Activity of Glycogen Phosphorylase in the Crystalline State*

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Glutaraldehyde cross-linked crystals of muscle phosphorylase a and b (α-1,4-glucan:orthophosphate transglucosylase, EC 2.4.1.1) in the tetragonal form have been shown to be catalytically active in the direction of saccharide synthesis. Precession x-ray photographs at 5.5 Å resolution of a single crystal of cross-linked phosphorylase b at the h01 zone indicate little change in the diffraction pattern when compared to non-cross-linked phosphorylase b under similar conditions. Non-cross-linked crystals crack and dissolve in the presence of both substrates, maltoheptaose and glucose-1-phosphate, although they are stable in the presence of each individually. These phenomena are prevented by treatment with glutaraldehyde, which causes a marked increase in mechanical stability and completely suppresses solubilization of the enzyme under our assay conditions. Diffusion of substrates into cross-linked microcrystals does not appear to be rate-limiting and assays of such crystals are linear with respect to both time and enzyme concentration. Kinetic constants for both substrates are reported. The maximal velocities of phosphorylase a are larger than those of phosphorylase b in both the soluble and crystalline states under our assay conditions, with the above substrates. It appears that crystallization (and cross-linking) reduces maximal velocities by about 11- to 50-fold in the case of phosphorylase b and 50- to 100-fold for phosphorylase a. Little or no differences were found between the $K_m$ values for maltoheptaose or glucose-1-phosphate in the soluble or crystalline states. Kinetic data suggest that substrate binding sites are similar in both states. Although loss of catalytic efficiency points to differences in the active site of the enzyme caused by crystallization, another explanation is that the crystal is restricting a conformational change that is an essential part of the catalytic cycle.

Studies of the activities of various enzymes in the crystalline state have been well documented. The work on ribonuclease S (1), chymotrypsin (2), carboxypeptidase A (3, 4), ribonuclease A (5), and papain (6) indicates that each of these enzymes is catalytically active in the crystalline state. On the other hand, the tetragonal crystals of lysozyme, on which were carried out the classical x-ray structure analysis, exhibit steric hindrance of part of the substrate binding site caused by the arrangement of the protein molecules in the crystal lattice, whereas all the substrate binding subsites of triclinic crystals of lysozyme are accessible (7).

The recent studies of Lipscomb and co-workers comparing the conformation of carboxypeptidase in the crystalline state to solution studies (8) and those of Vallee and his group examining the kinetic properties of crystalline and solution carboxypeptidase (9) lead one to the conclusion that the conformation of the active site of an enzyme may be a function of the physical state. It is possible that crystallization may select one of the conformations of the enzyme that exists in solution and the equilibrium between these conformations may be different in the crystal than in solution. Studies on enzymes in the crystalline state have important implications for the understanding and delineation of enzyme mechanisms based on the three-dimensional crystal structure.

The availability of tetragonal crystals of glycogen phosphorylase b and a in this laboratory, upon which low resolution crystal structure determinations have been completed (10, 11) and high resolution studies have been undertaken, has encouraged us to examine these crystal forms for activity in the crystalline state. Such a study is of particular interest in the case of an allosteric enzyme such as phosphorylase, since it is possible that the enzyme conformation in the tetragonal crystal might be totally or partially unrelated to the “active” conformation.

Quirocho and Richards (3, 4) have described in great detail the factors and difficulties associated with the study of the enzymatic activity of crystals of carboxypeptidase A. We have applied this information to muscle phosphorylase a and b and demonstrate in this report that both enzyme forms are active in the tetragonal crystal state after stabilization by the bifunctional reagent glutaraldehyde. Some of the kinetic properties of cross-linked microcrystals of phosphorylase are also discussed in an attempt to relate the activities of soluble and crystalline enzyme.

MATERIALS AND METHODS

Rabbit muscle phosphorylase b was prepared by the method of Fischer and Krebs (12) and recrystallized at least three times. Prior to use, the phosphorylase crystals were dissolved in 1 mM EDTA, 5 mM dithiothreitol, and 10 mM Bes buffer (pH 6.7) and purified on a

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† The abbreviations used are: Bes, 2-[bis(2-hydroxyethyl)amino]ethanesulfonic acid; glucose-1-P, α-D-glucopyranose-1-phosphate.
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Typical yields of pure maltoheptaose were 8 to 10%.

RESULTS

Preliminary assays of tetragonal crystals of phosphorylase b in the presence of the activator IMP, and substrates glucose-1-P and maltoheptaose showed that the crystals shattered and dissolved although stable in the presence of each substrate individually. It was therefore decided that glutaraldehyde cross-linking of the crystals would be carried out under conditions similar to those described for crystalline carboxypeptidase (3, 4) and for solution phosphorylase (13). With large crystals of phosphorylase b, 0.05% glutaraldehyde as described by Wang and Tu (13) caused severe cracking of the crystals. A lower glutaraldehyde concentration (0.03%) as described under “Materials and Methods” eliminated this problem. The resulting cross-linked crystals showed increased mechanical stability to physical manipulation. Experiments in which cross-linked microcrystals of phosphorylase b were incubated with maltoheptaose and glucose-1-P simultaneously indicated no detectable soluble enzyme activity in the supernatant fluid after the crystals were removed by centrifugation. Other experiments attempted to identify soluble enzyme in the supernatant fluid of cross-linked microcrystal stock suspensions at 10 times the concentration present in a normal assay. No activity was detectable in our system which we estimate would have detected 0.5% of the activity of the crystal suspension. Similar results were found with crystals of phosphorylase a.

Precession x-ray photographs of single crystals of glutaraldehyde cross-linked phosphorylase b at the hol zone and the non-cross-linked control are compared under identical conditions in Fig. 1. Only slight changes in the diffraction patterns were caused by cross-linking. A single cross-linked crystal of phosphorylase a was mounted on a diffractometer and 2,500 unique reflections, to a resolution of 6 Å, were collected by the methods published earlier (11). The root-mean-square difference between the native and cross-linked phosphorylase a structure amplitudes was 5.5%. When a difference Fourier map was calculated only a single peak of positive density was found, which, by comparison with the electron density map of phosphorylase a at 3.0 Å resolution, was located in the solvent region between monomers of different dimers. The extra peak of electron density lay adjacent to a long side chain from one monomer and extended toward a side chain of the other monomer. This x-ray analysis suggests that the glutaraldehyde moieties are distributed throughout the crystal lattice, not just at the surface, and that they may provide intermolecular cross-links. It also suggests why, by preliminary amino acid analysis of cross-linked and NaBH₄-reduced cross-linked microcrystals, we could not detect any loss of lysine residues. One modified lysine out of 46 in each monomer would not be a significant change in the amino acid analyses of the crystal suspensions. Details of the x-ray analysis will appear in a later publication.³

Tetragonal microcrystals of phosphorylase a and b were prepared primarily to limit kinetic effects of diffusion of substrates and products in the crystal lattice as has been discussed by others (1, 4, 9). Assays of suspensions of the smallest cross-linked microcrystals of phosphorylase b, whose average size was about 1 x 1 x 10 μm indicated that assays

³ R. J. Fletterick, and J. Syverson, personal communication.

² R. J. Fletterick, P. J. Kasvinsky, N. B. Madsen, and J. Syverson, manuscript in preparation.
were linear with time (Fig. 2). However, specific activities of larger crystals, whose average minimum dimension was about 5 μm, showed slight curvature as a function of time (Fig. 2). Nevertheless, the specific activities of the two sizes of crystals, as indicated by the slopes of the lines in Fig. 2, are identical within experimental error. As pointed out by Quiocho and Richards (4) a study of enzyme activity on both a weight and crystal surface basis, when related to crystal size can indicate the influence of diffusion on activity measurements. A constant specific activity as a function of crystal size indicates that activity measurements are free of diffusion effects. An inverse relationship between activity (on a surface area basis) and crystal size indicates an influence of diffusion on activity. In Table I we present studies of the effect of crystal size on activity. The relative activities on a weight basis were essentially identical even though the relative surface area increased 10-fold as a function of decreasing size. The results indicate no diffusion effects for crystals whose minimum dimension is less than 10 μm. The work on carboxypeptidase (4, 9) and papain (1), indicates that the activities of crystals whose minimum dimension is about 5 μm or less are not affected by the diffusion of substrate. Furthermore, the results of studies of the reaction of azide with myoglobin crystals, gave no evidence of diffusion limitation with minimum crystal dimensions in the range 2 to 5 μm (26).

Additional information which is of importance relative to the question of crystal surface activity is the finding that microcrystals exhibited significant activity with glycogen as the saccharide acceptor. Quiocho and Richards (4) have, without success, attempted to estimate the contribution of the surface layer to the activity of crystals of carboxypeptidase, by the use of the synthetic block copolymer of glutamic acid and tyrosine, which is a potent inhibitor of the enzyme. With phosphorylase some of the nonreducing chains of glycogen are presumably able to bind and undergo chain extension at the crystal surface. In the presence of 27 mM maltose and 50 mM glucose-1-P, specific activity of 2.34 (micromoles per h per mg); with 1% glycogen and 50 mM glucose-1-P, specific activity = 0.28; with all components, specific activity = 2.61. In the presence of glucose-1-P and either maltose or glycogen no detectable activity was present in the supernatant solution obtained after sedimentation of the crystals. In solution, phosphorylase b activities with glycogen or glycogen plus maltose are the same; equal to

![Fig. 1. A split ho1 precession photograph at 5.5 Å resolution for the native b (left) and glutaraldehyde cross-linked enzyme.](image)

**Fig. 2.** The linearity of assay of cross-linked microcrystals of phosphorylase b as a function of time with 14 mM maltose, 12.6 mM glucose-1-P, and 2 mM IMP. Assays are described in the text. Average minimum crystal dimensions are about 1 μm for the upper (---) line and 5 μm for the lower (—) curve. The vertical coordinates have been staggered for clarity. Horizontal bars indicate the standard error of the mean for triplicate samples. The regression line (---) was calculated using a Hewlett Packard-65 standard program and gave a coefficient of determination, \( r^2 = 0.99 \). For a perfect fit to a straight line, \( r^2 = 1.00 \).**

| Average crystal size (μm) | Protein (μg/ml) | Specific activity (μmol/h/mg) | Relative activity | Relative area (cm²/mg) | Relative activity (μmol/h/cm²) |
|---------------------------|----------------|-------------------------------|------------------|------------------------|-------------------------------|
| 1 x 1 x 10                | 7.91 ± 0.01    | 0.523 ± 0.028                | 1.05             | 10                     | 0.1                           |
| 2.5 x 2.5 x 25            | 15.49 ± 0.04   | 0.543 ± 0.005                | 1.09             | 4                      | 0.3                           |
| 5 x 5 x 40                | 9.98 ± 0.33    | 0.556 ± 0.038                | 1.11             | 2                      | 0.6                           |
| 10 x 10 x 70              | 12.43 ± 0.40   | 0.499 ± 0.052                | 1.00             | 1                      | 1.0                           |

*Activities determined in the presence of 13.7 mM maltose, 12.5 mM glucose-1-P, and 2.0 mM IMP.

*Limits shown are standard errors.

*Comparisons of activity on a weight or area basis are relative to the largest crystals.

*Based on density = 1.299 g/ml for crystals containing 52% protein.
that with glycogen alone and indicate the preference of phosphorylase for the superior priming activity of glycogen (27). Since the activity of the crystals due to maltoheptaose and that due to glycogen are additive when both substrates are present, it is likely that only the surface of the crystals is active with the latter substrate. The maltodextrin, however, is apparently able to penetrate into the crystal matrix. Approximately 2.5% of the dimer molecules of phosphorylase b are exposed to solvent at the surface of the microcrystal preparation used. This value is similar to the estimate that 0.7% of the total expected activity of microcrystals was contributed by glycogen alone (0.28 μmol/h/mg), assuming the crystal $V_{\text{max}}$ (Table II, 42 μmol/h/mg) with maltoheptaose approximates the total expected activity. It would appear that the surface of the crystals is active with glycogen. The maltodextrin, however, enters the interior of the microcrystal and the activity found is not related to surface activity alone.

Figs. 3 and 4 show that assays of microcrystals of phosphorylase a are linear as a function of both time and enzyme concentration. On the basis of these preliminary studies it appeared that it would be possible to characterize the kinetic properties of cross-linked microcrystals and compare these properties to those of the native enzymes in solution under similar conditions.

Initial studies of microcrystals indicated that there were large reductions of the specific activity when compared to enzyme in solution. Such a reduction in activity could be caused by the effects of crystallization on either the binding of substrates or catalytic efficiency. In an attempt to differentiate between these possibilities we have carried out activity studies of enzyme in the crystalline and soluble states as a function of substrate concentration. The results of these studies using maltoheptaose and glucose-1-P as variable substrates are shown in Figs. 5 to 10. Due to the limited availability of maltoheptaose we were unable to utilize high concentrations of primer in these studies. Nevertheless, experimental results were reasonable when the large extrapolations to $K_m$ and $V_{\text{max}}$ which resulted and the inherent difficulties encountered in the assays of crystal suspensions were taken into consideration.

Comparison of Lineweaver-Burk plots for soluble and crystalline phosphorylase b under identical conditions with maltoheptaose as variable substrate did not show any difference in kinetics over the range of substrate concentrations studied (Fig. 5). Phosphorylase a, however, showed curvature at low concentrations of maltoheptaose (Fig. 6) while the Hill plot of this data indicated cooperativity in solution and slightly less cooperativity in the crystalline state (Fig. 7).

With the variable substrate glucose-1-P, however, there were obvious differences between the solution and crystal kinetic patterns obtained. Phosphorylase a exhibits homotropic cooperativity for glucose-1-P with the acceptor glycogen (28). In solution, with the acceptor maltoheptaose we found similar results (Figs. 8 and 10). Upon crystallization, however, co-

**Fig. 3.** The linearity of assay of glutaraldehyde cross-linked microcrystals of phosphorylase a as a function of time with 27 mM maltoheptaose and 50 mM glucose-1-P. Minimum crystal dimensions are less than 2 μm. The regression line for triplicate samples gave $r^2 = 0.99$.

**Fig. 4.** The linearity of assay of crystals of phosphorylase a as a function of protein concentration (1 μl = 0.4 μg). Enzyme and conditions are as in Fig. 3 ($r^2 = 0.97$).

**Fig. 5.** Activities of glutaraldehyde cross-linked microcrystals of phosphorylase b (Phos b) and the native enzyme in solution as a function of maltoheptaose concentration with 50 mM glucose-1-P and 2 mM IMP. Average minimum crystal dimensions are about 1 μm. Assay conditions are described in the text. The coefficient of determination ($r^2$) for crystalline and soluble enzyme is 0.96 and 0.99, respectively.
operative interactions are no longer apparent and linear Michaelis-Menten type kinetics result (Fig. 8). The kinetics observed for phosphorylase b and glucose-1-P were linear with both crystal and soluble enzymes (Fig. 9).

Kinetic parameters for the various enzyme forms are presented in Table II. The refined \( K_m \) and \( V_{max} \) values were obtained by a fit of the data in its hyperbolic form to the Michaelis-Menten function (29). Only data from the linear portions of Lineweaver-Burk plots were utilized. Apparent dissociation constants in brackets were also evaluated from Hill plots where the former showed cooperativity. The results

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**Fig. 6.** Activities of cross-linked microcrystals of phosphorylase \( a \) (Phos \( a \)) and the native enzyme in solution as a function of maltoheptaose concentration with 50 mM glucose-1-P. Minimum crystal dimensions are less than 2\( \mu \)m. Assay conditions are described in the text.

**Fig. 7.** A Hill plot of the data of Fig. 6. The apparent \( K_m \) values (mM maltoheptaose) were 129 and 130 for the respective crystalline and soluble forms. The coefficient of determination \( (r^2) \) of the respective forms is 0.99 and 0.97. The Hill coefficients \( (n) \) are indicated.

**Fig. 8.** Activities of glutaraldehyde cross-linked microcrystals of phosphorylase \( a \) (Phos \( a \)) and the native enzyme in solution as a function of glucose-1-P concentration with 27 mM maltoheptaose present. The same crystal preparation as in Figs. 6 and 7 was used. The coefficient of determination \( (r^2) \) for the crystal data is 0.99.

**Fig. 9.** Activities of glutaraldehyde cross-linked microcrystals of phosphorylase \( b \) (Phos \( b \)) and the native enzyme in solution as a function of glucose-1-P concentration with 14 mM maltoheptaose and 2 mM IMP present. The same crystal preparation as in Fig. 8 was used. The coefficient of determination \( (r^2) \) for both the crystal and soluble enzyme data is 0.99.

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Note: The figures and graphs are not available in this text format. The text provides a description of the experiments and results. The figures and graphs are intended to support the described experiments and should be viewed in the original publication for a full understanding of the data.
suggest that there is little change in $K_m$ for either maltoheptaose or glucose-1-P upon crystallization and glutaraldehyde cross-linking of phosphorylase. Even when the kinetic pattern was different in the soluble and crystal form, as in Fig. 8, $K_m$ was unaffected. Comparison of $V_{\text{max}}$ values obtained for the various enzyme forms (Table II) indicates that crystallization (and cross-linking) has its greatest effect on this kinetic parameter. Maximal velocity is reduced by as much as 100-fold.

In the course of this investigation we found that the specific activity of cross-linked microcrystals of phosphorylase a decreases as the crystals age over a period of weeks or months (75% loss in 2 months). This correlated with a second observation, that the physical appearance of crystals of native phosphorylase a changes, darkening to an amber color from an initial pale yellow, as a function of age. We ascribe this darkening of the yellow color to perturbations of the pyridoxal-5'-P binding in the native crystal, resulting in increased concentrations of the Schiff's base form. A solution of sodium borohydride at neutral pH discharged the amber color, presumably by reduction of the Schiff's base. There was no affect of aging on the specific activity or physical appearance of cross-linked or native crystals of phosphorylase b. The observation of the effect of aging of crystals of phosphorylase a on both physical appearance and specific activity might suggest that high resolution crystal structure determinations would show differences between the "aged" and "young" crystals. Such differences might be correlated with the conformation of the active site.

**DISCUSSION**

Large reductions in the specific activity of crystals of phosphorylase when compared to soluble enzyme do not appear to be the result of diffusion limitation of substrates' entrance into the crystal matrix; nor do the activities obtained seem to be limited to the surface of the crystals. These results, however, could have been due to the affects of crystallization (and cross-linking) on catalytic efficiency ($V_{\text{max}}$) or on binding ($K_m$) of substrates. The finding that assays of microcrystals were linear with both time and enzyme concentration allowed us to investigate the possibility that one or both of these kinetic parameters had been altered. The results indicate that the primary effect of crystallization (and cross-linking) is on maximal velocity which is reduced 11- to 100-fold. Effects on $K_m$ were slight. The kinetic data with the crystals were compared to similar data for the native enzymes. We have not attempted to compare the activity of cross-linked crystals to similarly treated soluble enzyme, since it is clear that the cross-linking reaction in the crystal matrix is considerably different from that in solution. Wang and Tu (13) have described a soluble species of glutaraldehyde-modified phosphorylase b which had 10% of its lysines modified by the cross-linking reagent. Results of the preliminary amino acid analysis of microcrystals of phosphorylase b showed no detectable loss of lysine caused by the cross-linking reagent. Such a result would seem to indicate that the modified species in solution is considerably different from that in the crystal where only one intermolecular glutaraldehyde-lysine bond could stabilize the structure and still remain undetected by differ-

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**Table II**

Kinetic parameters for solutions and crystals of muscle phosphorylase

Refined Michaelis constants and $V_{\text{max}}$ values were obtained from the data yielding linear portions of Lineweaver-Burk plots (Figs. 5, 6, 8, and 9) according to the method of Wilkinson (29) as programmed for the HP-65 computer. * Limits shown are standard error.

| Enzyme  | Form | Variable substrate | $K_m$  $M \times 10^3$ | $V_{\text{max}}$  $M \times 10^3$ | $V/V_{\text{max}}$  |
|---------|------|-------------------|----------------|----------------|-----------------|
| Phos b  | Soln. | Maltoheptaose  | 178 ± 20 | 7.5 ± 1.1 | 0.09 |
| Phos b  | Cryst. | Maltoheptaose  | 175 ± 21 | 0.7 ± 0.1 | 0.09 |
| Phos a  | Soln. | Maltoheptaose  | 172 ± 23 (130) | 200 ± 30 | 0.02 |
| Phos a  | Cryst. | Maltoheptaose  | 150 ± 38 (129) | 5.8 ± 1.2 | 0.02 |
| Phos b  | Soln. | Glucose-1-P  | 37 ± 8 | 4.0 ± 0.5 | 0.02 |
| Phos b  | Cryst. | Glucose-1-P  | 36 ± 8 | 0.07 ± 0.01 | 0.02 |
| Phos a  | Soln. | Glucose-1-P  | 4.4 ± 0.6 (6.2) | 37.6 ± 1.1 | 0.01 |
| Phos a  | Cryst. | Glucose-1-P  | 3.6 ± 0.6 | 0.21 ± 0.01 | 0.01 |

* $V/V_{\text{max}}$ (crystal)/$V_{\text{max}}$ (solution).
* The abbreviations used are: Phos b, phosphorylase b; Phos a, phosphorylase a; Soln., solution; cryst., crystal.
* With 50 mM glucose-1-P. Data from Figs. 5 to 7.
* With 14 mM maltoheptaose. Data from Fig. 9.
* With 27 mM maltoheptaose. Data from Fig. 8.
ences in amino acid analyses. The x-ray difference Fourier analysis would appear to support this conclusion.

As indicated above, the maximal velocity was the only kinetic parameter of phosphorylase to be affected by crystallization and cross-linking. The fact that glutaraldehyde modification of soluble phosphorylase b had almost no effect on $K_m$ and reduced $V_{\text{max}}$ of the modified enzyme by only 35% (13) is compatible with this finding. Furthermore, it is likely that the large diminution of $V_{\text{max}}$ in crystals of phosphorylase a is primarily the result of crystallization. Similar results have been reported for carboxypeptidase, where cross-linking reduced activity by 2-fold (4). The kinetics of the cross-linked crystals, however, indicated that $k_{\text{cat}}$ was reduced by 20- to 50-fold (9). In the report of Sluyterman and De Graaf (6), papain was shown to be fully active in the crystalline state and it was suggested that ribonuclease S (1) and chymotrypsin (30) might also have exhibited full activity if diffusion effects had been eliminated by the use of microcrystalline preparations of these enzymes.

The finding that the maximal velocity of phosphorylase a with the substrate maltotetraose was 250 μmol/min/mg was quite surprising in the light of the recently reported value of 26.5 μmol/min/mg by Hu and Gold (18). Under our assay conditions using 68 mM glucose-1-P and 1% glycogen as the acceptor we found that the catalytic activity for soluble phosphorylase a was 83.1 μmol/min/mg; Hu and Gold (18) report a $V_{\text{max}}$ of 27.1 μmol/min/mg in the presence of AMP. In the absence of bovine serum albumin in our assay up to 90% of phosphorylase activity (depending upon enzyme concentration) was lost, presumably through contact denaturation. Although much of the quantitative difference between our results and those of Hu and Gold (18) is undoubtably due to variations in assay conditions it would still appear that the observation of elevated maximal velocities in the presence of oligosaccharide acceptor molecules is not confined to our laboratory.

The specific activities obtained with both soluble and crystalline phosphorylase b were considerably lower than those of phosphorylase a. This result is probably related to the increased nucleotide requirement of the enzyme when the glycogen acceptor is replaced by oligosaccharides. Smith (37) has reported that the apparent $K_m$ for AMP is 0.8 mM with maltopentaose; a 27-fold greater requirement over that with glycogen. A similar result with maltotetraose and the replacement of AMP in our experiments by IMP, to maintain crystallization conditions, could account for the activities and maximal velocities observed. The large $K_m$ for glucose-1-P exhibited by soluble phosphorylase b (37 mM, Table II) under our assay conditions (compared to 4.4 mM for phosphorylase a) is consistent with this interpretation. Black and Wang (32) have reported that the $K_m$ of phosphorylase b for glucose-1-P is between 32 and 37 mM in the presence of 0.1 to 4 mM IMP. Furthermore, the $K_m$ of 3.6 mM glucose 1-P for phosphorylase a in the absence of AMP (28) compares favorably with the present data.

Perhaps the most interesting implication of the present results is that the binding sites for maltotetraose and glucose-1-P in the cross-linked crystal are very similar to those in the soluble enzyme. Essentially no change in $K_m$ was observed for either substrate with phosphorylase a or b upon crystallization. Loss of catalytic efficiency, however, as reflected in diminished maximal velocities of the crystal, clearly points to differences in the active site of the enzyme caused by crystallization. This may not be a static difference but, rather, the crystal lattice may restrict a conformation change that is an essential part of the catalytic cycle so that it takes more energy to achieve the enzyme structure required for the transition state. This possibility is supported by the fact that, since the crystals crack on catalysis when not cross-linked, a fairly large conformational change must be essential for catalysis.

It is interesting that the Hill coefficients for the homotropic cooperativity of maltotetraose observed for both solutions and crystals of phosphorylase a were similar (Fig. 7). The possibility that allosteric interactions of the enzyme in solution are also present in the crystal is both remarkable and intriguing. Such a result might imply that there is considerable conformational flexibility, even in the crystal matrix. Failure to observe cooperativity for glucose-1-P binding in crystalline phosphorylase a as expected from the solution studies (Fig. 8) could be related to the differential in size, number of binding contact points, and binding energies between maltotetraose and glucose-1-P.

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