Biosynthesis of Bacterial Glycogen. III. The Adenosine Diphosphate–Glucose:α-4-Glucosyl Transferase of *Escherichia coli B**

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ABSTRACT: An enzyme transferring glucose from adenosine diphosphate–glucose to a primer to form α-1,4-glucosyl linkages has been partially purified from *Escherichia coli* B. Only the sugar nucleotides, adenosine diphosphate–glucose and deoxyadenosine diphosphate–glucose, could serve as the glucosyl donor. Other sugar nucleotides tested were inactive. The primer could be an α-1,4 glucan or an oligosaccharide of the maltodextrin series. The kinetic properties of the enzyme were studied; its pH optimum was from 7 to 9.5. The enzyme was inhibited by *p*-mercuribenzoate and by adenosine diphosphate.

The importance of adenosine diphosphate[1] (ADP)–glucose in the synthesis of α-1,4-glucans of plants and bacteria is well documented. Recondo and Leloir (1961) reported that ADP–glucose was a better glucosyl donor to starch than was UDP–glucose. The transfer of glucose from ADP–glucose to starch granules of rice grains (Murata *et al.*, 1964) and leaves (Murata and Akazawa, 1964) has been reported. Recently the presence of soluble transferases from extracts of spinach chloroplasts (Doi *et al.*, 1964; Ghosh and Preiss, 1965), tobacco leaves, potato tubers, sweet corn (Frydman and Cardini, 1964a, 1964b), and glutinous rice grains (Murata *et al.*, 1965) have been established.

In recent years many investigators have observed the accumulation of glycogen in various species of bacteria (Holmes and Palmistierna, 1956; Strange *et al.*, 1961; Stanier *et al.*, 1959; Mulder *et al.*, 1962). Glycogen accumulation also was shown to occur in certain strains of *Escherichia coli* which were deficient in the enzyme UDP–glucose pyrophosphorylase, thus suggesting that UDP–glucose was an unimportant precursor of glycogen in these strains (Sigal *et al.*, 1964). It has recently been shown that extracts of *Aerobacter aerogenes*, *Agrobacterium tumefaciens*, *Arthrobacter* sp. NRRL B1973, *Escherichia coli* B, *Micrococcus lysodeiktikus*, and *Rhodospirillum rubrum* catalyze the transfer of glucose from ADP–glucose to the ends of a glycogen primer (Greenberg and Preiss, 1964). UDP–glucose and TDP–glucose could not substitute for ADP–glucose as the glucosyl donor. The *Arthrobacter* ADP–glucose:glycogen transglucosylase was partially purified (Shen *et al.*, 1964; Greenberg and Preiss, 1965) and was shown not to be active with other glucose-containing nucleotides such as UDP–glucose, TDP–glucose, CDP–glucose, GDP–glucose, and IDP–glucose. Deoxy-ADP–glucose was the only other sugar nucleotide found that could substitute for ADP–glucose in the reaction. This paper reports the solubilization and partial purification of the ADP–glucose:α-1,4-glucan-4-glucosyl transferase of *E. coli* B. This study reveals that the properties of the *E. coli* enzyme are essentially similar to the *Arthrobacter* enzyme.

Experimental Section

Escherichia coli B was grown on nitrogen-limiting conditions with glucose as the carbon source (Sigal *et al.*, 1964). After the cells reached stationary phase they were harvested and frozen as a paste at −15°. Yeast mannan was prepared by the method of Peat *et al.* (1961). α-1,6-Dextrans were obtained from the Pharmachem Corp., Bethlehem, Pa. *E. coli* glycogen was a gift from Dr. Irwin H. Segel of the University of California, Davis. Panose (α-1,4-glucosylisomaltose) was a gift from Dr. S. C. Pan of the Squibb Institute, New Brunswick, N. J. Laminaribiose was obtained from Dr. David Feingold of the University of Pittsburgh, and sophorose was a gift from Dr. Gilbert Ashwell of the National Institutes of Health, Bethesda, Md. All other nonradioactive chemicals were either obtained from commercial sources or prepared as described earlier (Shen and Preiss, 1965). *Bacillus subtilis* crystalline α-amylase was obtained from Sigma Chemical Co., St. Louis, Mo., and β-amylase from Worthington Enzymes, Freehold, N. J. Glucose-^{14}C-1-P and GDP–glucose-^{14}C were obtained from the International Chemical and Nuclear Corp., City of Industry, Calif. ADP–glucose-^{14}C, deoxy-ADP–glucose-^{14}C, CDP–glucose-^{14}C, UDP–glucose-^{14}C, and TDP–glucose-^{14}C were prepared as previously described (Shen and Preiss, 1965). The glucose-^{14}C moiety of the above compounds was uniformly labeled.

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[1] Abbreviations used: ADP, UDP, TDP, CDP, GDP, and IDP, adenosine, uridine, thymidine, cytidine, guanosine, and inosine diphosphates, respectively; GSH, glutathione.

TABLE I: Partial Purification of the ADP–Glucose:Glycogen Transglucosylase.

Fraction	Volume (ml)	Activity (units/ml)	Total Activity (unit)	Protein (mg/ml)	Specific Activity (μmoles/mg)
32,000*g* particulate	21	9.7	202	25.0	0.39
32,000*g* supernatant fluid	80	31	2480	25.8	1.20
105,000*g* particulate	23	78.4	1795	0.34	2.3
105,000*g* supernatant fluid	110	4.5	495	11.2	0.40
Ammonium sulfate fractions particulate	19	4.8	91.5	14.0	0.34
Supernatant fluid	9.8	93.2	910	5.8	16.1

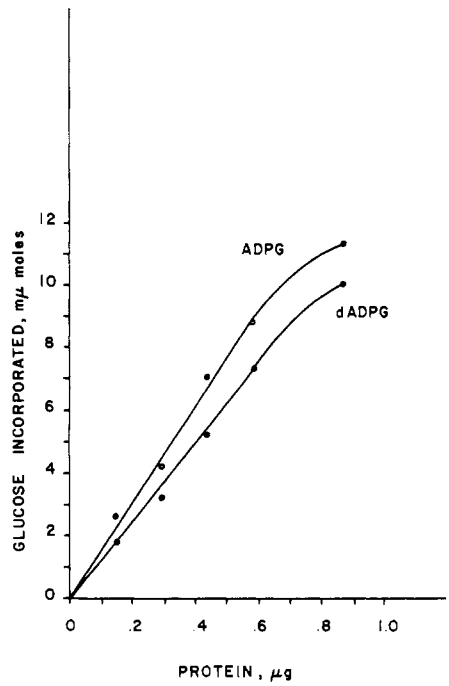

FIGURE 1: Linearity of transglucosylase activity with protein concentration. The conditions of the experiment were those in assay A.

TABLE II: Requirements for Transglucosylase Activity.[a]

Sugar Nucleotide	Omissions	Glucose-^{14}C Incorporated (mμmoles)
ADP–glucose	None	6.5
	–GSH	2.9
	–KCl	5.0
	–Bovine plasma albumin	5.5
	–GSH, KCl, albumin	1.2
	–Enzyme	<0.04
	–Glycogen	0.04
	–Enzyme + denatured enzyme	<0.04
Deoxy-ADP–glucose	None	4.7
	–Glycogen	0.04
	–Enzyme	<0.04
	–GSH, KCl, albumin	0.68

[a] The conditions of the experiment were those in assay A. The denatured enzyme was prepared by heating in a boiling water bath for 30 sec. The specific activities of ADP–glucose-^{14}C were 7.58×10^5 and 4.53×10^5 cpm/μmole, respectively.

The following solvent systems were used in descending paper chromatography on Whatman No. 1 filter paper: solvent A, ethyl acetate–pyridine–water (3.7:1.0:1.15); solvent B, 1-butanol–pyridine–water (6:4:3); solvent C, 1-propanol–ethyl acetate–water (7:1:2).

Assay of Transglucosylase. ASSAY A. Transglucosylase activity was studied by following the transfer of glucose-^{14}C from ADP–glucose-^{14}C or deoxy-ADP–glucose-^{14}C to glycogen which was isolated by methanol precipitation (Greenberg and Preiss, 1964). The reaction mixture contained 5 μmoles of glycine–NaOH, pH 8.5, 40 mμmoles of ADP–glucose-^{14}C or deoxy-ADP–glucose-^{14}C (3–14 $\times 10^5$ cpm/μmole), 5 μmoles of KCl, 0.5 mg of rabbit liver glycogen, 2 μmoles of glutathione, 100 μg of bovine plasma albumin, and enzyme in a total volume of 0.2 ml. The reaction was stopped by the addition of 2 ml of a 75% methanol solution containing 1% KCl. The mixture was then centrifuged and the glycogen precipitate was washed twice with 2 ml of the methanol–KCl solution. The glycogen was dissolved in 1 ml of water; a 0.5-ml portion of the solution was counted with 10 ml of Bray's solution (Bray, 1960) in a liquid scintillation spectrometer. Controls containing no enzyme usually registered 20–40 cpm over background which was 15 cpm. One unit of enzyme activity was defined as being equal to the transfer of 1 μmole of glucose-^{14}C to glycogen in 15 min at 37°.

ASSAY B. Transglucosylase activity was also followed by the formation of ADP or deoxy-ADP. The reaction

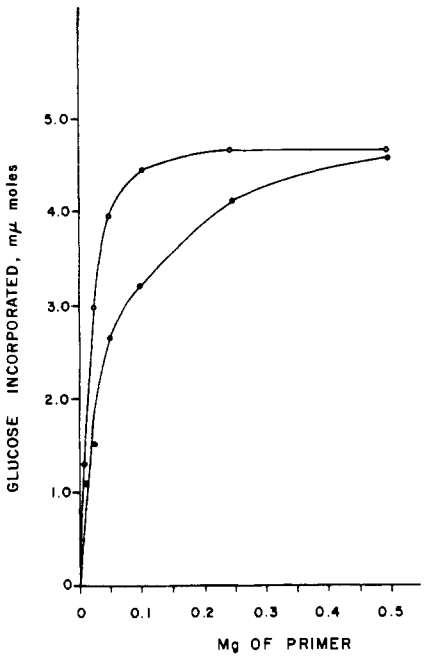

FIGURE 2: The effect of primer concentration on glucose incorporation. The conditions of the experiment were those in assay A. The filled circles represent values obtained with rabbit liver glycogen while the open circles are values obtained with *E. coli* glycogen.

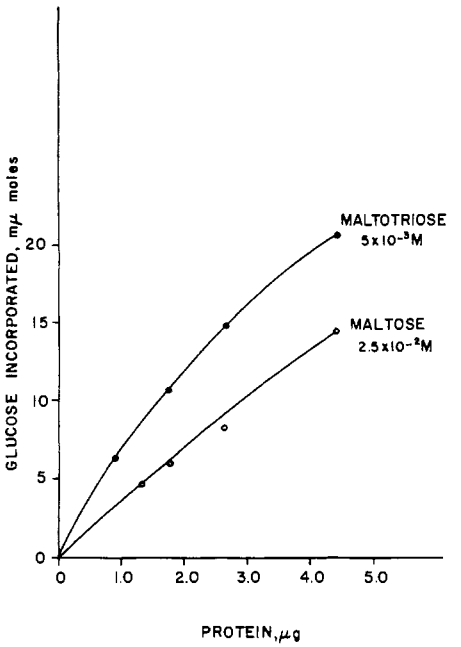

FIGURE 3: The effect of enzyme concentration on the rate of transfer of glucose from ADP–glucose to maltose and maltotriose. The conditions of the experiment are described in the text.

mixture which contained 25 μmoles of glycine–NaOH, pH 8.5, 0.15 μmole of deoxy-ADP–glucose or ADP–glucose, 25 μmoles of KCl, 2.5 mg of glycogen, 200 μg of bovine plasma albumin, 10 μmoles of glutathione, and purified enzyme in a total volume of 1.0 ml was incubated for 15 min at 37°. After the reaction was stopped by heating in a boiling water bath for 30 sec, 0.7 ml of the reaction mixture was assayed for nucleoside diphosphate (Kornberg and Pricer, 1950). About 1.5–3 mμmoles of nucleoside diphosphate was detected in controls containing no glycogen or enzyme.

Results

Preparation of the Partially Purified Transglucosylase. The preparation of the French Press extract has been described (Greenberg and Preiss, 1964). The extract obtained from 20 g of cells (wet weight) was centrifuged for 15 min at 1200*g*. The supernatant fluid then was centrifuged for 15 min at 32,000*g*. The precipitate obtained from this centrifugation was resuspended in 20 ml of a buffer containing 0.05 M Tris, pH 7.5, 0.005 M glutathione (GSH), and 0.01 M $MgCl_2$ (buffer A) and recentrifuged at 32,000*g*. The resulting particulate fraction was resuspended in 20 ml of buffer A and called the 32,000*g* particulate fraction. The 32,000*g* supernatant fluid was centrifuged for 1 hr at 105,000*g*. The particulate fraction obtained from this centrifugation was resuspended in 20 ml of buffer A, and termed the 105,000*g* particulate fraction. Table I shows the distribution of the transglucosylase in the various fractions. In the 105,000*g* particulate fraction 72% of the transglucosylase activity was present.

Ammonium Sulfate Fractionation. A saturated solution of ammonium sulfate was added to the 105,000*g* particulate fraction until 40% saturation was reached. After 10 min, the resulting suspension was centrifuged for 10 min at 30,000*g*. The precipitate was suspended in a buffer containing 0.05 M Tris–HCl, pH 7.5, and 0.01 M GSH and dialyzed overnight against 500 ml of the same buffer. The dialyzed suspension was then centrifuged at 105,000*g* for 1 hr. The particulate fraction was suspended in the 0.05 M Tris–HCl, pH 7.5, and 0.01 M GSH buffer solution. About 40–70% of the transglucosylase was now found in the supernatant solution and was about 10–20-fold more purified than the 32,000*g* supernatant fluid (Table I). No 5′-adenylate kinase, glycogen phosphorylase, or amylase can be detected in the ammonium sulfate fraction. Figure 1 shows the linearity of transglucosylase activity with respect to protein concentration using either deoxy-ADP–glucose-^{14}C or ADP–glucose-^{14}C as the glucosyl donor. Protein was determined by the method of Lowry *et al.* (1951).

Properties of the Transglucosylase

Requirements. Table II shows that glucose transfer from ADP–glucose is dependent on the presence of a primer and enzyme. Glutathione, KCl, and bovine plasma albumin consistently stimulated the enzymatic

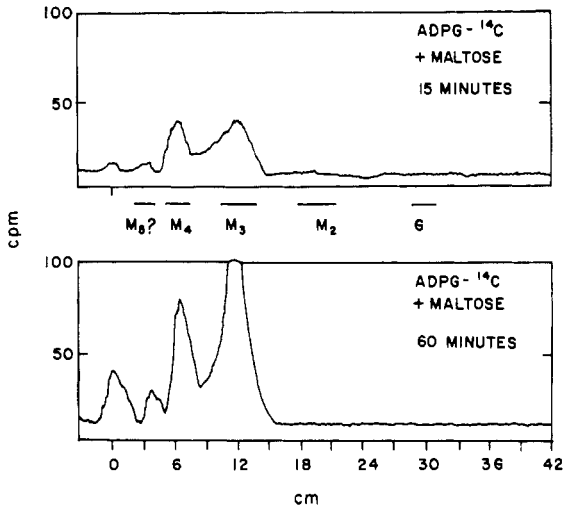

FIGURE 4: Transfer of glucose-^{14}C from ADP-glucose to maltose and maltotriose. The conditions of the experiment were those described for assay C. The filtrates obtained from the Dowex 1-X8 (Cl^-) treatment were concentrated, chromatographed in solvent B, and then counted in a paper strip counter. The omission of enzyme or oligosaccharide from the incubation resulted in the absence of radioactivity in the filtrates. The migration of the maltose (M_2), maltotriose (M_3), maltotetraose (M_4), and glucose (G) standards are seen in the figure. Maltopentaose would be expected to migrate at position M_5.

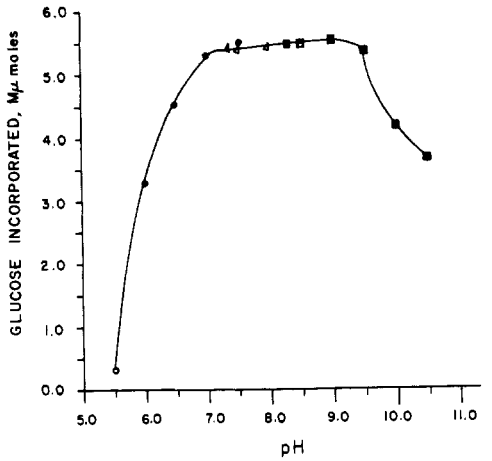

FIGURE 5: pH optimum of the transglucosylase. The conditions of the experiment are those of assay A. The open circle represents reaction mixtures containing sodium acetate buffer; the closed circles represent reactions containing phosphate buffer. The boxes are for reactions containing glycine–NaOH buffer, and the triangles are for reactions containing Tris–HCl buffers.

activity 16–55%. If all three were omitted simultaneously, the activity was reduced about 80%. The same results were obtained with deoxy-ADP-glucose-^{14}Cas the glucosyl donor. Incorporation of glucose into glycogen from both sugar nucleotides was inhibited by more than 95% if 2 μg of α-amylase or 10 μg of β-amylase was added.

Specificity of Sugar Nucleotide Donor. The transglucosylase was specific for ADP-glucose and deoxy-ADP-glucose. In assay A neither UDP-glucose-^{14}C, TDP-glucose-^{14}C, GDP-glucose-^{14}C, CDP-glucose-^{14}C, nor UDP-galactose-^{14}C could substitute for ADP-glucose-^{14}C. Glucose-^{14}C-1-P incorporation in the absence or presence of 5′-adenylate was not observed under the conditions of assay A. In assay B, IDP-glucose, ADP-mannose, and ADP-galactose were inactive. Deoxy-ADP-glucose was 72% effective as ADP-glucose in both assays A and B. Nucleoside diphosphate formation from either ADP-glucose or deoxy-ADP-glucose was absolutely dependent on the presence of an α-1,4-glucan primer and transglucosylase. It was inhibited completely by the presence of α- or β-amylase in the reaction mixture. Simultaneous addition of the two active sugar nucleotides, ADP-glucose and deoxy-ADP-glucose, produced activity not appreciably greater than would be expected from ADP-glucose alone. This would suggest that the same enzyme catalyzed the transfer of glucose from both sugar nucleotides.

Specificity of Primer. Figure 2 shows the effect of primer concentration of *E. coli* or rabbit liver glycogen on the rate of glucose transfer from ADP-glucose. Other primers that were active in assay A were corn amylopectin, soluble corn amylose, shell fish glycogen, and soluble potato starch. Other polymers tested for priming activity and found inactive were α-1,6-dextran (isolated from *Leuconostoc mesenteroides* NRRL-B512F), inulin, and yeast mannan. To determine whether oligosaccharides could serve as primers, the following assay was used. The reaction mixture in assay A was used except that various oligosaccharides were used as primers in place of the rabbit liver glycogen. The reaction mixture was incubated for 20 min at 37°; the reaction was terminated by heating for 30 sec in a boiling water bath. Solid Dowex 1-X8 (Cl^-), 170 mg, then was added to adsorb the unreacted ADP-glucose-^{14}C. The reaction mixture was filtered through a Millipore filter; 0.5 ml of the filtrate which contained the neutral primer plus the labeled product was counted in 10 ml of Bray's solution (Bray, 1960) in a liquid scintillation spectrometer. This assay will be referred to as assay C. Omission of enzyme or oligosaccharide acceptor resulted in the absence of radioactivity in the filtrate. Figure 3 shows the effect of enzyme concentration on the rate of transfer of glucose from ADP-glucose to maltose and maltotriose. Isomaltose, isomaltotriose, glucose, trehalose, cellobiose, gentiobiose, turanose, sophorose, laminaribiose, and panose were all inactive as primers when tested at concentrations of 5.0×10^{-3} M and 2.5×10^{-2} M.

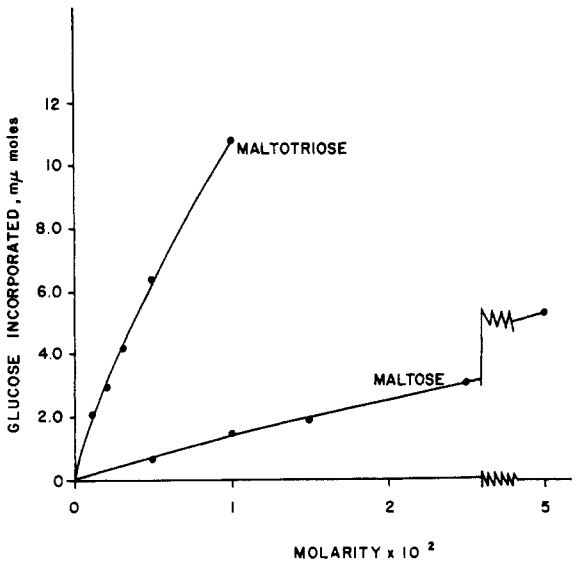

FIGURE 6: The effect of maltose and maltotriose concentrations on transglucosylase activity. The conditions of the experiment were those of assay C.

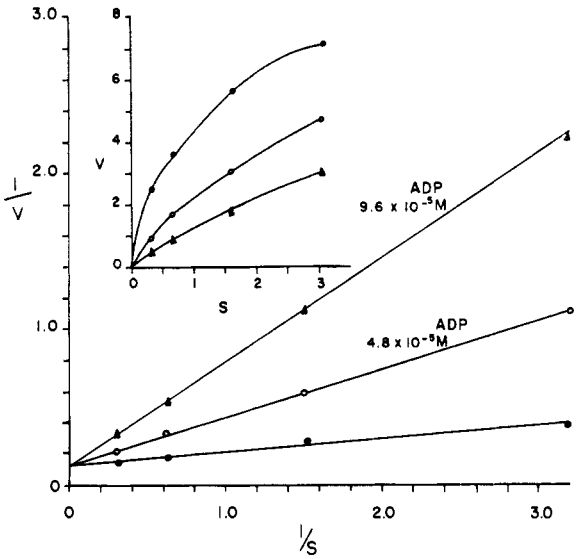

FIGURE 7: Competitive inhibition of the transglucosylase by ADP. V equals mμmoles of glucose incorporated in 15 min and S (ADP-glucose) equals M × 10^{-4}. The concentrations of inhibitor are indicated in the figure. The conditions of the experiment were the same as in assay A.

Identification of Products. The formation of an α-1,4-glucosidic linkage by the transfer of the glucose from ADP-glucose to the primer was shown by the fact that, when the radioactive alcohol-insoluble product was treated with α- or β-amylase, a 75% alcohol-soluble product was obtained. When the β-amylase-digested material was chromatographed in solvent systems B and C, all the radioactivity cochromatographed with maltose and had the same rate of migration as did maltose in paper electrophoresis in 0.05 M borate buffer, pH 9.4. Hydrolysis of the radioactive transglucosylase product or its β-amylase digest with 1 N HCl for 2 hr gave a radioactive product which cochromatographed with glucose in three solvent systems (Table III). The radioactive β-amylase product (1.4 × 10^5 cpm) was deionized by passage through a Dowex 50-X2 (H^+) column. Authentic maltose·H_2O· (2 g) was added and the resulting water mixture was converted to the β-maltosyl octaacetate (Wolfrom and Thompson, 1962). Table IV shows that, on recrystallization of the β-

TABLE III: Chromatography of ^{14}C Products.[a]

Compound	Paper Chromatography Solvent A	Solvent B	Solvent C	Paper Electrophoresis (cm)
Glucose	1.00	1.00	1.00	29.0
Maltose		0.70	0.55	4.0
Maltotriose		0.50	0.32	3.0
Cellobiose		0.67	0.50	2.3
Mannose	1.14	1.16	1.14	18.5
Galactose	0.81	0.85	0.89	24.0
^{14}C product	0.0	0.0	0.0	
^{14}C product hydrolyzed in 1 N HCl	0.99	1.00	1.00	28.0
^{14}C product treated with β-amylase		0.70	0.53	4.2

[a] The solvents are described in the text. The times of development were: solvent A, 16–24 hr.; solvents B and C, 40–48 hr. The paper chromatographic data refer to the rate of migration of the compounds relative to that of glucose. The electrophoretic data are expressed as centimeters traveled in 60 min in 0.05 M borate buffer with a voltage gradient of 80 v/cm of paper.

TABLE IV: Specific Activity of β-Maltose Octaacetate.[a]

Crystallization	Sample (mg)	Sample (cpm)	Sample (cpm/mg)
First	24.2	920	38.0
Second	20.8	834	40.1
Third	24.4	910	37.3
Fourth	30.0	1143	38.1

[a] β-Maltose-^{14}C octaacetate was prepared as indicated in the text. Its melting point was 157–160° (lit. mp 159–160°). The octaacetate was crystallized and recrystallized from 95% ethanol. The ^{14}C was measured in a scintillation counter with a background of 15 cpm.

TABLE V: Stoichiometry of Transglucosylase Reaction.[a]

Time (min)	Expt 1 (mμmoles) Glucose Incorporated	ADP	Expt 2 (mμmoles) Glucose Incorporated	Deoxy-ADP
0	0.0	6.5	0.0	5.8
15	62.0	71.8	57.2	66.6
Δ	62.0	65.3	57.2	60.8

[a] In expt 1 the reaction mixture was the same as in assay B with ADP-glucose-^{14}C as the glucose donor. The reaction was incubated for 15 min at 37° and then terminated by placing in a boiling water bath for 1 min. A portion of the reaction mixture (0.2 ml) was taken for assay of glucose incorporation (assay A); 0.6 ml of the mixture was analyzed for ADP formation (Kornberg and Pricer, 1950). Controls containing no enzyme or no glycogen were run simultaneously and gave values similar to the zero time control. In expt 2 the reaction mixture was the same as assay B except that 110 mμmoles of deoxy-ADP-glucose was present. Glucose incorporation and nucleoside diphosphate formation was measured as above.

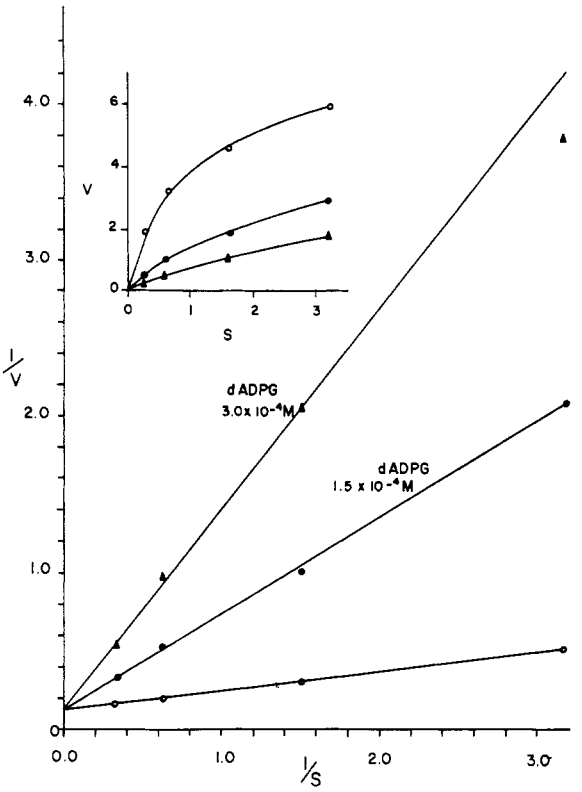

FIGURE 8: Competitive inhibition of the transglucosylase by deoxy-ADP-glucose. V is mμmoles of glucose incorporated from ADP-glucose-^{14}C in 15 min and S equals M $\times$ 10^{-4}. The conditions of the experiment were the same as in assay A.

maltosyl octaacetate, the specific activity of the octaacetate remained constant, indicating that the β-amylase product was indeed maltose.

The incubation of maltose and ADP-glucose-^{14}C with transglucosylase led to formation of both maltotriose-^{14}C and maltotetraose-^{14}C (Figure 4). If incubations were carried out for longer periods of time, formation of higher maltodextrins were also observed (Figure 4). Incubation of ADP-glucose-^{14}C with maltotriose-^{14}C led to formation of only maltotetraose-^{14}C.

pH Optimum of the Transglucosylase. The pH optimum of the transglucosylase was between 7 and 9.5 (Figure 5). The use of different buffers between 7 and 9 did not affect the activity of the transglucosylase.

Kinetics. The K_m of the *E. coli* glycogen was 0.1 mg/ml, which is equal to 5.6 $\times$ 10^{-4} anhydroglucose unit. Since 10% of the glucose in *E. coli* glycogen is terminal (Sigal *et al.*, 1964), the K_m in terms of glucose end units was 5.6 $\times$ 10^{-5} M. These end units presumably are the acceptors of glucose from ADP-glucose. The K_m values of ADP-glucose and of deoxy-ADP-glucose were 8.1 $\times$ 10^{-5} M and 3.8 $\times$ 10^{-5} M, respectively. Figure 6 shows the effect of maltotriose and maltose concentrations on the rate of glucose transfer from ADP-glucose to these primers. Saturation of the transglucosylase by these oligosaccharides was not achieved in the range of 1 to 5 $\times$ 10^{-2} M.

Inhibitors and Activators. ADP was an inhibitor of the transglucosylase and appeared to be competitive with ADP-glucose; its K_i was 1.5 $\times$ 10^{-5} M (Figure 7). Deoxy-ADP-glucose also inhibited the transfer of glucose-^{14}C from ADP-glucose-^{14}C, and this inhibition appeared to be competitive (Figure 8). The K_i was 3.4 $\times$ 10^{-5} M and was similar to the value obtained for the K_m of deoxy-ADP-glucose-^{14}C. *p*-Mercuribenzoate was found to be a potent inhibitor of the transglucosylase in the absence of glutathione. The reaction was inhibited 80% by 2.5 $\times$ 10^{-6} M *p*-mercuribenzoate. However, inhibition by even 10^{-3} M *p*-mercuribenzoate could be counteracted by 10^{-2} M glutathione. No activation or inhibition of transglucosylase (105,000*g* particulate fraction or ammonium sulfate fractions) was observed when glucose, glucose-6-P, fructose 1,6-diphosphate, ribose-5-P, phosphoenolpyruvate, 3-phosphoglycerate, 2,3-diphosphoglycerate, pyruvate, inorganic phosphate, 3,5-cyclic adenylate, L-leucine, L-lysine, L-alanine, L-aspartate, L-glutamate, or L-histidine were added to the reaction mixture at a concentration of 1 $\times$ 10^{-3} M.

Stoichiometry. Table V shows that 1 μmole of ADP was formed for every μmole of glucose incorporated into glycogen from ADP-glucose. Experiment 2 in Table V shows that for each μmole of deoxy-ADP formed from deoxy-ADP-glucose there was 1 μmole of glucose incorporated into glycogen.

Discussion

The above evidence indicates that synthesis of glycogen in *E. coli* B occurs by transfer of glucose from ADP-glucose to the chain ends of a glycogen primer. The presence of ADP-glucose pyrophosphorylase, the enzyme responsible for ADP-glucose synthesis, in *E. coli* extracts has been previously reported (Preiss *et al.*, 1965). It is interesting to note that certain mutant strains of *E. coli* which cannot synthesize UDP-glucose still accumulate glycogen (Sigal *et al.*, 1964) and contain both ADP-glucose:glycogen transglucosylase and ADP-glucose pyrophosphorylase.[2] This would minimize the importance of UDP-glucose in *E. coli* glycogen synthesis. The widespread occurrence of ADP-glucose:glycogen transglucosylase and ADP-glucose pyrophosphorylase in bacteria (Greenberg and Preiss, 1964; Shen and Preiss, 1965) seem to indicate that glycogen synthesis in bacteria is mediated by transfer of glucose from ADP-glucose to glycogen. Formation of the branch chains in the glycogen molecule is presumably catalyzed by an amylo-1,4-1,6-transglucosidase (branching enzyme) (Cori and Cori, 1943). Zevenhuizen has shown the presence of branching enzyme in extracts of the bacterium, *Arthrobacter globiformis* (Zevenhuizen, 1964).

Whereas the *Arthrobacter* transglucosylase is found in the soluble portion of the crude extract (Greenberg and Preiss, 1965), the *E. coli* transglucosylase seems to be mainly in the 105,000*g* particulate fraction. Despite this difference, the properties of both enzymes are almost identical. Both enzymes can only use deoxy-ADP-glucose and ADP-glucose as glucosyl donors. The pH optima of both enzymes are the same; both are inhibited by its product, ADP, and stimulations by GSH, KCl, and serum albumin have been observed with both enzymes. Both enzymes are also extremely sensitive to inhibition by *p*-mercuribenzoate.

Unlike the mammalian or yeast UDP-glucose:glycogen transglucosylases (Leloir and Goldenberg, 1960; Algranati and Cabib, 1962) the *E. coli* or *Arthrobacter* ADP-glucose:glycogen transglucosylases were not stimulated by glucose-6-P. In fact none of the glycolytic intermediates tested could stimulate the bacterial transglucosylase activities. The possibility remains though that a form of the transglucosylase which is stimulated by glycolytic intermediates was destroyed during the preparation of the crude extracts of these bacteria. However, it has been reported that certain glycolytic intermediates can activate the ADP-glucose pyrophosphorylases of *Arthrobacter* and *E. coli* (Preiss *et al.*, 1965; Shen and Preiss, 1964) and it thus appears that at least part of the control of bacterial glycogen synthesis might occur at the level of ADP-glucose synthesis.

[2] I. H. Segel and J. Preiss, unpublished results.

References

Algranati, E. D., and Cabib, E. (1962), *J. Biol. Chem. 237*, 1007.

Bray, G. A. (1960), *Anal. Biochem. 1*, 279.

Cori, G. T., and Cori, C. F. (1943), *J. Biol. Chem. 151*, 57.

Doi, A., Doi, K., and Nikuni, Z. (1964), *Biochim. Biophys. Acta 92*, 628.

Frydman, R. B., and Cardini, C. E. (1964a), *Biochem. Biophys. Res. Commun. 14*, 353.

Frydman, R. B. and Cardini, C. E. (1964b), *Biochem. Biophys. Res. Commun. 17*, 407.

Ghosh, H. P., and Preiss, J. (1965), *J. Biol. Chem. 240*, 961.

Greenberg, E., and Preiss, J. (1964), *J. Biol. Chem. 239*, PC 4313.

Greenberg, E., and Preiss, J. (1965), *J. Biol. Chem. 240*, 2341.

Holmes, T., and Palmistierna, N. (1956), *Acta Chem. Scand. 10*, 1557.

Kornberg, A., and Pricer, W. E. (1950), *J. Biol. Chem. 182*, 763.

Leloir, L. F., and Goldenberg, S. H. (1960), *J. Biol. Chem. 235*, 919.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

Mulder, E. G., Dienema, M. H., Van Neen, W. L., and Zevenuizen, L. P. T. M. (1962), *Rec. Trav. Chim. 81*, 797.

Murata, T., and Akazawa, T. (1964), *Biochem. Biophys. Res. Commun. 16*, 6.

Murata, T., Sugiyama, T., and Akazawa, T. (1964), *Arch. Biochem. Biophys. 107*, 92.

Murata, T., Sugiyama, T., and Akazawa, T. (1965), *Biochem. Biophys. Res. Commun. 18*, 371.

Peat, S., Whelan, W. J., and Edwards, T. E. (1961), *J. Chem. Soc.*, 29.

Preiss, J., Shen, L., and Partridge, M. (1965), *Biochem. Biophys. Res. Commun. 18*, 180.

Recondo, E., and Leloir, L. F. (1961), *Biochem. Biophys. Res. Commun. 6*, 85.

Shen, L., Ghosh, H. P., Greenberg, E., and Preiss, J. (1964), *Biochim. Biophys. Acta 89*, 370.

Shen, L., and Preiss, J. (1965), *J. Biol. Chem. 240*, 2334.

Sigal, N., Cattaneo, J., and Segel, I. H. (1964), *Arch. Biochem. Biophys. 108*, 430.

Stanier, R. Y., Doudoroff, M., Kunisawa, R., and Contopoulou, R. (1959), *Proc. Natl. Acad. Sci. U. S. 45*, 1246.

Strange, R. E., Dark, F. A., and Neww, A. G. (1961), *J. Gen. Microbiol. 25*, 61.

Wolfrom, M. L., and Thompson, A. (1962), *Methods Carbohydrate Chem. 1*, 334.

Zevenhuizen, L. P. T. M. (1964), *Biochim. Biophys. Acta 81*, 608.