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Association of rabbit muscle glyceraldehyde-3-phosphate dehydrogenase and 3-phosphoglycerate kinase

The biochemical and electron-microscopic evidence

Maks V. Sukhodolets, Vladimir I. Muronetz, Valery L. Tsuprun, Alla S. Kaftanova and Natalia K. Nagradova

A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 119899, USSR

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Rabbit muscle glyceraldehyde-3-phosphate dehydrogenase covalently bound to Sepharose was shown to form a complex with soluble 3-phosphoglycerate kinase. The strength of the association appeared to depend upon the functional state of both enzymes. The holoform of the dehydrogenase exhibited a lower affinity for the kinase than the enzyme-3-phosphoglycerol·NADH complex. The substrate-free 3-phosphoglycerate kinase associated much stronger with the acylated dehydrogenase than the kinase in complex with 1,3-diphosphoglycerate. Electron-microscopic evidence for the association of the soluble acyl-glyceraldehyde-3-phosphate dehydrogenase·NADH complex and 3-phosphoglycerate kinase was also obtained.

Glyceraldehyde-3-phosphate dehydrogenase; 3-Phosphoglycerate kinase; Bienzyme complex; Electron-microscopic study

1. INTRODUCTION

An increasing body of evidence suggests that intracellular enzymes can form specific functionally significant associates. Together with relatively stable multienzyme complexes which can be readily isolated (i.e. the pyruvate dehydrogenase complex), transient complexes are probably also formed between enzymes catalysing consecutive reactions of metabolism. In particular, glycolytic enzymes in muscle cytoplasm are supposed to form transient complexes wherein a direct transfer of metabolites can occur [1,2]. Numerous attempts have been made to prepare such complexes in vitro. The first studies carried out on the pig muscle GPDH·PGK system have shown no interaction between the two enzymes [3]. However, the results of Weber and Bernhard [4], who demonstrated a direct transfer of 1,3-diphosphoglycerate between the halibut muscle enzymes, suggested that a physical enzyme-enzyme interaction occurred. The evidence for GPDH·PGK complex formation was obtained in our studies on yeast enzymes [5] as well as on the enzymes isolated from rabbit muscle [6,7]. Using the technique of enzyme immobilization on a solid support, we could show a specific association between matrix-bound GPDH and soluble PGK under the conditions when the enzymes were functioning. The present study was performed to characterize the factors which control the strength of protein-protein interactions in the bienzyme complex and to obtain direct electron-microscopic evidence for the existence of the complex.

Correspondence address: N.K. Nagradova, A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 119899, USSR

Abbreviations: GPDH, glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12); PGK, 3-phosphoglycerate kinase (EC 2.7.2.3); Mops, 3-*N*-morpholinopropanesulfonic acid; DTT, 1,4-dithiothreitol

2. MATERIALS AND METHODS

Glyceraldehyde-3-phosphate, Sepharose 4B, NADH, Mops

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were obtained from Sigma (USA), NAD^+, 3-phosphoglycerate from Reanal (Hungary), EDTA, dithiothreitol from Serva (FRG). GPDH and PGK were isolated from rabbit skeletal muscle by the affinity elution method [8] with slight modifications [6]. The preparations were electrophoretically homogeneous and exhibited specific activities of 120–140 U (GPDH) and 650 U (PGK). Protein concentrations were determined by the method of Bradford [9]. Immobilization of glyceraldehyde-3-phosphate dehydrogenase on Sepharose 4B was performed as previously described [10]. Before an experiment, the immobilized enzyme was washed with 5 vols of 20 mM Mops, pH 7.0. The preparation contained about 2 mol of NAD^+ per mol tetramer, determined after enzymatic reduction and titration of the remaining SH-groups with 5,5′-dithio(bis)nitrobenzoate. Titration of immobilized GPDH with soluble PGK was performed as follows. A series of samples was prepared, each containing 2 ml of the packed gel (500 μg of immobilized enzyme). Increasing concentrations of PGK were added to the samples, the total volume of each sample being constant (3 ml). After 30 min incubation at 20°C the gel was settled by centrifugation (1 min at 1000 × g) and the protein concentration was determined in the supernatant. An exclusion volume of the immobilized enzyme suspension was determined in a separate experiment using the following system: Sepharose-bound bovine serum albumin-soluble PGK. The exclusion volume was found to be 0.2 ± 0.01 ml/ml of the packed gel.

Electron microscopy studies of GPDH and PGK and their complexes were performed with a Philips EM 400 electron microscope operated at 80 kV. Measurements were taken at a magnification of 50000. Samples (0.1 mg protein in 1 ml 20 mM Mops, 1 mM EDTA, 10 mM K_3PO_4, pH 7.0) contained GPDH, PGK or their mixtures with no other additions or in the presence of 0.23 mM glyceraldehyde-3-phosphate. The presence of substrate ensured acylation of the dehydrogenase at two active sites per tetramer, since the tetrameric enzyme contained 2 equivalents of firmly bound NAD^+. The samples were applied on a thin carbon support grid; the grid was allowed to air dry at 20°C, and the specimens were negatively stained with 2% phosphotungstic acid at pH 7.0. Alternatively, some preparations were stained with a 1% uranyl acetate.

3. RESULTS AND DISCUSSION

3.1. *A functional state of glyceraldehyde-3-phosphate dehydrogenase determines its affinity for 3-phosphoglycerate kinase*

In preliminary experiments it was established that GPDH and PGK have a rather low capability of interacting in the absence of substrates. It seemed reasonable to assume that conformational changes accompanying catalysis can increase the ability of these enzymes to form a complex. Our attention was first focused on GPDH which can be stabilized in different functional states, namely, in the form of the enzyme · NAD^+ complex and in the form of the enzyme-3-phosphoglyceroyl · NADH complex. Immobilized GPDH containing about 2 mol NAD^+ per tetramer was prepared and allowed to react with an excess of glyceraldehyde-3-phosphate. This resulted in formation of the enzyme-3-phosphoglyceroyl · NADH complex at two active sites of the tetramer. Fig.1 shows the titration pattern of these two enzyme forms with PGK, which suggests that the transition from the enzyme · NAD^+ conformation to the enzyme-3-phosphoglyceroyl · NADH conformation results in an increase in GPDH affinity to PGK.

The existence of a complex between the two enzymes was also substantiated by electron-microscopic studies. Fig.2 shows microphotographs of the samples containing free GPDH (a) and free PGK (b). Tetrameric molecules of GPDH have mainly rectangular or triangular contours. This is consistent with the idea that the molecules having 222 symmetry were stabilized on the support. The outline of a PGK molecule (fig.2b) is about 5 nm, which is compatible with the M_r value of this enzyme of 47000. The microphotograph given in fig.2c demonstrates the

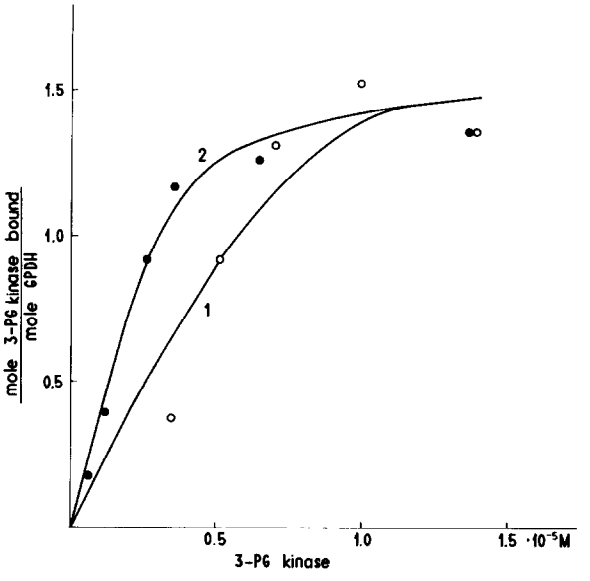

Fig.1. Titration of immobilized GPDH with PGK. Soluble PGK was added to a suspension of immobilized GPDH in 20 mM Mops, 10 mM K_3PO_4, 1 mM EDTA, pH 7.0, at 20°C. Each point represents a separate sample (see section 2 for details). Curves: 1, 500 μg of GPDH containing 2 molecules of bound NAD^+ per tetramer; 2, the same as 1, but 0.23 mM glyceraldehyde-3-phosphate was added.

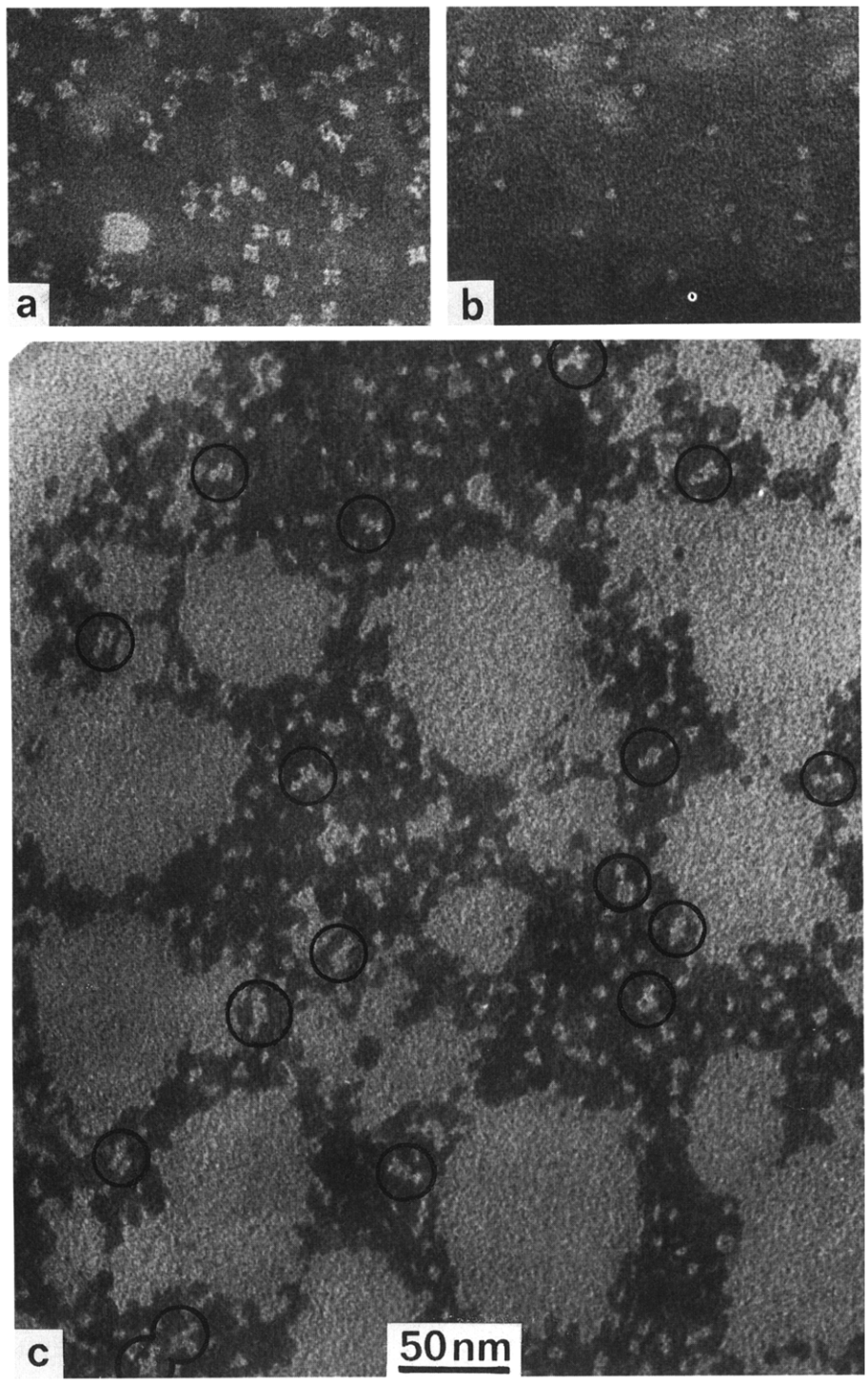

Fig.2. A general view of negatively stained preparations of GPDH (a), PGK (b) and acylated GPDH preincubated with PGK (c). The bienzyme complexes are circled.

results obtained after incubation of GPDH, acylated at nearly two active sites per tetramer, with PGK. In this case, it is seen that complexes of the two enzymes appear to contain one or two molecules of PGK per tetramer of the dehydrogenase. In samples containing mixtures of a non-acylated dehydrogenase and PGK very few bienzyme complexes appeared, in agreement with the results of the studies on immobilized enzymes. The different types of images of the bienzyme complexes detected in our experiments are shown in fig.3. One or two kinase molecules are seen to be bound to a tetrameric dehydrogenase molecule. Fig.3 (a and b) demonstrates rectangular projections of the dehydrogenase molecules in a complex with one molecule of kinase situated at the vertex

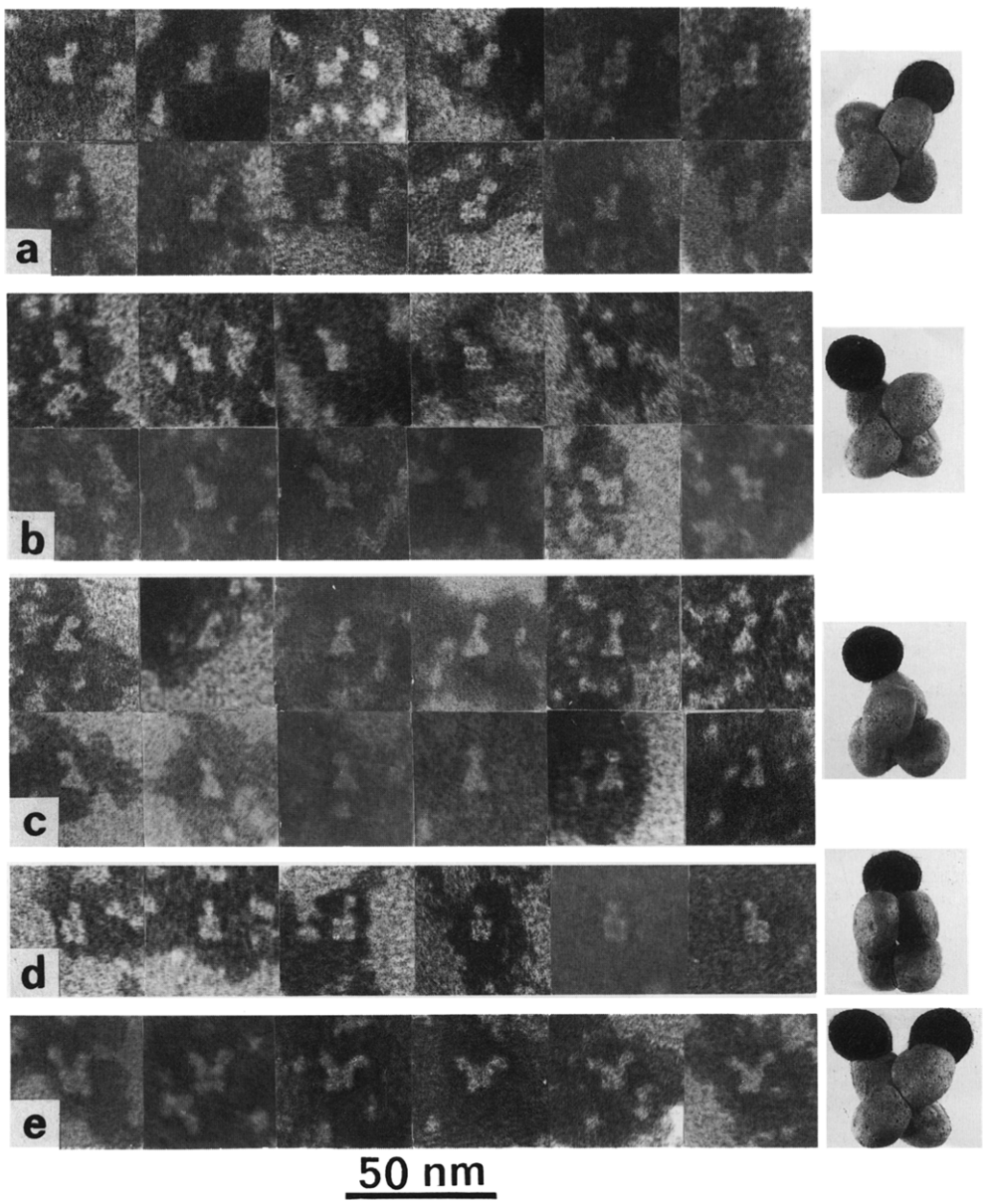

Fig.3. Characteristic types of GPDH·PGK complex images. The model of the complex of GPDH with PGK is shown on the right.

of the rectangle (two mirror variants of these images are seen in microphotographs) or in the middle of the rectangle's side (fig.3d). One can also see the images of the complexes with triangular projections of GPDH molecules (fig.3c) as well as the images of complexes containing two PGK molecules (fig.3e).

Thus, the results of the electron-microscopic experiments appear to be consistent with the data obtained using immobilized enzymes. The main conclusion which follows from these studies is that GPDH and PGK can exist in an associated state and that the bienzyme complex is relatively stable in vivo. In reality, a large proportion of rabbit muscle GPDH was shown to be present in the cytoplasm in an acylated form [11]. Under the conditions favoring phosphorolysis of the 3-phosphoglyceroyl-enzyme bond, a direct transfer of 1,3-diphosphoglycerate probably occurs between the dehydrogenase and the kinase active sites, in accordance with the data of Weber and Bernhard [4].

3.2. *On the role of 3-phosphoglycerate kinase conformation in the stability of the bienzyme complex*

To obtain more information about structural requirements for the bienzyme association, we carried out another series of experiments. Immobilized GPDH (1×10^{-6} M) was incubated in the presence of constant saturating concentrations of glyceraldehyde-3-phosphate and P_i and different concentrations of NAD^+: the reaction being carried out until equilibrium was established. The concentration of NADH which had accumulated in the samples was then determined. Since equal amounts of NADH and 1,3-diphosphoglycerate are formed in the course of the reaction, the data reflected the concentrations of 1,3-diphosphoglycerate present in the reaction mixtures at equilibrium. Therefore, it became possible to investigate the process of GPDH·PGK complex formation in reaction mixtures containing different 1,3-diphosphoglycerate concentrations. As shown in fig.4, marked differences were observed in the patterns of GPDH titration with PGK in the presence of low (curve 1) and high (curve 2) concentrations of 1,3-diphosphoglycerate. Given the very high affinity of PGK to 1,3-diphosphoglycerate [4] we can expect that

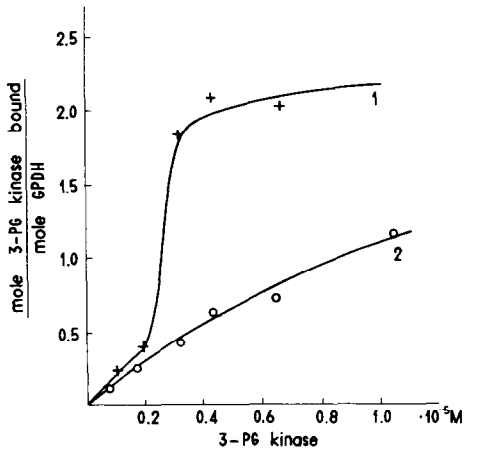

Fig.4. Association of GPDH and PGK in the presence of substrates of the dehydrogenase. Curves: 1, a sample containing 420 μg of immobilized GPDH was supplemented with 15 μM NAD^+ and after 10 min incubation PGK was added. Following another incubation (30 min), 0.23 mM glyceraldehyde-3-phosphate was added and the system was allowed to equilibrate for 10 min before centrifugation (see section 2); 2, the same as 1, but 167 μM NAD^+ was added. The concentrations of 1,3-diphosphoglycerate at equilibrium were found to be 0.21×10^{-5} M (1) and 0.9×10^{-5} M (2). Each point corresponds to a separate sample. Other details are given in fig.1 and in section 2.

under the conditions depicted in fig.4, stoichiometric binding of 1,3-diphosphoglycerate by PGK occurred. In such a case no substrate-free kinase will remain in solution in the samples of the series (curve 1) until the concentration of the kinase exceeds that of 1,3-diphosphoglycerate, i.e. becomes higher than 0.21×10^{-5} M. It is noteworthy that precisely in this range of kinase concentrations the titration curve changes its profile indicating a sharp increase in the affinity of this enzyme to GPDH. These results suggest that the kinase·1,3-diphosphoglycerate complex has a rather low affinity for acylated GPDH. The data of fig.4, curve 2 are in line with this idea. Actually, the concentration of 1,3-diphosphoglycerate in this series of experiments was high enough to convert all the kinase molecules into the form incapable of forming stable complexes with acylated GPDH. Taken together, the results of this study suggest that under intracellular conditions with low levels of free intermediates [12], GPDH and PGK can be bound in a complex while catalyzing the consecutive reactions of glycolysis.

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