) the phosphoester moiety. To date, only ligands of the latter class have been employed in the purification of glycosyltransferases. In addition to the synthetic methods for producing such compounds, the preparation of ligands containing uridine–5′-phosphate or pyrophosphate linked to a diaminohexyl spacer arm through a phosphoamide bond has been reported[37]. The following section will discuss the use of specific nucleotide derivatives in the purification of glycosyltransferases.

Derivatives of cytidine

Derivatives of cytidine–5′-diphosphate (CDP) containing an alkyl spacer linked to the β-phosphate can be conveniently synthesized by the reaction of an aminoalkylphosphoryl-imidazolide with cytidine–5′-monophosphate (CMP) to form the corresponding phosphodiester. Thus, CDP–hexanolamine is formed by reaction of N-trifluoroacetyl-O-phosphoryl-6-amino-1-hexanol imidazolide with CMP, followed by hydrolysis of the trifluoroacetyl group which serves to block the amino-group during the synthesis[38] (Fig. 1). An analogue containing a 2-carbon arm, CDP–ethanolamine, has been prepared similarly, and is also commercially available (Sigma, St. Louis, MO, U.S.A.). Linked to cyanogen bromide-activated agarose, these ligands have been used in the purification of three sialyltransferases: a β-galactoside α2 → 6 sialyltransferase from bovine colostrum[38], and both a β-galactoside α2 → 3 sialyltransferase and an α-N-acetylgalactosaminide α2 → 6 sialyltransferase from porcine submaxillary gland[39,40].

The colostrum β-galactoside α2 → 6 sialyltransferase has been purified upon either CDP–ethanolamine–agarose or CDP–hexanolamine–agarose, utilizing non-specific elution with sodium chloride and specific elution with CDP in separate steps[38]. The reported method completely resolves the sialyltransferase from the galactosyltransferase component of lactose synthase that is also present in colostrum, despite the minimal structural differences between cytidine, which has an amino group at the 4-position, and uridine, which has a keto group at the same location. As indicated below, the galactosyltransferase can be similarly resolved and purified to homogeneity on a UDP–hexanolamine–agarose adsorbent[34] (see *Derivatives of uridine*).

The porcine submaxillary gland β-galactoside α2 → 3 sialyltransferase and the α-N-acetylgalactosaminide α2 → 6 sialyltransferase were solubilized from tissue membranes with Triton X-100 and adsorbed to CDP–hexanolamine–agarose. The two sialyltransferases were partially resolved by sodium chloride gradient elution, and the β-galactoside α2 → 3 sialyltransferase was further purified by cytidine 5′-

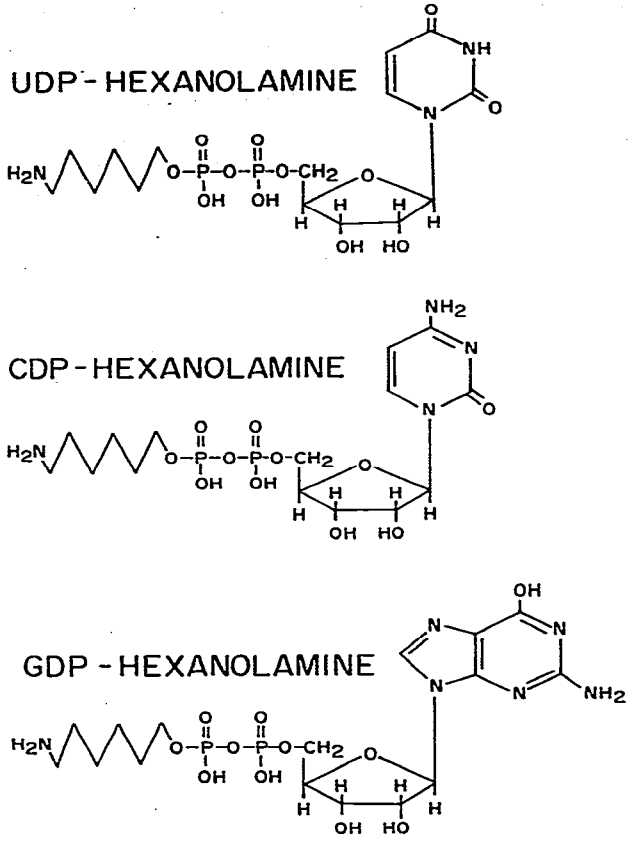


Fig. 1. Structures of the nucleoside–diphospho–hexanolamine ligands.

triphosphate (CTP) gradient elution from CDP–hexanolamine–agarose. Two forms of the enzyme having identical enzymic properties were resolved by gel filtration chromatography. One form has a hydrophobic site and could not be eluted from CDP–hexanolamine–agarose in the absence of detergent, suggesting that hydrophobic interactions with the chromatographic matrix (probably the hexyl spacer arm) must be minimized before effective biospecific elution can occur. Ionic forces are important to the specific interaction of both forms of the enzyme with the negatively charged CDP-ligand, but also contribute to the non-specific binding of contaminating proteins. To some extent, there is non-specific ionic interaction of the sialyltransferase with CDP–hexanolamine–agarose as well, and these forces must be neutralized to permit specific elution of the sialyltransferase with cytidine nucleotides. CTP is an extremely potent inhibitor of the sialyltransferase reaction with a K_I of 0.22 μM, but in buffers of low ionic strength, 0.3 mM CTP eluted the sialyltransferase in low yield and with little purification, as shown in Table II. The remaining adsorbed sialyltransferase could be recovered by elution with 1.0 M sodium chloride. However, development of the column with buffer containing a moderate concentration of sodium chloride (150 mM) did not elute the sialyltransferase, but removed some contaminating proteins. Subsequently, elution with 0.3 mM CTP together with 100

m*M* sodium chloride gave a very high purification of the enzyme in good yield. Thus, the failure of CTP alone to specifically elute the enzyme from a matrix containing an electrically charged, hydrophobic ligand did not indicate a lack of biospecific adsorption, but reflected associated non-specific interactions that masked the desired specific effect. By proper choice of elution conditions, the specific interaction was allowed to predominate, permitting effective biospecific chromatography[39].

TABLE II

ELUTION OF β-GALACTOSIDE α2 → 3 SIALYLTRANSFERASE FROM CDP–HEXANOLAMINE–AGAROSE WITH SELECTED BUFFERS

Partially purified sialyltransferase was adsorbed to a column of CDP–hexanolamine–agarose in 10 m*M* sodium cacodylate, pH 6.5, 1% (w/v) Triton X-100, and eluted with the same buffer containing the constituents indicated above. Data of ref. 56.

Eluent	*Percent eluted*	*Step purification*
0.3 m*M* CTP	20	7
150 m*M* NaCl	<5	≪1
100 m*M* NaCl + 0.3 m*M* CTP	63	93

The α-N-acetylgalactosaminide α2 → 6 sialyltransferase was also further purified by CTP gradient elution from CDP–hexanolamine–agarose. In contrast to the β-galactoside α2 → 3 sialyltransferase, it did not require Triton X-100 for efficient chromatography[40]. Interestingly, unlike the colostrum β-galactoside α2 → 6 sialyltransferase discussed above, neither submaxillary sialyltransferase would adsorb to CDP–ethanolamine–agarose[41].

Derivatives of uridine

Two classes of uridine derivatives have been employed in the purification of glycosyltransferases that utilize UDP–glycose donor substrates. Barker *et al.*[34] described the synthesis of UDP–hexanolamine [P′-(6-amino-1-hexyl)-P^2-(5′-uridine)-pyrophosphate] (Fig. 1) and utilized this ligand, attached to cyanogen bromide activated-agarose, for the purification of the bovine milk N-acetylglucosaminide β1 → 4 galactosyltransferase and the rabbit muscle glycogen synthetase. This same adsorbent has subsequently been used in the purification of a porcine submaxillary (fucosyl α1 → 2) galactoside α1 → 3 N-acetylgalactosaminyltransferase[33], a rat liver glucuronyltransferase[42,43], and a bovine colostrum[21], rabbit liver[19] and porcine liver[20] α1 → 3 mannoside β1 → 2 N-acetylglucosaminyltransferase. Derivatives of UDP–glucose and UDP–galactose prepared by carbodiimide coupling of UDP–glucuronic acid or UDP–galacturonic acid to aminohexyl agarose were used in the purification of the collagen glucosyl[14] and galactosyltransferases[44].

The N-acetylglucosaminide β1 → 4 galactosyltransferase from bovine milk was purified over 160-fold by a single chromatography on UDP–hexanolamine–agarose[34]. Manganous ion, a necessary cofactor for the enzyme, was required for effective adsorption of the transferase. Activity could be eluted by several means, but maximal yield and purification were obtained by elution with buffer containing EDTA. The eluted transferase was then purified further to homogeneity by affinity chromatography on α-lactalbumin agarose (see the section Biospecific ligands that

are not substrate analogues). Three molecular weight forms of the purified enzyme were observed on sodium dodecyl sulfate (SDS)–polyacrylamide gels. In contrast, only one major species of the enzyme was observed for the transferase purified by conventional techniques[45,46]. Similar size heterogeneity has been reported for several other glycosyltransferases purified by affinity chromatography[6] and probably reflects the ability of the adsorbents to separate enzymes solely on the basis of biological activity rather than on the basis of physical parameters such as size, shape, charge, solubility, etc.

The general utility of the UDP–hexanolamine adsorbent for purifying enzymes that use UDP–glycose donor substrates was tested using rabbit muscle glycogen synthetase[34]. A partially purified enzyme preparation was tightly adsorbed to the column but was not eluted with UDP. However, the transferase was eluted when glycogen was included in the wash buffer. The kinetic basis for the elution by the acceptor substrate was not investigated.

The porcine submaxillary gland (fucosyl $\alpha 1 \rightarrow 2$)galactoside $\alpha 1 \rightarrow 3$ N-acetylgalactosaminyltransferase was solubilized with Triton X-100 and purified by affinity chromatography on UDP–hexanolamine–agarose[33]. A micro batch desorption procedure was described for determining optimal conditions for affinity chromatography. Thus, rational conditions for such factors as pH, ionic strength, metal cofactor concentration and nucleotide inhibitor concentration could be predicted so as to minimize non-specific adsorption to the column and to maximize yield and purification of the transferase. The purification procedure reported included both non-specific elution of the transferase with 2 *M* NaCl and specific elution with UMP. Manganous ion was required for binding to the adsorbent, and Triton was required for effective elution.

A rat liver glucuronyltransferase involved in drug detoxification and steroid conjugation was purified to homogeneity on UDP–hexanolamine–agarose[42]. A partially purified enzyme preparation was adsorbed to the column and the activity was eluted with buffer containing UDP–glucuronic acid. A similar purification of glucurosyltransferase from rat liver after induction of the enzyme with phenobarbital has been reported[43]. A single step purification of over 40-fold was achieved.

The $\alpha 1 \rightarrow 3$ mannoside $\beta 1 \rightarrow 2$ N-acetylglucosaminyltransferase from bovine colostrum[21] and rabbit liver[19] were extensively purified on the UDP–hexanolamine–agarose adsorbent. After a preliminary fractionation on CM-Sephadex, the colostrum enzyme was purified another 65-fold by adsorption to the affinity resin and elution with UDP–GlcNAc. The rabbit liver enzyme, obtained by Triton X-100 extraction of an acetone powder, was purified to near homogeneity by chromatography on UDP–hexanolamine–agarose, utilizing non-specific elution with sodium chloride and specific elution with a UDP gradient. The transferase was separated from the $\alpha 1 \rightarrow 6$ mannoside $\beta 1 \rightarrow 2$ N-acetylglucosaminyltransferase which did not bind to the affinity adsorbent. A similar purification has been reported for the porcine liver transferase[20].

The collagen β-galactoside $\alpha 1 \rightarrow 2$ glucosyltransferase from chick embryos was purified on a column of UDP–glucuronic acid coupled to aminohexyl–agarose via the carboxyl group[14]. A single step purification of 1200-fold was achieved by eluting the enzyme from the adsorbent with acceptor substrates[9], dialyzable peptides prepared by collagenase digestion of collagen. Manganous ion was required for binding to the

column. Elution could also be achieved using EDTA or UDP–glucose but the yield and purification obtained were not as high. Chromatography on the UDP–glucuronic acid adsorbent was combined with affinity chromatography on collagen–agarose (see *Derivatives of collagen*) and concanavalin A-agarose (see the section Biospecific ligands that are not substrate analogues) to give a homogeneous enzyme preparation nearly 40,000-fold purified[17]. In this procedure, non-specific elution using sodium chloride was used in place of specific elution with collagen peptides.

Interestingly, the collagen hydroxylysyl β-galactosyltransferase from chick embryo did not bind to the UDP–glucuronic acid–agarose adsorbent[14]. Kinetic analysis revealed that the K_m values for UDP–glucose and UDP–galactose for the respective glycosyltransferases were similar. However, the K_I for UDP–glucuronic acid in the galactosyltransferase reaction is 3 to 4 times higher than the K_I for UDP[44]. Thus, while both enzymes might be expected to bind to an adsorbent containing only the nucleotide moiety, specificity is apparently conferred by including the sugar moiety. As expected, the collagen galactosyltransferase was bound weakly to a UDP–galacturonic acid adsorbent[44], but this step was not useful in the purification.

Derivatives of guanosine

A modification of the procedure described by Barker *et al.*[34] for synthesis of UDP–hexanolamine was employed for the synthesis of the corresponding guanosine 5′-diphosphate (GDP) derivative [P′-(6′amino-1-hexyl)-P²-(5′-guanosine)-pyrophosphate] (Fig. 1)[47]. The affinity adsorbent prepared by coupling this ligand to cyanogen bromide-activated agarose has been used in the purification of a porcine submaxillary β-galactoside α1 → 2 fucosyltransferase[47] and the copurification of N-acetylglucosamide α1 → 3 and α1 → 4 fucosyltransferase activities from human milk[48].

The H blood group β-galactoside α1 → 2 fucosyltransferase from porcine submaxillary glands was solubilized with Triton X-100 and purified to homogeneity utilizing affinity chromatography on the GDP–hexanolamine–agarose adsorbent combined with gel filtration and ion-exchange chromatography[47]. An improved procedure in which the latter steps were replaced by affinity chromatography on concanavalin A-agarose has also been reported[49] (see the section Biospecific ligands that are not substrate analogues). Both procedures utilized non-specific elution from the GDP–hexanolamine–agarose adsorbent with sodium chloride in an early step and specific elution with guanosine 5′-monophosphate (GMP) in a later step. Concentration of the transferase on a small GDP–hexanolamine–agarose column was used as the final step in the purification. Such a procedure is often useful in obtaining enzyme of high specific activity, particularly when using purification procedures based on physical properties such as gel filtration or ion exchange, since these methods do not discriminate between active and inactive enzyme.

The fucosyltransferase is activated approximately 10-fold by manganous ion, but inclusion of manganese chloride in the column buffers resulted in decreased affinity for the adsorbent and a poorer purification[50]. This is consistent with kinetic analysis of the enzyme which showed that saturating concentrations of manganous ion caused a 4-fold increase in the K_m for GDP-fucose[51].

Two fucosyltransferase activities in human milk, the Lewis blood group N-acetylglucosaminide α1 → 4 fucosyltransferase and the N-acetylglucosaminide α1 →

3 fucosyltransferase, were copurified over 500,000-fold utilizing affinity chromatography on GDP–hexanolamine–agarose[48]. Effective elution of the enzyme could only be achieved by including both sodium chloride (0.8 *M*) and GMP (5 m*M*) in the elution buffer. The concentration of the affinity ligand on the adsorbent was critical for the purification. At concentrations greater than 4 μmol per ml of agarose, high concentrations of sodium chloride (≈2 *M*) that caused inactivation of the enzyme were required for effective elution, but at concentrations less than 1 μmol per ml of agarose the enzyme was not adsorbed efficiently.

BIOSPECIFIC LIGANDS THAT ARE NOT SUBSTRATE ANALOGUES

Biospecific interactions with substances other than acceptor and donor substrate analogues have been employed in the purification of several glycosyltransferases. Lectin affinity chromatography has been particularly useful with these enzymes. In view of their localization within the endoplasmic reticulum and Golgi apparatus[24], it is not surprising that most, if not all, of the glycosyltransferases are glycoproteins[6]. Thus, it seems likely that affinity chromatography on immobilized lectins may be of general utility in the purification of these enzymes.

Purification procedures based on adsorption to immobilized concanavalin A have been reported for the collagen galactosyl[44] and glucosyltransferases[17] from chick embryos and for the β-galactoside α1 → 2 fucosyltransferase from porcine submaxillary gland[49]. The latter enzyme can also be purified on a wheat germ agglutinin adsorbent[50]. Strategies for specific adsorption and elution on these columns are determined by the biological properties of the immobilized lectin rather than the enzymatic properties of the transferase. The use of immobilized lectins in affinity chromatography has been reviewed[52].

The N-acetylglucosaminide β1 → 4 galactosyltransferase of lactose synthase was purified on an adsorbent containing the specifier protein of the lactose synthase complex, α-lactalbumin, attached to agarose[45,53]. The enzyme was not adsorbed unless an acceptor substrate, glucose or N-acetylglucosamine, was included in the buffer. Elution was achieved using buffer without the acceptor substrate. This behavior is in accord with the kinetic properties of the enzyme which indicate a 350-fold increase in the affinity of α-lactalbumin for the transferase in the presence of N-acetylglucosamine[54]. While α-lactalbumin is found only in the mammary gland, the β1 → 4 galactosyltransferase from a variety of nonmammary tissues can also be purified on the α-lactalbumin adsorbent[6].

Schwartz *et al.*[55] reported a specific complex formation between the chondroitin sulfate core protein β-xylosyltransferase and the β-xyloside β1 → 4 galactosyltransferase which catalyzes the subsequent step in the synthesis of the core region of these polysaccharides. A unique affinity purification of the galactosyltransferase on the immobilized xylosyltransferase was reported which exploited this interaction[11]. The partially purified galactosyltransferase was adsorbed to the column from dilute detergent solution and was eluted by increasing the potassium chloride and detergent concentration in the buffer. A purification of approximately 25-fold was obtained on the column.

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