THE ENZYMATIC SYNTHESIS OF CELLULOSE BY THE HIGHER PLANT LUPINUS ALBUS

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The cell free synthesis of cellulose from glycosyl donors has been reported for bacteria (Glaser, 1958) and mung beans (Elbein et al, 1964). The glucosyl donors were uridine diphosphate glucose (UDPG) for the bacterial preparation and guanosine diphosphate glucose (GDPG) for the mung bean system. UDPG did not serve in this capacity for the latter enzyme. This communication presents evidence that a particulate enzyme preparation from etiolated lupin hypocotyls forms cellulose from UDPG, forms it in greater amounts from this substrate than from GDPG at concentrations of 10^{-3} M and higher under the assay conditions employed, and that cellulose synthesis from these two donors appears to be catalyzed by separate enzymes.

Experimentally, seeds of lupinus albus were soaked in distilled water overnight and grown in the dark for 8 to 10 days on moist sand. The excised hypocotyls were ground in a mortar with sand along with 1.0 gram of $KHCO_3$ per 100 grams of hypocotyls. After straining through cheese cloth, the brei was centrifuged at 10,000 x g for 25 minutes. The pellet was suspended in 0.05 M Tris buffer, pH 8.0 and centrifuged as before. The combined supernatant fractions, centrifuged at 140,000 x g for 2 hours yielded a pellet which, when resuspended in the same buffer and recentrifuged, gave a final pellet which served as the enzyme source. UDPG-C^{14} was prepared enzymatically (Glaser, 1958; Rabinowitz and Goldberg, 1963) from uniformly labelled glucose-C^{14}. GDPG-C^{14} was synthesized by the method of Khorana,

(Roseman, et al 1961) from C^{14} labelled glucose-1-phosphate which had been prepared by the enzymatic hydrolysis (Brummond and Gibbons, 1962) of UDPG-C^{14}. Both nucleotides were separated chromatographically (Glaser, 1958) on Dowex-1, and further purified by paper chromatography (Roseman, et al 1961).

The reaction mixtures were incubated for one or two hours at 30°, after which time they were heated to 100° for 3 minutes, lyophylized to dryness, and 250 mg Whatman powdered cellulose added along with 1.2 ml. of a mixture of 1 part by volume acetic anhydride and 0.09 parts sulfuric acid (Braun, 1947). After 8 days incubation at 35° the cellobiose octa-acetate was isolated and recrystallized and counted 4 or 5 times. Radio-activity determinations were carried out at infinite thickness under a micromil window gas flow counter. A factor for the determination of the percent glucose-C^{14} converted to cellulose C^{14} was obtained by combustion of samples of glucosyl donor and of cellobiose octaacetate to CO_2 and counting the respective $BaCO_3$ plates.

The results of two experiments are presented in Table I. In experiment I, a comparison was made of the extent of C^{14} glucose incorporation from UDPG and GDPG at several substrate concentrations. In experiment II a comparison of the extent of cellulose synthesis from these substrates alone and in combination was made. In experiment I, 3 to 7% of the glucose added as UDPG-C^{14} was incorporated into cellulose while from 2 to 8% of that from GDPG was incorporated. Zero time controls showed no incorporation. In experiment II, in which about 2.5% of the added C^{14} from UDPG was incorporated into cellulose, it was observed that the C^{14} incorporation from these two substrates was nearly additive. That this increase in counts arose via independent synthesis from both substrates rather than by stimulation attributable to the presence of the other nucleotide is indicated by the lack of stimulating effect of added unlabelled UDPG and GDPG, lines 9 and 10.

TABLE I*

Substrate	c/m observed in cellobiose octaacetate	μmoles glucose incorporated (calculated)
Experiment I		
1. .001 M UDPG-C^{14}	406	.070
2. .003 M UDPG-C^{14}	242	.120
3. .006 M UDPG-C^{14}	183	.180
4. .00065 M GDPG-C^{14}	518	.050
5. .003 M GDPG-C^{14}	171	.080
6. .0052 M GDPG-C^{14}	106	.100
Experiment II		
7. .001 M UDPG-C^{14}	154	.025
8. .001 M GDPG-C^{14}	90	.013
9. .001 M UDPG-C^{14} + .001 M GDPG	115	.020
10. .001 M UDPG + .001 M GDPG-C^{14}	97	.016
11. .001 M UDPG-C^{14} + .001 M GDPG-C^{14}	203	.034

*Experiment I

The reaction mixture contained in a volume of 1.0 ml the following ingredients: 100 μmoles of Tris buffer, pH 8.0, 20 μmoles of $MgCl_2$, 10 μmoles cysteine, UDPG and GDPG at the concentrations indicated, UDPG-C^{14}, 375,000 c/m in tubes 1, 2 and 3 and GDPG-C^{14}, 400,000 c/m in tubes 4, 5 and 6, 34 mg of soluble cellulose dextrans and 0.30 ml. of enzyme representing 6.7 ml of filtered brei which contained about 4 mg of protein. The mixture was allowed to react for 2 hours at 30°; stopped by heating to 100° for 3 minutes, dried, and cellobiose octaacetate prepared in the presence of carrier cellulose.

Experiment II

The reaction mixture contained the same ingredients as in experiment I, but 54 mg. of cellulose dextrans were present in all tubes except #8, to which none was added, 400,000c/m of UDPG-C^{14} was present in each tube containing UDPG-C^{14}, and 0.4 ml of enzyme representing 8 ml. of filtered brei with about 4 mg. protein. The reaction mixture was incubated for 1 hour at 30°, and cellulose synthesis determined as in Experiment I.

That the C^{14} detected in the cellobiose octaacetate was not a contaminant was established by several independent procedures. Five additional recryatallizations did not affect the specific activity. Deacetylation (Braun, 1947) and recrystallization of the cellobiose 4 times yielded a preparation with radioactivity very close to that of the octaacetate. Hydrolysis of the cellobiose with emulsin (Worthington), stopped when 85 % complete, yielded glucose which, when isolated as its osazone, and recrystallized 3 times had an activity which again was almost identical to that of

the octaacetate. The emulsin hydrolysis served to confirm that the synthesized material was of a β configuration. Further characterization of the water insoluble dextrans produced by this enzymatic reaction with UDPG-C^{14} as substrate was afforded by the degradation according to Wolfrom et al (1959). The glyoxylic acid (2,4 dinitro phenylhydrazone) and the D-erythronic acid (1-4 lactone) were isolated in the presence of carrier cellulose. Once more the expected radioactivity was retained in the derivatives. As a final check on the potential effect of contamination by coexisting synthesized laminarin, a sample of C^{14} labelled laminarin prepared by a spinach leaf enzyme as described by Feingold et al (1958) was added to a reaction mixture, cellobiose octaacetate prepared and recrystallized 4 times. Less than 0.2% of the counts from the laminarin were carried into the final product.

Of the synthesized polysaccharide obtained from UDPG about 1/3 to 1/2 was not soluble in water. Half of the water insoluble fraction was not solubilized by heating at 100° for 5 minutes with 2% NaOH.

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