EFFECT OF ANIONS ON THE STRUCTURE OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE FROM RAT SKELETAL MUSCLE

N.K.Nagradova and M.K.Guseva

Laboratory of Bioorganic Chemistry, Lomonosow State University, Moscow, USSR

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SUMMARY

Glyceraldehyde-3-phosphate dehydrogenase from rat skeletal muscle undergoes reversible partial dissociation into 4.2-4.5 S fragments at +4°C and at protein concentrations as high as 8-9 mg/ml, depending upon the anion species present. The order of effectiveness of various anions in dissociating activity corresponds to their position in the Hofmeister series: bromide > nitrate > chloride > acetate > sulfate > phosphate=citrate. Quite similar is the order of activity of anions in their influence on the thermal stability of the dehydrogenase.

INTRODUCTION

We have isolated glyceraldehyde-3-phosphate dehydrogenase (GAPD) from rat skeletal muscle and found it to be different from rabbit muscle GAPD in some properties [1] . Unlike the rabbit enzyme, GAPD from rat skeletal muscle appeared to be able to undergo a reversible partial dissociation into 4.2-4.5 S fragments in solution of sufficiently high protein concentration (8-9 mg/ml). The dissociation (and inactivation) took place only at low temperature (0- +4°C) and was strongly dependent on the ionic composition of the medium. Monovalent anions increased the dissociation, while the polyvalent anions had the opposite effect. The order of effectiveness of various anions in dissociating activity conformed to the Hofmeister series of ions. Quite similar was the order of activity of anions in their influence on the thermal stability of GAPD. It is assumed, that

the effect of anions on the dissociation and inactivation of GAPD is the consequence of their specific interactions with peptide and amide groups of the enzyme molecule leading to the formation of complexes with different solubility in surrounding medium.

MATERIALS AND METHODS

Isolation, characterization and enzyme assay of rat skeletal muscle GAPD are described in the previous paper [1] . All reagents were analytical grade commercial preparations. Sedimentation velocity experiments were performed in a Beckman-Spinco Model E ultracentrifuge at 56,100 r.p.m. using Schlieren optics.

RESULTS AND DISCUSSION

Suspension of crystals of GAPD was dissolved in 5 mM Tris-Cl buffer, pH 7.5-7.8 containing 4 mM β-mercaptoethanol, 5 mM EDTA to give a final protein concentration of 8 mg/ml and dialyzed against the same buffer at +4°C. After 12-16 hours of dialysis 30-40% of enzyme activity was lost and analysis in ultracentrifuge revealed the heterogeneity of the material- in addition to the main 7.4 S peak a slowly moving component appeared. The amount of this component significantly increased if 0.15 M NaCl was added to the sample, enzymatic activity diminished, and the protein sedimented as two peaks - 7.4S and 4.2-4.5 S. Sedimentation coefficient of 7.4 S is characteristic of tetrameric GAPD isolated from various sources [2,3] . So we assume that the faster moving peak in our experiments must represent a native tetrameric GAPD. As to the 4.5 S component, we believe it to be a dimer, a product of partial dissociation of GAPD. This conclusion is supported by the observation that the

amount of 4.5 S component increased as the protein concentration lowered. Moreover, 4.2-4.5 S fragment observed by several authors in the study of rabbit muscle GAPD dissociation was considered as a dimer [4-6] .

Dissociation of rat muscle GAPD under conditions of our experiments was markedly influenced by the presence of several salts which differed in effectiveness depending on the character of anion. While polyvalent anions were able to prevent the dissociation (and inactivation) nearly completely, monovalent anions increased both the dissociation and inactivation (Fig.1, Table 1). The data presented in Table 1 clearly show that the ionic strength effect must be excluded as a possible cause of the low temperature salt-dependent inactivation of the enzyme. In fact, GAPD inactivation could be observed in the presence of 0.15 M NaCl; the effect increased at 0.5 M concentration of

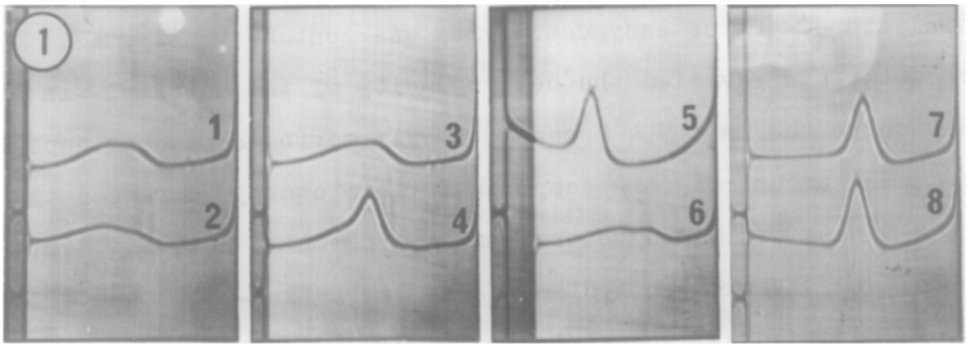

Fig.1. Schlieren patterns of GAPD dialyzed against solutions of various salts (for conditions of dialysis see table 1). Sedimentation from left to right at +4°C. Pictures were taken 110 min (1-6) and 120 min. (7,8) after a rotor speed of 56,100 r.p.m. had been attained. Protein concentration 8 mg/ml. 1,3 - sodium chloride (a), 2 - potassium bromide, 4 - sodium acetate, 5 - sodium citrate, 6 - potassium nitrate, 7 - ammonium sulfate, 8 - potassium phosphate.

this salt. On the other hand, 0.15 M sodium citrate completely protected GAPD from dissociation and inactivation.

The influence of salts could be best explained assuming some specific interaction of protein functional groups with

TABLE 1

Effect of Anions on the Stability of GAPD from Rat Skeletal Muscle at different temperatures.

Salt	Dialysis at +4°C * % of activity	Incubation at +37°C** % of activity
KJ	-	22
KBr	1.6	56
KNO_3	3.2	73
KCl	7.1	-
NaCl (a)	7.1	81
NaCl (b)	3.0	-
Sodium acetate	43	92
$(NH_4)_2SO_4$	95	93
Potassium phosphate	114	107
Sodium citrate	118	95

* Crystalline suspension of GAPD was dissolved in a small volume of 5 mM EDTA, 4 mM β-mercaptoethanol pH 7.5 to give a protein concentration of 15 mg/ml and dialyzed for 12 hours at +4°C against 0.15 M salt solution containing 5 mM EDTA and 4 mM β-mercaptoethanol. A protein precipitate formed during dialysis was removed by centrifugation, the protein solution was diluted with an appropriate salt solution at a concentration of 8 mg protein/ml and analyzed in the ultracentrifuge (Fig.1). The values of activity, given in the table, were measured after the sedimentation in the ultracentrifuge. The activity of an nondialyzed sample served as a control. NaCl (a) - 0.15 M. NaCl (b) - 0.5 M.

** Crystalline suspension of GAPD was dissolved in 5 mM potassium phosphate pH 7.6, 5 mM EDTA, 5 mM β-mercaptoethanol to give a protein concentration 7.6 mg/ml and dialyzed against the same buffer for 5 hours at +4°C. A sample containing 0.5 mg of the dialyzed protein was incubated at +37°C for 9 min in the presence of 0.3 M solution of the appropriate salt, 5 mM EDTA, 5 mM β-mercaptoethanol pH 7.8, after which the activity was determined. For 100% was assumed the enzyme activity before incubation.

anions. Depending on the character of the anion, such an interaction would favor dissociation or inhibit it. Effect of monovalent anions might be due to their interaction with some reactive groups which are exposed to the medium and come in contact with the solvent when a low temperature dissociation occurs. The effectiveness of various anions in dissociating activity was: bromide > nitrate > chloride > acetate > sulfate > phosphate=citrate. This progression conforms to the Hofmeister series [7] . As seen in Table 1, the order of activity of anions in their influence on the thermal stability of GAPD was nearly the same. Such a correlation allowed us to suppose some general mechanism in both cases. We suggest that such a mechanism might be similar to that supposed by Robinson and Jencks in their study of the effect of anions on the solubility of a synthetic peptide [8]. Anionic effects could be explained by specific interactions with peptide and amide groups of the protein, followed by for-

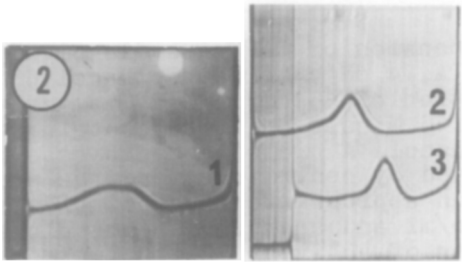

Fig.2. Reassociation and reactivation of GAPD. Schlieren patterns of GAPD; 1 - after 12 hr dialysis at +4°C against 0.15 M NaCl, 5 mM EDTA, 4 mM β-mercaptoethanol, pH 7.8. Protein concentration 8 mg/ml, activity 7%.2 - the same protein solution after 2 hr incubation at +22°C with 8 mM β - mercaptoethanol. 3 - the same as in 2, but in the presence of 0.15 M potassium phosphate. Activity 80%. Sedimentation from left to right at +4°C (I) and at + 20°C (2,3). Pictures were taken 110 min (I) and 60 min (2,3) after a rotor speed of 56,100 r.p.m. had been attained.

mation of complexes with different solubility in surrounding medium.

We believe, that low temperature induces some destabilization of tetrameric GAPD caused by the weakening of hydrophobic interactions. This may lead to unmasking of some additional amide and peptide groups and their interaction with anions. The effectiveness of various anions depends presumably, upon their relative polarizability, thus explaining different effects on the solubility of the newly exposed groups. Effect of anions on the dissociation is, therefore, due to a shift of an equilibrium between a tetrameric form of the enzyme stabilyzed by polyvalent anions and subpolymers which are produced in the presence of monovalent anions. Warming the solution of a partly-dissociated GAPD to $+22^{\circ}$ and addition of phosphate results in 80% reactivation and concomitant reassociation of the enzyme, as is shown in Fig.2.

It seems reasonable that the effect of anions should manifest itself not only under conditions of low temperature protein destabilization, but also in some other cases - when the native protein structure is somewhat weakened. Thermal stability of GAPD in the presence of various salts is an example (Table 1).

Reversible dissociation and inactivation of rat skeletal muscle GAPD observed in our experiments, occured at rather high protein concentration (8-9 mg/ml) and at salt concentrations as low as 0.15 M. A possibility therefore exists, that this effect might be one of the mechanisms to control the level of the enzyme *in vivo*.

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