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THE STRUCTURAL REQUIREMENTS OF GLUCOSE FOR PHOSPHORYLATION BY PHOSPHOGLUCOMUTASE

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Summary

During catalysis, the phosphoryl group of phosphoglucomutase (α-D-glucose-1,6-bisphosphate:α-D-glucose-1-phosphate phosphotransferase, EC 2.7.5.1) is transferred through a nucleophilic displacement reaction to the monophosphate substrates to form the diphosphate. Some non-phosphorylated analogs of glucose have been shown to serve as effective acceptors of the active phosphate albeit at a much reduced rate. Several other analogs exhibit little or no reactivity. The relative reaction rates of the reactive analogs follow the order: thioglucose > α- or β-D-glucose > D-xylose, > L-arabinose > *myo*-inositol. The rate of transfer increased with the increased concentration of glucose or its analogs. The products of the reaction may be acid stable ester phosphate or acid labile glycosyl phosphate as well as inorganic phosphate. *S*-phosphoryl (hemiacetal) thioglucose was identified as a product of the 1-thioglucose reaction.

It was possible to define certain steric requirements for the orientation of the hydroxyl groups in all the reacting sugars. These requirements are limited to 3 hydroxyl groups and pertain to loci or receptors on the active site of the enzyme. These would correspond in topography to carbons 2, 3 and 4 of the glucose molecule in the enzyme substrate complex. These hydroxyl groups should be oriented equatorially and project below, above and below the plane of the pyranose ring for C-2, C-3 and C-4, respectively.

Introduction

Phosphoglucomutase (α-D-glucose-1,6-bisphosphate:α-D-glucose-1-phosphate phosphotransferase, EC 2.7.5.1) catalyzes the overall reversible interconversion of glucose-1-P[1] and glucose-6-P. The enzyme transfers its phosphoryl group to

Abbreviations: Glucose-1-*P*, glucose 1-phosphate; glucose-6-*P*, glucose 6-phosphate; glucose-1,6-P_2, glucose 1,6-diphosphate.

either C-1 or C-6 of the appropriate substrate to form glucose-1,6-P_2. The enzyme then accepts either phosphoryl group of glucose-1,6-P_2 to form monophosphates [1,2]. The reaction is relatively specific for glucose phosphates, but other sugar phosphates and even unphosphorylated sugars have been shown to react albeit at a slower rate [2—4].

It was proposed earlier that the active transfer of phosphate to the glucose phosphate substrate involves an enzyme facilitated nucleophilic attack by either the C-1 or C-6 hydroxyl oxygen of the sugar molecule on the electron deficient phosphorus atom [2]. The demonstration, in this laboratory, that nucleophilic compounds such as cysteine, hydroxylamine, and fluoride ion, under the usual mild conditions of enzymatic reactions, are capable of dephosphorylating the native enzyme, made this all the more plausible [5,6]. The demonstration that nonphosphorylated sugars [4,7] are also acceptors of the phosphoryl moiety from the phosphoenzyme led us to explore this reaction further. The main objective is to determine the orientations of the hydroxyl function that are acquired for enzymatic activity. The absence of the phosphoryl group in these sugars eliminates the complicating factor of strong ionic interaction with the enzyme which could possibly override the weaker interaction of the nonphosphorylated substrate with the enzyme. It appears that the phosphoryl bond is rendered unusually electron deficient by the topography of the active site since denatured phosphoglucomutase is utterly inert under these conditions. The purpose of this article is to show that the enzyme transfers its active phosphate to underivatized sugars of seemingly diverse types but whose conformation points to a unique structural requirement which is necessary for maximal transfer. The products of the reaction vary with the sugar analog. A preliminary account of this work has been reported [4].

Materials and Methods

All chemicals were reagent grade and used without further purification. These were obtained as follows: β-D-glucose, α-D-glucose, 1-thio-β-D-glucose, L-arabinose, D-xylose, β-methyl-D-glucoside, α-methyl-D-xyloside, D-mannose, D-arabinose, D-ribose, α-methyl-D-glucoside, L-glucose, β-methyl-D-xyloside, 3-*O*-methyl-D-glucose, D-gluconic acid, D-galactosamine hydrochloride, D-glucosamine hydrochloride, L-xylose, xylitol, α-D-fucose, α-L-fucose, *myo*-inositol-2-monophosphate (Sigma); D-mannosamine hydrochloride, *myo*-inositol, α-methyl-D-galactoside, 2-deoxyglucose, and 2-deoxyribose (Calbiochem); L-lyxose, D-lyxose, L-mannose, β-phenyl-D-glucoside, and sorbitol (Nutritional Biochemicals); D-galactose (Fisher Scientific Company); 5-thio-D-glucose (Pfanstiel) [γ-^{32}P]ATP (specific radioactivity approx. 25 Ci/mmol) and Aquasol (New England Nuclear); hexokinase (Boehringer-Mannheim) and bovine serum albumin (Pentex).

The enzyme was crystallized from rabbit skeletal muscle as previously described to yield a homogeneous peak by ultracentrifugation [8]. It was activated 4—6-fold by preincubation in 1 mM $MgCl_2$/40 mM imidazole, pH 7.5 [2]. ^{32}P-labeled enzyme was prepared as before by allowing exchange of [^{32}P]glucose-6-*P* and phosphorylated phosphoglucomutase according to the mechanism of the reaction [2].

The phosphorylation reaction between [^{32}P]phosphoglucomutase and various sugars

The reaction consisted of mixing a solution of [^{32}P]phosphoglucomutase in 5 mM Tris/HCl (pH 7.5) with an equal volume of the sugar analog that had been dissolved in 80 mM imidazole HCl buffer (pH 7.5)/2 mM $MgCl_2$. This mixture was incubated at 37° C for appropriate time intervals. The reaction was terminated by the addition of bovine serum albumin (final concentration 3 mg/ml) and immediately followed by trichloroacetic acid, final concentration 8%. Appropriate aliquots of the supernatant were then either counted directly in 15 ml Aquasol in a Nuclear Chicago liquid scintillation counter or used subsequently for phosphate partitioning in the phosphomolybdate reaction [5,9].

The amount of transferable phosphate from enzyme to substrate was obtained from the amount of acid soluble counts released by a very large molar excess of glucose-1-*P*. Such excess causes virtually complete transfer of ^{32}P from the enzyme and reflects its molar concentration. Controls containing only buffer yield negligible production of inorganic phosphate not exceeding 2% per h. Boiled enzyme does not phosphorylate any of the analogs under the conditions used. Acid labile phosphate was determined by hydrolysis in 1 M HCl at 100° C for 7 min.

Purity of the sugar analogs used

As will be evident below, rather large concentrations of the sugar analogs were used. Consequently, it became important to establish their purity as relates to phosphorylated and nonphosphorylated derivatives.

Purity was verified as follows:

(a) Chromatography on acetate impregnated silica gel in ethyl acetate/isopropanol/water (100 : 60 : 30, v/v) and in ethyl acetate/acetic acid/methanol/water (60 : 15 : 05 : 10, v/v) showed only one spot as observed with alkaline silver nitrate spray except for thioglucose which exhibited a small amount of a less mobile contaminant which proved to have no dephosphorylating ability. The contaminant was not identified but was probably the disulfide of thioglucose.

(b) A highly reactive phosphorylated derivative might well be too small a contaminant to be detected by chromatography. Consequently, all analogs were dissolved in distilled water and passed through a Dowex-1 formate column. The phosphate free effluent was used for reaction with the enzyme. The rates of transfer of the phosphate from the enzyme to the particular sugar before and after Dowex treatment were identical. This was true with all the acceptor analogs. Consequently, all experiments reported herein are with untreated analogs.

(c) Identification of the phosphorylated products of the enzyme reaction was done in various ways which appear in the appropriate place.

Results

Phosphorylation of sugar analogs

The active sugar analogs were D-glucose, 1-thio-D-glucose, 5-thio-D-glucose, D-xylose, L-arabinose and *myo*-inositol. The rate of transfer of the enzyme

phosphate to the sugar acceptor varies with the sugar. At 0.1 mM enzyme and 0.1 M sugar, the initial rate of reaction with 1-thio-β-D-glucose is many times faster than with any other analog including glucose (Fig. 1). The initial rate varied directly with the concentration of only three of the analogs, namely xylose, arabinose and inositol. This conclusion is supported by reciprocal plots of xylose, arabinose and inositol concentration versus time which yield lines that pass through the origin. By contrast, glucose and thioglucose exhibited saturation kinetics as seen in Fig. 2. The K_m values obtained were 0.7 and 0.022 M for glucose and thioglucose, respectively. A possible interpretation of these differing kinetics is discussed below. Suffice it to indicate at this point that reciprocal plots are sufficiently flexible as to preclude accurate K_m values when the concentrations used are uncommonly large and the intercept close to the origin as is the case with these analogs.

Inactive glucose analogs

Many of the glucose analogs tested were either completely inactive or so poorly reactive that they could well be conveniently classified as inactive. These include D-allose, L-glucose, L-mannose, D-mannose, α-D-fucose, α-L-fucose, D-arabinose, L-xylose, L-lyxose, D-lyxose, D-glucosamine, D-galactosamine, D-mannosamine, α-methyl-D-galactoside, 3-*O*-methyl-D-glucose, 2-deoxyglucose, 2-deoxyribose, α-methyl-D-glucoside, β-methyl-D-glucoside, β-phenyl-D-glucoside, β-methyl-D-xyloside, inositol-2-phosphate, xylitol, sorbitol and gluconate. D-galactose and D-ribose show very slight activity (Fig. 1).

Metal ion requirements

The enzyme as prepared does not require added metal ions for these reactions. However, it is stimulated about 100% by $MgCl_2$ with maximal stimulation occurring at 2—3 mM (Fig. 3).

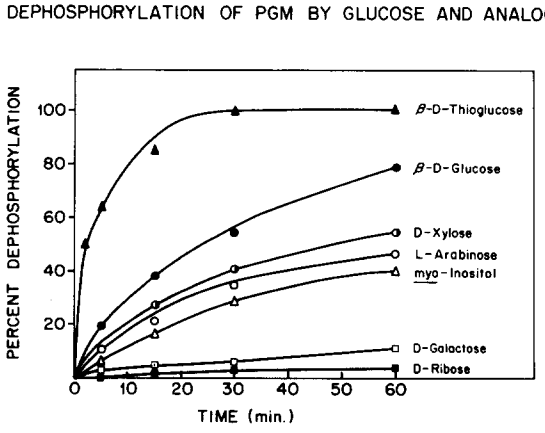


Fig. 1. The phosphorylation of D-glucose and analogs by phosphoglucomutase: 100 μl of 200 mM substrate in 80 mM imidazole, and 2 mM $MgCl_2$ pH 7.5 were added to 100 μl of 0.2 mM phosphoglucomutase that was previously dialyzed against 5 mM Tris-HCl, pH 7.5 and incubated at 37°C for the times indicated. 50 μl of 6% bovine serum albumin were added followed immediately by 100 μl of 25% trichloroacetic acid. After centrifugation, 200 μl of supernatant were counted. The extent of phosphate transfer is expressed as the percent of acid soluble counts released from the labeled enzyme. Controls containing only buffer yield negligible production of inorganic phosphate not exceeding 2% per h.

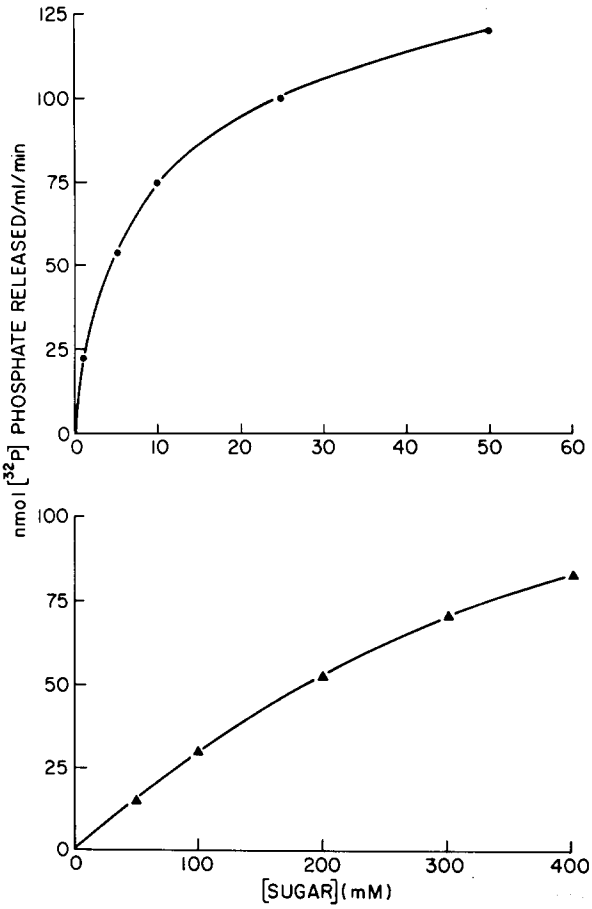


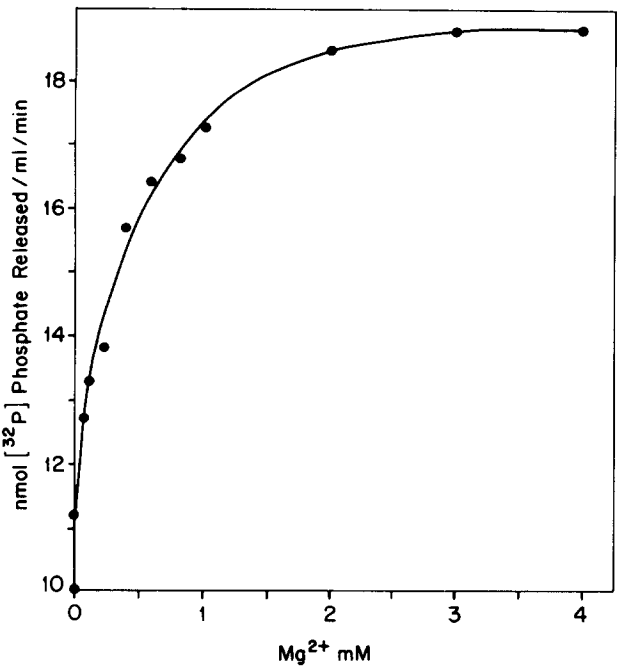
Fig. 2. Saturation kinetics of the reaction of glucose and thioglucose with phosphoglucomutase: 100 μl of the sugar in 80 mM imidazole and 2 mM $MgCl_2$ pH 7.5 were incubated for 1 min with 100 μl of 0.2 mM phosphoglucomutase previously dialyzed against 0.5 mM Tris HCl, pH 7.5. The reaction was stopped and assayed as in Fig. 1; glucose ▲, thioglucose ●.

Products of phosphorylation

The products of phosphorylation can be characterized in a general manner as either (a) acid stable organic phosphates, acid-stable phosphate, (b) acid labile organic phosphates, acid-labile phosphate, or (c) inorganic phosphate (P_i). The acid-stable phosphate:acid-labile phosphate:P_i ratios vary with substrate and with the duration of the reaction. A typical distribution for a 60-min reaction time is illustrated in Table I. As can be observed, several combinations of acid-stable phosphate, acid labile phosphate and P_i are obtained. There are, as expected, three products of glucose phosphorylation: glucose-6-*P*, glucose-1-*P*, glucose-1,6-P_2. These products were characterized as described below:

Glucose-6-*P* was characterized (a) by its stability to hydrolysis in 1 M HCl at 100°C for 7 min as compared to an authentic sample of glucose-6-*P*; (b) by chromatography on Dowex-1 formate eluting as a monophosphate (Fig. 4). (c) by the production of NADPH as catalyzed by glucose-6-*P* dehydrogenase.

Glucose-1-*P* was characterized (a) by its rapid rate of hydrolysis (5 min) in



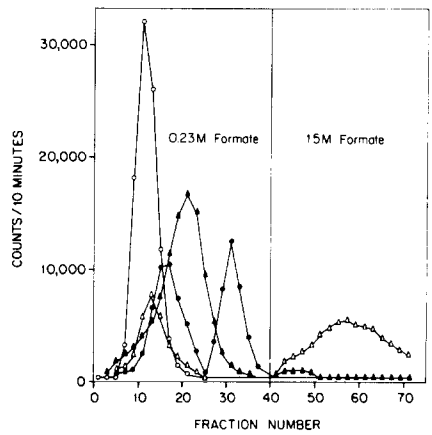


Fig. 3. The stimulation of glucose phosphorylation by magnesium: The enzyme was dialyzed exhaustively against 5 mM Tris-HCl pH 7.5 before initiation of the reaction. The final concentration of glucose and phosphoglucomutase were 50 and 0.1 mM respectively. The mixture was incubated at 37°C for 8 min and the reaction was stopped and assayed as in Fig. 1. Under these conditions 37% of the total phosphate transferred was found to be acid labile.

Fig. 4. Dowex-1 chromatography of the products of sugar phosphorylation. 500 μl containing 200 mM sugar, 80 mM imidazole and 2 mM $MgCl_2$, pH 7.5 were incubated at 37°C for 60 min with 500 μl of 0.2 mM phosphoglucomutase. The reaction was terminated with trichloroacetic acid as in Fig. 1. The trichloroacetic acid was then extracted 3 times with 2 vols. of ether. The ether was removed by bubbling nitrogen through the solution. The aqueous phase was then applied to a 0.55 X 8 cm Dowex-1 formate column and eluted with 0.23 M formate (4 parts formic acid and 1 part ammonium formate) to remove the monophosphates and inorganic phosphate. The elution was continued with 1.5 M formate (same proportions as above) to remove the diphosphate. Fraction volume was 0.5 ml. △ glucose; ▲, thioglucose; ○, inositol; ●, arabinose. With arabinose the first peak (Fractions 10—25) is arabinose phosphate and the second peak (Fractions 25—38) corresponds to the elution volume of inorganic phosphate.

1 N HCl at 100° C as compared to an authentic sample of glucose-1-*P*; (b) by its elution pattern from Dowex-1 formate appearing as a single peak with the monophosphate fraction.

Glucose-1,6-P_2 was characterized by (a) chromatography on a Dowex-1 formate column (Fig. 4); (b) by hydrolysis in HCl resulting in the release of half the radioactivity as inorganic phosphate, the other half being acid stable.

Phosphorylation of 1-thioglucose yields some P_i in addition to the corresponding mono- and diphosphates. These latter correspond to the mono and diphosphates of glucose and have similar properties.

1-*S*-phosphothioglucose (thioglucose-1-phosphate), (a) is acid hydrolyzable at a more rapid rate than glucose-1-*P*, (b) differs from glucose-1-*P* in its instability in the reaction mixture being converted entirely to inorganic phosphate,

TABLE I

DISTRIBUTION OF ^{32}P AMONG PRODUCTS OF DEPHOSPHORYLATION BY REACTIVE ANALOGS

The reaction was carried out as described in Fig. 1. All reactions were run for 60 min. After trichloroacetic acid preparation of the protein, 2-equal aliquots were taken from the supernatants. One was analyzed for acid-stable protein (ASP), acid-labile protein (ALP), and P_i according to the phosphomolybdate partioning procedure (5.9). The other was first hydrolyzed with 1 M HCl at 100°C for 7 min then partitioned as before. The isobutanol/benzene (I-B) extract of the unhydrolyzed sample represented $^{32}P_i$. The amount of ALP is the difference between the P_i and that extracted into I-B after acid hydrolysis. ASP is that ^{32}P which remains unextracted in the latter.

Substrate	Percent of total ^{32}P released		
	ALP	ASP	P_i
β-D-glucose	40—45	60—55	0
1-thio-β-D-glucose	10—20	84—70	6—10
5-thio-D-glucose	42	52	6
D-xylose	100	0	0
L-arabinose	0	60	40
myo-inositol	0	100	0

(c) since 1-thioglucose is the most reactive acceptor (Fig. 1), its reactivity is not likely to be due to contamination with any of the five reactive analogs reported herein.

1-Thioglucose 6-phosphate is by contrast (a) acid stable, (b) does not reduce NADP through the action of glucose-6-dehydrogenase, (c) like the 1-*S*-phosphothioglucose, it chromatographs over Dowex-1 with the monophosphate fraction (Fig. 4).

1-Thioglucose diphosphate (a) appears with the elution volume of glucose diphosphate, (b) elutes earlier than glucose diphosphate, (c) is produced in much smaller quantities as compared to the two 1-thioglucose monophosphates. All these characteristics clearly indicate that these phosphorylated products are indeed those of thioglucose and not the products of a possible glucose contaminant. The relative amounts of the products, inorganic phosphate, the two monophosphates, and the diphosphate, change during the course of incubation (fig. 5). There is a rapid decline in acid-labile phosphate which runs parallel with the increase in P_i. This reciprocal relationship is maintained until all acid-labile phosphate disappears with the concomitant cessation of P_i formation. At about this point, the level of acid-stable phosphate remains constant. The amount of acid-labile phosphate, which includes the thiohemiacetal monophosphate as well as the diphosphate varies considerably with the enzyme preparation. The reason for this is not clear. Chromatography of the reaction products (Fig. 4) after a 2-min incubation yields a single monophosphate peak containing acid stable and acid labile monophosphates along with a smaller peak of diphosphate.

Phosphorylation of xylose has been reported previously [4,7]. The product of the reaction is almost all acid-labile phosphate (98%). This has been identified as xylose 1-phosphate because of the following characteristics: (a) It chromatographs on Dowex-1 formate with the monophosphate fraction as a single peak (not shown). (b) All the product is easily hydrolyzable in acid. (c) No acid

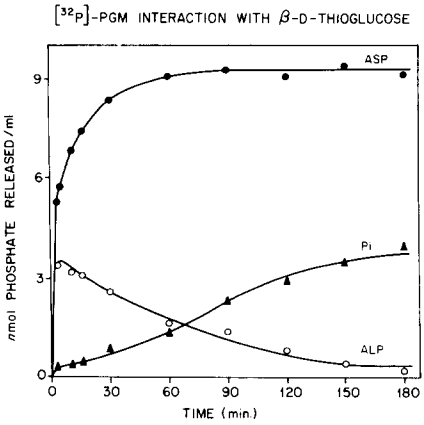


Fig. 5. The variation of the distribution of 1-thioglucose phosphates formed with time: 1.4 ml of 200 mM 1-thioglucose in 80 mM imidazole and 2 mM $MgCl_2$ pH 7.5 were incubated with 1.4 ml of 0.01 mM phosphoglucomutase in 5 mM Tris HCl, pH 7.5 at 37° for a maximum of 3 h. At the indicated times, 200-μl aliquots were removed and the reaction stopped as in Fig. 1. The supernatant of each aliquot was assayed with the phosphomolybdate partitioning reaction for acid-stable, acid-labile and organic phosphates. The release of 14 nmol of labeled phosphate is equivalent to complete dephosphorylation.

stable derivative is obtained. All of these three characteristics are unique to xylose 1-phosphate and do not apply to any of the phosphates of the other sugars used in this study.

Inositol, not a glycose, is quite unlike the three previous sugars in several characteristics. (a) Its product is an acid stable phosphate. This is not unex-

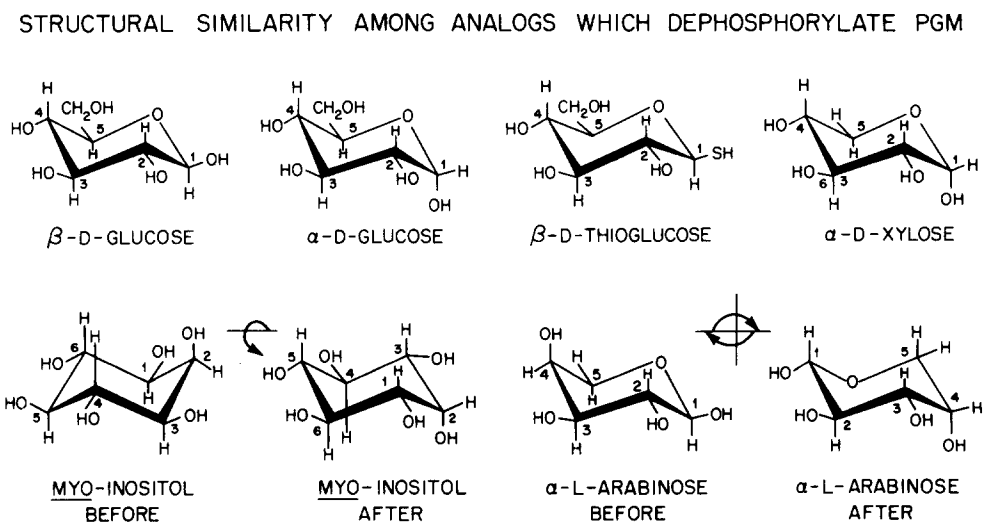


Fig. 6. Structural similarities among analogs which can be phosphorylated by phosphoglucomutase: Each analog is drawn as a pyranose (cyclohexane ring for inositol) in the chair form according to IUPAC conventions [11]. These are the conformations known to exist in the unsubstituted substrates. The structures are drawn and rotated (arabinose and inositol) to bring the 3 equatorial hydroxyl groups to an orientation below, above and below the plane of the ring such that they correspond to carbons 2, 3 and 4, respectively in the glucose molecule.

pected since it is not a hemiacetal. (b) Unlike glucose and xylose it yields little or no inorganic phosphate. (c) The product does not reduce NADP in the glucose-6-*P* dehydrogenase reaction. (d) The product also chromatographs on Dowex-1 formate as a monophosphate. (e) There is no detectable diphosphate formation nor is any expected since added *myo*-inositol-2-phosphate does not react with [^{32}P]phosphoglucomutase (see above). None of these characteristics indicate the exact location of the phosphate group on the inositol ring. No attempt has been made to characterize this compound any further. Many of the various phosphorylated derivatives of *myo*-inositol are not available and have not been characterized.

Phosphorylation of L-arabinose yielded a unique products of considerable interest. (a) Unlike D-xylose, L-arabinose yields acid-stable phosphate but no acid-labile phosphate. This indicates that no hemiacetal phosphate is formed. (b) Its elution pattern on Dowex-1 formate shows one organic phosphate peak corresponding to that of monophosphate sugars. (c) There is no detectable formation of diphosphate. (d) Unlike inositol, it produces considerable amounts of inorganic phosphate. (e) Upon prolonged incubation with phosphoglucomutase, it is completely converted to inorganic phosphate as the only product. Thus all these characteristics are uniquely the property of an L-arabinose phosphate.

Discussion

Phosphorylation of glucose and various analogs by [^{32}P]phosphoglucomutase has enabled us to specify the essential as well as the permissive orientations of the component parts of the substrate. Diverse as these substrates may appear, those that react with the enzyme have common structural requirements. The similarity of D-glucose and D-thioglucose is obvious, i.e. they are structurally the same except at carbon 1 where sulfur is substituted for oxygen. D-xylose has the same pyranose structure as D-glucose but lacks C-6. The similarity of L-arabinose to D-glucose is not immediately apparent. To facilitate the discussion of this matter, it is necessary first to delineate the required structure and orientation of certain groups in the substrate analogs. The reader would be well advised to refer to Fig. 6 periodically. There are 3 structural requirements for successful reaction of the nonphosphorylated sugars with the enzyme: (a) One requirement is a 6-membered ring such as the pyranose structure of sugars where an oxygen atom forms a part of the ring. The oxygen can be replaced by a sulfur atom with no loss of activity such as in 5-thio-D-glucose. Another favorable ring form is the cyclohexane structure of inositol where a carbon atom replaces the oxygen. The pyranose form is the dominant form of nonphosphorylated sugars under the conditions of the experiments described herein. At this point, it should be recalled that the phosphorylated sugars in the furanose form are to some degree reactive such as ribose-1-phosphate and ribose-5-phosphate are examples [2,10]. (b) The C-1 hydroxyl or sulfhydryl group must be unsubstituted and may be of the α or β orientation. (c) A sequence of 3 equatorial hydroxyl groups corresponding to the positioning C-2, C-3 and C-4 of glucose on the active site of the enzyme is required. They are oriented alternately below, above and below the plane of the ring, respectively. These are the

minimum requirements for reaction of non-phosphorylated substrate analogs. Phosphorylated substrates do not lend themselves to such fine subtleties since the highly charged phosphate group is so strongly attracted to the histidinium side chain at the active site [2] that in many instances these requirements can be overridden.

Viewed as usually depicted in Fig. 6, L-arabinose does not seem to follow the dictum relative to carbons 2, 3 and 4 if it binds to the active site as would glucose. However, it does fulfill the requirements if it binds the enzyme in the orientation obtained when it is flipped end over end (180°) until the axial hydroxyl group of C-4 coincides with the enzyme site that would normally be occupied by the C-1 hydroxyl group of α-D-glucose. Through this manipulation, C-3, C-2 and C-1 would then be positioned at the loci that C-2, C-3 and C-4 of glucose occupy. C-4 of arabinose would then be the only possible phosphate acceptor because it occupies the same site as that of C-1 of glucose.

myo-Inositol, like arabinose, does not appear to fit the required orientation of hydroxyl groups. As it appears in Fig. 6, its conformation and numbering do not conform with those of the pyranoses. However, it was drawn as usual according to the IUPAC convention [11] for conformation and nomenclature of cyclitols. When such a structure is turned 180° side on side, it can then be positioned such that the hydroxyls of C-1, C-6 and C-5 occupy the same sites on the enzyme surface as those of glucose carbons 2, 3 and 4. C-2 would then be the recipient of the phosphate group. An alternative positioning is obtained if after having completed the side-on-side turn required above, the molecule is rotated in the plane of the paper such that C-5, C-4 and C-3 are aligned on the enzyme surface corresponding to C-2, C-3 and C-4 of glucose, respectively. This orientation places C-6 of inositol in the proper position as phosphate acceptor.

Each of the nonphosphorylated substrates that was found to be substantially reactive with the enzyme satisfies all three criteria. In addition, all the sugars that proved less reactive or inactive fail to satisfy these criteria in one aspect of another. Thus, D-mannose, which has an axial hydroxyl at C-2, D-ribose with an axial hydroxyl at C-3 and D-galactose with an axial hydroxyl at C-4 do not conform to the requirements and exhibit minimal activity at best (Fig. 1).

A further look at the reactive analogs makes it clear that the positions on the enzymatic surface corresponding to C-1, C-5 and ring-oxygen positions of D-glucose are not imperative structural determinants in phosphate transfer. This statement is based on the fact that (a) C-1 hydroxyls in any orientation, α or β, are equally reactive, (b) xylose, a reactive pentose in the pyranose form lacks a hydroxyl group at C-5, (c) *myo*-inositol possesses a carbon in place of the ring oxygen and suffers no loss of reactivity, (d) L-arabinose, after being flipped to the reactive orientation has its C-5 occupying the position that is ordinarily occupied by the ring-oxygen of D-glucose in the reactive enzyme glucose complex.

D-Mannose, the C-2 epimer of glucose, is not phosphorylated nor is 2-deoxyglucose or glucosamine. These facts stress the sensitivity of the enzyme to an alteration of the substituents on C-2. α or β-methyl glycosides of hexoses are not phosphorylated indicating a sensitivity of the enzyme to an increase in the bulk of the substrate at C-1 which apparently does not allow the proper positioning of the nonimperative C-1. Increased bulk may also explain why inositol-

2-phosphate, and 3-*O*-methyl-D-glucose are unreactive, in addition to the fact that in these compounds the critical C-2 and C-3 hydroxyls are substituted. The methyl glycosides of pentoses are not expected to react and did not since the only site of phosphorylation, C-1, is substituted. Straight chain compounds such as xylitol, sorbitol and gluconate are inactive indicating the importance of the ring structure.

While the preferred orientations of C-2, C-3 and C-4 equatorial hydroxyls are below, above and below the plane of the ring respectively, the other constituents of the ring are necessary only insofar as they determine the gross structure of the molecule, i.e. the chair form for both the pyranose and the cyclohexane ring. The exact localisation of the acid stable arabinose phosphate has yet to be determined. This applies equally to phosphorylated inositol.

1-Thioglucose yields acid stable and acid labile phosphate. By analogy to glucose, these are assumed to be 1-thioglucose-6-phosphate and 1-*S*-phosphothioglucose and 1-thioglucose-1,6-diphosphate. The distinguishing properties of the three phosphorylated products delineated in the experimental section makes it most unlikely that these could be other than the thioglucosyl ester, the 6 ester and the 1-6-diphosphate of 1-thioglucose.

Two pertinent points are worthy of discussion. Saturation kinetics were not obtained for the reaction of *myo*-inositol, L-arabinose or xylose with the enzyme. By contrast, the reaction with glucose and its thio analog can be shown to be saturable albeit at very high concentrations. With the latter two substrates, the reaction is complicated by the fact that the first products, the glycosyl and ester monophosphates are also substrates.

It should be pointed out also that saturation kinetics were obtained only with those analogs that produce a sugar diphosphate, glucose and thioglucose. This conforms to the established fact that the limiting step in phosphoglucomutase catalysis which determines the K_m of the reaction is between the glucose-1,6-P_2 and the dephosphoenzyme [12].

Acknowledgements

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References

1 Najjar, V.A. and Pullman, M. and Pullman, M. (1954) Science 119, 631—634
2 Najjar, V.A. (1962) in The Enzymes (Boyer, P.D., ed.), Vol. 6, pp. 161—178, Academic Press, New York
3 Lowry, O.H. and Passoneau, J.V. (1969) J. Biol. Chem. 244, 910—916
4 Layne, P.P. and Najjar, V.A. (1975) Fed. Proc. 34, 680
5 Layne, P.P. and Najjar, V.A. (1975) J. Biol. Chem. 250, 966—972
6 Layne, P.P. and Najjar, V.A. (1973) Fed. Proc. 32, 667
7 Long, J.W. and Ray, W.J., Jr. (1972) Fed. Proc. 31, 419
8 Najjar, V.A. (1955) Methods Enzymol. 1, 294—299
9 Martin, J.B. and Doty, D.M. (1949) Anal. Chem. 21, 965—967
10 Klenow, H. (1953) Arch. Biochem. Biophys. 46, 186
11 Anderson, L. (1972) in The Carbohydrates (Pigman, W. and Horton, D., eds.), Vol. IA, 2nd Edn. pp. 519—579, Academic Press, New York
12 Najjar, V.A. and McCoy, E.E. (1958) Fed. Proc. 17, 1141