INCORPORATION OF D-XYLOSE-C^{14} INTO GLYCOPROTEIN BY PARTICLES FROM HEN OVIDUCT

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D-Xylose, once thought to be restricted to polysaccharides of plants and microorganisms, has recently been found in a number of macromolecules of animal origin. This pentose occupies a unique position in chondroitin sulfate and heparin, where it joins the polysaccharide and protein moieties through a xylosyl-serine linkage (Gregory et al., 1964; Lindahl and Rodén, 1965; Rodén and Lindahl, 1965). Free xylosyl-serine has been isolated from human urine (Tominaga et al., 1965). Xylose[*] has been reported to be a constituent of mucopolysaccharides of mammalian brain (Wardi et al., 1965), and of glycoproteins from human lung (Masamune et al., 1957), cancerous ascitic fluid (Sugimoto, 1956), placenta (Tomoda and Murayama, 1965) and aorta (Klemer and Burbaum, 1965), and from cockroach plasma (Lipke et al., 1965). UDP-Xylose, the probable precursor of such glycosidically-bound xylose, has been isolated from the milk of sows and goats, and from cow colostrum (Kobata and Ziro, 1965), while its biosynthesis by decarboxylation of UDP-glucuronic acid has been demonstrated in preparations from hen oviduct (Bdolah and Feingold, 1965). The present communication describes a particulate system from the oviduct of laying hens, which catalyzes the transfer of xylose from UDP-xylose into glycoprotein.

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[*]The sugars are of the D-configuration.

METHODS

Laying hens were decapitated and the oviduct used immediately or after storage at -15°. In early experiments, the enzyme was prepared from the isthmus section, which produces sulfated mucopolysaccharides for the egg membrane (Romanoff and Romanoff, 1949). The albumen-secreting portion was subsequently found to yield more enzyme, and was used for the studies reported here.

The tissue was minced into cold 0.1 M Tris-HCl, pH 7.0, and ground in a Tenbroeck homogenizer. The homogenate was centrifuged at very low speed to remove coarse debris. The supernatant plus the large, loose white layer of particulate material were decanted from the small pellet of unhomogenized tissue, filtered through a fine mesh wire gauze and centrifuged at 20,000 g for 15 minutes. The resulting precipitate was suspended in the above buffer (1 ml per g of tissue). These preparations could be stored at -15° for several months and withstood repeated freezing and thawing. Before use, the precipitate was centrifuged, washed, and resuspended in the same volume of buffer.

UDP-Xylose-C^{14} (104 μc/μmole) was prepared by decarboxylation of UDP-glucuronic acid-C^{14} (New England Nuclear Corp.) catalyzed by an enzyme from wheat germ (Ankel and Feingold, 1965), and was purified by paper chromatography in ethanol-1 M ammonium acetate, pH 7.5 (75:30).

RESULTS

Evidence for the transfer of xylose from UDP-xylose into material insoluble in 5% trichloroacetic acid (TCA) is summarized in Table I. The incorporation required Mn^{++} (optimal concentration, 6×10^{-3} M), and was markedly stimulated by the addition of glutathione (optimal concentration, 10^{-2} M; 5×10^{-2} M was completely inhibitory). Highest incorporation was obtained at pH 7 ± 0.5. The half-maximal rate of incorporation, measured while linear (10 minutes incubation), occurred at a concentration of UDP-xylose of 2×10^{-6} M. The rate usually diminished after 30 minutes, due in part to the hydrolysis of UDP-xylose.

TABLE I

Incorporation of Xylose-C^{14} into TCA Precipitate

The complete system includes 6,000 cpm UDP-xylose (68 µµmoles); 6 x 10^{-3} M $MnCl_2$, 10^{-2} M glutathione, 200 µl of enzyme suspension in 0.1 M Tris-HCl, pH 7.0, in a total volume of 260 µl. Incubation was for one hour at 37°. The reaction was stopped by the addition of 2 ml of 5% TCA. The precipitate was washed 2 times with 2 ml of 5% TCA, dehydrated by successive washes in 95% ethanol, 100% ethanol, ethanol-ether (1:1) and ether, and dissolved in one ml of hyamine hydroxide. This solution was mixed with 10 ml of toluene-phosphor for liquid scintillation counting.

System	cpm Incorp.
Complete	1394
-Mn^{++}	109
-Mn^{++}, +Mg^{++}	144
-Glutathione	440
-Mn^{++}, -glutathione	103
-UDP-xylose, +hydrolyzed UDP-xylose*	6
Complete, +hydrolyzed UDP-xylose*	1420
-UDP-xylose, +xylose 1-phosphate**	10
Boiled enzyme	7
Zero time	6

*UDP-xylose was hydrolyzed at 100°, pH 2.0, for 15 minutes.

**α-D-Xylose 1-phosphate was prepared by treatment of UDP-xylose with venom of Crotalus adamanteus and purified by paper chromatography.

All the radioactivity could be removed from TCA-insoluble material by hydrolysis in 1.0 N HCl at 100° for 15 minutes, and identified as xylose by two-demensional co-chromatography with unlabeled sugar in butanol-acetic acid-water (52:13:35) and 90% phenol. (The hydrolysate was deionized by passage through Amberlite MB-3 prior to chromatography.)

From 30 to 60% of the xylose which had been incorporated into the TCA

insoluble pellet could be rendered TCA-soluble by treatment with pronase.** The amount released varied with different oviduct preparations. In one instance, papain was substituted for pronase and found to give similar solubilization. The radioactive TCA-soluble material released by pronase was resolved by paper electrophoresis at pH 2.0 into four positively charged bands which migrated in the close vicinity of unlabeled glycopeptides, each containing several amino acids and sugars, that had also been released during the digestion. These results are taken as evidence for the association of part (up to 60%) of the incorporated xylose with protein.

The transfer of xylose into glycoprotein occurred in the absence of protein synthesis, as demonstrated by the inability of the particles to incorporate serine-C^{14} or leucine-C^{14} into TCA-precipitable material under conditions which were optimal for xylose incorporation. Puromycin, up to 10^{-2} M, did not inhibit the transfer.

To determine whether xylose was transferred to a serine residue to give a xylosyl-serine linkage such as is found in heparin or chondroitin sulfate, a search was made for labeled xylosyl-serine among the glycopeptides released by pronase digestion of radioactive TCA pellets. No xylosyl-serine was found in chromatography on paper or on Dowex-50, nor in paper electrophoresis. This result is equivocal, however, since it is possible that pronase is unable to hydrolyze all of the peptide bonds proximal to the sugar residue.

**Pronase digestions were carried out as follows: TCA-insoluble pellets (ca. 15 mg dry weight) from each incubation carried out as described for the complete system in Table I were washed twice with 5% TCA, then with water until neutral. One mg of pronase was added in 0.2 ml of 0.1 M Tris buffer, pH 7.6, containing 0.001 M $CaCl_2$ and 7% ethanol. After 5 hours at 37°, additional pronase (1 mg) was added, and incubation continued for 12 hours.

The nature of the material not digested by pronase is not known; it was not solubilized by additional pronase nor by incubation with RNAase, DNAase, collagenase, hyaluronidase, β-glucosidase, α-amylase, or xylanase, nor was it extractable with lipid solvents. The kinetics of incorporation do not encourage the hypothesis that the xylose in the pronase-digestible fraction might serve as precursor to the xylose in the indigestible residue, or vice-versa.

In spite of the absence of xylosyl-serine among the products of pronase digestion, existence of such linkages is suggested by the alkali lability of part of the radioactive xylose incorporated into the TCA pellet (Table II). Xylosyl-serine is known to undergo a β-carbonyl elimination of the carbohydrate from the hydroxyl group of serine in the presence of alkali (Anderson et al., 1965). Of the radioactivity released by alkali containing $NaBH_4$ in experiment A (Table II), three-quarters was identified as xylitol by two-dimensional co-chromatography in propanol-ethyl acetate-water (7:1:2) and 90% phenol.

TABLE II

Treatment of Labeled Product with Alkali

The radioactive pellets, obtained by incubation of enzyme with UDP-xylose-C^{14} under conditions indicated in Table I, were washed three times with 5% TCA, then with water until neutral. Different preparations of enzyme were used for A and B. The pellets were dissolved in 0.5 ml of 0.2 N or 0.5 N NaOH, containing 5 mg $NaBH_4$. At the indicated time, the solutions were neutralized and an equal volume of 10% TCA added. The radioactivity remaining in solution was measured in Bray's scintillation fluid (Bray, 1960), while the precipitate was prepared for counting as described in Table I; aliquots of all washes were counted, for after 3 days in alkali the bulk of the TCA-insoluble material (labeled and unlabeled) had become soluble in acid ethanol.

Experiment	Concentration of NaOH (N)	Time (days)	Radioactivity made TCA-soluble (%)
A	0.2	0.1	14
	0.2	1	17
	0.2	3	20
	0.2	7	22
B	0.2	3	39
	0.5	3	37

Though the release of xylitol by alkali-borohydride may be taken as tentative evidence for the attachment of some radioactive xylose to serine or threonine residues, the same data indicate that the bulk of the xylose was incorporated into a different (alkali-stable) position. It is apparent that the product of xylosyl transfer is heterogeneous with respect to both pronase digestibility and alkali lability. The relative proportion of the fractions

varied among different oviduct preparations, implying the existence of more than one transferase. This may be of interest in view of the reports, cited above, of the occurrence of xylose in a variety of polymers of animal origin.

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