BIOSYNTHESIS OF THE METHYL ESTER GROUPS OF PECTIN BY TRANSMETHYLATION FROM S-ADENOSYL-L-METHIONINE*

H. Kauss,[1] A. L. Swanson and W. Z. Hassid

Department of Biochemistry, University of California, Berkeley, California 94720

Received December 28, 1966

The carboxyl groups of the polygalacturonic acid chains of plant pectin are partly or fully esterified with methanol (Deuel and Stutz, 1958). In vivo experiments have shown that this methyl group can be derived from L-methionine (Sato et al., 1958); however, the mechanism of methylation has not been demonstrated. We have shown in this communication that a particulate enzyme preparation from mung beans (Phaseolus aureus) contains a transferase capable of transferring the methyl group of S-adenosyl-L-methionine to the polygalacturonic acid which is present in the particles.

The particulate enzyme preparation was prepared essentially in the same manner as that which was found capable of synthesizing polygalacturonic acid from UDP-D-galacturonic acid (Villemez et al., 1966). Three day old mung bean shoots (25 g) were ground with sand in 25 ml 0.05 M potassium phosphate buffer pH 7.3 containing 1% albumin, the material was squeezed through cheesecloth and centrifuged at 1000 g

*Supported in part by Research Grant A-1418 from the National Institute of Health and by Research Grant G-23763 from the National Science Foundation.

[1]Stipendiat des Deutschen Akademischen Austauschdienstes. Permanent address: Institut für Angewandte Botanik der TH München, Germany.

for 15 minutes. The supernatant liquid was centrifuged at 34,000 g for 45 minutes, the pellet suspended in 20 ml of the buffer which was used for grinding and centrifuged again at 34,000 g for 45 minutes. The particles were suspended in 0.5 ml 0.05 M potassium phosphate buffer, pH 6.8, containing 1% albumin and 0.4 M sucrose.

For assaying the enzyme, 50 µl of this preparation was mixed with 50 µl of 0.1 M cacodylate-HCl buffer containing 1% albumin and 0.4 M sucrose and 5 µl of S-adenosyl-L-methionine-methyl-^{14}C (1.96 mµmoles, 140,000 CPM, obtained from New England Nuclear Corporation). The mixture was incubated at 30°, inactivated by addition of 100 µl 20% TCA and then diluted with 1 ml of water. The precipitate was centrifuged and washed 2 times with 0° water, 1 mg of citrus pectin (Exchange Lemon Products) in 0.2 ml of water was added, and the pectin extracted 2 times with 1 ml 0.5% ammonium oxalate at 100° for 10 minutes. Sufficient absolute ethanol was added to the solution to bring the concentration to 75% (v/v) and the precipitate was collected by centrifugation after standing for 1 hour at room temperature.

The pectin was solubilized in 1 ml of water and aliquots of 200 µl were applied on strips of chromatography paper. Parallel control strips with 200 µl of the solution were wetted with 7.5 N NH_4OH and kept for 1 hour in a closed container over 7.5 N NH_4OH to saponify the methyl ester. The strips were dried with a fan and counted in toluene with Liquifluor in a scintillation counter. The values for the incorporation of ^{14}C-methyl groups into the polygalacturonic acid chain were obtained by subtracting the values of the alkali treated strips and those from the zero time controls.

As shown in Fig. 1, the introduction of the methyl ester group into pectin is practically linear up to 10 minutes. The progressive decrease of the reaction rate may be due to partial inactivation of the enzyme or to the limited amount of polygalacturonic acid present

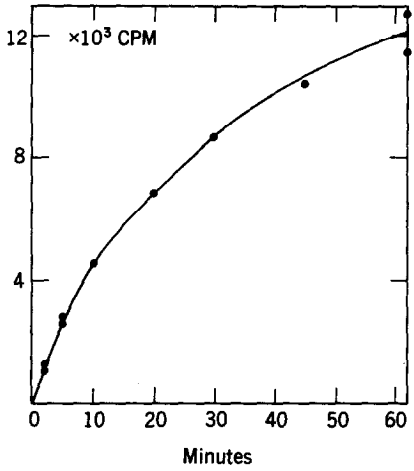

Fig. 1: Incorporation of alkali labile ^{14}C-methyl groups with increasing time of incubation. Standard assay mixture, pH 6.6.

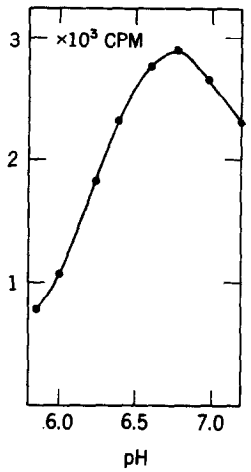

Fig. 2: Incorporation of alkali labile ^{14}C-methyl groups at different pH values. Standard assay mixture, 5 minutes incubation time.

in the particles. Fig. 2 shows that the transferase has an optimal pH value of 6.6 - 7.0.

The transfer reaction does not require divalent cations, since the rate of incorporation is not changed if $MgCl_2$ or $MnCl_2$ (2.5×10^{-3} M) is added. The addition of other salts, such as $NiCl_2$, $ZnCl_2$, $FeCl_2$, $AlCl_3$, $CoCl_2$, and $CaCl_2$, causes a decrease of 70 - 10% in comparison with the standard assay mixture. Thus, the addition of EDTA (2×10^{-3} M) increases the incorporation rate to 125% by complexing metal ion traces.

The identity of the product of the reaction as ^{14}C-methyl esterified pectin is established by the following criteria: The polymer is soluble in 0.5% ammonium oxalate and is precipitated in 75% ethanol, indicating that it has a high molecular weight.

The alkali-liability of the methyl group (Table I) shows that it is linked as an ester rather than an ether. Since the radioactive constituent volatilyzes from alkaline solution, it must be a neutral,

Table I

The Liberation of the ^{14}C-Methyl Group by Pectin Methyl Esterase or Alkali

Samples of radioactive pectin containing 1 mg citrus pectin carrier in 100 µl of water were incubated at 37° with 100 µl tomato pectin methyl esterase (Nutritional Biochemical Corporation, 1 unit/mg, 1 mg/ml 0.2 M sodium phosphate buffer, pH 6.7). The reaction was stopped on the planchets by addition of 1 ml absolute ethanol. In the experiment with 0.05 N NaOH + 0.2% dimedone, the reaction mixture was adjusted to pH 5.0 with acetic acid after 2 hours. The planchets were dried and the radioactivity measured in a Nuclear Chicago flow counter.

	CPM remaining on the planchets
Starting material	320
After treatment with:	
4 N NH_4OH, 2 hours	11
0.05 N NaOH + 0.2% dimedone, 2 hours	9
Boiled pectin esterase, 2 hours	330
Pectin esterase, 15 minutes	16
Pectin esterase, 2 hours	15

low, molecular substance. This substance cannot be formaldehyde, because it was not trapped by dimedone (Table I).

The volatile compound formed by alkaline hydrolysis was further identified as methanol by radio gas chromatography[2] in a Aerograph A 700 with a Carbowax 300 column (He 16 ml/min, 85°) in combination with a Nuclear Chicago counting set. A sample of dry radioactive pectin con-

[2]Made possible by courtesy of Dr. C. E. Ballou and Dr. T. S. Stewart of this Department.

taining 0.2 mg of citrus pectin carrier was solubilized in 10 µl of 0.5 N NaOH. After 30 minutes 5 µl of methanol were added and the mixture injected into the gas chromatograph. The radioactive compound and the authentic methanol carrier had the same retention time of 5.0 minutes.

Direct evidence for the identity of the product with methyl ester labeled pectin is given in Table I. The methyl group is readily liberated by the enzyme pectin methyl esterase. This enzyme is specific for the methyl ester of polygalacturonic acid (Deuel and Stutz, 1958).

As can be seen from Table I, the radioactive methyl group is not completely liberated by alkali or pectin esterase. The proportion of the alkali stable methyl groups is about 5% after incubation of the assay mixture at pH 6.6 in the absence of Co^{++}. It can be as high as 60% after incubation at pH 8.1 and in the presence of 2.5×10^{-3} M $CoCl_2$. This indicates that the alkali stable labeling is most likely due to the formation of methyl ether groups. It was recently shown that the introduction of the methyl ether group into the 4-O-methyl-D-glucuronic acid unit of corn cob hemicellulose B is optimal at pH 8.1 and is stimulated by Co^{++} (Kauss and Hassid, unpublished data).

Albersheim (1963, 1965) and Loewus (1965) formerly suggested that the methylation reaction in the biosynthesis of pectin may occur prior to polymerization, involving a nucleotide bound methyl-D-galacturonic acid. Villemez et al. (1966) previously found that under conditions in which the uronic acid moiety of UDP-galacturonic acid is transferred to form polygalacturonic acid, the respective carboxyl methyl ester is not incorporated into high polymer products. The present data constitute further evidence that the incorporation of the methyl ester groups of pectin occurs at the macromolecular level.

The radioactivity incorporated after 1 hour of incubation (Fig. 1)

represents about 0.2 μmole of methanol. It is very unlikely that the washed particulate enzyme preparation contains such a considerable amount of endogenous low molecular precursor which could be the acceptor for the methyl groups and serve after the methylation as a donor of radioactive D-galacturonic acid methyl ester residues. Polygalacturonic acid, however, which may serve as acceptor for the methyl ester groups is present in mung bean particulate preparations (Bailey, et al., 1966).

Different types of macromolecules of biological interest can be altered enzymically by introduction of methyl groups. This reaction is well-known for the C- and N-methyl groups of RNA and DNA (Borek and Srinivasan, 1966); such a transfer was recently shown for the O-methyl ether group of the 4-O-methyl D-glucuronic acid unit of hemicellulose B (Kauss and Hassid, unpublished data).

The carboxyl groups are of considerable significance for the ion-exchange and water binding capacity of pectin and for the formation of cross linkages by cations, ester linkages and hydrogen bonds. These properties are important for the biological function of pectin as building material of the primary plant cell walls and are determined to a large extent by the degree of methylation of the pectin molecules. Thus, the action of the pectin methyl transferase must have significant influence on the formation and the properties of the primary plant cell walls.

REFERENCES

Albersheim, P., J. Biol. Chem., 238, 1608 (1963).
Albersheim, P., in Plant Biochemistry, p. 305, Academic Press (1965).
Bailey, R. W., Haq, S. and Hassid, W. Z., Phytochemistry, in press (1966).
Borek, E. and Srinivasan, P. R., Ann. Rev. Biochem., 35, 275 (1966).

Deuel, H. and Stutz, E., Adv. Enzym., 20, 341 (1958).

Kauss, H. and Hassid, W. Z., J. Biol. Chem., unpublished data.

Loewus, F., Fed. Proc., 24, pt. 1, p. 855 (1965).

Sato, C. S., Byerrum, R. U., Albersheim, P. and Bonner, J., J. Biol. Chem., 233, 128 (1958).

Villemez, C. L., Jr., Swanson, A. L. and Hassid, W. Z., Arch. Biochem. Biophys., 116, 446 (1966).