Preliminary Notes

The enzymic synthesis of cellulose by *Acetobacter xylinum*

Acetobacter xylinum has been shown to produce cellulose from glucose in resting cultures[1] and in suspensions of non-viable lyophilized cells[2]. This note reports the synthesis of cellulose by a cell-free particulate system from *Acetobacter xylinum* (American Type Culture Collection, No. 10821).

Cells were grown and freed of cellulose as described by HESTRIN AND SCHRAMM[2] and were ruptured by 30 min treatment in a 9 kc magnetostriction oscillator. Cell debris was removed by centrifugation at 12,000 × *g* for 12 min and the turbid supernatant fluid centrifuged at 140,000 × *g* for 1 h. The high speed pellet was washed by centrifugation in 0.05 *M* tris(hydroxymethyl)aminomethane (Tris)-0.01 *M* $MgCl_2$-0.001 *M* versene. Finally it was suspended in the same buffer and used in the experiments described below.

As shown in Table I, incubation of the enzyme with ^{14}C-glucose-labelled uridine diphosphoglucose (UDPG) gave rise to a water-insoluble, alkali-insoluble material which was radioactive. ^{14}C-labelled α-glucose-1-phosphate (G-1-P) and ^{14}C-labelled glucose were inactive in this system.

TABLE I

THE ENZYMIC SYNTHESIS OF CELLULOSE

Radioactive substrate	*Time of incubation (min)*	*^{14}C in cellulose (c.p.m.)*
UDPG 3.5 μmoles, 83,500 c.p.m.	0	6.2
UDPG 3.5 μmoles, 83,500 c.p.m.	120	1220
G-1-P 5.1 μmoles, 87,000 c.p.m.	120	22
Glucose 7.1 μmoles, 78,000 c.p.m.	120	0

Reaction mixture: Cellodextrins, 12 mg; Tris, 80 μmoles; $MgCl_2$, 7 μmoles; versene, 0.7 μmole; enzyme and radioactive substrate in a final volume of 1.8 ml, pH 8.2, 28° C. After addition of 10 mg carrier cellulose (Whatman cellulose powder), the reaction was stopped by heating at 100° C for 5 min. The denatured protein and cellulose were removed by centrifugation and washed with 2 ml water. The residue was then suspended in 2 ml 1% NaOH and heated at 100° C for 5 min. The insoluble material after alkaline digestion was washed by centrifugation 3 times with 2 ml water and a suitable aliquot of an aqueous suspension was plated and counted.

For the identification of the ^{14}C-labelled material as cellulose, the product of a large scale incubation was partially acid hydrolyzed by the procedure of ZECHMEISTER[3]. The partial acid hydrolysate was shown to contain by paper chromatography (butanol/pyridine/H_2O, 6:4:3) glucose, cellobiose, and higher molecular weight material. The cellobiose eluted from the paper was crystallized after addition of authentic cellobiose as carrier, and its specific activity remained essentially constant during four recrystallizations.

The formation of insoluble cellulose has been found to be stimulated from 5- to 20-fold by the addition of high molecular weight soluble cellodextrins prepared by the method of ZECHMEISTER[3]. In addition to insoluble cellulose the enzyme appears to form soluble cellodextrins.

The enzyme catalyzing the synthesis of UDPG from G-1-P and uridine triphosphate[4] has been shown to be present in the supernatant fraction, after centrifugation at 140,000 × *g*.

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Received May 27th, 1957