Rate Studies of Polysaccharide Biosynthesis from Guanosine Diphosphate α -D-Glucose and Guanosine Diphosphate α -D-Mannose

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With enzyme preparations from *Phaseolus aureus* seedlings, the initial rate of ^{14}C -labelled polysaccharide formation from GDP- α -D- ^{14}C glucose is not increased by additions of GDP- α -D-mannose. However, final incorporation is increased by addition of GDP- α -D-mannose, since the total reaction-time is extended. In contrast, the initial rate of ^{14}C -labelled polysaccharide formation from GDP- α -D- ^{14}C mannose is increased by all concentrations of GDP- α -D-glucose that are less than that of the GDP- α -D- ^{14}C mannose. Maximum stimulation of the initial rate occurs at a GDP- α -D- ^{14}C mannose/GDP- α -D-glucose concentration ratio of about 4:1. However, eventual incorporation from GDP- α -D- ^{14}C mannose is decreased by the addition of GDP- α -D-glucose, since the reaction rate falls off sharply after about 2 min. Reciprocal plots of ^{14}C -labelled polysaccharide formation from GDP- α -D- ^{14}C mannose result in biphasic graphs. The two straight-line portions of the plot are joined by a curved line in the concentration range between 2–3 and 50 μM . Extrapolated K_m values for the two linear components are 0.4–1.0 and 700–1500 μM . The effect of GDP- α -D-glucose on the kinetics of ^{14}C -labelled polysaccharide formation from GDP- α -D- ^{14}C mannose is complex, and depends on relative concentrations of the two sugar nucleotides. ^{14}C -labelled polysaccharide formation from GDP- α -D- ^{14}C glucose also results in biphasic reciprocal plots. One component appears to have K_m about 2–3 μM , the other about 200–400 μM . In this reaction, GDP- α -D-mannose appears to be a competitive inhibitor with K_i 20–30 μM . With particulate preparations of *P. aureus*, GDP- α -D- ^{14}C glucose appears to be a precursor for the synthesis of one polysaccharide, a glucomannan, the mannose moieties of which are derived from an intermediate existing in the particulate preparation. From the rate results, GDP- α -D- ^{14}C mannose appears to be a precursor for at least two polysaccharides, one of which is a glucomannan.

Particulate enzyme preparations from *Phaseolus aureus* seedlings catalyse the incorporation of radioactivity from GDP- α -D- ^{14}C glucose and GDP- α -D- ^{14}C mannose into polysaccharide (Barber, Elbein & Hassid, 1964; Elbein, 1969). The complete structure of the resulting polysaccharide has not been determined. The particulate enzyme preparations contain a large quantity of polysaccharide relative to the amount of radioactive sugar incorporated (Bailey, Haq & Hassid, 1967). Consequently, the structural information that can be obtained about the newly synthesized polysaccharide is limited to the immediate vicinity of the radioactive sugar. Any contribution of sugar moieties from the particulate enzyme preparation to the newly formed radioactive polymer can easily go unnoticed, since these contributions would not be radioactive.

As a result, conclusions regarding the function *in vivo* of the *P. aureus* glycosyl transferases must consider the properties of the enzyme system as well as the structural information that can at present be obtained. Barber *et al.* (1964) concluded that GDP- α -D-glucose was the precursor of cellulose and glucomannan in *P. aureus*. The rate results presented in the present paper indicate that GDP- α -D-glucose is probably not the precursor of cellulose, but only of a glucomannan. A preliminary note, discussing one of these experiments, has been published (Villemez & Heller, 1970).

Flowers, Batra, Kemp & Hassid (1969) reported that the formation of water-insoluble ^{14}C -labelled polysaccharide from GDP- α -D- ^{14}C glucose was not linear with respect to time. Consequently, they could not determine the initial rate of the reaction.

Villemez & Clark (1969) observed that the water- and lipid-insoluble material formed from GDP- α -D-[^{14}C]glucose contained not only polysaccharide, but also a phenol-soluble saccharide derivative that is rapidly labelled. When this rapidly labelled component is removed, the formation of ^{14}C -labelled polysaccharide from GDP- α -D-[^{14}C]glucose is linear with respect to time until the reaction ceases. Under these circumstances, the initial rate can be determined, and accurate kinetic studies were possible. The rate constants are reported to facilitate further experimentation, and a working hypothesis has been formulated to explain the kinetic information.

MATERIALS AND METHODS

GDP- α -D-[U- ^{14}C]glucose (sp. radioactivity 124 or 191 $\mu\text{Ci}/\mu\text{mol}$) was obtained from New England Nuclear Corp., Boston, Mass., U.S.A. GDP- α -D-[U- ^{14}C]mannose (sp. radioactivity 65 or 151 $\mu\text{Ci}/\mu\text{mol}$) was obtained from International Chemical and Nuclear Corp., Irvine, Calif., U.S.A. Unlabelled sugar nucleotides were purchased from Calbiochem., Los Angeles, Calif., U.S.A.

The particulate enzyme system from *P. aureus* seedlings was prepared in a manner similar to that described by Villemez & Clark (1969). The shoots, with hypocotyls 4–5 cm long, were homogenized in a cold mortar with sand, in the presence of an equal weight of potassium phosphate buffer (0.05 M, pH 7.3, 0°C) containing 1% bovine serum albumin (Sigma Chemical Co., St Louis, Mo., U.S.A.) and 1 mM-MgCl₂. The crude homogenate was strained through Miracloth (Calbiochem) and the filtrate was centrifuged at 1000g for 10 min. The material that sedimented from the 1000g supernatant solution when the latter was centrifuged at 49000g for 15 min was resuspended in homogenizing buffer and resedimented at 49000g. The final pellet was suspended in 0.05 M-potassium phosphate buffer, pH 7.3 (0.5 ml/10 g of shoots), that contained 0.01 M-MgCl₂, 0.4 M-sucrose and 1% bovine serum albumin. This suspension was stored at –20°C for later use. The enzyme suspension was thawed at 0°C and homogenized with a loose-fitting Potter homogenizer before use. Portions (50 μl) of the enzyme preparation, containing 0.1–0.2 mg of plant protein, were used in the assay.

All reactions were carried out at 22°C. The temperature of the enzyme preparation was raised from 0 to 22°C by immersion in a 22°C water bath for 1 min before initiating the reaction. Pre-incubation for this length of time did not affect the catalytic activity of the enzyme preparation. To initiate the reaction, radioactive sugar nucleotide and any other additives (total volume 50 μl ; 22°C) were added to the enzyme suspension with sufficient velocity to cause essentially instantaneous mixing. When higher concentrations of substrate were used, they were produced by mixing radioactive and non-radioactive substrate in known quantity ratios. Therefore all radioactivity measurements fell within a tenfold range, whereas concentration ranges were greater than 1000-fold. The reactions were terminated by the addition of 50 μl of an aqueous 15% (w/v) solution of trichloroacetic acid in the same manner as the substrate was added. Reaction times

for the determination of initial rate were approx. 6 s. The timing was performed manually, read to 0.01 s and rounded to the nearest 0.1 s. The same initial rate values could be obtained from any reaction time up to 1 min. After termination, the samples were held at 0°C until that stage of the analysis where the trichloroacetic acid had been removed.

Approx. 10 mg of powdered cellulose was added to each sample to provide a supporting medium. In the following extractions, soluble and insoluble materials were separated by centrifugation. The insoluble material was extracted twice with 1 ml samples of water at 0°C to remove unchanged sugar nucleotide and water-soluble products of the reaction. The water-insoluble material was then extracted twice for 10 min with 1 ml portions of aq. 45% (w/v) phenol at 21–25°C to remove water-insoluble saccharide derivatives (Villemez & Clark, 1969). The remaining insoluble polysaccharide was washed sequentially with 1 ml of chloroform-methanol (1:1, v/v) and 1 ml of acetone. The pellet was then suspended in 0.5 ml of 1 M-methanolic Hyamine hydroxide and placed in a scintillation counting vial. Toluene (15 ml), containing 5 g of diphenyloxazole and 0.1 g of 1,4-bis(4-methyl-5-phenyloxazol-2-yl)benzene/l was added and radioactivity was determined. Quench corrections were made on all radioactivity determinations.

The kinetic constants *K* and *V* were reproducible $\pm 10\%$ for the same enzyme preparation. For different enzyme preparations the variations were larger. For this reason the values reported are ranges obtained from three replicate experiments with different enzyme preparations.

RESULTS

The synthesis of ^{14}C -labelled polysaccharide from GDP- α -D-[^{14}C]glucose comes to an abrupt halt within a few minutes after starting the reaction (Fig. 1). The exact duration of the reaction time depends on the conditions, including the temperature and the individual enzyme preparation. With samples from the same enzyme preparation, the reaction stops in progressively less time as the temperature is increased from 20 to 37°C. However, the amount of ^{14}C -labelled polysaccharide eventually formed in each case is the same. Pre-incubation of the enzyme without substrate at the reaction temperature for 5–10 min does not affect the initial rate or the final amount of ^{14}C -labelled polysaccharide. Aqueous extracts of the reaction mixture taken after ^{14}C -labelled polysaccharide formation has stopped contain GDP- α -D-[^{14}C]glucose. Addition of more GDP- α -D-[^{14}C]glucose after the cessation of ^{14}C -labelled polysaccharide formation does not promote synthesis of additional ^{14}C -labelled polysaccharide. On the other hand, addition of fresh enzyme to the reaction mixture does promote additional synthesis of ^{14}C -labelled polysaccharide. Also, the presence of unlabelled GDP- α -D-mannose extends the reaction (Fig. 1), but does not increase the initial rate at any concen-

Fig. 1. Effect of GDP- α -D-mannose on ^{14}C -labelled polysaccharide formation from GDP- α -D- ^{14}C]glucose. Either GDP- α -D- ^{14}C]glucose (20000 c.p.m., 94 pmol) alone (O, initial rate 2474 c.p.m./min), or GDP- α -D- ^{14}C]glucose and GDP- α -D-mannose (280 pmol) together (●, initial rate 2346 c.p.m./min), were added to the enzyme preparation at time zero. Reactions were stopped at the times indicated. Other assay and analytical procedures are described in the text.

Fig. 2. Effect of GDP- α -D-glucose on ^{14}C -labelled polysaccharide formation from GDP- α -D- ^{14}C]mannose. Either GDP- α -D- ^{14}C]mannose (42000 c.p.m., 560 pmol) alone (●, initial rate 1345 c.p.m./min), or GDP- α -D- ^{14}C]mannose and GDP- α -D-glucose (560 pmol) together (O, initial rate 1600 c.p.m./min), were added to the enzyme preparation at time zero. Reactions were stopped at the times indicated. Other assay and analytical procedures are described in the text.

Fig. 3. Effect of the complementary sugar nucleotide on initial rate of ^{14}C -labelled polysaccharide formation from GDP- α -D- ^{14}C]mannose or GDP- α -D- ^{14}C]glucose. Either GDP- α -D- ^{14}C]glucose (final concn. $1\ \mu\text{M}$) (O) or GDP- α -D- ^{14}C]mannose (final concn. $2\ \mu\text{M}$) (●) was added to the enzyme in combination with the indicated quantities of GDP- α -D-mannose or GDP- α -D-glucose respectively. Initial rates of the reactions were determined as indicated in the text.

tration. I have also performed these experiments with the alkali-insoluble polysaccharide fraction. The same results were found.

The synthesis of ^{14}C -labelled polysaccharide from GDP- α -D- ^{14}C]mannose was linear with respect to time for reaction times of about 10 min (Fig. 2) depending on the individual enzyme preparation. In the presence of unlabelled GDP- α -D-glucose the formation of ^{14}C -labelled polysaccharide from GDP- α -D- ^{14}C]mannose proceeds at the initial rate for only 2–3 min, after which time the reaction rate decreases rapidly. Although the pattern is slightly different, the same phenomena are observed with the alkali-insoluble polysaccharide fraction.

The initial rate of ^{14}C -labelled polysaccharide formation from GDP- α -D- ^{14}C]glucose as a function of GDP- α -D-mannose concentration is given in Fig. 3. All concentrations of GDP- α -D-mannose inhibit the initial rate of ^{14}C -labelled polysaccharide

Fig. 4. Kinetics of ^{14}C -labelled polysaccharide formation from $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{mannose}$. Initial rates were determined as indicated in the text. Velocity (v) is given as $\mu\text{mol/s}$ per $50\ \mu\text{l}$ of enzyme suspension. Substrate concentration (s) is given in mol/l .

formation from $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{glucose}$, the inhibition becoming increasingly severe at $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{glucose}/\text{GDP-}\alpha\text{-D-mannose}$ concentration ratios less than 0.1. In contrast, $\text{GDP-}\alpha\text{-D-glucose}$ increases the initial rate of ^{14}C -labelled polysaccharide formation from $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{mannose}$, provided that the $\text{GDP-}\alpha\text{-D-glucose}$ concentration is less than that of the $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{mannose}$. The $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{mannose}/\text{GDP-}\alpha\text{-D-glucose}$ concentration ratio that provides maximum stimulation of the initial rate is 4:1. All concentrations of $\text{GDP-}\alpha\text{-D-glucose}$ greater than that of the $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{mannose}$ inhibit the initial rate of ^{14}C -labelled polysaccharide formation.

Reciprocal plots of the initial rate of ^{14}C -labelled polysaccharide formation from $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{glucose}$ or from $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{mannose}$ are biphasic. An example of such a plot is given for $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{mannose}$ in Fig. 4. Linear reciprocal plots are obtained if substrate concentration ranges are restricted to either specific low- or specific high-concentration ranges. For example, with $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{mannose}$ these ranges are (a) below $2\text{--}3\ \mu\text{M}$ and (b) above $50\ \mu\text{M}$. A curved reciprocal plot is obtained from the $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{mannose}$ concentration range $2\text{--}50\ \mu\text{M}$. Therefore the reaction can be described most concisely with two sets of kinetic constants, representing the low- and high-concentration linear components of the reciprocal plots respectively. For $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{mannose}$ the apparent K_m values for these components are $0.4\text{--}1.0$ and $700\text{--}1500\ \mu\text{M}$ and for $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{glucose}$, the apparent K_m values are $2\text{--}3$ and $200\text{--}400\ \mu\text{M}$.

Fig. 5. Kinetics of ^{14}C -labelled polysaccharide formation with low concentrations of $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{glucose}$. The conditions are those given in the legend to Fig. 4. For each curve there is an additional point at a lower concentration than that encompassed by the horizontal axis. These points fell precisely on the lines drawn, and were deleted for convenience in presentation. ●, $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{glucose}$ alone; ×, $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{glucose} + 10\ \mu\text{M GDP-}\alpha\text{-D-mannose}$; ○, $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{glucose} + 0.1\ \text{mM GDP-}\alpha\text{-D-mannose}$.

$\text{GDP-}\alpha\text{-D-mannose}$ appears to be a competitive inhibitor of ^{14}C -labelled polysaccharide formation from lower concentrations of $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{glucose}$ (Fig. 5). The apparent K_i of $\text{GDP-}\alpha\text{-D-mannose}$ is $20\text{--}30\ \mu\text{M}$, which is about 10 times greater than the K_m for the low-concentration linear component of $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{glucose}$. I have not determined the effect of $\text{GDP-}\alpha\text{-D-mannose}$ on the high-concentration component of $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{glucose}$, but from other results would expect $\text{GDP-}\alpha\text{-D-mannose}$ to be a competitive inhibitor in that case as well. The effect of $\text{GDP-}\alpha\text{-D-glucose}$ on the kinetics of ^{14}C -labelled polysaccharide formation from $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{mannose}$ is more complex, as might be predicted from the results in Fig. 3. When the $\text{GDP-}\alpha\text{-D-glucose}$ concentration is higher than that of $\text{GDP-}\alpha\text{-D-}[^{14}\text{C}]\text{mannose}$, the apparent K_m and V

Table 1. *Kinetic constants of ^{14}C -labelled polysaccharide formation from GDP- α -D- ^{14}C mannose*

Reaction component	Addition		
	None	GDP-glucose (10 μM)	GDP-glucose (50 μM)
Low concentration			
K_m ($\mu\text{mol/l}$)	0.4	4.4	18.2
V (pmol/s per 50 μl of enzyme)	28.0	53.0	91.0
High concentration			
K_m ($\mu\text{mol/l}$)	1540	600	540
V (pmol/s per 50 μl of enzyme)	3700	2200	1700

are increased (Table 1), but when GDP- α -D-glucose concentration is lower than that of GDP- α -D- ^{14}C mannose, the apparent K_m and V are decreased.

DISCUSSION

The above results might appear to contradict earlier results (Barber *et al.* 1964; Elbein, 1969). There is actually no contradiction: the results presented here represent an amplification of previous data. However, as a consequence of the additional information, considerably different conclusions can be drawn.

Barber *et al.* (1964) reported that the addition of GDP- α -D-mannose stimulated the production of radioactive polysaccharide from GDP- α -D- ^{14}C glucose, and interpreted this as an indication of an additional polysaccharide being formed. This conclusion seemed to be supported when partial acid hydrolysis of the product resulted in additional oligosaccharides, compared with the polysaccharide synthesized from GDP- α -D- ^{14}C glucose alone.

With regard to the stimulation of incorporation from GDP- α -D- ^{14}C glucose when GDP- α -D-mannose is present, these investigators measured incorporation at a single time-point: after 15 min of incubation. To be the result of the catalytic action of a different enzyme system, the observed increase in incorporation should be due to an increase in initial rate of the reaction. From Fig. 1 it is apparent that the addition of GDP- α -D-mannose causes an increase in incorporation from GDP- α -D- ^{14}C glucose, but this is not due to an increase in initial rate. The enhanced incorporation is the result of an increase in the length of time that incorporation is maintained. That this is not the result of an adverse concentration ratio is demonstrated by the fact that no concentration of GDP- α -D-mannose stimulates the initial rate of ^{14}C -labelled polysaccharide formation from GDP- α -D-

^{14}C glucose (Fig. 3). Also, the effect of GDP- α -D-mannose on the initial-rate kinetics of GDP- α -D- ^{14}C glucose incorporation (Fig. 5) exhibits no anomalies, and GDP- α -D-mannose appears to be a simple competitive inhibitor of the reaction. There is additional evidence that only one type of polysaccharide is being formed from GDP- α -D- ^{14}C glucose. Both the total ^{14}C -labelled polysaccharide and alkali-insoluble ^{14}C -labelled polysaccharide exhibit similar kinetic patterns as a function of GDP- α -D-mannose concentration. Also, the oligosaccharide patterns that result from partial acid hydrolysis or acetolysis of the radioactive polysaccharides formed from GDP- α -D- ^{14}C glucose are identical with regard both to distribution and percentage composition of the oligosaccharide mixture, for ^{14}C -labelled and alkali-insoluble ^{14}C -labelled polysaccharide (Flowers *et al.* 1969). These workers also demonstrated that radioactive oligosaccharides containing mannose were produced on acetolysis of the ^{14}C -labelled polysaccharide formed from GDP- α -D- ^{14}C glucose alone, but these oligosaccharides were not present in the oligosaccharide mixture that resulted from partial acid hydrolysis. The mannose moiety in these oligosaccharides had only a trace of radioactivity, indicating that it was derived primarily from the particulate enzyme preparation, and only a very small proportion from GDP- α -D- ^{14}C mannose resulting from epimerization of the glucose moiety of GDP- α -D- ^{14}C glucose. Barber *et al.* (1964) did not observe these mannose-containing oligosaccharides from degradation of ^{14}C -labelled polysaccharide formed from GDP- α -D- ^{14}C glucose alone since they used partial acid hydrolysis. However, these were the additional oligosaccharide peaks that they observed when GDP- α -D-mannose and GDP- α -D- ^{14}C glucose together were used as substrates for the ^{14}C -labelled polysaccharide that was subjected to partial acid hydrolysis. In the latter case the increased amount of ^{14}C -labelled polysaccharide and proportion of mannose that was produced due to the addition of GDP- α -D-mannose undoubtedly allowed the mannose-containing oligosaccharides to be observed even though they were more sensitive to the hydrolytic conditions than were the β -1,4-linked glucose oligosaccharides. Therefore I conclude that GDP- α -D-glucose is the natural substrate for a glucomannan in the *P. aureus* system, and is not a natural substrate for cellulose synthesis. The glucose moiety is probably transferred *in vitro* from GDP- α -D-glucose to a mannose-containing intermediate, and the final polymerization reaction uses this intermediate as a substrate. When only GDP- α -D- ^{14}C glucose is supplied to the enzyme preparation the amount of polymer formed would be limited by the amount of the intermediate pre-existing in the preparation. That some limiting

factor, that is not substrate or enzyme, is inherent in the particulate preparation is obvious from the present results. Fig. 1 shows that this limitation can be alleviated to some extent by GDP- α -D-mannose. Liu & Hassid (1970) reported that the addition of cellulose, cellodextrins, or other oligosaccharides expected to serve as primers for the reaction, did not enhance the rate of polymer formation from GDP- α -D-[^{14} C]glucose by using a solubilized, ammonium sulphate-purified enzyme. This suggests that polymer formation may not occur by single sugar additions to a growing polysaccharide chain. Finally, we have reported evidence to indicate that membrane-bound intermediates in the synthesis of *P. aureus* polysaccharides do indeed exist (Villemez & Clark, 1969).

Elbein (1969) reported that the addition of GDP- α -D-glucose severely inhibited the formation of ^{14} C-labelled polysaccharide from GDP- α -D-[^{14} C]mannose. The incorporation was measured at a single time-point, 15 min after the initiation of the reaction. The present results demonstrate that for concentrations of GDP- α -D-glucose less than that of GDP- α -D-[^{14} C]mannose, the initial rate is actually increased (Figs. 2 and 3). The lower eventual incorporation as a result of GDP- α -D-glucose addition is due to a substantial decrease in rate about 2 min after initiation of the reaction (Fig. 2). The increase in initial rate of ^{14} C-labelled polysaccharide formation from GDP- α -D-[^{14} C]mannose when GDP- α -D-glucose is added is consistent with GDP- α -D-[^{14} C]mannose serving as substrate for two enzyme systems forming two different polysaccharides, one of which should contain glucose. It has been shown that GDP- α -D-[^{14} C]mannose serves as a precursor for a glucomannan in *P. aureus* (Elbein, 1969). Also, the change in rate when GDP- α -D-glucose is present occurs at a point in the course of the reaction that correlates well with the cessation of ^{14} C-labelled glucomannan synthesis from GDP- α -D-[^{14} C]glucose (Fig. 1), considering the differences between individual enzyme preparations. In addition, the effect of GDP- α -D-glucose on the initial-rate kinetics of GDP- α -D-[^{14} C]mannose incorporation into polysaccharide is very complex (Table 1). These kinetics could be easily explained if the initial rate being measured were the sum of two reactions, one of which was stimulated by GDP- α -D-glucose (at lower concentrations of GDP- α -D-glucose than GDP- α -D-[^{14} C]mannose) and for both of which GDP- α -D-glucose served as a competitive inhibitor at higher relative concentrations of GDP- α -D-glucose. Preliminary results (C. L. Villemez, unpublished work) obtained by separation into alkali-soluble and -insoluble fractions suggest that two polysaccharides are indeed being formed, but that complete separation cannot be achieved simply by alkali extraction.

Elbein (1969) reported a K_m of 100 μ M for the synthesis of radioactive alkali-insoluble polysaccharide from GDP- α -D-[^{14} C]mannose. The concentration range that was used (25–900 μ M) fell primarily in the linear high-concentration component that I report (K_m 700–1500 μ M), but missed entirely the low linear concentration component (K_m 0.4–1.0 μ M). The lower result that was reported (Elbein, 1969) for the linear high-concentration component is probably due partially to the fact that the lower concentrations that were used fell into the curved concentration area (3–50 μ M) of the reciprocal plot reported here which would give a lower apparent K_m . It may also be partially due to the fact that alkali extraction removes one of the polysaccharide components formed from GDP- α -D-[^{14} C]mannose to a greater extent than it does the other, so that Elbein (1969) may have been measuring a slightly different mixture of the two radioactive polysaccharides as product.

It should be noted that biphasic kinetics, observed for the formation of polysaccharide from GDP- α -D-glucose or GDP- α -D-mannose, have also been reported for the synthesis of polygalacturonic acid (Villemez, Swanson & Hassid, 1966) and of galactan (McNab, Villemez & Albersheim, 1968) with *P. aureus* enzyme preparations. The disproportionate increase in rate at higher substrate concentrations, which produces the biphasic reciprocal plot, is probably due to a portion of the enzyme systems being bound to membranes that have been damaged in the isolation of the particulate fraction. It is well known that enzymes that normally function when attached to membranes exhibit considerably different kinetics in a soluble form or when the membranes are damaged (Munkres & Woodward, 1966). By using low concentrations of GDP- α -D-[^{14} C]glucose, and particulate enzyme preparations, Flowers *et al.* (1969) presented results from which a K_m for GDP- α -D-[^{14} C]glucose of about 1–5 μ M can be estimated. This correlates well with the value (2–3 μ M) I have determined for the linear low-concentration component of the reciprocal plot for GDP- α -D-[^{14} C]glucose. Liu & Hassid (1970) also reported a 16–80-fold higher value for GDP- α -D-[^{14} C]glucose as substrate when a digitonin-solubilized enzyme preparation was used.

The rate studies presented in this report, in conjunction with previous work, can be explained most readily if a glucose- and mannose-containing intermediate is an obligatory precursor of glucomannan biosynthesis in *P. aureus*. It is impossible to prove this with kinetic data. However, these rate results do indicate strongly that this would be the most profitable working hypothesis on which to base further investigation. The individual enzymes catalysing the reactions must be isolated and the intermediates chemically characterized.

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