THE INVESTIGATION OF REGULATORY TRANSITION IN D-GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE QUATERNARY STRUCTURE

D. S. MARKOVICH, L. D. KRAPIVINSKAYA
Institute of Biophysics, Academy of Sciences USSR, Pushchino-on-Oka, 142292, USSR

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1. Introduction

Regulatory behaviour of oligomeric enzymes is to a very large extent determined by monomer interactions. There is reason to believe that the dissociation of oligomers is hardly important in regulatory mechanisms in vivo owing to high local enzyme concentrations and the highly ordered structure of multienzyme systems. We suppose that regulatory mechanisms involve mainly nondissociative transitions in the quaternary structure such as oligomer symmetry changes. These rearrangements have been observed by X-ray analysis of hemoglobin [1] and some dehydrogenases [2,3], as a result of their interactions with coenzyme and substrate. There might be a change in the interactions between monomers in the course of an enzymatic reaction to provide the co-ordinate of the functions of several active sites. These changes might also be caused by different effectors, some of them exerting a direct influence on the contacts between monomers, as 2,3-DPG in hemoglobin [4] and possibly pyruvate in lactate dehydrogenase [5]. We have previously shown, by the fluorescent label method, that the inhibitor of glycolytic pathway, ATP, produces slow changes (within 7–15 min) in D-glyceraldehyde-3-phosphate dehydrogenase (GPD) involving quaternary structure rearrangements [6]. There was no correlation between ATP-induced, very slow dissociation and inactivation processes (hours) [7] and the rearrangements mentioned above [6]. We have previously proposed nondissociative changes in the quaternary structure [6]. The investigation of subtle quaternary structure changes by most physical methods is highly restricted. In the present study the molecular hybridization method is used to characterize ATP-induced changes in GPD quaternary structure. It is known that hybrid formation occurs between muscle and yeast GPD [8] and also between GPD from various strains of micro-organisms [9]. A single hybrid species was observed in these studies, a result in agreement with the view that GPD consists of two functional dimers (the dimer of dimers model). If all the monomers are identical, it is necessary somehow to modify a part of protein so that hybridization can be observed and separation of hybrid performed. In this study the specific alkylation of a single sulfhydryl group of GPD active sites (SH_{149}) was used [10]. This modification does not appreciably alter the enzyme conformation; moreover, the modified molecule is somewhat similar to the enzyme–substrate complex. GPD tetramers with four carboxymethylated SH groups can be easily separated by electrophoresis from native molecules and hybrid ones containing various numbers of modified monomers (from one to three).

2. Materials and methods

GPD was isolated from swine muscles according to Elodi and Szorenyi [11] and additionally purified on DEAE-cellulose column [13]. Apoenzyme preparation and alkylation procedure by iodacetate (IA) have been described previously [12]. The hybridization was performed by mixing the native and modified apoenzymes in equal concentrations ($3–6 \times 10^{-5}$ M). The incubations are detailed in the legends to the figures. Analytical gel electrophoresis was performed in 7% polyacrylamide at 0°C, during 3–3.5 h (0.0125 M Tris–HCl, pH 8.9).

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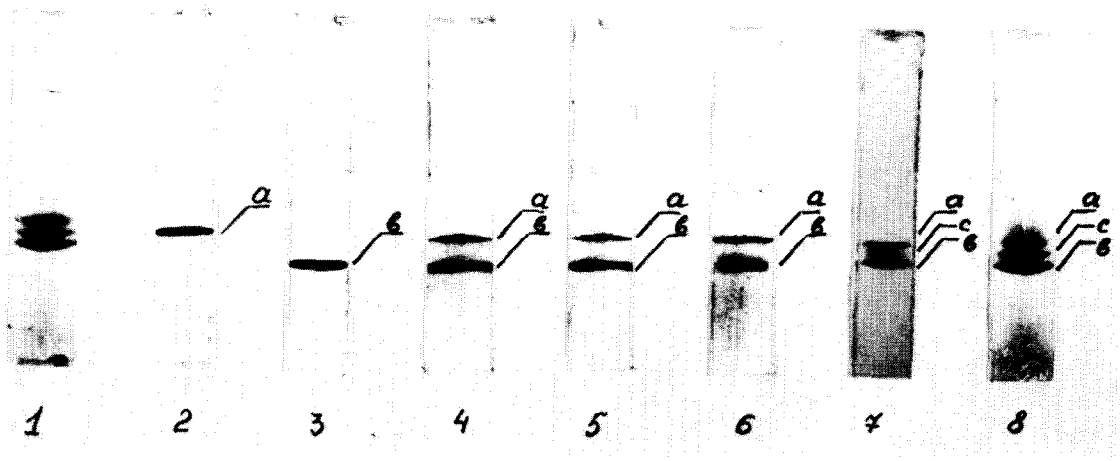

Fig.1. Polyacrylamide gel electrophoresis of pig muscle GPD. gel 1, native apo-GPD; gel 2, apo-GAPD with NAD (10^{-3} mole NAD per mole GPD); gel 3, IA-treated apo-GPD (alkylation conditions; 10 mole IA per mole GPD in 0.1 M Tris–HCl, pH 8.0; incubation for 1 h at 0°C); gel 4, mixture of native and IA-treated GPD, NAD was added at the beginning of hydridization; gel 5, 5 min hybridization; gel 6, 15 min hybridization; gel 7, 20 min hybridization; gel 8, 30 min hybridization. In all experiments hybridization was stopped by NAD addition in molar excess (1000/1). Electrophoresis conditions see in Methods. (a), native apo-GPD; (b), IA-treated GPD; (c) hybrid fraction.

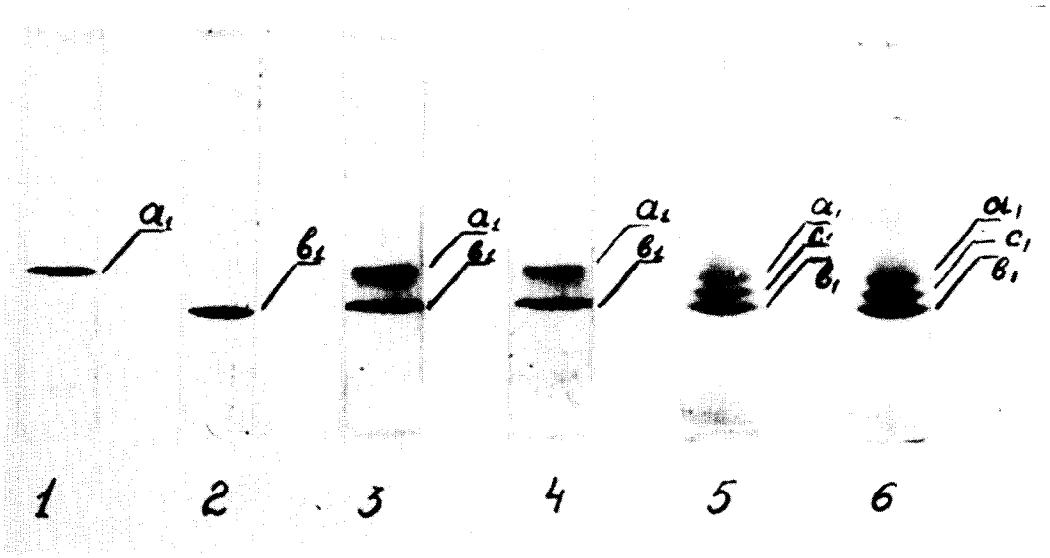

Fig.2. Polyacrylamide gel electrophoresis of pig muscle GPD in the presence of 1 mM ATP gel 1, native apo-GPD with 1 mM ATP (incubation time was varied from 10 min to 3 hours); gel 2, IA-treated GPD with 1 mM ATP. gel 3; native + IA-treated GPD (enzyme concentration 3-5 mg/ml; 0.1 M Tris–HCl; pH 8.0; at 0°C), preincubation time (with 1 mM ATP) was 10 min; NAD was added simultaneously with the beginning of hybridization; gel 4, the same as 3, but 30 min incubation with ATP; gel 5, 3 h incubation with ATP; gel 6, 5 min hybridization of native and IA treated GPD, 10–30 min preincubation with ATP. (a), apo-GPD; (b), IA treated GPD; (c) hybrid fraction.

Results and discussion

The apoenzyme is quite unstable: dissociation and inactivation occur easily in the course of electrophoresis (see [13] and fig.1(1) in this paper). The addition of NAD in large molar excess (1000 moles per mole of GPD) prior to electrophoresis eliminates the heterogeneity (fig.1(2). It is well-known that NAD hinders GPD inactivation and dissociation [7,14,15].

Hence NAD addition was used in all experiments to stop hybridization and to hinder the dissociation in the course of electrophoresis. Fig.1. [4–6] shows that the hybrid band cannot be observed during the first 15 min of hybridization. In most experiments a single hybrid band occurs after more prolonged hybridization (see fig.1 (gels 7 and 8). The addition of NAD simultaneously with mixing of native and modified GPD completely blocks hybrid formation. The existence of a single band of hybrid is in agreement with recent X-ray data on GPD: the tetramer is composed of two dimers with the active sites closely approximated [16].

Preincubation of native and modified GPD with 1 mM ATP during 10–30 min caused a marked acceleration of hybridization: the hybrid band can be observed as early as 5 minutes after mixing (fig.2). The change of hybridization rate indicates an alteration of monomer interactions i.e. quaternary structure changes. This process may or may not be followed by marked dissociation. NAD induces a rapid shift of equilibrium towards tetramers and blocks hybridization, hence the addition of coenzyme simultaneously with the beginning of hybridization might cause the formation of hybrids only when marked dissociation in the presence of ATP takes place, the hybrids being formed by association of dissociated molecules of the two species.

Fig.2 shows that hybrid does not appear in this experiment after 10–30 min incubation with 1 mM ATP. On the contrary, the same experiment gives a significant amount of hybrid molecules when GPD was preincubated with ATP for 3 h (fig.2) that causes dissociation of tetramers [7,17,18].

It is apparent from these studies that:

(1) 10–30 min incubation with ATP does not produce a marked dissociation of GPD tetramer,

(2) within the same time the rate of hybridization is increased markedly.

It may be concluded that the slow (7–15 min) changes induced by ATP involve rearrangement of GPD quaternary structure that is not followed by dissociation of tetramer. Insofar as ATP binds to active site regions [12] which are in close proximity to contact areas between monomers [17], a direct influence of ATP on monomer interactions is probable.

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