The quaternary structure of phosphoglycerate mutase from yeast

Evidence against dissociation of the tetrameric enzyme at low concentrations

Nicholas C. Price and Rainer Jaenicke +

Department of Biochemistry, University of Stirling, Stirling FK9 4LA, Scotland and + *Institut für Biophysik und Physikalische Biochemie, Universität Regensburg, D-8400 Regensburg, FRG*

Received 3 May 1982

1. INTRODUCTION

Phosphoglycerate mutase (EC 2.7.5.3) catalyses the interconversion of 3-phosphoglycerate and 2-phosphoglycerate. The enzyme from yeast is a tetramer of $M_r \sim 110\,000$ [1, 2]; the three-dimensional structure [3] and part of the amino acid sequence [4] of this enzyme are both known. As a result of ultracentrifuge studies [5], yeast enzyme at < 0.1 mg/ml was shown to be dissociated into subunits; it was concluded that the subunits were probably the active from of the enzyme observed in steady state kinetic experiments [5].

We have investigated the behaviour of yeast phosphoglycerate mutase as a function of concentration using ultracentrifugation (sedimentation equilibrium and sedimentation velocity) and gel filtration. There is no evidence of dissociation of the tetrameric enzyme even at 5 μg/ml. Cross-linking experiments using glutaraldehyde [6] show that at 20 μg/ml the enzyme exists as a tetramer.

2. MATERIALS AND METHODS

Assays of phosphoglycerate mutase activity were performed using the enolase-coupled assay procedure [7]. Concentrations of protein during purification were determined as in [8], using bovine serum albumin as a standard. The concentration of purified phosphoglycerate mutase was determined spectrophometrically using the value $E_{280}^{0.1\%} = 1.45$ [2].

The purification of phosphoglycerate mutase from baker's yeast was performed as in [9] with the following modification. After the acetone precipitation step, fraction III was fractionated with ammonium sulphate. The protein precipitating between 55–66% saturation was redissolved in a small volume of 10 mM Tris–HCl buffer (pH 8.0) and dialyzed against this buffer. It was then applied to a DEAE-cellulose column and after washing with 10 mM Tris–HCl (pH 8.0) to remove unbound proteins, the enzyme was eluted by applying a linear gradient formed by using volumes of 10 mM Tris–HCl (pH 8.0) and the same buffer containing 0.2 M NaCl. The phosphoglycerate activity emerged as a single peak and the specific activity of the fractions was 1220 μmol 3-phosphoglycerate consumed . min^{-1} . mg $protein^{-1}$ [7]. The enzyme was stored at 4°C in 0.05 M sodium phosphate buffer to which ammonium sulphate was added to 75% saturation. The preparation showed a single band on SDS–polyacrylamide gel electrophoresis with a subunit 28 000 ± 2000. For the experiments in which the behaviour of the enzyme was studied as a function of concentration, the buffer used in [5] was employed: namely 11 mM KH_2PO_4, 13 mM Na_2HPO_4, 1 mM EDTA (disodium salt), 50 mM NaCl (pH 7.0).

Gel filtration was performed at 20°C and at 3°C using a column of Sephacryl S-300 (85 cm × 1.7 cm). The flow rate was 20 ml/h and fractions of 2 ml were collected. The column was calibrated using proteins of known M_r (rabbit muscle aldolase, hog

Published by Elsevier Biomedical Press
001457593/82/0000–0000/$2.75

muscle lactate dehydrogenase, bovine serum albumin, ovalbumin and chymotrypsinogen). Blue dextran 2000 and thionitrophenol were used to determine the elution volumes of compunds which are completely excluded and completely included, respectively. The distribution coefficient, K_d, for a given protein was calculated as in [10]. Elution of enzyme was monitored by performing assays of enzyme activity and in addition spectrophotometrically at 280 nm (for 0.5 mg/ml).

Ultracentrifugation experiments were performed in a Beckman Spinco Model E analytical ultracentrifuge equipped with a high intensity light source and a UV scanning system. Double sector cells (12 mm and 30 mm pathlength) with sapphire windows were used in AnF-Ti, AnG and AnE rotors. To detect possible concentration-dependent dissociation, heterogeneity and non-ideality, the meniscus depletion technique [11, 12] was applied over a wide range of concentrations (5 μg/ml–10 mg/ml), fill-heights and rotor speeds (12 000–18 000 rev./min); scanning wavelengths were 230, 240, 280, 293, 300 and 310 nm. At > 1 mg/ml, Schlieren optics were employed in addition to UV absorption optics. The temperature in all ultracentrifuge experiments was 20 ± 1°C. *s*-Values were calculated from plots of log *r* vs *t*. Sedimentation equilibrium data were plotted in the form of ln *y* vs r^2.

Cross-linking with glutaraldehyde was performed at 20°C as in [6]. Glutaraldehyde levels employed ranged from 0.5–5% (w/v) and the reaction time was 2 min.

3. RESULTS AND DISCUSSION

The ultracentrifuge experiments (fig.1) do not show any concentration-dependent alteration of the quaternary structure of the enzyme. High speed

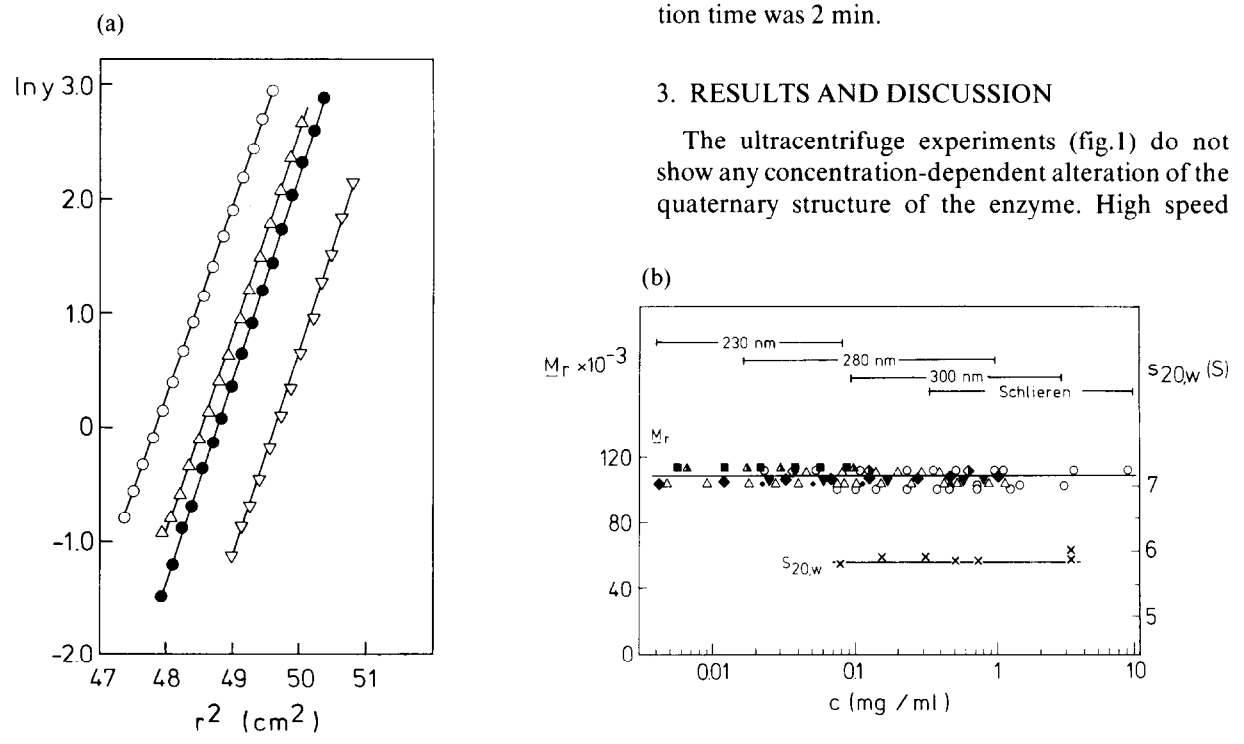

Fig.1. Ultracentrifugation of phosphoglycerate mutase. Experiments were performed at 20°C in 11 mM KH_2PO_4, 13 mM Na_2HPO_4, 1 mM EDTA (disodium salt), 50 mM NaCl, (pH 7.0).

(a) Semilogarithmic plots of data from a high speed meniscus depletion sedimentation equilibrium run at 18 000 rev./min. The initial enzyme concentration was 0.32 mg/ml. Scans were made at the following wavelength: (○) 230 nm, (●) 240 nm, (△) 280 nm, (▽) 293 nm. The different wavelengths were scanned with different full scale deflections.

(b) Variations of relative molecular mass ($\bar{M}_r$) and sedimentation coefficient ($s_{20,w}$) with enzyme concentration. The M_r data refer to a number of experiments with different initial concentrations, fill heights, rotor speeds, and scanning wavelengths as described in the text. Initial concentrations (mg/ml): (○) 3.3, (○) 0.7, (▽) 0.5, (△) 0.3, (□) 0.15, (◇) 0.075. Open symbols, half-filled and filled symbols refer to rotor speeds of 12 000, 16 000 and 18 000 rev./min., respectively. Sedimentation coefficients (+) were determined at a rotor speed of 44 000 rev./min.

Table 1

Elution behaviour of yeast phosphoglycerate mutase from Sephacryl S-300

Temp.	Enzyme applied (mg/ml)	K_d
3°C	0.01_3	0.34
	0.06	0.33
	0.12	0.33
	0.49	0.34
	1.82	0.34
20°C	0.01_1	0.34
	0.05	0.33
	0.16	0.34
	0.43	0.34
	1.00	0.34
	2.00	0.33

K_d is the distribution coefficient defined in [10]. The accuracy of K_d is estimated to be ± 0.01. K_d-Values for chymotrypsinogen, bovine serum albumin and rabbit muscle aldolase were 0.53, 0.38 and 0.30, respectively.

sedimentation equilibrium experiments at different fill-heights and initial concentrations of the enzyme (0.05–3.3 mg/ml) lead to identical standard M_r moments. This holds over the entire concentration range accessible to evaluation (5 μg/ml–10 mg/ml) proving homogeneity as well as the absence of a dissociation–association equilibrium in the given concentration range (fig.1b). Using 0.74 ml/g for the partial specific volume [2], the M_r of the enzyme is calculated to be 107 000 ± 4000 (mean ± SD of separate determinations at 6 different initial enzyme concentrations).

Homogeneity and stability of the native quaternary structure is also suggested by the occurrence of a single symmetrical peak in high speed sedimentation velocity experiments making use of the radial displacement of the sedimentation boundary over the total radical length of the ultracentrifuge cell. The sedimentation coefficient showed very little concentration dependence over 0.08–3.3 mg/ml: $s_{20,w} = (5.91_1 - 0.006_6\, c) \times 10^{-13}$ s (c in mg/ml) (fig.1b).

These conclusions were confirmed by gel filtration experiments. Over 2–0.011 mg enzyme/ml applied to the Sephacryl S-300 column, there was no difference in the elution behaviour of the enzyme (table 1). The elution volume corresponded to M_r 120 000 ± 10 000. In all cases the recovery from the column was > 90% of the applied activity. Similar results were obtained at 3°C (table 1).

Cross-linking experiments with glutaraldehyde also showed that at 20 μg/ml, phosphoglycerate mutase exists exclusively as a tetramer within the experimental error (± 5%) of the method. The above results prove clearly that yeast phosphoglycerate mutase does not dissociate into subunits at < 1 mg/ml. We cannot be certain why dissociation was observed in [5], but an explanation may be found in the statement [5] that:

'storage of the dialyzed enzyme for 10 days at 40°C was accompanied by indefinite aggregation and degeneration of the protein.'

By contrast, a sample of our dialyzed enzyme (0.7 mg/ml) stored at 4°C for 11 days lost < 5% activity and no sign of turbidity could be detected. After incubation at 20°C for 5 days the loss in activity was < 25%. It seems probable therefore that dissociation of the enzyme reported [5] was an artifact of the enzyme preparation studied. Since our studies were made down to the concentration range employed in steady state kinetic assays, we conclude that the tetramer is the active species of phosphoglycerate mutase.

ACKNOWLEDGEMENTS

We wish to thank the Deutsche Forschungsgemeinschaft (R. J.) and the Science and Engineering Research Council (N.C.P.) for financial support. N.C.P. also wishes to thank the Alexander von Humboldt Stiftung for the award of a fellowship. We would also like to thank Ingrid Heider for excellent technical assistance and Alan Scobbie who performed some preliminary gel filtration experiments.

REFERENCES

[1] Ray, W.J. jr and Peck, E.J. jr (1972) The Enzymes 6, 3rd edn, pp. 407–477.
[2] Edelhoch, H., Rodwell, V.W. and Grisolia, S. (1957) J. Biol. Chem. 228, 891–903.
[3] Campbell, J.W., Watson, H.C., and Hodgson, G.I. (1974) Nature 250, 301–333.

[4] Winn, S.I., Watson, H.C., Fothergill, L.A. and Harkins, R.N. (1977) Biochem. Soc. Trans. 5, 657–659.
[5] Spragg, S.P., Roche, J.J. and Wilcox, J. (1977) Biochem. J. 163, 543–555.
[6] Hermann, R., Jaenicke, R. and Rudolph, R. (1981) Biochemistry 20, 5195–5201.
[7] Grisolia, S. (1962) Methods Enzymol. 5, 236–242.
[8] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[9] Morena, E. de la, Santos, I. and Grisolia, S. (1968) Biochim. Biophys. Acta 151, 526–528.
[10] Andrews, P. (1970) Methods Biochem. Anal. 18, 1–53.
[11] Yphantis, D.A. (1964) Biochemistry 3, 297–317
[12] Roark, D.E. and Yphantis, D.A. (1969) Ann. NY Acad. Sci. 164, art. 1, 145–278.