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ENZYMIC 4-*O*-METHYLATION OF GLUCURONIC ACID LINKED TO GALACTOSE IN HEMICELLULOSE POLYSACCHARIDES FROM *PHASEOLUS AUREUS*

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Abstract—A particulate enzyme preparation from mung bean shoots can transfer the ^{14}C-labelled methyl groups from *S*-adenosyl-L-methionine to the glucuronic acid unit of polysaccharides present in the particles. The labelled polysaccharides can be partly extracted by ammonium oxalate solution and partly by dilute NaOH (hemicellulose B). On partial acid hydrolysis, both fractions give an uronic acid mixture consisting predominantly of MeGlcUA-Gal besides some MeGlcUA-Xyl and MeGlcUA. This indicates that the products of the transfer reaction consist mainly of polysaccharides in which 4-*O*-methylglucuronic acid is linked to galactose, presumably methylglucuronogalactans. It is concluded that the mode of biosynthesis of the 4-*O*-methyl-D-glucuronic acid unit by transfer of methyl groups to preformed polysaccharides is the same regardless of the type of polysaccharide to which the glucuronic acid is linked.

IT HAS been recently shown[1] that particulate preparations from growing corn cobs contain enzymes which transfer methyl groups from *S*-adenosyl-L-methionine to the hydroxyl groups at C-4 of the glucuronic acid moieties in the glucuronoxylans which are present in the particulate material. Aldobiouronic acids containing 4-*O*-methyl-D-glucuronic acids linked to sugars other than xylose are known from the hemicelluloses and gums of many plants.[2] This indicates that 4-*O*-methyl-D-glucuronic acid can form single residue side-chains in heteropolysaccharides of quite different structure. It was of interest, therefore, to see whether or not the mode of biosynthesis of the 4-*O*-methyl-D-glucuronic acid unit of other polysaccharides was the same as has been shown for the glucuronoxylans.

During an investigation of the biosynthesis of the methyl ester groups of pectic substances[3] it was noticed that a particulate preparation from mung bean shoots (*Phaseolus aureus*) was also able to incorporate labelled methyl group of *S*-adenosyl-L-methionine as methyl ether groups into other unknown polysaccharides. These polysaccharides can be partly extracted from the incubated particles with ammonium oxalate solution, which is normally used for the isolation of pectic substances (Table 1). The greater part, however, is soluble only after treatment with dilute alkali, which is typical for hemicellulose B. From both polysaccharide

Abbreviations:

GlcUA = D-glucuronic acid.
MeGlcUA = 4-*O*-methyl-D-glucuronic acid.
GlcUA-Gal = 6-*O*-β-D-glucopyranuronosyl-D-galactose.
MeGlcUA-Xyl = 2-*O*-(4-*O*-methyl-α-D-glucopyranuronosyl)-D-xylose.
MeGlcUA-Xyl-Xyl = *O*-(4-*O*-methyl-α-D-glucopyranuronosyl)-(1 → 2)-*O*-β-D-xylopyranosyl)-(1 → 4)-D-xylose.
SAM = *S*-adenosyl-L-methionine.

[1] H. KAUSS and W. Z. HASSID, *J. Biol. Chem.* **242**, 1680 (1967).
[2] R. W. BAILEY, *Oligosaccharides*, p. 132, Pergamon Press, Oxford (1965).
[3] H. KAUSS, A. L. SWANSON and W. Z. HASSID, *Biochem. Biophys. Res. Commun.* **24**, 234 (1967).

fractions, radioactive uronic acid mixtures can be obtained by acid hydrolysis, which were analysed by paper chromatography as shown in Fig. 1. The uronic acid-containing substances from the two polysaccharide fractions showed a qualitatively identical pattern. Two of the

TABLE 1. FRACTIONATION OF THE RADIOACTIVE POLYSACCHARIDES FORMED FROM *S*-ADENOSYL-L-METHIONINE -^{14}C-METHYL BY A PARTICULATE ENZYME PREPARATION FROM MUNG BEAN SHOOTS

Polysaccharide fraction*	Total counts/min × 10^{-3} Exp. I	Exp. II
Ammonium oxalate soluble	15	13
Uronic acids obtained by acid hydrolysis	5	4
Hemicellulose B	27	19
Uronic acids obtained by acid hydrolysis	7	8

* Details of the methods used for isolation and hydrolysis of the polysaccharides are given in the Experimental Section.

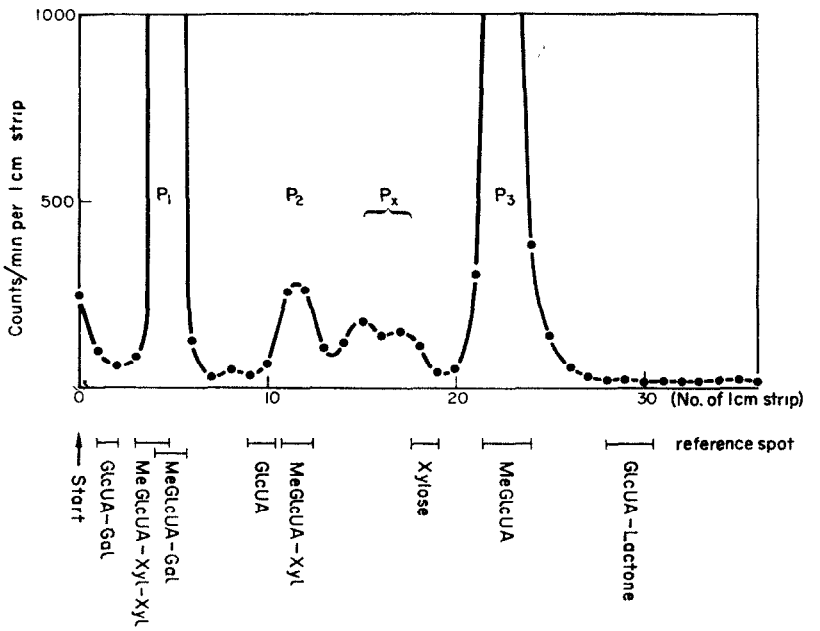

FIG. 1. PATTERN OF RADIOACTIVITY OF THE URONIC ACID MIXTURE RESULTING FROM HYDROLYSIS OF ^{14}C-METHYLATED HEMICELLULOSE B ENZYMICALLY FORMED BY THE PARTICULATE ENZYME PREPARATION FROM MUNG BEAN SHOOTS.

Same uronic acid mixture as in Experiment II, Table 1. Solvent mixture I.

three main peaks were the same as found in the methylated hemicellulose from corn cobs.[1] On co-chromatography with authentic standards in solvent systems I, II, III and V, P_3 was shown to be MeGlcUA and P_2 MeGlcUA-Xyl. The identity of the main peak P_1, however, was unknown.

In the case of corn cob hemicellulose B[1] P_1 only occurs as a very minor constituent (Table 2). Although the radioactive compound in the same area had a similar R_f value as MeGlcUA-Xyl-Xyl in solvent I, it did not fully coincide with this substance (Fig. 1) and was clearly separated in solvent II. Furthermore, no MeGlcUA-Xyl could be found after acid hydrolysis of P_1 (1 N HCl, 4 hr). This result suggested that the substance P_1 might be an aldobiouronic acid containing methyl labelled MeGlcUA bound to a sugar other than xylose.

A larger amount of the aldobiouronic acid fraction from an unlabelled mung bean hemicellulose B was therefore prepared, and the acid having R_f values like P_1 isolated. With aniline-phosphoric acid it gave the same yellow-white colour as GlcUA-Gal, whereas aldobiouronic acids containing xylose show a typical orange-red colour. After complete acid hydrolysis the sugars were identified by paper chromatography as galactose and MeGlcUA. It was found that the radioactive material of P_1 fully coincided with this aldobiouronic acid in both one-dimensional co-chromatography in the solvent mixtures I, II, III (30 hr), IV (24 hr) and V (48 hr) and two-dimensional chromatography in II (30 hr) followed

TABLE 2. PROPORTION OF THE DIFFERENT RADIOACTIVE URONIC ACIDS DERIVED BY ACID HYDROLYSIS FROM THE METHYLATED POLYSACCHARIDES

Polysaccharide fraction		% of the radioactivity in *			
		P_1	P_2	P_3	P_x
Ammonium oxalate soluble (mung bean enzyme)	Exp. I	56·9	13·7	21·9	4·0
	Exp. II	51·9	18·5	23·5	2·7
Hemicellulose B (mung bean enzyme)	Exp. I	60·4	5·0	19·7	12·9
	Exp. II	52·6	5·0	34·3	5·4
Hemicellulose B (corn cob enzyme, Ref. 1)		3·8	80·1	15·1	0·2

* The number of the peaks refer to Fig. 1. P_1 = MeGlcUA-Gal, P_2 = MeGlcUA-Xyl, P_3 = MeGlcUA, P_x = unknown.

by I. In all cases the compound was clearly different from MeGlcUA-Xyl-Xyl and it is, therefore, clear that P_1 represents MeGlcUA-Gal.

The occurrence of this galactose-containing aldobiouronic acid in the hydrolysates from mung bean polysaccharides indicates that the methylated product has a different structure from the ^{14}C-methylated polysaccharides formed by the corn cob enzyme. In the latter case MeGlcUA-Xyl was almost the only aldobiouronic acid found (Table 2) and in this case, therefore, the methyl ether groups appear to be preferentially incorporated into glucuronoxylans. Such polysaccharides also appear to occur in the mung bean fractions and the ammonium oxalate fraction especially shows the presence of MeGlcUA-Xyl (Table 2). The predominant acceptors for the methyl ether groups in the mung bean, however, must be D-glucuronic acid molecules linked to the galactose moiety of other polysaccharides. That such polysaccharides are present in mung bean particulate preparations is demonstrated by the isolation of D-glucuronosyl-D-galactose from partial hydrolysates.[4] Little, however, is known about their structure. The isolation of 6-*O*-β-D-galactosyl-D-galactose from the same source[4] can possibly be taken as an indication that the polysaccharides are glucuronogalactans.

[4] R. W. BAILEY, S. HAQ and W. Z. HASSID, *Phytochem.* 6, 293 (1967).

EXPERIMENTAL

The particulate fraction containing the enzyme activity was prepared from 4-day-old mung bean shoots (*Phaseolus aureus*) as described previously but without addition of EDTA.[3,5] 1·0 ml of this enzyme suspension was incubated with 1·0 ml buffer (tris-HCl, 0·1 M, pH 8·4, containing 0·4 M sucrose, 1% albumin), and 30 μl *S*-adenosyl-L-methionine-^{14}C-methyl (59 mμmoles, $5{\cdot}4 \times 10^6$ cpm, final pH 8·1) at 30° for 30 min, and 2 ml of 5% TCA was then added. The precipitate was washed by centrifugation two times with cold water, extracted two times with 1·0 ml 0·5% ammonium oxalate at 100° for 10 min, and then with 1·0 ml of 2% NaOH under the same conditions. The polysaccharides were precipitated from the ammonium oxalate solution by addition of 4 vol. EtOH. The NaOH solution was brought to pH 4·6 with acetic acid and the precipitate (hemicellulose A plus proteins and lipoproteins) removed. To the supernatant, 5 vol. EtOH were added, and, after 2 hr at 2°, hemicellulose B was collected by centrifugation. Hydrolysis was carried out with 1 N HCl for 4 hr at 100° in a sealed tube.

The general methods for the analysis of products and for counting were carried out as described previously[1] and the following solvent mixtures were employed: (I) ethyl acetate–acetic acid–HCOOH–H_2O = 18:3:1:4; (II) 88% (w/w) phenol–H_2O–1 M EDTA–acetic acid = 840:160:1:10; (III) ethyl acetate–pyridine–H_2O = 2:1:2 (org. phase); (IV) n-BuOH–pyridine–H_2O–acetic acid = 60:40:30:3; (V) ethyl acetate–acetic acid–H_2O = 9:2:2. The radioactive spots on two-dimensional chromatograms were located using radioautography. A hemicellulose B fraction was prepared from 150-g shoots of 4-day-old mung bean seedlings by blending them in EtOH (600 ml), boiling for 30 min and then again treating with 95% EtOH for 30 min. The residue was filtered off, dried (P_2O_5 *in vacuo*) and extracted for 18 hr at room temperature with 300 ml of 2 N NaOH under N_2. The solution was neutralized with acetic acid and cleared by centrifugation. The hemicellulose B was precipitated by addition of 5 vol. of EtOH, washed with 80% EtOH and dried. Aldobiouronic acids were isolated after hydrolysis of these polysaccharides[6] and purified by paper chromatography in solvents I and II The individual aldobiouronic acid fractions were freed from neutral glycosides on small columns of Dowex 1 acetate.[6] Samples of the aldobiouronic acids were hydrolysed with 100 μl of 1 N HCl at 100° for 6 hr and dried (P_2O_5) in vacuum.

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[5] H. KAUSS and W. Z. HASSID, *J. Biol. Chem.* **242**, 3449 (1967).
[6] P. M. RAY and D. A. ROTTENBERG, *Biochem. J.* **90**, 646 (1964)