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BIOSYNTHESIS OF CELLULOSE IN GROWING COTTON HAIRS

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Abstract—The utilization of UDPG-^{14}C and glucose-^{14}C as precursors of cellulose has been studied by incorporation experiments with cotton hairs at different stages of growth. It could be shown that the sugar nucleotide UDPG serves as an excellent substrate for cellulose biosynthesis *in vivo*. This is in agreement with results previously obtained *in vitro*. Free glucose is incorporated to a lesser extent.

INTRODUCTION

THE SUGAR nucleotide uridinediphosphate glucose (UDPG) has been isolated from a great number of growing plant tissues which are actively forming cellulosic cell walls, e.g. from seedlings of *Phaseolus aureus*,[1] and of *Avena sativa*,[2] and also from growing cotton hairs.[3]

Since it has been shown that UDPG serves as a glucosyl donor in the *in vitro* synthesis of cellulose using enzymes from higher plants,[4–6] it seemed of interest to obtain information about the *in vivo* biosynthesis of cellulose by radioactive incorporation studies of the same nucleotide.

RESULTS AND DISCUSSION

Andrews, Hough and Picken[7, 8] were able to demonstrate the incorporation of radioactive glucose into the cellulose and hemicellulose fractions of plum leaves. Isolated cotton hairs are a more useful material for the study of cellulose biosynthesis, since they consist mainly of cellulose and contain only very little pectin and hemicelluloses. They can easily be obtained in a living, although physiologically somewhat altered state, by opening the cotton bolls and separating the hairs from the seeds to which they are attached.

It was first shown that the rate of incorporation of the glucose moiety of the UDPG was approximately proportional to the amount of the acceptor (cotton hairs) offered (Fig. 1). All further experiments were carried out using 5 mg of the hairs.

The rates of incorporation of both UDPG-^{14}C and glucose-^{14}C were compared under identical conditions (Fig. 2). It was shown that the glucose moiety of UDPG-^{14}C was incorporated to a much larger extent into the alkali-insoluble residue of cotton hairs than was free glucose-^{14}C. The rate of incorporation of glucose from UDPG increases up to 60 min.

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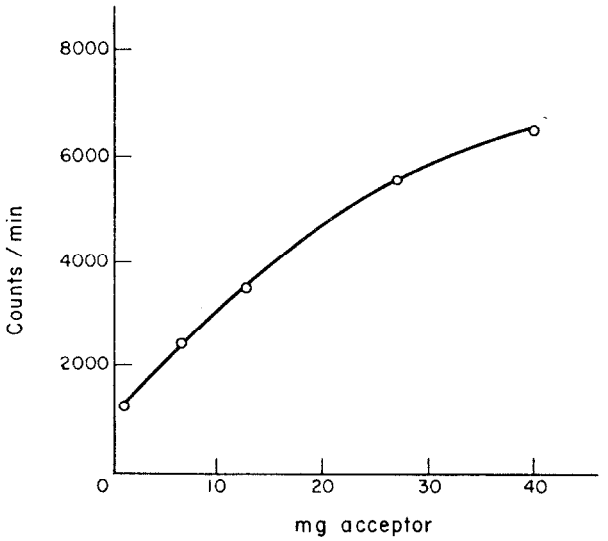

FIG. 1. INCORPORATION OF A FIXED AMOUNT OF UDPG-^{14}C INTO THE ALKALI-INSOLUBLE RESIDUE OF COTTON HAIRS WITH INCREASING WEIGHT OF HAIRS. THE HAIRS WERE TAKEN FROM THE SAME 25-DAY-OLD COTTON BOLL.

Time of incubation: 30 min. The incubation mixture contained 50 μl tris-maleate buffer, pH 6·5, 10 μl 0·1 M $MgCl_2$, $2{\cdot}5 \times 10^{-4}$ μM UDPG-^{14}C made up to a final volume of 100 μl.

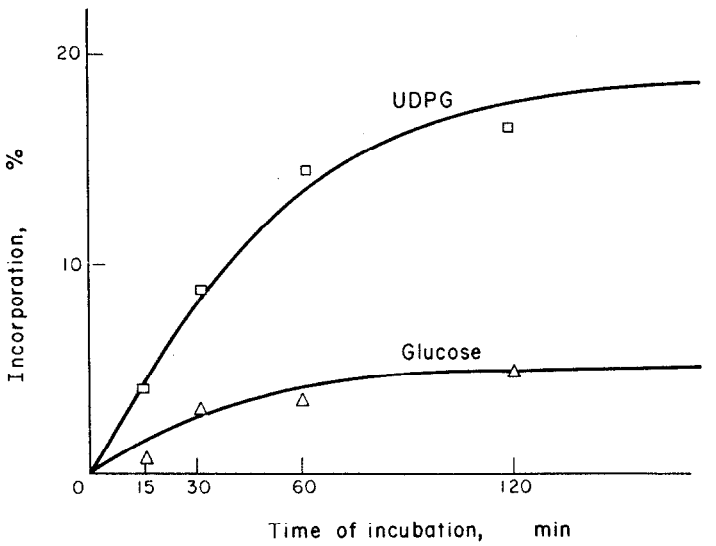

FIG. 2. RATE OF INCORPORATION OF UDPG-^{14}C AND GLUCOSE-^{14}C INTO 5 mg OF THE ALKALI-INSOLUBLE RESIDUE OF COTTON HAIRS (25-DAY-OLD).

Incubation mixture as in Fig. 1.

After this time the curve flattens out and after 120 min shows no further rise. This might be due to inactivation of the cellulose synthetase, since the physiological condition of the cotton hairs is changed after detachment from the plant.

The alkali-insoluble residue, obtained from 25-day-old cotton hairs which had been

incubated for 30 min with UDPG-^{14}C, was subjected to total acid hydrolysis, and the only radioactive sugar released was glucose (Fig. 3A). After 60 min incubation, however, both galactose and xylose, derived from a small amount of alkali-insoluble hemicelluloses, were also found to be radioactive (Fig. 3B).

When the material from the 30-min incubation was subjected to a partial acid hydrolysis several compounds, including cellobiose, were released (Fig. 4). Cellobiose was identified by co-chromatography and co-electrophoresis and by the identification of glucose on hydrolysis.

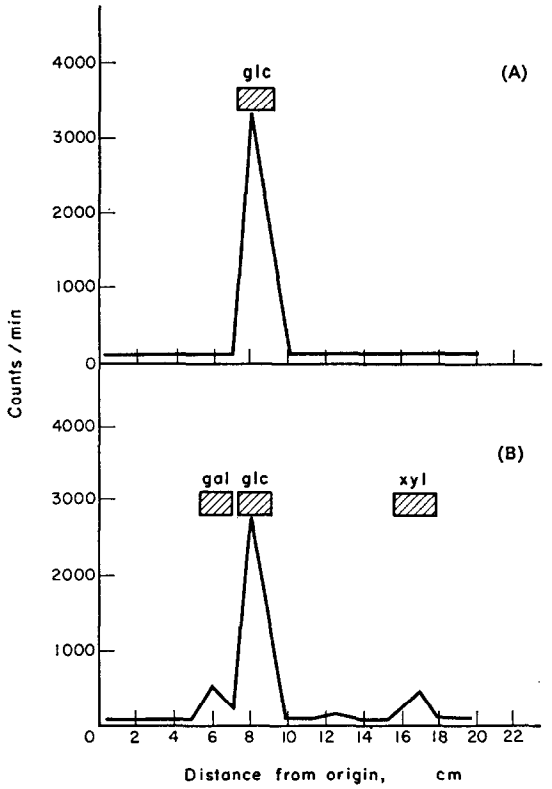

FIG. 3. DISTRIBUTION OF RADIOACTIVITY IN THE HYDROLYSATE OF THE ALKALI-INSOLUBLE RESIDUE OF COTTON HAIRS AFTER INCUBATION WITH UDPG-^{14}C AND SEPARATION BY PAPER CHROMATOGRAPHY IN SOLVENT I.

A: 30 min incubation; B: 60 min incubation.

Laminaribiose, which had been reported to be present in the partial hydrolysate of the alkali-insoluble residue after *in vitro* synthesis of cellulose with UDPG-^{14}C as glucosyl donor,[5] was not found in the present investigation (Fig. 4).

The incorporation of the radioactive substrates was also studied at different stages of growth of the cotton hairs. During the formation of the primary wall, which lasts about 25 days after flowering, cellulose is only formed in relatively small amounts. After 25–35 days the formation of the secondary wall takes place. This consists almost entirely of cellulose.[9] Incorporation of the glucose moiety of UDPG-^{14}C and of glucose-^{14}C into the alkali-insoluble part of 5-day-old cotton hairs is nearly the same at identical substrate concentrations

[9] P. SAKOSTSCHIKOFF and G. A. KORSHENIOVSKY, *Faserforschung* **9**, 249 (1931).

(Fig. 5). Thereafter with increasing age the incorporation of glucose-^{14}C hardly changes whilst that of the glucose moiety of UDPG-^{14}C increases greatly. The increased incorpora-

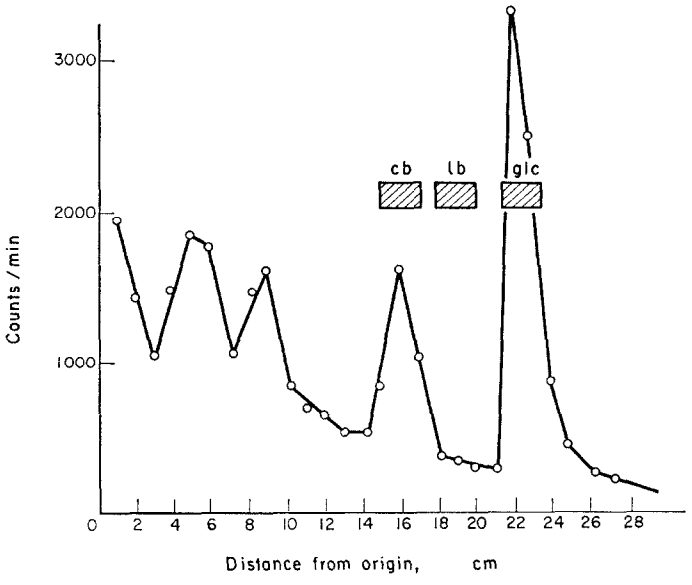

FIG. 4. DISTRIBUTION OF RADIOACTIVITY IN THE PARTIAL HYDROLYSATE OF THE ALKALI-INSOLUBLE RESIDUE OF COTTON HAIRS AFTER INCUBATION WITH UDPG-^{14}C AND SEPARATION BY PAPER CHROMATOGRAPHY IN SOLVENT II.

Reference sugars: cb=cellobiose; lb=laminaribiose; glc=glucose.

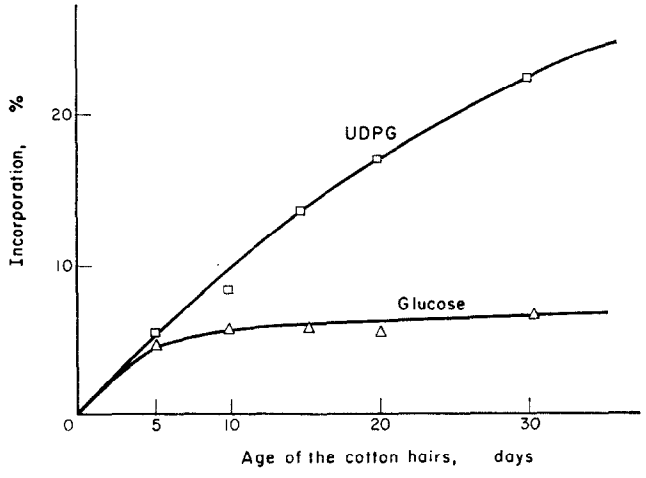

FIG. 5. INCORPORATION OF UDPG-^{14}C AND GLUCOSE-^{14}C INTO THE ALKALI-INSOLUBLE RESIDUE OF COTTON HAIRS DURING DIFFERENT STAGES OF GROWTH.

The composition of the incubation mixture is the same as in Fig. 1. Time of incubation: 60 min.

tion of glucose from UDPG can be explained by the growing activity of the cellulose synthetase during the late stages of primary wall formation and especially at the beginning of the secondary wall formation. Similar results were obtained with the *in vitro* biosynthesis of

cellulose using particulate enzyme preparations of *Phaseolus aureus* seedlings at different ages.[10]

It might be questioned why as much radioactivity is incorporated into the alkali-insoluble material from glucose-^{14}C as from UDPG-^{14}C in very young cotton hairs. The reason is possibly that free glucose and UDPG are equally good precursors of the non-cellulosic polysaccharides that form a large proportion of the 4% NaOH-insoluble residue of very young cotton hairs. These non-cellulosic materials consist mainly of xylans and pectic substances.[3]

EXPERIMENTAL

Radioactive glucose-^{14}C (320 mc/mM) and UDPG-^{14}C (194 mc/mM) were purchased from the Radiochemical Centre, Amersham, England.

Cotton hairs during different stages of growth were prepared from freshly harvested cotton bolls. The cotton bolls were opened, the hairs detached from the seeds and directly transferred to the incubation medium, which consisted of 50 μl tris/maleate buffer, pH 6·5, 10 μl 0·1 M $MgCl_2$ and the radioactive substrate made up to a total volume of 100 μl. The incubation was carried out at 30° and terminated by adding 2 ml hot EtOH to the mixture. After centrifugation of the incubation mixture the cotton hairs were washed several times, first with EtOH and then with H_2O, centrifuged, suspended in 4% NaOH and heated for 10 min at 100°. The alkali-insoluble residue was washed successively with H_2O and EtOH, collected and dried.

For measurements of the radioactivity, the ^{14}C-labelled material was burnt in O_2[11] and the $^{14}CO_2$ produced trapped in ethanolamine/MeOH mixed with the scintillator solution, 0·8% butyl-phenylbiphenyl-oxadiazole in toluene and measured in a Tri-Carb liquid scintillation spectrometer (model 314-AX, Packard Instrument Co.). For the determination of radioactivity on paper chromatograms, the paper was cut into 1 cm strips, which were burned and counted by the above method.

For total hydrolysis of the alkali-insoluble residue of the cotton hairs, the material was dissolved in 72% H_2SO_4 by heating at 30° for 60 min. The solution was diluted with water to 4% H_2SO_4 and heated at 120° for 1 hr. After neutralization ($Ba(OH)_2$) the hydrolysate was chromatographed on paper in the solvent system ethyl acetate–pyridine–water, 8:2:1 (v/v) (solvent I). Partial hydrolysis was carried out in fuming HCl saturated at −18°.[12] The partial hydrolysate was dried (KOH) *in vacuo* and chromatographed in the solvent systems ethyl acetate–pyridine–water, 2:1:2 (v/v) upper phase (solvent II), and *n*-propanol–ethyl acetate–water, 7:1:2 (v/v) (solvent III). Paper electrophoresis was carried out at 50 V/cm in 0·5 M borate buffer (pH 10).

Acknowledgement—The Tri-Carb liquid scintillation spectrometer was a generous gift of the Sandoz Stiftung zur Förderung der medizinisch biologischen Wissenschaften.

[10] G. Franz, *Verhandl. Schweiz. Naturforsch. Ges.* **147**, 134 (1968).
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[12] D. S. Feingold, E. F. Neufeld and W. Z. Hassid, *J. Biol. Chem.* **233**, 783 (1959).