Evidence for a change in catalytic properties of glyceraldehyde 3-phosphate dehydrogenase monomers upon their association in a tetramer

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1. INTRODUCTION

Oligomeric structure is not a prerequisite for catalytic activity of glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12). Using the technique of matrix immobilization we prepared isolated monomeric [1], dimeric [2] and trimeric [3] enzyme forms. Their specific activities assayed in a standard mixture containing saturating concentrations of NAD^+ and substrate were nearly equal and close to the activity of the original tetramer. This indicated that subunit cooperativity known to exist in oligomeric enzyme species [2–5] played no detectable role in enzyme functioning under the conditions employed. It became interesting to compare catalytic properties of monomeric and oligomeric enzyme forms in assay mixtures of different composition, i.e., containing non-saturating concentrations of specific ligands. As shown here, dissimilarities between catalytic activities of monomeric and tetrameric enzyme forms really emerge when activity is measured in presence of low coenzyme concentrations. The monomeric and tetrameric enzyme species were also shown to catalyze the reverse reaction (reductive dephosphorylation of 1,3-diphosphoglycerate) with different velocities.

2. MATERIALS AND METHODS

Glyceraldehyde 3-phosphate dehydrogenase was isolated from baker's yeast and immobilized on CNBr-activated Sepharose as in [2]. The preparation of matrix-bound monomers wase done as in [3]. Packed gel volumes were determined after centrifugation at 100 × *g* for 3 min in graduated centrifuge tubes. The stock suspension of a matrix-bound derivative was prepared by mixing equal volumes of gel and buffer: 0.1 M sodium phosphate, 5 mM EDTA, 2 mM dithiothreitol (pH 8.3) in studies on glyceraldehyde 3-phosphate oxidation or 10 mM imidazole, 2 mM dithiothreitol (pH 7.5) in experiments with the reverse reaction.

Lactate dehydrogenase from pig heart (Reanal) was immobilized on Sepharose as in [6], using 50 mg CNBr/ml Sepharose to activate the gel. The content of the matrix-bound protein was determined spectrophotometrically [7]. Glyceraldehyde 3-phosphate was prepared as in [8]. 1,3-Diphosphoglycerate was prepared as in [9,10].

The standard assay mixture to measure the activity of glyceraldehyde 3-phosphate dehydrogenase in the forward direction contained 50 mM Tris–HCl (pH 7.9), 5 mM sodium arsenate, 5 mM EDTA, 2 mM NAD^+, 1.5 mM glyceraldehyde 3-phosphate and 0.1–0.2 ml of a suitably diluted suspension of matrix-bound enzyme in 3 ml final vol. The reaction was done at 25°C and followed spectrophotometrically for 15–60 s. The assay mixture used to determine the dehydrogenase activity at low coenzyme concentrations was supplemented with lactate dehydrogenase and pyruvate to regenerate NAD^+ from NADH. In this case the reaction was monitored by the disappearance of glyceraldehyde 3-phosphate. The procedure was as follows: 2 ml 50 mM sodium pyrophosphate, containing 5 mM sodium arsenate (pH 8.0) were mixed with 0.4 ml immobilized lactate dehydrogenase suspension (2.4 activity units) and 0.4 ml immobilized glyceraldehyde 3-phosphate dehydrogenase suspension (0.8 activity units). The latter enzyme was

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present in a tetrameric or monomeric form. To achieve equality in the number of active centers and the amount of Sepharose in samples containing tetrameric and monomeric forms, a control Sepharose suspension was added to the suspension of immobilized tetramers at 3:1. The reaction was started by simultaneous addition of glyceraldehyde 3-phosphate and NADH (0.05 ml each). The final concentration of glyceraldehyde 3-phosphate in the assay mixture thus achieved was 1.0 mM; [NADH] varied from 0.6–57.7 μM. The incubation was carried out at 25°C under continuous gentle stirring and stopped by transferring the mixture on a glass filter to separate immobilized enzymes from the solution. The concentration of glyceraldehyde 3-phosphate was then determined enzymatically using soluble glyceraldehyde 3-phosphate dehydrogenase. A control sample was run in each experiment containing 0.4 ml Sepharose suspension instead of immobilized glyceraldehyde 3-phosphate dehydrogenase.

To follow the reaction in the direction of NADH oxidation, 0.1–0.2 ml immobilized glyceraldehyde 3-phosphate dehydrogenase suspension (5–10 μg protein) were added to a mixture of 10 mM Tris–SO_4, 100 mM KCl, 1 mM EDTA (pH 7.0), containing 0.3 mM NADH and different amounts of 1,3-diphosphoglycerate, the final volume of the sample being 2.0 ml. The reaction was started by the addition of 1,3-diphosphoglycerate and carried out at 18°C, the disappearance of NADH being measured. Other reagents and materials were as in [1–3].

3. RESULTS AND DISCUSSION

3.1. *Study of the forward reaction*

We demonstrated [1] that immobilized monomeric and tetrameric yeast glyceraldehyde 3-phosphate dehydrogenase, assayed at saturating coenzyme and substrate concentrations, did not differ in V_{max}-values. This indicated that the rate-limiting step of the reaction, i.e., phosphorolysis of acyl-enzyme preceded by the substitution of NADH for NAD^+ [11] was not influenced by subunit interactions. Consequently, NAD^+ concentrations employed in these experiments were concluded to be sufficient to effectively displace NADH tightly bound to acylated enzyme sites, both in monomeric and tetrameric forms of the dehydrogenase. It became apparent to us that to reveal the participation of subunit interactions in enzyme funtioning, catalytic properties of monomeric and oligomeric species should be compared using the physiological ranges of coenzyme concentrations. To this end, the following experimental procedure was elaborated. The assay mixture containing low NADH concentrations was supplemented with lactate dehydrogenase and pyruvate to regenerate the oxidized coenzyme. The reaction was carried out in presence of sodium arsenate and followed by the disappearance of glyceraldehyde 3-phosphate. Control experiments established that it proceeded linearly in time (measurements were made after 5, 10 and 15 min incubation) and was proportional to the enzyme concentration.

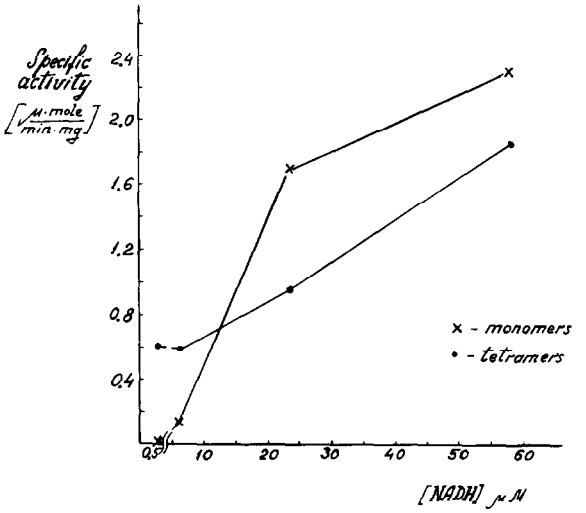

Fig.1. Specific activity of immobilized glyceraldehyde 3-phosphate dehydrogenase monomers (X) and tetramers (•) as a function of coenzyme concentration. Assay mixture contained glyceraldehyde 3-phosphate, sodium arsenate, NADH, lactate dehydrogenase and pyruvate. For details see section 2.

Fig.1 shows that under these conditions the difference in catalytic efficiency between monomeric and tetrameric enzymes is quite clear. That concerns first of all the activity in presence of 0.6–6.0 μM coenzyme. As seen in fig.1, the activity of monomeric form is negligible or absent under these conditions in contrast to the behavior of tetrameric enzyme. The most likely explanation of this result is that the affinity of monomeric enzyme for NAD^+ is

lowered. This makes an isolated subunit incapable of binding coenzyme present at <6 μM. These concentrations seem however to be sufficient to ensure a partial saturation of the active sites of the tetrameric enzyme, since it displays a measurable activity (fig.1).

Another difference between the monomeric and oligomeric enzyme is the markedly higher catalytic activity of the monomer, exhibited over 20–60 μM coenzyme (fig.1). This difference disappears at saturating [NAD^+]. Thus, in presence of 2 mM NAD^+, specific activities of monomeric and tetrameric forms were found to be similar and equal to 60 μmol . min $^{-1}$. mg $^{-1}$. These results demonstrate that subunit interactions are involved in regulation of enzyme activity.

The decrease in specific activity of individual subunits upon their association in a tetramer suggests that the rate-limiting step of the reaction was affected. We may assume that the binding of NADH in the ternary complex with the acyl-enzyme becomes stronger in the tetrameric enzyme as compared with its binding with a monomer. This would explain the observation that the difference in catalytic efficiency between the monomeric and tetrameric enzyme forms depends upon the NAD^+ concentration in the assay mixture. Fig.1 delineates the conditions under which only $\sim 1/2$ of the active centers of the tetramer are functioning simultaneously. It seems likely that subunit cooperativity plays an important role in catalysis under these conditions, the functioning of one of the subunits within a dimer being dependent upon the conformational state of the adjacent subunit [12].

3.2. *Study of the reverse reaction*

The reaction of 1,3-diphosphoglycerate with NADH to yield glyceraldehyde 3-phosphate was shown to involve subunit cooperativity [11–14]. Binding of NAD^+ at 2 of the 4 active sites of the enzyme is required for catalytic reduction with NADH at the acylated sites [14]. The NAD^+-dependent activation of the reverse reaction was suggested to result from a conformational change transmitted via the intersubunit contacts [14]. These results together with [11,13,15], preshow the participation of NAD^+ as a co-catalyst in the reductive dephosphorylation of 1,3-disphosphoglycerate.

Such an effect would obviously not take place if a monomeric enzyme form were used. Ability of an isolated subunit to catalyze the reverse reaction would therefore indicate that the allosteric effect of NAD^+ is not necessary for catalysis. We investigated the reaction of reductive dephosphorylation of 1,3-diphosphoglycerate using monomeric and tetrameric enzyme species. The results of this study shown in fig.2 can be summarized as follows:

(1) An isolated subunit is capable of catalyzing the reverse reaction. This indicates that subunit interactions are not necessary for reductive dephosphorylation of 1,3-diphosphoglycerate.
(2) Specific activity of a monomer appears to be considerably higher than that of a tetrameric form of the enzyme.

To evaluate the possible role of diffusion limitations as a factor responsible for different catalytic activities of immobilized monomers and tetramers, we investigated the pH profile of the reaction. Monomeric and tetrameric forms exhibit similar pH dependencies of activity (fig.3). This would not have been observed if diffusion effects had been significant. The more likely explanation of the results is

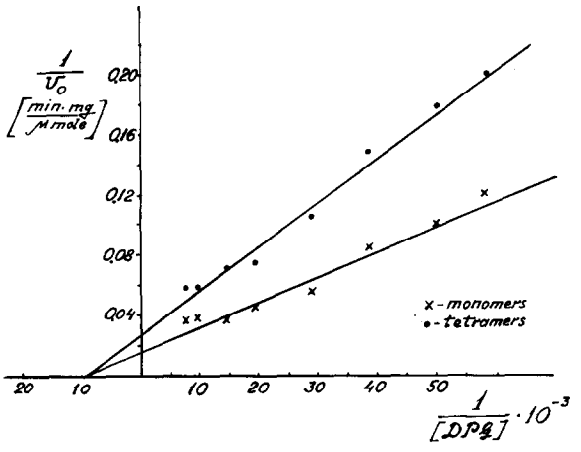

Fig.2. Double reciprocal plot of 1,3-diphosphoglycerate saturation curves in the reactions catalyzed by monomeric (X) or tetrameric (•) enzyme forms. The assay conditions are described in section 2. The straight lines were obtained by linear regression analysis of experimental points. Specific activities calculated from the extrapolated V_{max} are 37.6 and 58.8 μmol NADH . min $^{-1}$. mg $^{-1}$ for the tetrameric and monomeric forms, respectively; K_M for 1,3-disphosphoglycerate equals 1.14 . 10^{-4} M and 1.01 . 10^{-4} M. DPG, 1,3-diphosphoglycerate.

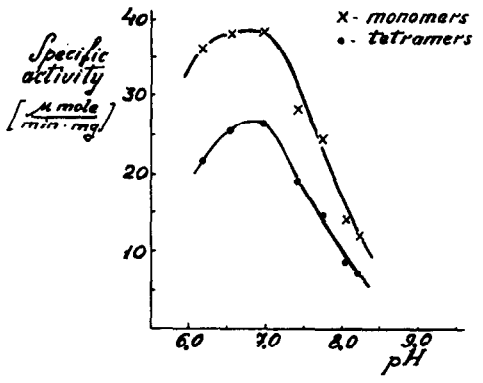

Fig.3. Dependence of activity of immobilized monomers (X) and tetramers (•) on pH. Reductive desphosphorylation of 1,3-diphosphoglycerate was studied; 1,3-diphosphoglycerate was 1.3 mM.

that a monomeric enzyme form actually exhibits a higher activity than an oligomer. The results of these experiments suggest that only ~1/2 of the active centers of the tetrameric yeast enzyme function simultaneoulsy in the reverse reaction, in agreement with [13], obtained in kinetic studies on sturgeon soluble glyceraldehyde 3-phosphate dehydrogenase. This is also true in the case of the forward reaction under certain conditions (see above).

Thus, changes in catalytic properties of isolated subunits upon their association in a tetramer appear to be directly demonstrated. These changes are probably significant for regulation of enzyme activity under physiological conditions. Due to subunit cooperativity, oligomeric enzyme can utilize very low [NAD^+], which are insufficient for it to be bound to a monomer. The partial 'inhibition' of activity in presence of moderate [NAD^+] (only 1/2 of the active sites are functioning) is lifted under the conditions when the coenzyme concentration is sharply increased.

Our results are consistent with the idea that the major function of the tetrameric structure of glyceraldehyde 3-phosphate dehydrogenase is to coordinate the activity of individual subunits with the conditions existing in the cell.

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REFERENCES

[1] Ashmarina, L.I., Muronetz, V.I. and Nagradova, N.K. (1980) Biochem. Int. 1, 47–54.
[2] Muronetz, V.I., Zueva, V.S. and Nagradova, N.K. (1979) FEBS Lett. 107, 277–280.
[3] Ashmarina, L.I., Muronetz, V.I. and Nagradova, N.K. (1981) FEBS Lett. 128, 22–26.
[4] Stallcup, W.B. and Koshland, D.E. jr (1972) Biochem. Biophys. Res. Commun. 49, 1108–1114.
[5] Cook, B.A. and Koshland, D.E. jr (1970) Biochemistry 9, 3337–3342.
[6] Ashmarina, L.I., Muronetz, V.I. and Nagradova, N.K. (1981) Biochem. Int. 3, 415–424.
[7] Golovina, T.O. Cherednikova, T.V., Mevkh, A.I. and Nagradova, N.K. (1977) Analyt. Biochem. 83, 778–781.
[8] Szewezuk, A., Wolna, E., Wolny, M. and Baranowski, T. (1961) Acta Biochim. Pol. 8, 201–207.
[9] Negelein, E. and Brömel, H. (1939) Biochem. Z. 303, 132–144.
[10] Furfine, C.S. and Velick, S.F. (1965) J. Biol. Chem. 240, 844–855.
[11] Duggleby, R.G. and Dennis, D.T. (1974) J. Biol. Chem. 249, 175–181.
[12] Malhotra, O.P., Bernhard, S.A. and Seydoux, F. (1981) Biochimie 63, 131–141.
[13] Kellershohn, N. and Seydoux, F.J. (1979) Biochemistry 18, 2465–2470.
[14] Schwendimann, B., Ingbar, D. and Bernhard, S.A. (1976) J. Mol. Biol. 108, 123–138.
[15] De Vijlder, J.J.M., Hilvers, A.G., Van Lis, J.M.J. and Slater, E.C. (1969) Biochim. Biophys. Acta 191, 221–228.