Further Characterization of Collagen Galactosyltransferase from Chick Embryos

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(Received 23 June 1977)

Optimum extraction of collagen galactosyltransferase activity from chick embryos required relatively high concentrations of detergent and salt. The activity was inhibited by concanavalin A, and the enzyme had a high affinity for columns of this lectin coupled to agarose; these results suggest the presence of carbohydrate units in the enzyme molecule. Collagen galactosyltransferase was highly labile, and only 1% of the originally bound enzyme activity could be eluted from the concanavalin A-agarose column with a buffer containing methyl glucoside and ethylene glycol. The purification of the activity over the original supernatant of chick embryo homogenate was 250–300-fold, with the optimum reaction conditions for the purified transferase differing somewhat from those for crude enzyme preparations. The reaction was inhibited by glucose-free basement-membrane collagen, UDP and galactosylhydroxylysine, and also by Co^{2+} and a number of compounds resembling UDP-galactose. Hydroxylysine was also a weak inhibitor. Immobilized hydroxylysine and UDP-glucuronic acid did not bind the collagen galactosyltransferase, but the enzyme was retarded in a column of UDP-galacturonic acid linked to agarose.

Collagen galactosyltransferase catalyses the formation of galactosylhydroxylsyl residues in collagen by transferring the monosaccharide moiety from UDP-galactose to hydroxylsyl residues present in the collagen polypeptide chain (Spiro & Spiro, 1971; for reviews on collagen biosynthesis see Bornstein, 1974; Kivirikko & Risteli, 1976; Prockop *et al.*, 1976). There are only a few reports on the purification of this enzyme, and its lability has been clearly demonstrated (Risteli *et al.*, 1976*a,b*). Collagen galactosyltransferase was prepared initially at a relatively low degree of purity from guinea-pig skin (Bosmann & Eylar, 1968), rat kidney cortex (Spiro & Spiro, 1971) and chick-embryo cartilage (Myllylä *et al.*, 1975), and some of its properties were examined in a preparation from chick embryos purified about 100-fold (Risteli *et al.*, 1976*a*). An affinity-column procedure on collagen linked to agarose was introduced, which gave 1000-fold purification of collagen galactosyltransferase (Risteli *et al.*, 1976*b*).

In the present study the chick collagen galactosyltransferase and the reaction catalysed by this enzyme are examined in more detail and some new affinity-chromatography approaches for the purification of this enzyme are suggested.

Experimental

Materials

Crude $(\text{NH}_4)_2\text{SO}_4$ -precipitated enzyme (0–55%

Abbreviation used: Mops, 4-morpholinepropane-sulphonic acid.

satn.) was prepared from 14- or 15-day-old embryos of white Leghorn chickens (Siipikarjanhoitajien liitto r.y., Hämeenlinna, Finland) as described by Risteli *et al.* (1976*a*).

Calf-skin gelatin substrate was prepared as reported by Myllylä *et al.* (1975). Glomerular basement membrane was prepared from human kidney (Kefalides, 1971, 1974) and the glucose removed by mild acid hydrolysis (0.2M-HCl at 110°C for 3h) (Askenasi, 1973). Glucosylgalactosylhydroxylysine was prepared and purified from sponge collagen (Katzman *et al.*, 1972; Anttinen & Kivirikko, 1976), and then converted into galactosylhydroxylysine by hydrolysis with 0.05M- H_2SO_4 at 100°C for 24h (Spiro & Fukushi, 1969; Anttinen & Kivirikko, 1976). The galactosylhydroxylysine was further purified by gel filtration on Sephadex G-15, and the final preparation was over 95% pure when examined with an amino acid analyser (Anttinen & Kivirikko, 1976).

UDP-D- $[^{14}\text{C}]$ galactose (274 Ci/mol) was purchased from New England Nuclear Corp. (Boston, MA, U.S.A.), and non-radioactive UDP-galactose from Sigma Chemical Co. (Kingston-upon-Thames, Surrey KT2 7BH, U.K.). The radioactive glycoside was diluted with the non-labelled compound to a final specific radioactivity of 10 Ci/mol. All the other nucleotides and nucleotide sugars were purchased from Sigma. The MnCl_2 , a Merck analytical reagent, was further purified with dithizone (Morrison & Uhr, 1966) for use in kinetic experiments. Sepharose 4B, aminohexyl-agarose (AH-Sepharose 4B) and Concanavalin A-Sepharose 4B were purchased from Pharmacia Fine Chemicals AB (Uppsala, Sweden).

Citrate-soluble rat skin collagen (Gallop & Seifter, 1963) was coupled to Sepharose 4B by the CNBr-activation method (Cuatrecasas & Anfinsen, 1971), as described in detail by Risteli *et al.* (1976b). This material will be termed collagen-agarose. UDP-galacturonic acid, UDP-glucuronic acid and hydroxylysine were coupled from their carboxyl groups to free amino groups of AH-Sepharose 4B by using the carbodi-imide method (Cuatrecasas & Anfinsen, 1971), as described by Anttinen & Kivirikko (1976). About 300mg of hydroxylysine was used per 10ml of packed gel.

Assays

Collagen galactosyltransferase activity was assayed as described previously (Risteli *et al.*, 1976a; Risteli, 1977; slightly modified from Myllylä *et al.*, 1975). To make sure that Mn^{2+} was not bound by the buffer, 50mM-Mops buffer was used instead of 50mM-Tris buffer in experiments in which the effects of reactant concentrations were being tested.

Units of collagen galactosyltransferase activity were as previously defined (Risteli *et al.*, 1976a).

The protein concentration was measured by peptide absorbance at 225nm by using bovine serum albumin as a standard, which gave an absorption coefficient of $A_{225}^{1mg/ml} = 7.40$ with a 1cm light-path.

Chromatography

All chromatography was performed at +4°C. The columns were equilibrated and washed with a buffer containing 0.2M-NaCl, 1% (v/v) glycerol, 0.5mM-dithiothreitol, 0.01M- $MnCl_2$ and 0.05M-Tris/HCl buffer adjusted to pH7.4 at +4°C. Before the chromatography the $(NH_4)_2SO_4$ -precipitated enzymes, stored at -20°C, were thawed and centrifuged for 30min at 15000g.

Results

Extraction of collagen galactosyltransferase from chick embryos

Treatment of chick embryo homogenate with 0.1% Triton X-100 is known to double the measurable collagen galactosyltransferase activity in the homogenate and also in the 15000g and 150000g supernatants (Risteli *et al.*, 1976a). By increasing the concentration of the detergent (either Triton X-100 or Nonidet P-40) a further increase of about 50% could be achieved in the soluble enzyme activity (Table 1). A higher salt concentration also increased the solubilization, but no additional effect was found when the salt concentration was increased in the presence of the higher detergent concentration. Slight increases were also noted with 20mM-dithio-

Table 1. *Extraction of collagen galactosyltransferase from chick embryos*

Chick embryos (15 days old) were homogenized in a cold solution containing 0.2M-NaCl, 50 μ M-dithiothreitol and 50mM-Tris/HCl, pH adjusted to 7.4 at +4°C (1 ml of solution/g of embryo). The homogenate was divided into eight equal portions, and each portion received an addition of 0.5ml of an appropriate stock solution per ml of the sample to give the final concentrations shown below. The samples were incubated at +4°C for 1h with shaking, then centrifuged at 15000g for 30min. The enzyme activity was assayed in the 15000g supernatants and the results are expressed as d.p.m. of galactosylhydroxylysine synthesized in a sample corresponding to 10mg wet wt. of embryo under standard assay conditions. In addition to the components shown, each solution contained 50 μ M-dithiothreitol and 50mM-Tris/HCl buffer, pH adjusted to 7.4 at +4°C. The values are the means of duplicate samples in one experiment, and similar results were also obtained in an additional experiment.

Solution	Galactosyltransferase activity	
	(d.p.m.)	(%)
0.1% Triton X-100/0.2M-NaCl	1990	100
0.5% Triton X-100/0.2M-NaCl	3110	156
0.1% Triton X-100/0.5M-NaCl	2520	127
0.5% Triton X-100/0.5M-NaCl	2670	134
0.5% Nonidet P-40/0.2M-NaCl	3100	156
0.5% Nonidet P-40/0.5M-NaCl	3010	151
0.1% Triton X-100/0.2M-NaCl/ 20mM-dithiothreitol	2900	146
0.1% Triton X-100/0.2M-NaCl/ 2M-urea*	2470	124

* This sample was dialysed before the assay against the same solution not containing urea.

threitol instead of 50 μ M and also with 2M-urea in the extraction mixture. Most enzyme preparations were carried out with 0.1% detergent in the extraction medium, since preparations extracted with higher detergent concentrations showed a tendency to aggregate during chromatography, resulting in substantially lower flow rates.

Stability of collagen galactosyltransferase

Collagen galactosyltransferase loses its activity rapidly when stored in the form of impure or partially purified preparations at +4°C (Table 2). In experiments in which urea, galactose or the detergent Triton X-100 or high concentrations of glycerol or ethylene glycol were added to the enzyme, none of these compounds clearly protected it against inactivation. In the frozen state the activity could usually be preserved for several weeks, but on a couple of occasions considerable losses were also noted during

Table 2. Effect of storage at +4°C on collagen galactosyltransferase activity

Crude chick-embryo (NH₄)₂SO₄-precipitated enzyme was thawed (day 0) and then kept at +4°C at a protein concentration of 10mg/ml. The buffer contained 0.2M-NaCl, 0.05M-Tris/HCl, pH adjusted to 7.4 at +4°C, 0.5mM-dithiothreitol, 1% glycerol and 0.01 M-MnCl₂.

	Enzyme activity/mg of protein	
	(d.p.m.)	(%)
Expt. 1		
Day 0	3330	100
Day 7, kept at -20°C	3020	90
Day 1	1770	53
Day 3	1060	32
Day 7	470	14
Expt. 2		
Day 0	4920	100
Day 5	1470	30
Expt. 3		
Day 0	5080	100
Day 6	750	15

storage at -20°C. In contrast with previous data (Spiro & Spiro, 1971), addition of 0.25M- or 0.1M-galactose or 25% (v/v) glycerol did not improve the stability of the enzyme in the frozen state.

Collagen galactosyltransferase purified 250–300-fold by affinity chromatography on collagen-agarose or concanavalin A-agarose (see below) and containing 50% (v/v) ethylene glycol was kept for several weeks at -20°C without any loss of activity.

The behaviour of collagen galactosyltransferase in chromatographical procedures is highly variable (see Risteli *et al.*, 1976b), and conclusions about the affinity of this enzyme for any given material can be drawn only after a series of experiments with several batches of the enzyme.

Evidence for carbohydrate residues in collagen galactosyltransferase

On incubating the enzyme with different concentrations of concanavalin A up to 600 µg/ml, inhibition of the enzyme activity occurred (Fig. 1), the magnitude of which was dependent on the lectin concentration, the maximum inhibition observed being about 45%. This inhibition could be prevented by 0.3M-methyl α-D-glucopyranoside (Fig. 1), and in part by several other sugars known to bind to concanavalin A, whereas galactose, a monosaccharide not recognized by the lectin, had no effect on the inhibition (Table 3). None of these sugars alone had any effect on the galactosyltransferase activity.

Fig. 1. Inhibition of collagen galactosyltransferase activity by concanavalin A

Collagen galactosyltransferase activity was measured in 10 µl of chick-embryo (NH₄)₂SO₄-precipitated enzyme (protein concentration 20mg/ml) in the absence (○) or presence (●) of 0.3M-methyl α-D-glucopyranoside.

In subsequent experiments the binding of collagen galactosyltransferase to concanavalin A-agarose was tested. Crude chick-embryo (NH₄)₂SO₄-precipitated enzyme was passed through a column (bed vol. 5ml) of this affinity material, in a protein concentration of 10mg/ml. Essentially all the protein, but only about 5% of the enzyme activity, was recovered in the column effluent of the first 150ml (Fig. 2, ○). In a similar experiment carried out simultaneously with the same batches of both enzyme and affinity-chromatography material but with the addition of 0.5M-methyl α-D-glucopyranoside to the enzyme preparation, about 80% of the enzyme activity passed through the column unretarded (Fig. 2, ●). These results suggest that collagen galactosyltransferase binds to concanavalin A through carbohydrate groups present in the enzyme.

Partial purification of collagen galactosyltransferase

Collagen galactosyltransferase could not be eluted from the concanavalin A-agarose column by 0.5M-methyl α-D-glucopyranoside in the equilibration buffer, addition of 50% ethylene glycol to the elution solution being required. The specific activity of collagen galactosyltransferase purified with the concanavalin A-agarose column was 250–300 times greater than in the initial 15000g supernatant of chick-embryo homogenate, the purification obtained in the affinity chromatography being 100–160-fold. The recovery was only about 1% of the enzyme activity originally bound.

Ethylene glycol (50%) also eluted collagen galactosyltransferase from the collagen-agarose column (Risteli *et al.*, 1976b), but the recovery was lower than when the elution was carried out with small

Table 3. *Effect of various sugars on the inhibition of the collagen galactosyltransferase reaction by concanavalin A*
The reaction was carried out as described in the Experimental section, with the additions indicated. The concentration of concanavalin A in the reaction mixture was 400 $\mu\text{g/ml}$. The enzyme used was $(\text{NH}_4)_2\text{SO}_4$ -precipitated crude chick-embryo enzyme.

Addition (M)		[^{14}C]Galactosylhydroxylysine formed (d.p.m.)		Inhibition with concanavalin A (%)
		-Concanavalin A	+Concanavalin A	
None		946	520	45
Galactose	0.1	938	497	47
	0.2	1181	646	45
Fructose	0.1	1054	819	22
	0.2	998	839	16
Mannose	0.1	1132	877	23
	0.2	1093	962	12
Glucose	0.1	980	633	35
	0.2	1091	774	29
Methyl α -D-glucopyranoside	0.1	1140	959	16
	0.2	1121	906	19
Methyl α -D-mannopyranoside	0.1	1201	892	26
	0.2	1035	933	10

Fig. 2. *Affinity chromatography of collagen galactosyltransferase on a column of concanavalin A-agarose*
Crude chick-embryo $(\text{NH}_4)_2\text{SO}_4$ -precipitated enzyme was passed through a 5-ml affinity column in a protein concentration of 10mg/ml at a flow rate of 10ml/h. The specific activity of the enzyme in the column effluent is expressed as percentage of that in the original $(\text{NH}_4)_2\text{SO}_4$ -precipitated enzyme. The experiment was carried out without (○) and with (●) the addition of 0.5M-methyl α -D-glucopyranoside in the enzyme preparation.

collagen peptides. The degree of purification was similar to that obtained with the concanavalin A-agarose column.

The experiments described below were carried out with enzymes purified by using either the concana-

valin A-agarose or the collagen-agarose column unless otherwise stated.

Metal cofactor of the reaction

Collagen galactosyltransferase from chick embryos requires Mn^{2+} (Myllylä *et al.*, 1975). The optimum Mn^{2+} concentration for the purified enzyme studied here was 2.0–2.5 mM, and with higher concentrations marked inhibition was noted. The K_m value was about 0.12 mM.

Addition of Mg^{2+} or Fe^{2+} in concentrations from 1 to 30 mM to the incubation mixture containing either 0.5 or 2.5 mM- Mn^{2+} did not affect the reaction, whereas Co^{2+} was clearly inhibitory, 50% inhibition being observed at about 6 mM- Co^{2+} .

Sugar nucleotide co-substrate and its analogues

The K_m value of UDP-galactose for the purified collagen galactosyltransferase was between 20 and 23 μM in various experiments. Several nucleotides and their derivatives inhibited the enzyme activity (Table 4). No inhibition was observed with 1.0 or 2.0 mM-uridine, but both UMP and UDP acted as inhibitors. Under the conditions used about 95% of the UDP was actually present in the form of a Mn^{2+} -UDP complex (see Khatra *et al.*, 1974). Compounds in which a sugar moiety is linked to the nucleotide were somewhat more effective inhibitors than the nucleotides alone, the most effective inhibitor being UDP-glucose. The enzyme also recognized the uronic acids derived from UDP-galactose and UDP-glucose, of which UDP-galacturonic acid was a better

inhibitor than UDP-glucuronic acid. The purine nucleotides ATP, ADP, AMP and GDP gave 50% inhibition with concentrations between 1.0 and 2.0 mM (results not shown). Free monosaccharides did not inhibit the enzyme activity (Table 3), nor was inhibition observed with 1 mM-lactose or -sucrose.

Two different patterns were observed when the type of inhibition caused by various nucleotides and their derivatives was tested with respect to the various reactants (Fig. 3). Both CDP and UDP-glucose showed linear non-competitive inhibition with respect to collagen substrate, and linear competitive inhibition with respect to UDP-galactose,

but with regard to Mn^{2+} the inhibition was either non-competitive (CDP) or uncompetitive (UDP-glucose).

About half of the collagen galactosyltransferase activity present in 40 ml of crude $(NH_4)_2SO_4$ -precipitated enzyme (protein concn. 10 mg/ml) became bound to a column (bed vol. 2.5 ml) of immobilized UDP-galacturonic acid, whereas immobilized UDP-glucuronic acid did not retard the enzyme activity.

Collagen substrate and its analogues

Concentrations of gelatinized collagen in excess of 60 mg/ml were found to inhibit the collagen galactosyltransferase reaction, and the K_m value of this substrate proved to be about 16 mg/ml.

Product inhibition of collagen galactosyltransferase was studied with free galactosylhydroxylysine and glomerular basement membrane from which the glucose had been removed to uncover the galactosylhydroxylysine residues (Table 5). Both substances caused some inhibition, but the high-molecular-weight inhibitor was more effective when the values were calculated per mol of the product sites. The enzyme does not transfer galactose to free hydroxylysine (Spiro & Spiro, 1971; Myllylä *et al.*, 1975), and thus it is noteworthy that the enzyme can recognize free galactosylhydroxylysine as an inhibitor. A slight inhibition was also observed with free hydroxylysine, but only with very high concentrations of the amino acid, and even then to a smaller extent than with galactosylhydroxylysine. Yet the effect of hydroxylysine was apparently not due to non-specific factors, since a similar concentration of lysine had no effect on the reaction (Table 5).

Hydroxylysine was coupled from its carboxyl group to AH-Sepharose 4B, thus preserving the free ϵ -amino group essential for acceptor activity (Spiro & Spiro, 1971). When the material was tested for the

Table 4. Inhibition of collagen galactosyltransferase by various nucleotide derivatives

The concentrations of the inhibitors were chosen to give about 50% inhibition.

Inhibitor (mM)		Enzyme activity (d.p.m.)	Inhibition (%)
None	—	801	—
Uridine	1.0	921	—
	2.0	810	—
UMP	0.5	549	31
	1.0	350	56
UDP*	0.06	505	37
	0.1	344	57
UDP-galacturonate	0.065	457	43
	0.13	320	60
UDP-glucuronate	0.2	406	49
	0.4	302	62
UDP-glucose	0.025	449	44
	0.05	376	53
CDP	0.13	424	47
	0.2	352	56

* Present in the form of a Mn^{2+} -UDP complex.

Fig. 3. Inhibition of collagen galactosyltransferase reaction by analogues of the nucleotide co-substrate

The concentrations of Mn^{2+} , UDP-galactose and collagen substrate were 2.0 mM, 50 μ M and 24 mg/ml respectively when not used as variable substrates. Velocities (v) are expressed as nmol of product formed in 45 min. The inhibition by CDP is shown with respect to UDP-galactose (a), collagen substrate (b) and Mn^{2+} (c), and the inhibition by UDP-glucose with respect to Mn^{2+} (d). The concentrations of CDP were none ($\circ$), 0.1 mM ($\bullet$), 0.2 mM ($\square$) and 0.4 mM ($\blacksquare$) and those of UDP-glucose, none ($\circ$), 25 μ M ($\bullet$), 50 μ M ($\square$) and 100 μ M ($\blacksquare$).

Table 5. *Effect of hydroxylysine, galactosylhydroxylysine and glucose-free basement-membrane collagen on the collagen galactosyltransferase reaction*

The reaction was carried out as described in the Experimental section, with 24 mg of collagen substrate/ml and the additions indicated. Values for galactosyltransferase activity are expressed in terms of the amount of product formed in 45 min and the relative activity.

Inhibitor	Amount (mg/ml)	Concentration of acceptor or product sites (μ mol/ml)	Galactosyltransferase activity	
			(pmol)	(%)
Expt. 1				
None	—	—	36.1	100
Hydroxylysine	10.0	50	22.4	62
Expt. 2				
None	—	—	27.6	100
Lysine	9.1	50*	27.3	99
Hydroxylysine	10.0	50	22.6	82
Expt. 3				
None	—	—	34.1	100
Galactosylhydroxylysine	1.8	5	23.9	70
	3.6	10	20.1	59
	7.2	20	14.7	43
	14.4	40	9.8	29
Expt. 4				
None	—	—	25.4	100
Glucose-free glomerular basement-membrane collagen	2.7	0.4	20.1	79

* Concentration of lysine.

affinity chromatography of collagen galactosyltransferase, essentially all the enzyme activity was always recovered in the column effluent, although the same enzyme preparations had a high affinity for collagen-agarose.

Discussion

Attachment to membrane structures of cells is a characteristic of many glycosyltransferases (Schwartz & Rodén, 1975), and vigorous procedures are often required for the solubilization of these enzymes. Considerable amounts of collagen galactosyltransferase activity can be extracted without detergent (Risteli *et al.*, 1976a), but the presence of 0.1% Triton X-100 doubles the measurable activity both in the homogenate and in its soluble fractions (Risteli *et al.*, 1976a). In the present study additional increases of 24–56% could be achieved with a number of compounds, the most effective being 0.5% detergent. Thus collagen galactosyltransferase is associated with cell membranes, in agreement with its presumed location within the cisternae of the endoplasmic reticulum (Brownell & Veis, 1975; Harwood *et al.*, 1975; Oikarinen *et al.*, 1976; Risteli *et al.*, 1976a).

Concanavalin A binds substances containing α -D-mannosyl, α -D-glucosyl and sterically similar residues, and the results on inhibition by this lectin and binding of the enzyme to a column of immobilized concanavalin A suggest that such carbohydrate units

are present in the enzyme. Interestingly, the enzymes participating in the intracellular events of collagen biosynthesis may all be glycoproteins, since evidence has been presented for the presence of carbohydrate in prolyl hydroxylase (Guzman *et al.*, 1976; Berg *et al.*, 1977) and lysyl hydroxylase (Ryhänen, 1976; Turpeenniemi *et al.*, 1977) and collagen glucosyltransferase (Myllylä *et al.*, 1977; Anttinen *et al.*, 1977). The inhibition of collagen galactosyltransferase activity by concanavalin A was about 50%. Thus the carbohydrate groups to which the lectin is bound may not be situated quite in the active centre of the enzyme. Yet over 90% of the enzyme activity became bound to immobilized concanavalin A, which seems to exclude the possibility of isoenzymes, part with carbohydrate groups and part without. Hydrophobic forces may be involved in the interaction of collagen galactosyltransferase with the lectin, since the enzyme could be eluted only with high concentrations of ethylene glycol (Davey *et al.*, 1976; Anttinen *et al.*, 1977).

With partially purified collagen galactosyltransferase preparations the optimum reaction conditions are somewhat different from those reported for crude chick enzyme (Myllylä *et al.*, 1975). Inhibition is noted with Mn^{2+} concentrations exceeding 2.5 mM, and with collagen substrate concentrations over 60 mg/ml. The K_m values for Mn^{2+} , UDP-galactose and collagen substrate were 0.12 mM, about 20 μ M and 16 mg/ml respectively, being smaller than those

reported previously (Myllylä *et al.*, 1975; Risteli *et al.*, 1976a). The optimum concentration obtained here for the metal cofactor is still more than 10^3 times greater than the physiological concentration of Mn^{2+} , and it remains to be settled whether some other cation also takes part in the reaction *in vivo*, a mechanism that has been suggested for the galactosyltransferase participating in lactose synthesis (Powell & Brew, 1976).

With crude collagen galactosyltransferase Mn^{2+} can be replaced to a small extent by Mg^{2+} , Co^{2+} and Fe^{2+} (Myllylä *et al.*, 1975), although the activity in the presence of any of these is not more than 10% of that obtained with Mn^{2+} . Of these three metals only Co^{2+} significantly inhibited the purified galactosyltransferase, probably by competing with Mn^{2+} . This inhibition is not likely to be due to contamination by other metals such as Pb^{2+} , since no inhibition of collagen glucosyltransferase activity was noted with the same batch of $CoCl_2$ (Myllylä, 1976).

The degree of substitution of the hydroxylysine residues in glomerular basement membrane collagen is high (Spiro, 1967, 1969), most of the carbohydrate being in the form of glucosylgalactosylhydroxylysine. When the glucose was removed from this material to uncover the galactosylhydroxylysine residues, an effective product inhibitor resulted. Another possible product, UDP, also inhibited the enzyme activity. Although the enzyme does not galactosylate free hydroxylysine (Spiro & Spiro, 1971; Myllylä *et al.*, 1975), it recognized galactosylhydroxylysine as an inhibitor. Some inhibition was also brought about by free hydroxylysine, which thus might occupy part of the binding site for the polypeptide substrate in the enzyme, but not firmly enough for the reaction to take place.

A number of nucleotides and their derivatives also inhibited the reaction, presumably by attaching to the same site in the enzyme as the co-substrate UDP-galactose or the product UDP. For most inhibitors the concentrations giving 50% inhibition are of the same order of magnitude as the K_i values of the same compounds for the galactosyltransferase operating in lactose synthesis (Khatra *et al.*, 1974). The patterns of inhibition caused by CDP and UDP-glucose with respect to the various reactants were somewhat different, in that the inhibition with respect to the metal cofactor was non-competitive for CDP and uncompetitive for UDP-glucose. This finding is similar to results obtained with collagen glucosyltransferase (Myllylä, 1976) and human milk galactosyltransferase (Khatra *et al.*, 1974). It is probable that free nucleotides act as analogues of the reaction product UDP rather than of the co-substrate UDP-galactose.

Owing to its tendency to aggregate and to lose its activity during storage and purification (Risteli *et al.*, 1976a) collagen galactosyltransferase has not yet

been obtained in pure form. These features are common to most glycosyltransferases (Schwartz & Rodén, 1975). Some new possibilities for the affinity chromatography of collagen galactosyltransferase are suggested in the present paper, although their use is seriously restricted by the tendency of the enzyme to become inactivated. It is predicted that effective affinity-chromatography matrices should be produced if the K_i of the ligand to be immobilized is about 1 mM (Graves & Wu, 1974). Thus immobilized hydroxylysine cannot be expected to bind collagen galactosyltransferase effectively, though it would theoretically be the most specific affinity-chromatography matrix for the enzyme. The difference noted in the binding of the enzyme to immobilized UDP-galacturonic acid and UDP-glucuronic acid is in agreement with the K_i values of these compounds.

Since the properties of the purified enzyme used in the present study differ considerably from those of the crude enzyme from the same source (Myllylä *et al.*, 1975), it is possible that the degree of purification of the enzyme protein is in fact higher than the apparent 200–300-fold purification of the enzyme activity.

This work was supported in part by a grant from the Medical Research Council of the Academy of Finland. I gratefully acknowledge the valuable suggestions and comments of Professor Kari I. Kivirikko, M.D., and the expert technical assistance of Miss Helmi Konola and Mrs. Raija Pietilä.

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