Dissociation of the tetrameric phosphoglycerate mutase from yeast by a mutation in the subunit contact region

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Phosphoglycerate mutases from different sources exhibit a variety of quaternary structures (tetramer, dimer and monomer). To perturb the tetrameric structure of yeast phosphoglycerate mutase we have prepared a mutant enzyme in which Lys-168 in the subunit-contact region has been replaced by proline. The K168P mutant enzyme undergoes dissociation to dimers at low concentrations; thus on lowering the concentration from 200 µg/ml to 5 µg/ml the proportion of tetramer falls from 85% to 53%. The tetrameric structure of the wild-type enzyme remains intact over this range of concentrations. The mutant enzyme has similar kinetic properties to the wild-type enzyme, with k_{cat} being reduced by 26%. Far-u.v. c.d. studies show that there has been

a small loss of helical structure in the mutant. Compared with wild-type enzyme, the K168P mutant enzyme is slightly less stable towards proteolysis by trypsin, but significantly less stable towards denaturation by guanidinium chloride, with the mid-point concentration of guanidinium chloride some 50% lower. After denaturation, the mutant enzyme could regain activity and quaternary structure when the guanidinium chloride concentration was lowered to 0.05 M. The properties of the mutant enzyme are discussed in terms of other dimeric phosphoglycerate and bisphosphoglycerate mutases which contain proline at position 168.

INTRODUCTION

The enzyme phosphoglycerate mutase (EC 5.4.2.1) catalyses the interconversion of 2- and 3-phosphoglycerates in the glycolytic/gluconeogenic pathways. The enzyme from *Saccharomyces cerevisiae* has been particularly well-characterized and its amino acid sequence and crystal structure have been determined (Watson, 1982; White and Fothergill-Gilmore, 1988; Fothergill-Gilmore and Watson, 1989). The structure of the tetrameric yeast enzyme is shown in Figure 1.

Phosphoglycerate and bisphosphoglycerate mutases from vertebrates have a similar subunit size to the yeast enzyme (M_r about 27000) and also show considerable sequence identity (about 50% overall). However, these enzymes are dimeric, in contrast to the tetrameric enzyme from yeast. A comparison of the available sequences (see Fothergill-Gilmore and Watson, 1989) shows that most of the residues involved in the subunit-contact region that is labelled D in Figure 1 are well-conserved. The residues in the other subunit-contact region (labelled T) are much more variable and in general show no clear pattern of variability between the dimeric and tetrameric enzymes. A notable exception is residue 168, which is a lysine in the tetrameric yeast enzyme, but is a proline in all the dimeric enzymes. This residue forms part of helix 6 and the substitution would be expected to disrupt the local secondary structure and hence weaken the interaction between pairs of dimers.

In an earlier paper (White and Fothergill-Gilmore, 1992) we described the development of an over-expression system for the yeast enzyme and demonstrated its applicability to the preparation of a mutant enzyme in which one of the two histidine side-chains at the active site (His-181) was replaced by an alanine. In this paper we examine the effects of the substitution of Lys-168 by proline on the catalytic properties, quaternary structure and stability of the enzyme. The mutant enzyme

(K168P) has similar kinetic constants to the wild-type enzyme, but shows a tendency to dissociate into dimers on dilution and is considerably less stable than the wild-type enzyme towards denaturation by guanidinium chloride (GdnHCl).

EXPERIMENTAL

Preparation of K168P mutant

The yeast phosphoglycerate mutase gene carried by a pVT-type shuttle phagemid (pVT-GPM) was subjected to site-directed mutagenesis as described previously (White and Fothergill-Gilmore, 1992). Expression was in a yeast strain in which the wild-type phosphoglycerate mutase gene had been deleted. Approx. 25% of the protein in a crude extract was the mutated phosphoglycerate mutase, as indicated by SDS/PAGE. The phagemid with the mutated gene was rescued from transformed yeast cells and the gene was sequenced to verify that only the single desired mutation was present. Purification of the K168P mutant enzyme was by ammonium sulphate and QA-cellulose fractionation, essentially as reported previously (White and Fothergill-Gilmore, 1992), with the addition of a final f.p.l.c. Superose-12 gel-filtration step. The Superose column was eluted with 20 mM Tris/HCl, pH 8.0.

The concentration of enzyme was determined spectrophotometrically using a value of 1.45 for the A_{280} of a 1 mg/ml solution (Edelhoch et al., 1957).

The enzyme was assayed for phosphoglycerate mutase activity in the direction of 2-phosphoglycerate synthesis, using the assay system in which enolase, pyruvate kinase and lactate dehydrogenase are used as coupling enzymes (White and Fothergill-Gilmore, 1992).

C.d. and fluorescence measurements were made using a JASCO J-600 spectropolarimeter and a Perkin-Elmer LS50 fluorimeter respectively (White et al., 1993). Molar ellipticities

Abbreviation used: GdnHCl, guanidinium chloride.

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Figure 1 Yeast phosphoglycerate mutase tetramer

The two sulphate ions are shown and it is assumed that the phospho groups of the ligands occupy the same positions as the sulphate ions in the unligated enzyme that has been crystallized (Fothergill-Gilmore and Watson, 1989). The C-terminal tail extends 14 residues beyond the C-terminus observed crystallographically, indicated by C. The subunit-contact region that is thought to be present in the tetrameric enzyme only is labelled T; the subunit-contact region present in dimers and tetramers is labelled D. The position of residue 168 is indicated in one subunit.

were calculated assuming a value of 112 for the mean residue weight (White and Fothergill-Gilmore, 1988). The stabilities of the K168P mutant and wild-type enzymes towards GdnHCl were measured using the changes in ellipticity at 225 nm and fluorescence emission at 330 nm with excitation at 290 nm (White et al., 1993). The concentrations of solutions of GdnHCl (Ultrapure, Gibco-RBL) were checked using refractive-index measurements (Nozaki, 1972).

Cross-linking of the enzymes by glutaraldehyde and subsequent analysis by SDS/PAGE were done using published procedures (Jaenicke and Rudolph, 1986; White et al., 1993). The reactivation and reassociation of the enzymes after denaturation in GdnHCl were studied using the methods of Hermann et al. (1983, 1985).

RESULTS

Purification of the K168P mutant

Substantial purification of the K168P mutant was achieved by ammonium sulphate and QA-cellulose fractionation, and the few remaining contaminants were removed by f.p.l.c. gel filtration on a Superose-12 column. The contaminants were eluted in two early peaks, followed by the major peak containing the tetrameric form of the K168P mutant. A subsequent smaller peak (approx. 10% of the major peak) corresponded with the dimeric form of the enzyme as shown by cross-linking studies. Both mutase peaks showed a single band in SDS/PAGE, with an M_r indistinguishable from authentic wild-type enzyme (results not shown).

Table 1 Kinetic constants of wild-type and K168P mutant phosphoglycerate mutases

The kinetic constants for the K168P mutant enzyme were derived from the analysis of Hanes plots as was done previously for the wild-type enzyme (White and Fothergill-Gilmore, 1992). The values of k_{cat} are expressed per subunit of enzyme. Abbreviations used: 3PGA, 3-phosphoglycerate; BPG, 2,3-bisphosphoglycerate.

	k_{cat} (s^{-1})	K_m (μM)		$k_{cat}/K_m(3PGA)$ ($M^{-1} s^{-1}$)
		3PGA	BPG	
Wild-type	490	740	8.1	6.62×10^5
K168P	360	480	1.7	7.50×10^5

Kinetic properties

The K168P mutant showed the two-substrate, ping-pong pattern characteristic of the wild-type enzyme (Britton et al., 1972; White and Fothergill-Gilmore, 1992). The kinetic constants, summarized in Table 1, show that the mutation has only relatively small effects on the catalytic and substrate-binding properties of the active sites; k_{cat} is reduced by around 26% and the K_m for 3-phosphoglycerate is reduced by 35%. The value of $k_{cat}/K_m(3\text{-phosphoglycerate})$ is increased by 13% in the mutant.

Spectroscopic properties

The c.d. spectra of the wild-type enzyme and of the K168P mutant enzyme are shown in Figure 2. The far u.v. c.d. spectra (Figure 2a) show that the mutation has led to a small reduction in the amount of secondary structure in the enzyme. Applying CONTIN analysis (Provencher and Glöckner, 1981) over the range 240 nm to 190 nm, the reduction in α -helix content is from $32 \pm 2\%$ to $28 \pm 1\%$. This small change in the α -helix content would be consistent with the perturbation of helix 6 by the substitution of Lys-168 by proline. The α -helix content of wild-type enzyme determined by c.d. is similar to that derived using X-ray crystallography (32%; Watson, 1982). CONTIN analysis also indicates that the β -sheet content is reduced in the mutant enzyme compared with the wild-type enzyme ($53 \pm 2\%$ compared with $60 \pm 2\%$). However, it is clear that the β -sheet content is grossly over-estimated compared with the value (6%) from the X-ray crystallographic data, possibly due to a neglect of the contribution of β -turns and to the smaller contributions to the c.d. spectra from β -sheet compared with α -helix.

Far-u.v. c.d. spectra of the K168P mutant enzyme and of the wild-type enzyme were recorded at concentrations ranging from 260 $\mu g/ml$ to 10 $\mu g/ml$ with no significant change (less than 3%) in the molar ellipticity values between 260 nm and 200 nm in each case. The high noise level associated with the use of long path-length cells (0.5 cm) prevented data being acquired below 200 nm at the lower end of this concentration range.

The near-u.v. c.d. spectra of wild-type enzyme and of the mutant enzyme (Figure 2b) show that there is a significant reduction in the ellipticity of the mutant enzyme. This would be consistent with the perturbation of the environment of one or more tryptophan side-chains, possibly including Trp-162, which is in the neighbourhood of the altered amino acid.

The intensity of the fluorescence of the mutant enzyme was reduced by about 15% compared with that of the wild-type enzyme, with no detectable change in the wavelength of maximum emission (334 nm) when excited at 290 nm (results not shown). This would be consistent with a small perturbation in the environment of one or more tryptophan side-chains.

Figure 2 C.d. spectra of wild-type and of K168P mutant phosphoglycerate mutases

Spectra were recorded at 20 °C in 50 mM sodium phosphate buffer, pH 7.0. Solid line, wild-type enzyme, dashed line, K168P mutant enzyme. (a) Far u.v. spectra, (b) near u.v. spectra. The concentrations of protein were in the range 0.3 to 0.5 mg/ml, and the cell path-lengths were 0.02 cm and 0.5 cm for a and b respectively. Molar ellipticities ($[\theta]$) are given in units of degrees · cm² · dmol⁻¹.

Stability

Incubation at 20 °C both of wild-type enzyme and of mutant enzyme at the low concentrations (140 ng/ml) that were employed for the assay of catalytic activity led to significant loss of activity (approx. 60 % loss over a 5 h period). This loss could be prevented either by increasing the concentration of protein to 20 µg/ml or by including 1 mg/ml of BSA. In each case, less than 5 % activity was lost over a 5 h period (results not shown). Similar observations have been reported for other enzymes such as citrate synthase (Zhi et al., 1992), and have been ascribed to a significant loss of protein by adsorption onto the surfaces of reaction vessels and either sacrificial protection by BSA or the formation of a complex between BSA and the protein of interest.

At a concentration of 20 µg/ml, incubation of wild-type or mutant enzymes with trypsin (20 µg/ml) led to the loss of 15–20 % activity over a 60 min period, with the mutant enzyme being slightly more susceptible to trypsin (results not shown). The relatively low rate of proteolysis (probably reflecting localized unfolding events) suggests that no additional susceptible bonds become exposed in the mutant enzyme.

Figure 3 Cross-linking data for K168P mutant phosphoglycerate mutase

Cross-linking with glutaraldehyde and subsequent analysis by SDS/PAGE were carried out as described in the text. (Δ) and (●) represent the percentages of the total protein as the tetramer and as the dimer respectively. No monomeric species were detected under these conditions. The solid lines are calculated assuming that the association constant for the $2D \rightleftharpoons T$ equilibrium is 10^7 M⁻¹. Over this range of concentrations, no dissociation of the tetrameric structure of wild-type enzyme could be observed.

Quaternary structure

The glutaraldehyde cross-linking technique (Hermann et al., 1983; Jaenicke and Rudolph, 1986; White et al., 1993) was used to study the quaternary structure of phosphoglycerate mutase. For the wild-type enzyme, a tetrameric structure was observed, as noted in previous studies. However, for the mutant enzyme, the cross-linking pattern observed depended on the concentration of the enzyme (Figure 3). Thus, at a concentration of 200 µg/ml, 85 % of the protein was in the form of a tetramer and 15 % as a dimer; however at 5 µg/ml (the lowest concentration at which cross-linking could be performed under these conditions) the proportions of tetramer and dimer were 53 % and 47 % respectively. There was no evidence over this range of concentrations for the existence of monomeric species of the K168P mutant or for dissociation of the tetrameric structure of the wild-type enzyme. The latter result confirms the earlier conclusion from ultracentrifuge studies (Price and Jaenicke, 1982) that the wild-type enzyme is still fully associated at 5 µg/ml.

As shown in Figure 3, the data for the K168P mutant enzyme over the range 5 µg/ml to 200 µg/ml can be described by an association constant of 10^7 M⁻¹ for the $2D \rightleftharpoons T$ equilibrium (where D, dimer; T, tetramer). This would correspond with a ΔG° of -39.2 kJ/mol at 20 °C.

In the present work, assays of enzyme activity were generally performed at concentrations of enzyme below 0.1 µg/ml. Under these conditions it is probable that the predominant species is the dimer, assuming that the presence of substrates does not have a major effect on the quaternary structure of the enzyme.

Denaturation by GdnHCl

The unfolding of the wild-type enzyme and of the mutant enzyme by GdnHCl was studied by measurements of far-u.v. c.d. spectra, protein fluorescence and the cross-linking pattern. Data for the wild-type enzyme have been reported previously (White et al., 1993); the comparative data for the wild-type and K168P mutant enzymes are summarized in Table 2. In the case of the mutant enzyme, the changes in fluorescence and the appearance of monomer run roughly in parallel, whereas the changes in the far-

Table 2 Unfolding of wild-type and K168P mutant enzymes by GdnHCl

Changes in the various parameters were measured at 20 °C using protein concentrations of 0.1 mg/ml, as described in the text. Abbreviation used: BPG, 2,3-bisphosphoglycerate.

	[GdnHCl] midpoint (M)	
	No BPG	+2.5 mM BPG
C.d. (ellipticity at 225 nm)		
wild-type	1.05	1.40
K168P	0.60	0.75
Fluorescence at 330 nm		
wild-type	1.05	1.35
K168P	0.48	0.60
Cross-linking (% of monomer)		
wild-type	1.05	1.40
K168P	0.47	0.65

Figure 4 Refolding of K168P mutant phosphoglycerate mutase after denaturation

The enzyme (at a concentration of 1.6 mg/ml) was incubated in buffer containing 4 M GdnHCl to achieve complete denaturation. Refolding was initiated by an 80-fold dilution into buffer (50 mM sodium phosphate, pH 7.0). (a) Regain of quaternary structure as monitored by cross-linking. (○) Percentage of monomer, (△) that of dimer and (□) that of tetramer. (b) Regain of activity and tetrameric structure. (●) Enzyme activity relative to a non-denatured control sample. (○) Enzyme activity assayed after a 1 min incubation with trypsin (20 µg/ml). (□) Percentage of protein as the tetramer. The data shown represent the average values from three separate experiments, the variation being less than 10% between the individual values observed.

u.v. c.d. spectrum lag behind. These data suggest that monomeric species with some residual secondary structure persist over the range of GdnHCl concentrations from about 0.8 M to 1.5 M.

The cross-linking data for the K168P mutant enzyme show that the proportions of both tetrameric and dimeric species decline as the GdnHCl concentration is raised. It can therefore be concluded that the rise in the proportion of the monomeric species over the range of GdnHCl concentrations from 0 M to 0.6 M is due to the dissociation of both tetrameric and dimeric species.

As shown in Table 2, the K168P mutant is significantly less stable than the wild-type enzyme towards denaturation by GdnHCl, with the mid-point concentrations of the transitions around 45%–55% lower depending on the parameter monitored. Addition of 2.5 mM 2,3-bisphosphoglycerate leads to a similar degree of stabilization for the two enzymes as measured by the ratio of the mid-point concentrations in the presence and absence of cofactor.

Refolding after denaturation

The refolding of wild-type phosphoglycerate mutase after denaturation in GdnHCl has been studied extensively (Hermann et al., 1983, 1985). The refolding process involves the rapid formation of folded monomeric species followed by associative steps to yield successively the dimer and the tetramer. The monomeric and dimeric species were each shown to possess about 35% of the specific activity of the tetramer, but this activity, unlike that of the tetramer, was sensitive to trypsin and to other proteinases (Hermann et al., 1985; Johnson and Price, 1986).

Analogous experiments have been performed with the K168P mutant enzyme. As with the wild-type enzyme, the mutant enzyme (at a concentration of 1.6 mg/ml) was incubated with buffer containing 4 M GdnHCl to achieve complete denaturation; refolding was initiated by an 80-fold dilution into buffer. The regain of quaternary structure is shown in Figure 4(a). Over the first 20 min there is a rapid rise in the proportion of dimeric species. Up to 240 min the proportion of dimer then remains approximately constant, in contrast to the behaviour of the wild-type enzyme (Hermann et al., 1983), where the proportion of dimer declines as a result of essentially irreversible association to form the tetramer. In the case of the mutant enzyme, the proportions of dimeric and tetrameric species that are observed after 240 min are similar to those that are observed at 20 µg/ml protein before denaturation (Figure 3); no further changes in these proportions are observed over a further 20 h period.

The regain of activity of the K168P mutant enzyme runs ahead of the regain of tetrameric structure in the early part of the refolding process (Figure 4b), consistent with the proposal that the dimeric species is enzymically active. Over the first hour, part of the activity is sensitive to trypsin, but this proportion declines as the proportion of tetramer increases (Figure 4b). These results suggest that the activity of the dimeric species is at least partially susceptible to trypsin. Under these conditions, the activity observed in the very early phase of the refolding process, when the monomeric species is predominant, is small, suggesting that, in contrast to the wild-type enzyme, the folded monomeric form of the K168P mutant enzyme possesses little if any activity.

DISCUSSION

The introduction of the lysine to proline mutation at position 168 in yeast phosphoglycerate mutase was designed to weaken the contacts between the pairs of dimers in the enzyme. Our results

show that this is indeed the case, the substantial dissociation of the enzyme occurs as the concentration is lowered from 200 $\mu\text{g/ml}$ to 5 $\mu\text{g/ml}$ (Figure 3). From the results of far-u.v. c.d., this dissociation does not appear to be accompanied by any substantial change in the secondary structure of the mutant enzyme.

In the case of the wild-type enzyme, we have been unable to demonstrate any dissociation of the tetramer at 5 $\mu\text{g/ml}$, either by cross-linking or by ultracentrifuge techniques (Price and Jaenicke, 1982). Assuming that a maximum of 1% of the tetramer is dissociated at this concentration, the association constant for the $2D \rightleftharpoons T$ equilibrium would be $> 5 \times 10^{10} \text{ M}^{-1}$. Comparison with the value of 10^7 M^{-1} for the K168P mutant shows that the shift in the equilibrium towards the dimer is > 5000 -fold, corresponding to a change in ΔG° of $> 20 \text{ kJ/mol}$ at 20 $^\circ\text{C}$. This would be consistent with the loss in the mutant enzyme of a small number of hydrogen bonds or other interactions stabilizing the tetrameric structure across this subunit interface. Values for the strengths of hydrogen bonds range from about 2 kJ/mol to about 17 kJ/mol, depending on whether the donor/acceptor pair is uncharged or charged (Fersht et al., 1985).

It is apparent from Figure 1 that the tetrameric structure of yeast phosphoglycerate mutase contains two types of inter-subunit contact region. One type (labelled D in Figure 1) involves extensive interactions and connects the central β -sheet cores of the two subunits to form an extended sheet with 12 strands. The other type of contact region (labelled T) involves interactions between the side-chains of the loop preceding helix 5 (residues 139–144) in one subunit which fit between side-chains in helix 6 (residues 165–172) and an adjoining loop (residues 214–224) of the symmetry-related subunit. It is likely that the D-type contacts persist in the dimeric forms of phosphoglycerate mutase, whereas the T-type contacts are only found in the tetrameric enzymes (such as that from yeast). This can be justified: (1) D-type contacts are more extensive than T-type contacts, (2) D-type contacts involve a stretch of five residues (74–78) that are highly conserved between dimeric and tetrameric enzymes, and (3) there is considerable variation in the sequences and in the lengths of the residues 139–144 loop in various dimeric vertebrate enzymes (Fothergill-Gilmore and Michels, 1993). The variation of this residues 139–144 loop would be consistent with the suggestion that it no longer contributes to inter-subunit contacts in the dimeric forms of the enzyme, and that it is part of the surface where sequence changes can be tolerated without the loss of function.

The position of residue Lys-168, in the middle of helix 6 of the yeast enzyme, is indicated in Figure 1. Replacement of this residue by a proline (as occurs in the dimeric forms of the enzyme) would have the inevitable consequence of disrupting the helix and thus significantly weakening the subunit contacts in this region. This is consistent with the observation that the tetrameric form of the K168P mutant enzyme is destabilized by at least 20 kJ/mol relative to the wild-type enzyme. Far-u.v. c.d. measurements show that only relatively small differences exist between the secondary structures of the mutant enzyme and of the wild-type enzyme. This is not unexpected since the substitution occurs in a portion of the enzyme that (in the dimeric form) would be located on the surface. Presumably alterations to the region corresponding to helix 6 do not have a major impact on the other helices or on the β -sheet core in the overall structure.

The value for the association constant (10^7 M^{-1}) for the $2D \rightleftharpoons T$ equilibrium for the mutant enzyme falls within the range of values that have been measured for interactions that occur between subunits during the assembly of various oligomeric proteins (Table 3), but is considerably lower than the association

Table 3 Association constants of steps in the association of oligomeric proteins

Note that in the cases of lactate dehydrogenase and aspartate carbamoyltransferase the dimeric species undergo further association to yield tetrameric and trimeric species respectively; these steps are essentially irreversible. Abbreviations used: PGAM, phosphoglycerate mutase; LDH, lactate dehydrogenase; ACTase, aspartate carbamoyltransferase; M, monomer; D, dimer; T, tetramer.

Protein	Association step	$K (\text{M}^{-1})$	Reference
PGAM (yeast)			
Wild-type	$2M \rightleftharpoons D$	1.04×10^6	Hermann et al. (1983)
	$2D \rightleftharpoons T$	$> 5 \times 10^{10}$	Price and Jaenicke (1982); this paper
K168P mutant	$2D \rightleftharpoons T$	1×10^7	This paper
LDH (pig muscle)	$2M \rightleftharpoons D$	3×10^8	Hermann et al. (1981)
ACTase (<i>E. coli</i> ; catalytic unit)	$2M \rightleftharpoons D$	1.33×10^7	Burns and Schachman (1982)

constant for the step that completes the final native quaternary structure (for example $2D \rightleftharpoons T$ for wild-type phosphoglycerate mutase). Indeed, for most of the enzymes whose assembly has been subject to detailed kinetic analysis, the reverse reaction of this final step is kinetically insignificant (Jaenicke, 1987).

At the concentrations of enzyme that were used for the assay ($< 1 \mu\text{g/ml}$), the K168P mutant enzyme is likely to be effectively completely dissociated to dimers. Such dissociation occurs with little change in the kinetic parameters compared with the wild-type enzyme (Table 1). In this connection it is interesting to note that the cofactor-dependent phosphoglycerate mutases occur in a variety of quaternary structures (Fothergill-Gilmore and Watson, 1989), that is monomer (*Schizosaccharomyces pombe*), dimer (mammals, birds and *Escherichia coli*) and tetramer (*S. cerevisiae*). Despite this diversity, the k_{cat} and K_m values do not vary over a wide range. For the enzymes from *Sch. pombe*, chicken breast muscle and *S. cerevisiae*, the K_m values for 3-phosphoglycerate are 500 μM , 600 μM and 740 μM respectively, and the corresponding k_{cat} values are 96, 400 and 490 s^{-1} expressed per active site (Grisolia and Carreras, 1975; Price et al., 1985; White and Fothergill-Gilmore, 1992). It is therefore clear that the tetrameric structure of the yeast enzyme is not required for the expression of catalytic activity, although studies of the properties of intermediates during the assembly of the enzyme show that the tetrameric structure confers stability towards proteolysis (Hermann et al., 1983; 1985). Consistent with this, we have found that the K168P mutant enzyme is more sensitive than the wild-type enzyme towards denaturation by GdnHCl and (to a lesser degree) towards proteolysis by trypsin.

The phosphoglycerate mutase from *Streptomyces coelicolor* has 64% sequence identity with the yeast enzyme, but has a proline instead of a lysine at position 168 (White et al., 1992). On the basis of limited gel-filtration data, the enzyme was reported to be a tetramer. It would be of considerable interest to examine the quaternary structure of the *Strep. coelicolor* enzyme over a range of concentrations to assess whether the strength of the interactions between the dimers are as strong as in the yeast enzyme. On the basis of the results reported in this paper, we would expect a significant dissociation of the *Strep. coelicolor* enzyme at low concentrations.

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REFERENCES

- Britton, H. G., Carreras, J. and Grisolia, S. (1972) *Biochemistry* **11**, 3008–3014
- Burns, D. L. and Schachman, H. K. (1982) *J. Biol. Chem.* **257**, 8638–8647
- Edelhoch, H., Rodwell, V. W. and Grisolia, S. (1957) *J. Biol. Chem.* **228**, 891–903
- Fersht, A. R., Shi, J.-P., Knill-Jones, J., Lowe, D. M., Wilkinson, A. J., Blow, D. M., Brick, P., Carter, P., Waye, M. M. Y. and Winter, G. (1985) *Nature (London)* **314**, 235–238
- Fothergill-Gilmore, L. A. and Michels, P. A. M. (1993) *Prog. Biophys. Mol. Biol.* **59**, 105–235
- Fothergill-Gilmore, L. A. and Watson, H. C. (1989) *Adv. Enzymol. Relat. Areas Mol. Biol.* **62**, 227–313
- Grisolia, S. and Carreras, J. (1975) *Methods Enzymol.* **42**, 435–450
- Hermann, R., Jaenicke, R. and Rudolph, R. (1981) *Biochemistry* **20**, 5195–5201
- Hermann, R., Rudolph, R., Jaenicke, R., Price, N. C. and Scobbie, A. (1983) *J. Biol. Chem.* **258**, 11014–11019
- Hermann, R., Jaenicke, R. and Price, N. C. (1985) *Biochemistry* **24**, 1817–1821
- Jaenicke, R. (1987) *Prog. Biophys. Mol. Biol.* **49**, 117–237
- Jaenicke, R. and Rudolph, R. (1986) *Methods Enzymol.* **131**, 218–250
- Johnson, C. M. and Price, N. C. (1986) *Biochem. J.* **236**, 617–620
- Nozaki, Y. (1972) *Methods Enzymol.* **26**, 43–50
- Price, N. C. and Jaenicke, R. (1982) *FEBS Lett.* **143**, 283–286
- Price, N. C., Duncan, D. and Ogg, D. J. (1985) *Int. J. Biochem.* **17**, 843–846
- Provencher, S. W. and Glöckner, J. (1981) *Biochemistry* **20**, 33–37
- Watson, H. C. (1982) Protein Data Bank Entry: Phosphoglycerate Mutase (Yeast) (3PGM), Brookhaven, New York
- White, M. F. and Fothergill-Gilmore, L. A. (1988) *FEBS Lett.* **229**, 383–387
- White, M. F. and Fothergill-Gilmore, L. A. (1992) *Eur. J. Biochem.* **207**, 709–714
- White, P. J., Nairn, J., Price, N. C., Nimmo, H. G., Coggins, J. R. and Hunter, I. S. (1992) *J. Bacteriol.* **174**, 434–440
- White, M. F., Fothergill-Gilmore, L. A., Kelly, S. M. and Price, N. C. (1993) *Biochem. J.* **291**, 479–483
- Zhi, W., Landry, S. J., Gierasch, L. M. and Srere, P. A. (1992) *Protein Sci.* **1**, 522–529