Volume 244, number 2, 276–278 FEB 06815 February 1989

Heat and cold denaturation of phosphoglycerate kinase (interaction of domains)

Yu.V. Griko, S.Yu. Venyaminov and P.L. Privalov

Institute of Protein Research, Academy of Sciences of the USSR, Pushchino, Moscow Region, USSR

Received 7 December 1988

It has been shown that the denaturation of phosphoglycerate kinase (PGK) can be observed not only when the solution is heated above 30°C, but also when it is cooled below this temperature. The disruption of the native PGK structure upon cooling and the subsequent formation of this structure upon heating both proceed in two distinct stages which correspond to the independent disruption or reformation of each of its domains. In contrast, the heat denaturation of PGK proceeds in one stage, showing that the two domains of the molecule are associated into a single complex which figures in the denaturation process as a cooperative unit. It follows that, at elevated temperature, there is a positive interaction between the domains, which disappears at lower temperatures. This might be due to hydrophobic interactions, which are known to be temperature dependent. The temperature decrease leads to a decrease in inter- and intradomain interactions, which results in an increase of the independence of the domains and a decrease in their stability.

Denaturation; Kinase; Domain interaction

1. INTRODUCTION

Phosphoglycerate kinase (PGK) belongs to the class of enzymes which are supposed to display large-scale structural rearrangements during their functioning [1,2]. Its single polypeptide chain, which contains 415 amino acid residues, is organized in two widely separated domains of almost equal size connected by a hinge [2]. The C-terminal domain contains a nucleotide-binding site, while the phosphoglycerate-binding site is situated in the N-terminal domain. These two sites are separated by a distance of 1.2–1.5 nm. Therefore, the reaction between substrates can be explained only by assuming that the hinge can bend, i.e. that it is flexible [3].

The postulated mobility of the domains implies their relative independence. However, a calorimetric study of the thermal unfolding of PGK, in both the absence and presence of substrates, has shown that this process is approximated by a two-state transition [4], which indicates that the structure of a PGK molecule represents a single cooperative system. In other words, it appears that there are strong positive interactions between the two domains which integrate them into a single cooperative unit. This apparent contradiction has been solved by studying PGK unfolding over a broad temperature range and observing not only its heat denaturation, but also its cold denaturation. The results of this study are discussed below.

Correspondence address: P.L. Privalov, Institute of Protein Research, Academy of Sciences of the USSR, Pushchino, Moscow Region, USSR

2. MATERIALS AND METHODS

Phosphoglycerate kinase (PGK) was isolated from yeast according to Scopes [5]. The homogeneity of PGK was checked by electrophoresis under native and denaturing conditions, as described in [6,7]. The protein concentration was measured using the extinction coefficient, $A_{280}^{1\%} = 4.95$ [8]. All measurements were made in 20 mM phosphate buffer, pH 6.5, containing 10 mM EDTA and different concentrations of GuHCl.

Calorimetric measurements were performed with a DASM-4 scanning microcalorimeter (Bureau of Biological Instrumentation of the Academy of Sciences of the USSR) at heating and cooling rates of 0.5 K/min. Calorimetric enthalpies for the

Published by Elsevier Science Publishers B.V. (Biomedical Division)

denaturational transition were obtained from the peak area as in [9].

Circular dichroism measurements were made using a Jasco-41 (Japan) spectropolarimeter, equipped with a temperature-controlled cell holder. Fluorescence measurements were made using an SPF-1000 CM spectrofluorimeter (Aminco, USA).

3. RESULTS AND DISCUSSION

Fig.1a represents the temperature dependence of PGK ellipticity in the far and near ultraviolet. Judging from the ellipticities at 222 and 277 nm, which reflect the contents of α-helical structure in the protein [10] and the asymmetry in the aromatic amino acid environment, respectively [11], a temperature decrease to below 30°C leads to an almost complete loss of helicity and symmetrization of the aromatic group environment in this protein. This change in the molecular structure is reversible, since on subsequent heating from −6°C, the initial helicity is regained and the specific environment of aromatic groups, peculiar to the unique native structure of PGK, is restored. This change is very similar to those observed in experiments with myoglobin, apomyoglobin and staphylococcal nuclease which, as has been shown, undergo cold denaturation on cooling [12–14]. In analogy to these proteins, we can regard the disruption of the native structure of PGK on cooling as its cold denaturation.

It is noteworthy that the changes in ellipticities of PGK in the far- and near-ultraviolet regions proceed simultaneously during heat denaturation and non-simultaneously during cold denaturation and renaturation. The change of ellipticity at 222 nm is usually supposed to be associated with a change of the secondary structure of the polypeptide chain [10], i.e. they reflect the change of the conformation of the entire molecule; as for the ellipticity at 277 nm, it is caused mainly by changes in the tryptophan environment. Since there are two tryptophans in PGK and both are in the C-terminal domain [15], it is possible to conclude that the two domains of these molecules undergo conformational changes non-simultaneously on cooling, i.e. these domains disrupt non-cooperatively over different temperature ranges, showing that they are independent and differ in stability against cold denaturation.

This conclusion is confirmed by the changes in the fluorescence spectrum of the two tryptophan

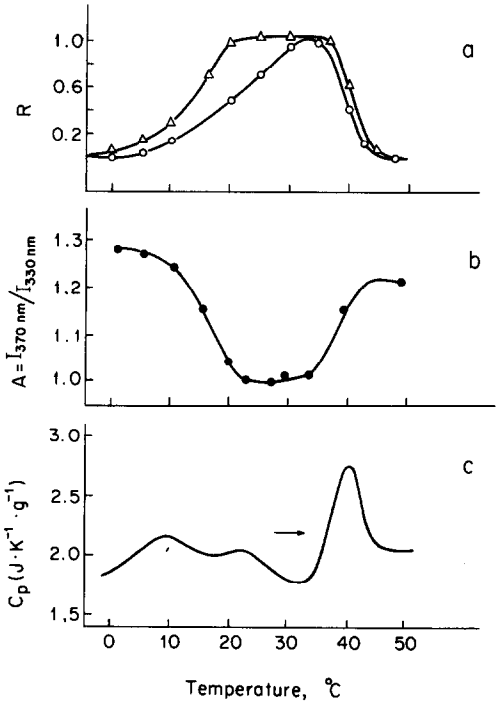

Fig.1. Temperature dependence of (a) relative changes, R, of PGK ellipticity at 222 nm (○) and 277 nm (△), (b) tryptophan emission spectrum maximum and (c) partial specific heat capacity in solution, containing 0.7 ml GuHCl.

residues. As seen from fig.1b, PGK fluorescence changes in the same temperature range as the ellipticity at 277 nm. Thus, at lower temperatures, the changes in PGK are definitely identified with those in the C-terminal domain and, therefore, the changes observed at higher temperatures should be identified with these in the N-terminal domain. On the other hand, the changes in ellipticity and fluorescence which take place above 30°C and proceed simultaneously in the same temperature range can be identified with the cooperative disruption of the two domains.

The calorimetric study also shows (see fig.1c) that, when the pre-cooled PGK solution is heated up to 30°C, the heat is absorbed in two distinct peaks: above 30°C it is absorbed in a single sharp peak. The latter corresponds to the heat denaturation of PGK. It undoubtedly represents a single two-state transition as the area of this peak, which is the calorimetric enthalpy of the process ($\Delta H = 586$ kJ/mol) is in perfect agreement with the van 't Hoff enthalpy ($\Delta H = 636$ kJ/mol) deter-

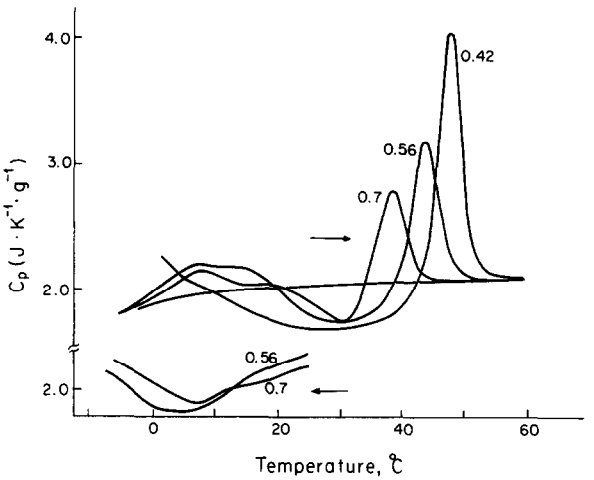

Fig. 2. Temperature dependence of the partial specific heat capacity of PGK upon cooling and subsequent heating in solution with various GuHCl concentrations.

mined from the sharpness of the peak (see [16]). The two partly overlapping peaks in the low-temperature zone, which are associated with renaturation of the cold denaturation domains, represent two independent processes, since they shift in temperature almost indepedently when the solvent conditions are varied (fig.2). It should be noted that the cold and heat denaturations of PGK domains proceed with thermal effects of opposite signs, as they should [12].

It follows from the above that, at elevated temperatures (above 30°C), a PGK molecule represents a single cooperative system in which two domains are associated into a single block and disrupt simultaneously upon denaturation. At low temperatures (below 30°C), PGK domains appear as independent subsystems. In other words, a temperature increase leads to an increase of positive interaction between the domains in a PGK molecule.

It is known that, among the various intramolecular interactions, only hydrophobic interactions are strongly dependent on temperature: they decrease in magnitude as the temperature decreases and can even change their sign at a sufficiently low temperature [17]. One can conclude, then, that there are hydrophobic interactions between PGK domains and, therefore, that the temperature should influence considerably the configuration of the domains in this molecule.

Acknowledgement: The authors are grateful to Dr S.N. Timasheff for helpful discussion of the manuscript in preparation.

REFERENCES

[1] Anderson, C.M., Zucker, F.H. and Steitz, T.A. (1979) Science 204, 375-380.
[2] Blake, C.C.F. and Rice, D.W. (1981) Phil. Trans. Roy. Soc. Lond. A 293, 93-104.
[3] Banks, R.D., Blake, C.C.F., Evans, P.R., Haser, R., Rice, D.W., Hardy, G.W., Merrett, M. and Philips, R.W. (1979) Nature 279, 773-777.
[4] Calderon, R.O., Stolowich, N.J., Gerlt, J.A. and Sturtevant, J.M. (1985) Biochemistry 24, 6044-6049.
[5] Scopes, R.K. (1971) Biochem. J. 122, 89-92.
[6] Ornstein, L. (1964) Ann. NY Acad. Sci. 121, 312-349.
[7] Laemmli, U.K. (1970) Nature 227, 680-685.
[8] Adams, B. and Pain, R.H. (1986) FEBS Lett. 196, 361-364.
[9] Privalov, P.L. and Potekhin, S.A. (1986) Methods Enzymol. 131, 4-51.
[10] Chen, Y.-H., Yang, J.T. and Martinez, H.M. (1972) Biochemistry 11, 4120-4131.
[11] Kahn, P.C. (1979) Methods Enzymol. 61, 339-378.
[12] Privalov, P.L., Griko, Yu.V., Venyaminov, S.Yu. and Kutyshenko, V.P. (1986) J. Mol. Biol. 190, 487-498.
[13] Griko, Yu.V., Privalov, P.L., Venyaminov, S.Yu. and Kutyshenko, V.P. (1988) J. Mol. Biol. 202, 127-138.
[14] Griko, Yu.V., Privalov, P.L., Sturtevant, J.M. and Venyaminov, S.Yu. (1988) Proc. Natl. Acad. Sci. USA 85, 3343-3347.
[15] Watson, H.C., Walker, N.P.C., Shaw, P.J., Bryant, T.N., Wendell, P.L., Fothergill, L.A., Perkins, R.E., Conroy, S.C. Dobson, M.J., Tuite, M.F. and Kingsman, S.M. (1982) EMBO J. 1, 1635-1640.
[16] Privalov, P.L. (1979) Adv. Protein Chem. 33, 167-241.
[17] Privalov, P.L. and Gill, S.J. (1988) Adv. Protein Chem., in press.