Phytochemistry, 1968, Vol. 7, pp. 1561 to 1564. Pergamon Press. Printed in England

SHORT COMMUNICATION

ENZYME-CATALYZED SYNTHESIS OF ^{14}C-GLUCOLIPID FROM UDP-GLUCOSE-^{14}C*

C. L. VILLEMEZ, JR,† B. VODAK‡ and P. ALBERSHEIM

Department of Chemistry, University of Colorado, Boulder, Colorado 80302

(*Received* 28 *February* 1968)

Abstract—Particulate enzymes of *Phaseolus aureus* seedlings catalyze the synthesis from UDP-glucose-^{14}C of two D-glucose-^{14}C containing compounds soluble in 1:1 chloroform–methanol. The distribution of this enzyme activity among a series of centrifugal fractions obtained in the range from 270 to 100,000 *g* is remarkably similar to the distribution of particles active in synthesizing cell-wall polysaccharides.

GLYCOLIPIDS have been shown to be intermediates in the formation of cell-wall components for several micro-organisms.[1–6] A glucolipid has been reported as an intermediate in the biosynthesis of cellulose by *Acetobacter xylinum*.[7] By use of a cellulose-synthesizing enzyme system from *Acetobacter*, Colvin[8] has observed the synthesis of cellulose from precursors extracted from plant sources with ethanol.

In contrast, from plants, the substrates for enzyme systems that have been reported to catalyze the synthesis of cell-wall polysaccharides[9–16] are glycosyl esters of nucleotides. Although the evidence does not preclude the existence of glycolipid intermediates, positive indication of their involvement in the biosynthesis of plant cell-wall polysaccharides has not been obtained as yet. In this report, we describe the formation of a glucolipid from UDPG, the synthesis of which is catalyzed by an enzyme from *Phaseolus aureus* that appears to be

* Supported in part by a grant from the U.S. Atomic Energy Commission No. AT(11-1)-1426.

† Present address: Department of Chemistry, Ohio University, Athens, Ohio 45701.

‡ NSF Undergraduate Research Participant, University of Colorada, 1967.

[1] J. S. ANDERSON, M. MATSUHASHI, M. A. HASKIN and J. L. STROMINGER, *Proc. Nat. Acad. Sci. U.S.* **53**, 881 (1965).
[2] M. MATSUHASHI, C. P. DIETRICH and J. L. STROMINGER, *Proc. Nat. Acad. Sci. U.S.* **54**, 587 (1965).
[3] I. M. WEINER, T. HIGUCHI, L. ROTHFIELD, M. SALTMARSH-ANDREW, M. J. OSBORN and B. L. HORECKER, *Proc. Nat. Acad. Sci. U.S.* **54**, 228 (1965).
[4] A. WRIGHT, M. DANKERT and P. W. ROBBINS, *Proc. Nat. Acad. Sci. U.S.* **54**, 235 (1965).
[5] A. WRIGHT, M. DANKERT, P. FENNESSEY and P. W. ROBBINS, *Proc. Nat. Acad. Sci. U.S.* **57**, 1798 (1967).
[6] Y. HIGASHI, J. L. STROMINGER and C. C. SWEELEY, *Proc. Nat. Acad. Sci. U.S.* **57**, 1878 (1967).
[7] J. R. COLVIN, *Nature* **183**, 1135 (1959).
[8] J. R. COLVIN, *Can. J. Biochem. Physiol.* **39**, 1921 (1961).
[9] D. S. FEINGOLD, E. F. NEUFELD and W. Z. HASSID, *J. Biol. Chem.* **233**, 783 (1958).
[10] G. A. BARBER, A. D. ELBEIN and W. Z. HASSID, *J. Biol. Chem.* **239**, 4056 (1965).
[11] D. A. BRUMMOND and A. P. GIBBONS, *Biochem. Z.* **342**, 308 (1965).
[12] C. L. VILLEMEZ, T. Y. LIN and W. Z. HASSID, *Proc. Nat. Acad. Sci. U.S.* **54**, 1626 (1965).
[13] C. L. VILLEMEZ, A. L. SWANSON and W. Z. HASSID, *Arch. Biochem. Biophys.* **116**, 446 (1966).
[14] R. W. BAILEY and W. Z. HASSID, *Proc. Nat. Acad. Sci. U.S.* **56**, 1586 (1966).
[15] C. L. VILLEMEZ, G. FRANZ and W. Z. HASSID, *Plant Physiol.* **42**, 1219 (1967).
[16] J. M. MCNAB, C. L. VILLEMEZ and P. ALBERSHEIM, *Biochem. J.* **106**, 355 (1968).

located in the same subcellular body[17] as that responsible for the synthesis of cell-wall polysaccharides.

The enzyme was found to be present in the 10,000 × *g* sediment of the extract from shoots of 2–3-day-old germinated *P. aureus*. The enzyme was incubated with ^{14}C UDPG and the chloroform–methanol-soluble compounds extracted and examined by TLC.

The radioactive lipid material was immobile when the chromatogram was developed with chloroform or with 70:30:1 hexane–ether–acetic acid. Development with 80:16:4 chloroform–methanol–acetone resulted in a single spot of radioactivity at the solvent front. However, development with 19:1 chloroform–methanol separated the radioactive lipid zone into two compounds (Fig. 1) having R_f values of 0·59 and 0·21 respectively. The entire lipid extract was then purified by this procedure, and the two radioactive components were examined separately.

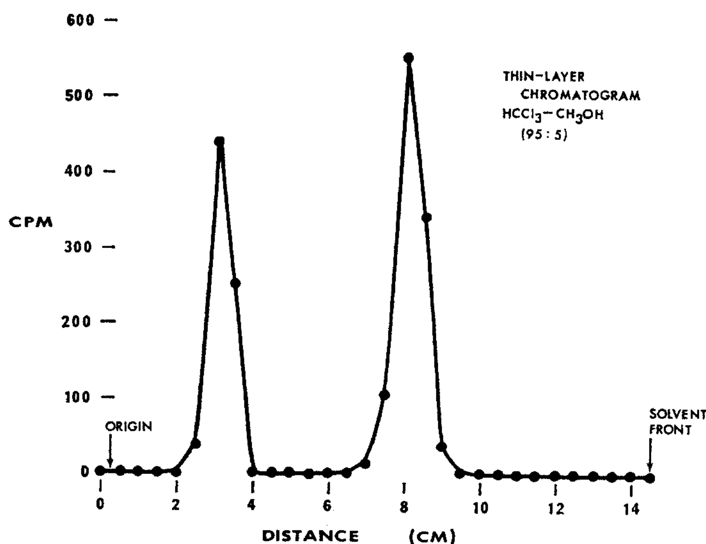

FIG. 1. CHROMATOGRAPHIC PURIFICATION OF GLUCOLIPID.

A small aliquot of glucolipid-^{14}C in chloroform was spotted on a thin layer of silica gel and developed with 19:1 chloroform–methanol. The chromatogram was cut into 0·5 cm strips and counted on a scintillation counter.

Samples were suspended in 40 per cent methanol and treated with various concentrations of HCl for 30 min at 95° in a sealed tube, and subjected to descending paper chromatography for 10–13 hr in 10:4:1 ethyl acetate–pyridine–water. In this solvent system, the untreated lipid moved with the solvent front, whereas D-galactose, D-glucose, and D-mannose were clearly separated from each other and from D-xylose. No hydrolysis occurred with 0·01 N or 0·1 N HCl; both compounds were hydrolyzed to *ca.* 75 per cent by 1 N acid, and hydrolysis was essentially complete with 2 N HCl for 2·5 hr. The major part of the radioactive, hydrolysis product from both lipids moved in a manner identical to that of authentic D-glucose. The only other product observed on hydrolysis of either lipid was methyl D-glucoside, which apparently resulted from reaction with methanol since authentic D-glucose produced approximately the same percentage of methyl D-glucosides under similar conditions.

[17] C. L. VILLEMEZ, J. M. MCNAB and P. ALBERSHEIM, *Nature*, in press.

Furthermore, hydrolysis of an aqueous suspension of each lipid with 2 N trifluoroacetic acid[18] resulted in only one hydrolytic product. This product had a chromatographic mobility identical to that of authentic D-glucose.

The single, radioactive, hydrolysis product from each lipid was then reduced with excess sodium borohydride for 1 hr at room temperature. After completion of the reaction, the solution was treated with Dowex 50 in the presence of methanol, and methyl borate was removed under vacuum. The reaction mixture was spotted on a paper chromatogram, and developed for 12 hr in 9 : 1 : 1 2-butanone–acetic acid–water presaturated with boric acid. The reaction product moved as a single spot, identical to that of authentic D-glucitol. Hence, the sole radioactive sugar present in each lipid is D-glucose.

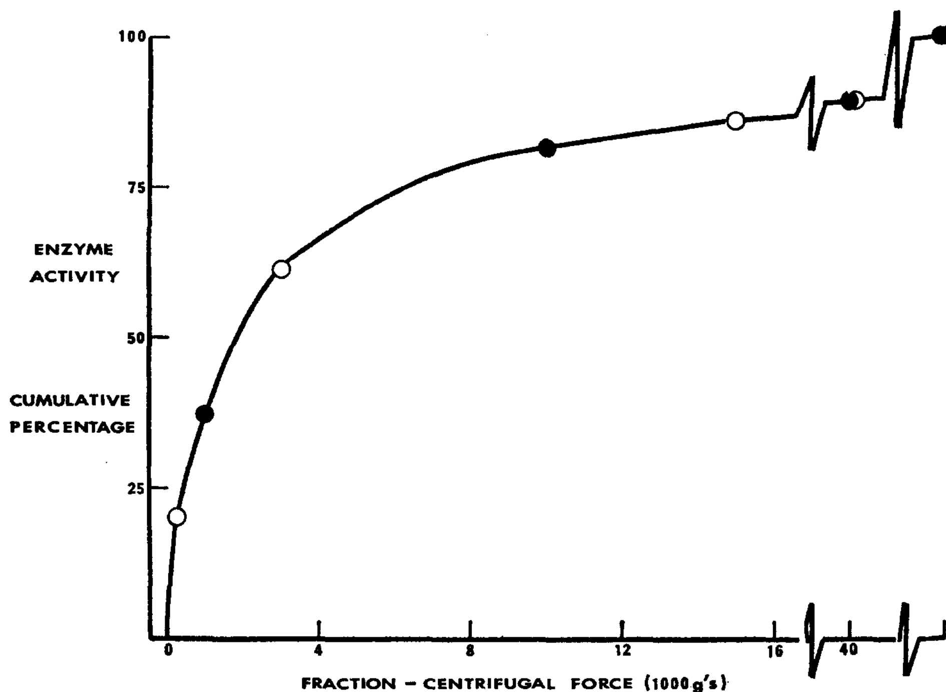

FIG. 2. DISTRIBUTION OF POLYGALACTURONIC ACID SYNTHETASE AND GLUCOLIPID-FORMING ENZYME AMONG PARTICULATE FRACTIONS.

The enzyme for glucolipid formation was prepared as described in the text, except that sequential centrifugal sediments at 1000, 10,000, 40,000 and 100,000 *g* were used as enzyme sources. A similar experiment for polygalacturonic acid synthetase was performed, using sequential sediments at 270, 3000, 15,000, 40,000 and 100,000 *g* as enzyme sources. Assay for glucolipid synthesis was performed as follows. To 0·1 ml of enzyme solution was added 0·18 μg (30,000 cpm) of UDPG-^{14}C to yield a mixture of 0·11 ml total volume. Each reaction mixture was incubated at 25° for the time indicated. The analysis was performed as indicated in the text. Assay for polygalacturonic acid synthetase has been reported.[11] The data (cumulative percentage) are reported as that per cent of enzyme activity (in the total homogenate) that has sedimented at or below the centrifugal forces indicated.

Polygalacturonic acid synthetase = ○
Glucolipid synthetase = ●

[18] P. ALBERSHEIM, D. J. NEVINS, P. D. ENGLISH and A. KARR, *Carbohyd. Res.* 5, 340 (1967).

Enzyme Characteristics

The enzyme system that forms glucolipid from UDPG is present exclusively in the particulate fraction of the suspension. As was observed with polygalacturonic acid synthetase,[13] removal of the supernatant solution enhances the enzyme activity. The enzyme is rapidly inactivated at room temperature and with about the same time factor as polygalacturonic acid synthetase.[13] The variation of reaction rate with substrate concentration demonstrates a disproportionate increase in rate at substrate concentrations higher than 5×10^{-4} M. This peculiarity has also been observed for polygalacturonic acid synthetase[13] and galactan synthetase.[16] Finally, the distribution of enzyme activity among the various centrifugal fractions is remarkably similar to the distribution of polygalacturonic acid synthetase (Fig. 2). The similarity between these properties of the enzyme that forms glucolipid and those of enzymes that synthesize cell-wall polysaccharides[17] suggests that these properties may be a consequence of their residing in the same particulate body. If this is so, physical proximity to the cell-wall forming mechanism could imply a functional role of the glucolipid in this process. However, a function cannot be definitely assigned to this glucolipid at the present time.

METHODS

Enzyme Preparation

Seeds of *Phaseolus aureus* were germinated for 2–3 days. The shoots were separated from the cotyledons, and ground with sand and an equal weight of pH 7·4 buffer at 0°. The concentrations of the buffer components were: 0·05 M potassium phosphate, 0·5 M sucrose, 0·005 M $MgCl_2$, and 1 per cent bovine serum albumin (crystalline, Armour). The crude suspension was filtered through cheesecloth, and centrifuged for 5 min at 1000 ***g***. The sediment was discarded, and the supernatant solution centrifuged for 15 min at 10,000 ***g***; the resulting sediment was resuspended in one-fifth of the original volume of the homogenizing buffer, and this suspension was used as the source of the enzyme.

Glucolipid Preparation

To 1 ml of enzyme suspension was added 5 μCi of UDPG-^{14}C (0·02 μmole) to make a total volume of 1·1 ml. The mixture was incubated for 30 min at 26°, and the reaction was terminated by the addition of 1 ml of 1 N HCl. The reaction mixture was cooled to 0°, 5 ml of H_2O and 5 mg of cellulose powder were added, and the suspension was centrifuged for 15 min at 35,000 ***g***. The supernatant solution was discarded, and the pellet was resuspended n 5 ml of H_2O. The resuspended pellet was centrifuged for 15 min at 10,000 ***g***, and the supernatant solution was discarded; this step was repeated. The pelleted material was then extracted with 5 ml aliquots of 1 : 1 chloroform–methanol until no further radioactive material was extracted; two extractions were usually sufficient. The lipid extracts were combined, and evaporated to dryness under vacuum. The dried lipid was redissolved in 1·5 ml of chloroform and stored at 0°.

Identification of Radioactive Sugar Moiety

The homogeneity of the radioactive lipid material was examined by thin-layer chromatography (silica gel, Eastman).