# GLYCEROL ACTIVATION OF GLUCAN SYNTHESIS IN CELL-FREE PREPARATIONS FROM LUPINUS ALBUS

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Cell-free systhesis of β-1,3 and β-1,4 glucans was reported by Feingold, Neufeld and Hassid (1958), Barber, Elbein and Hassid (1964), Brummond and Gibbons (1965), Ordin and Hall (1967), and Villemez, Franz and Hassid (1967). Feingold et al. obtained the best incorporation, in Phaseolus preparations, with additives laminaribiose or glucose. Glucose promoted synthesis without being incorporated into the β-glucan. Feingold (1966) suggested that the glucose may have an allosteric regulatory effect on synthethase enzymes. Attempting to improve recovery of the glucan synthesizing system from plants, we used glycerol as the solvent in a "non-aqueous" extraction. We found that glycerol is a powerful activator of glucan synthesis in cell-free preparations from plants, including Lupinus.

## METHODS AND MATERIALS

Lupinus albus plants were grown for 5 to 7 days on vermiculite in a dark growth chamber at 26°C. Hypocotyls were ground in a mortar with 1% (w/w) $NaHCO_3$ and 5% polyvinylpyrrolidone (PVP 40; Sigma Chemical Co.). The filtrate obtained by squeezing the brei through cheesecloth was centrifuged at 1000 × g for 10 min. The supernatant was centrifuged at 12,000 × g for 20 min, and the resulting pellet was washed 3 times by suspending in distilled water and recentrifuging at 34,800 × g for 20 min. The washed pellet, suspended in 0.1 M tris-HCl buffer, pH 8, is termed the "particulate transferase." Extractions were done at 5°C.

Each reaction tube received 0.5 ml of "particulate transferase" corresponding to 2 to 8 g fresh weight of tissue and containing 0.5 to 2 mg of protein, determined by the Folin method (Lowry et al., 1951). In addition each tube, unless otherwise specified, received 0.5 ml of incubation mixture containing UDP-glucose-$^{14}$C (glucose-$^{14}$C, uniformly labeled; 213 mc/mM; initial counts varying in different experiments between 5,000 and 6,000 cpm), $MgCl_2$ (15.2 mM), cysteine (6.8 mM) and unlabeled UDP-glucose (1.4 mM). Glycerol-2-$^{14}$C (10 mc/mM) was also used. Radioactive compounds were from New England Nuclear Corp.

Enzyme-substrate mixtures were incubated at 22°C for 1 hr on a shaker. The reaction was terminated by adding 1 ml of hot 4% NaOH to each tube, which was placed in a boiling water bath for 10 min to hydrolyse alkali-soluble polysaccharides. The insoluble material was collected on glass fiber filter discs (Reeve Angel, 934 AH), washed with 1 ml 1N HCl and two 1 ml lots of distilled water. The radioactivity of the dried filter discs was counted by liquid scintillation. Final counts were little affected by procedural modifications such as doubling the NaOH concentration, including a hot ethanol wash, or omitting the acid wash. The acid wash expedited filtration. Radioactivity was decreased by 70% if the product was incubated with glucanase (Aspergillus niger preparation from Worthington Biochemical Co., containing β-1,3- as well as β-1,4-glucanase).

## RESULTS AND DISCUSSION

Stimulation of glucan synthesis was obtained at glycerol concentrations between $1.36 \times 10^{-2}$M and 1.36 M (Fig. 1A). A glycerol concentration of 0.68 M was selected for subsequent experiments.

Incorporation of labeled substrate into glucan was time-dependent (Fig. 1B). The decline in radioactivity which follows a peak at 120 min suggests the presence of a particulate-bound hydrolytic enzyme like the β-1,3-glucanase found in a similar preparation from mung beans (Feingold et al., 1958). If the 1 hr

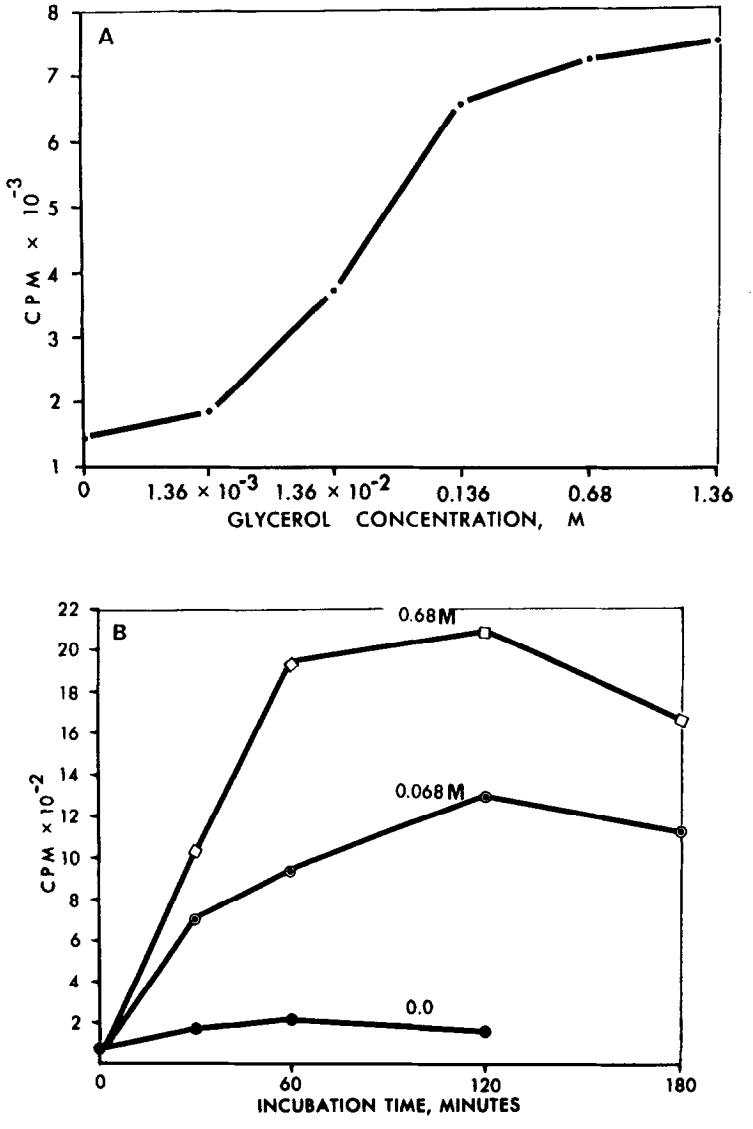


Fig. 1. Glycerol stimulation of glucan synthesis: (A) Effect of glycerol concentration on incorporation of UDP-glucose-$^{14}$C, plotted on an expanded scale; (B) Effect of glycerol concentration on the time course of glucan synthesis.

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incubation period was followed by a 4 hr incubation at 50°C, a temperature which promotes β-1,3-glucanase activity (Mandels, Parrish and Reese, 1967), then up to 80% of the glucan synthesized in the presence of glycerol was lost. Boiling prior to the 50°C incubation prevented glucan loss.

The effect of substrate concentration on polymer synthesis with and without added glycerol is shown in Fig. 2. Glycerol strongly enhanced the enzyme response to increasing substrate concentrations. In other experiments using both glycerol and unlabeled UDP-glucose and higher enzyme concentrations, incorporation of UDP-glucose-$^{14}$C into polymer approached 100% efficiency.

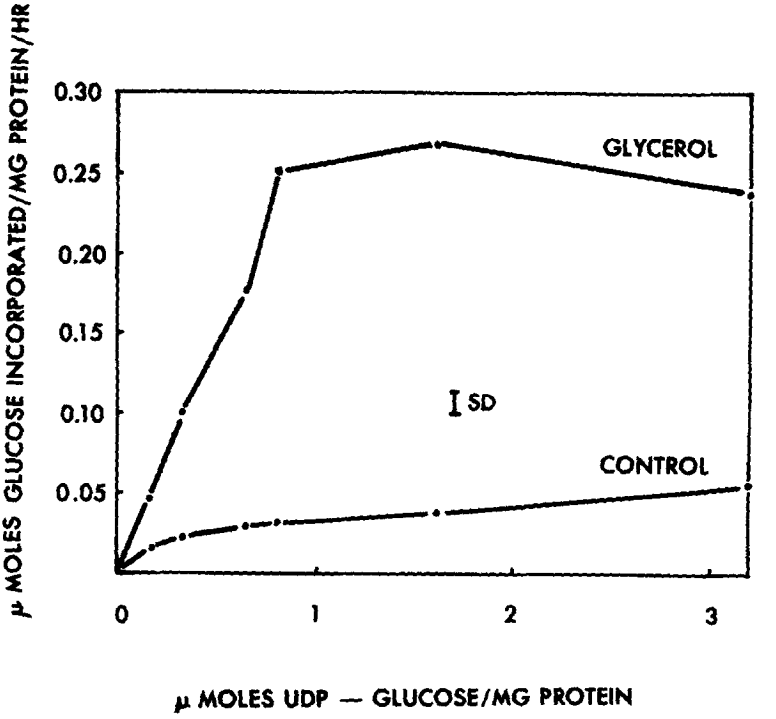


Fig. 2. Effect of UDP-glucose concentration on glucan synthesis in the presence and absence of glycerol (0.68M).

An experiment using glycerol-$^{14}$C (Table I) showed that while glycerol stimulates glucan synthesis, the amount of label from glycerol that becomes associated with the glucan pellet is small. In other experiments labeled glycerol added to the systems containing UDP-glucose-$^{14}$C did not increase the radioactivity of the product.

The glucan material synthesized in the presence of glycerol differs from that formed without glycerol. Glycerol-stimulated polymer was susceptible to hydrolysis by exogenous β-1,3-glucanase (from Rhizopus arrhizus QM 1032) or by the glucanase associated with the synthesizing system. Polymer made in the

TABLE I

TEST FOR INCORPORATION OF GLYCEROL INTO GLUCAN

| Radioactive Label | Glycerol Concentration, M | cpm incorporated per mg protein |
|---|---|---|
| Glycerol-$^{14}$C (2412 cpm/mg protein) | 0.0 | 13 ± 9* |
| " " | 0.68 | 14 ± 6 |
| UDP-glucose-$^{14}$C (2524 cpm/mg protein) | 0.0 | 690 ± 26 |
| " " | 0.68 | 2088 ± 195 |

* Means of 3 replicates, corrected for background ± standard deviation.

absence of glycerol was completely resistant to such hydrolysis.

Glycerol stimulation of glucan synthesis may account for the observation (Hall and Ordin, 1967) that plant wall preparations showed low UDP-glucose pyrophosphorylase activity when extracted in the presence of glycerol. The enzymatic activity was determined by measuring glucose-1-P formed from UDP-glucose and pyrophosphate. It is possible that the low activity they observed in the presence of glycerol was due to stimulation of glucan synthesis, which would compete for UDP-glucose.

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