Volume 297, number 3, 247–249 FEBS 10688 February 1992

Interaction between D-glyceraldehyde-3-phosphate dehydrogenase and 3-phosphoglycerate kinase and its functional consequences

Natalia A. Khoroshilova, Vladimir I. Muronetz and Natalia K. Nagradova

A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, USSR

Received 28 November 1991

E. coli D-glyceraldehyde-3-phosphate dehydrogenase covalently bound to Sepharose was shown to form a complex with soluble *E. coli* 3-phosphoglycerate kinase with a stoichiometry of 1.77±0.61 kinase molecules per tetramer of the dehydrogenase and an apparent K_d of 1.03±0.68 μM (10 mM sodium phosphate, 0.15 M NaCl). No interaction was detected between *E. coli* D-glyceraldehyde-3-phosphate dehydrogenase and rabbit muscle 3-phosphoglycerate kinase. The species-specificity of the bienzyme association made it possible to develop a kinetic approach to demonstrate the functionally significant interaction between *E. coli* D-glyceraldehyde-3-phosphate dehydrogenase and *E. coli* 3-phosphoglycerate kinase, which consists of an increase in steady-state rate of the coupled reaction.

D-Glyceraldehyde-3-phosphate dehydrogenase; 3-Phosphoglycerate kinase; Bienzyme complex

1. INTRODUCTION

The problem of specific interactions between various enzymes of the glycolytic pathway has attracted considerable interest over the last decade [1–3]. Weber and Bernhard were the first to suggest an association between D-glyceraldehyde-3-phosphate dehydrogenase (GPDH) and 3-phosphoglycerate kinase (PGK); they presented kinetic evidence for a direct transfer of 1,3-bisphosphoglycerate via an enzyme–substrate–enzyme complex and came to the conclusion that in the coupled two-enzyme reaction the 1,3-bisphosphoglycerate PGK complex serves as the substrate for GPDH [4]. Recent reinvestigations [5–7] produced no experimental support for this assumption. First, the dissociation of 1,3-bisphosphoglycerate from its complex with PGK was shown to be an intrinsically rapid reaction step that does not require the presence of GPDH. Second, the kinetics of 1,3-bisphosphoglycerate transfer from PGK to GPDH were consistent with prior dissociation of the complex according to a free-diffusion mechanism of metabolite transfer [6,7]. No kinetic evidence for the interaction between the two enzymes was obtained in these studies.

At the same time, the possibility of specific association between PGK and GPDH was demonstrated by physico-chemical methods [8–11]. The results obtained were in line with the suggestion that the strength of the bienzyme association varied depending on the source of the enzymes. It is therefore possible that both free and complexed enzymes may function within the cells. Is there any difference in their catalytic effectiveness?

To answer this question, one has to compare the kinetics of a coupled PGK–GPDH reaction performed by free enzymes with that performed by enzymes bound in a complex. Such an investigation was attempted in the present study. Advantage was taken of the fact that *E. coli* GPDH formed a complex with PGK isolated from the same source, but did not interact with rabbit muscle PGK. Thus, the kinetics of the coupled reaction catalyzed by the bienzyme complex could be studied using the system of the non-interacting enzymes as control. The results obtained provide evidence of changes in the kinetics of the coupled reaction catalyzed by the bienzyme complex suggesting a possibility of channeling of 1,3-bisphosphoglycerate from the catalytic site of PGK where it is synthesized, toward the catalytic site of GPDH.

Correspondence address: N.A. Khoroshilova, A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, USSR.

2. MATERIALS AND METHODS

NAD^+, NADH, ATP, human serum albumin, and glycine were purchased from Reanal (Hungary); 3-phosphoglycerate, Coomassie G-250, from Sigma (USA), Tris from Merck (Germany), EDTA, dithiothreitol and sodium phosphate, from Serva (Germany). D-Glyceraldehyde-3-phosphate was prepared from D-fructose-6-phosphate [12]. 1,3-Bisphosphoglycerate was prepared as described in [13]. Sepharose 4B was purchased from Pharmacia (Sweden). GPDH and PGK from rabbit skeletal muscle were prepared following the affinity elution method with slight modifications [9]; the enzymes from *E. coli* were purified using the procedure which will be published elsewhere. The enzymes appeared homogeneous when examined by polyacrylamide gel electrophoresis carried out according to Laemmli [14] and exhibited specific activities of 160–220 U/mg (GPDH) and 350–500 U/mg (PGK). Protein concentrations were determined by the method of Bradford [15]. Concentrations of all enzymes (tetrameric GPDH

and monomeric PGK) are reported as the active site concentrations. Stopped-flow kinetic experiments were performed at 20°C with an Aminco DW-2000 spectrophotometer; experimental conditions are specified in the legends to figures. Reactions were monitored at 340 *minus* 500 nm.

Immobilization of *E. coli* GPDH on Sepharose 4B was performed as previously described [16]. 1 g of wet Sepharose was activated with 30 mg CNBr. The amount of immobilized enzyme was estimated by the modified Bradford method [17]. Titration of immobilized GPDH with soluble PGK was performed as follows. A series of samples were prepared, each containing immobilized GPDH (1.0 μM). Increasing concentrations of soluble PGK (0–30 μM) were added to the samples, the total volume of each sample being constant (1.0 ml). The reaction was carried out in 10 mM sodium phosphate buffer, pH 7.6, containing 0.15 M NaCl. After gentle stirring, the samples were incubated for 30 min at room temperature. The gel was then settled by centrifugation and the protein concentration was determined in the supernatant. The exclusion volume of the immobilized enzyme suspension was determined in a separate experiment using the system of Sepharose-bound serum albumin-soluble PGK. The exclusion volume was found to be 0.2±0.01 ml/ml of the packed gel. The computations were performed on an IBM-XT compatible personal computer using the Graph PAD/In Plot/Version 3.0 N non-linear regression program.

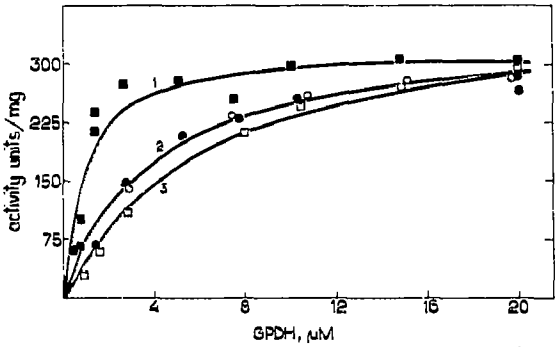

Fig. 2. Dependence of the steady-state rate of the coupled PGK–GPDH reaction on the concentration of the dehydrogenase. One syringe of the stopped-flow apparatus contained 80 mM Tris-HCl buffer, 0.5 mM NADH, 4.8 mM ATP, 30 mM 3-phosphoglycerate, 14.0 mM $MgSO_4$, 1 mM dithiothreitol, pH 7.6, in a volume of 2 ml. The other syringe contained 80 mM Tris-HCl buffer, 1.0 mM NAD^+, 1 mM dithiothreitol, pH 7.6, 2.0 μM *E. coli* PGK and varying concentrations of *E. coli* (1) or rabbit muscle (2) GPDH. Sample 3 = the same as sample 1, but 0.3 M NaCl was present. Curve 2, no NaCl was added (●); 0.3 M NaCl was present (○).

3. RESULTS

Fig. 1 demonstrates the results of titration of immobilized *E. coli* GPDH with soluble PGK. In two series of samples, the titration was performed with kinase isolated from *E. coli* (1) and from rabbit muscle (2). In the first case, complex formation was detected with an apparent K_d of 1.03 ± 0.68 μM and a stoichiometry of 1.77 ± 0.61 kinase molecules per tetramer of the dehydrogenase. Specific ligands (NAD^+, ATP or D-glyceraldehyde 3-phosphate) exhibited no effect on the strength of the bienzyme association. Fig. 1 also shows that no measurable interaction occurred between *E. coli* dehydrogenase and rabbit muscle kinase, suggesting a strong species-specificity of the bienzyme association. This finding proved rather valuable in our subsequent studies carried out with soluble enzyme preparations.

In these series of experiments, rapid kinetic studies were performed to follow the coupled reaction catalyzed by PGK and GPDH; the enzymes were taken at concentrations sufficiently high to ensure that they could associate in a complex. The experiments were aimed at comparing the kinetics of the coupled reaction catalyzed by two enzyme pairs: (1) *E. coli* PGK–rabbit muscle GPDH; and (2) *E. coli* PGK–*E. coli* GPDH. The concentration of PGK was equal in the two cases; saturating concentrations of Mg-ATP and 3-phosphoglycerate were used. Under these conditions, the steady-state rate of the overall reaction was observed to depend on the GPDH concentration (Fig. 2). As shown in Fig. 2, the character of the dependence was different with the two enzyme pairs. In the case of *E. coli* PGK–*E. coli* GPDH pair (curve 1), the maximal steady-state rate was attained at lower concentrations of the auxiliary en-

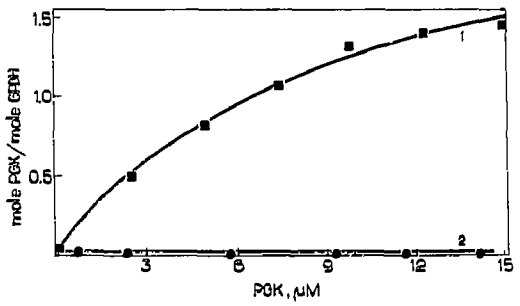

Fig. 1. Interaction of Sepharose-bound *E. coli* GPDH with soluble PGK from *E. coli* (1) and from rabbit muscle (2). 10 mM sodium phosphate buffer, pH 7.6, 0.15 M NaCl, 2 mM dithiothreitol, 1.0 μM matrix-bound *E. coli* GPDH. See section 2 for details. The data are consistent with the binding of 1.77±0.61 mol of PGK per mol of the tetrameric GPDH with an apparent K_d of 1.03±0.68 μM.

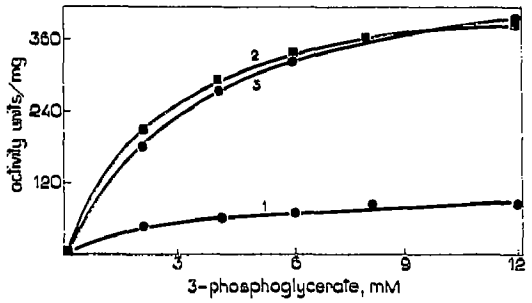

Fig. 3. Steady-state rate of the coupled PGK–GPDH reaction as a function of 3-phosphoglycerate concentration. One syringe of the stopped-flow apparatus contained 80 mM Tris-HCl buffer, 0.5 mM NADH, 1.0 mM NAD^+, 4.8 mM ATP, varying concentrations of 3-phosphoglycerate, 7 mM $MgSO_4$, 1 mM dithiothreitol, pH 7.6. The other syringe contained 80 mM Tris-HCl buffer, 1 mM dithiothreitol, 7 mM $MgSO_4$, pH 7.6, and varying concentrations of enzymes. (1) Rabbit muscle PGK 8 μM, *E. coli* GPDH 8 μM; (2) *E. coli* PGK 8 μM, *E. coli* GPDH 8 μM; (3) rabbit muscle PGK 8 μM, *E. coli* GPDH 80 μM.

zyme, than in the case of the non-interacting enzymes (curve 2). The effect could not be attributed to different affinities of *E. coli* and rabbit muscle GPDHases for 1,3-bisphosphoglycerate (the apparent K_m values for this substrate measured under the employed experimental conditions were 19.0 μM for *E. coli* and 16.7 μM for rabbit muscle dehydrogenase).

These results led us to conclude that some kinetic advantages arise from specific interactions between the *E. coli* enzymes. The results of experiments carried out at high ionic strength, i.e. under the conditions known to weaken enzyme–enzyme interactions, lend support to this conclusion. As seen in Fig. 2, curve 3, in this case the kinetic behaviour of the *E. coli* PGK–*E. coli* GPDH pair becomes nearly identical with that of the pair of non-interacting enzymes.

Another series of experiments was carried out using different combination of enzymes, with *E. coli* GPDH as an auxiliary enzyme. In this case, the kinetics of reaction carried out by rabbit muscle PGK–*E. coli* GPDH and *E. coli* PGK–*E. coli* GPDH were compared. Fig. 3 demonstrates that under the experimental conditions employed (the ratio of PGK active sites/GPDH active sites being equal to 1), the steady-state rate exhibited by the interacting enzymes (curve 2) was considerably higher than that observed in the case of the non-interacting ones (curve 1). These results suggest that in the absence of specific enzyme–enzyme interactions, when 1,3-bisphosphoglycerate must diffuse from rabbit muscle PGK to *E. coli* GPDH via the reaction medium, the concentration of the latter enzyme (under the conditions of Fig. 3), '1') is too low to ensure that the kinase reaction to the rate-limiting step; the effect produced by an excess of GPDH is seen in Fig. 3, '3'.

4. DISCUSSION

To obtain reliable information about the capability of a given enzyme pair of specifically interacting to form a complex, one has to apply an appropriate experimental approach. In the variety of physico-chemical methods used thus far to study associations between glycolytic enzymes, the technique based on matrix immobilization, first introduced by Beekmans and Kanarek [18], seems to be among the most appealing. Using this approach, we have demonstrated specific association between rabbit muscle GPDH and lactate dehydrogenase [19], as well as between GPDH and PGK isolated from baker's yeast [8] and rabbit muscle [9,10]. Comparison of the results obtained on enzymes isolated from different sources leads us to conclude that the strength of interaction between homologous GPDH and PGK is not the same in each case; it is weakest between mammalian muscle enzymes and strongest between bacterial ones.

It should be noted, however, that the values of K_d which characterize enzyme complexes immobilized on solid support, may differ from those of the soluble complexes. It seems plausible that non-covalent interactions with the matrix confer stability to a complex, lowering its K_d. Bearing in mind such considerations, we made no attempt to evaluate the extent of complexation between soluble GPDH and PGK under our experimental conditions. It seems likely that part of the enzyme molecules might have remained unbound and catalyzed the coupled reaction through a free-diffusion mechanism, but the results of kinetic studies indicate the existence of a functioning bienzyme complex. The large differences in steady-state kinetics between the associating and non-associating enzyme pairs can be explained by two different molecular mechanisms (or by a combination of them). Either there is channeling of the intermediate between the kinase and the dehydrogenase, or the enzymes in the complex have more favorable kinetic constants than those of the unassociated enzymes. Work is in progress to clarify this problem.

In conclusion, our data are consistent with the idea that functional consequences of specific interaction between PGK and GPDH may be an important factor contributing to the ability of enzymes to work efficiently. The role of this factor will increase under the conditions favoring enzyme–enzyme interactions.

REFERENCES

[1] Friedrich, P. (1985) in: Organized Multienzyme Systems (Welch, R.G. ed.) pp. 141–146. Academic Press, New York.
[2] Bernhard, S.A. and Srivastava, D.K. (1987) Biochem. Soc. Trans. 15, 977–981.
[3] Keleti, T. and Ovadi, J. (1988) Curr. Top. Cell Regul. 29, 1–33.
[4] Weber, J.P. and Bernhard, S.A. (1982) Biochemistry 21, 4189–4194.
[5] Landgraf, R. and Betts, G.F. (1989) Anal. Biochem. 176, 444–448.
[6] Kvassman, J. and Pettersson, G. (1989) Eur. J. Biochem. 186, 261–264.
[7] Kvassman, J. and Pettersson, G. (1989) Eur. J. Biochem. 186, 265–272.
[8] Ashmarina, L.I., Muronetz, V.I. and Nagradova, N.K. (1984) Biochem. Int. 9, 511–521.
[9] Sukhodolets, M.V., Muronetz, V.I. and Nagradova, N.K. (1987) Biokhimiya 52, 128–133.
[10] Sukhodolets, M.V., Muronetz, V.I., Tsuprun, V.L., Kaftanova, A.S. and Nagradova, N.K. (1988) FEBS Lett. 238, 161–166.
[11] Malhotra, O.P., Kumar, A. and Tikoo, K. (1987) Ind. J. Biochem. Biophys. 24, 16–20.
[12] Szewczuk, A., Wolna, E., Wolny, M. and Baranowsky, T. (1961) Acta Biochem. Polon. 8, 201–207.
[13] Furfine, C.S. and Velick, S.F. (1965) J. Biol. Chem. 240, 844–855.
[14] Laemmli, U.K. (1970) Nature 227, 680–685.
[15] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[16] Cherednikova, T.V., Muronetz, V.I. and Nagradova, N.K. (1980) Biochim. Biophys. Acta 613, 292–308.
[17] Asryants, R.A., Douzhenkova, I.V. and Nagradova, N.K. (1985) Anal. Biochem. 151, 571–574.
[18] Beekmans, S. and Kanarek, L. (1982) Appl. Biochem. Biotechnol. 7, 177–188.
[19] Sukhodoletz, M.V., Muronetz, V.I. and Nagradova, N.K. (1989) Biochem. Int. 19, 379–384.