INFLUENCE OF ENZYME CONCENTRATION ON THE REACTION OF RABBIT MUSCLE PHOSPHOFRUCTOKINASE WITH ANTIBODIES

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1. Introduction

In the absence of positive effectors, phosphofructokinase activity is not proportional to enzyme concentration [1]. This phenomenon could only be partially explained by concentration dependent inactivation of the enzyme, which is caused by dissociation. The data are consistent with a mechanism which involves different allosteric properties of the aggregated forms of the enzyme [2].

This study demonstrates that the formation of precipitable complexes between specific antibodies and phosphofructokinase also depends on the concentration of the enzyme. Moreover, the kinetics of precipitable and non-precipitable complexes of the enzyme are clearly different.

2. Materials and methods

The preparation of antibody-containing γ-globulin fractions and of crystalline rabbit-skeletal muscle phosphofructokinase was the same as described in [3]. In control experiments, antibody-free γ-globulins were used.

The following assays were used for phosphofructokinase activity determinations: system A:50 mM triethanolamine-HCl, 5 mM EDTA, 8 mM $MgSO_2$, 0.4 mM NADH, 3 mM fructose 6-phosphate, 1 mM ATP, 100 μg aldolase/ml, 30 μg triosephosphate isomerase/ml, 80 μg glycerolphosphate dehydrogenase/ml, pH 7.6. System B: 100 mM imidazole-HCl, 4 mM $MgCl_2$, 3 mM ATP, 1 mM phosphoenolpyruvate, 0.4 mM NADH, 200 μg pyruvate kinase/ml, 200 μg lactate dehydrogenase/ml, pH 6.8. The concentrations of fructose 6-phosphate were variable. Auxiliary enzymes were used in $(NH_4)_2SO_4$-free solution.

3. Results

Phosphofructokinase, which was preincubated in imidazole buffer, was activated by low concentrations of antibodies even when the reaction mixture was centrifuged before the assay in order to remove precipitable complexes between antibodies and enzyme. Fig. 1 correlates the specific activities of phosphofructokinase with increasing concentrations of the antibodies. The abscissa has been plotted in terms of the antibody/antigen ratio. The curves refer to different concentrations of phosphofructokinase. The extent of activation was more pronounced at low concentrations of phosphofructokinase, but a relatively higher amount of antibodies was required to reach optimum activity. At still higher antibody concentrations visible precipitation occurred and the enzymic activity in the supernatants decreased. Thus the fraction of precipitable enzyme–antibody complexes could be estimated by means of activity determinations.

The precipitating effect of antibodies was optimal at high phosphofructokinase concentrations. Similar to the activation, a relatively higher amount of antibodies was required at lower concentrations of the enzyme in order to obtain the same degree of precipitation.

When phosphofructokinase was diluted in a buffer system, which provided optimum specific activity, e.g. in 0.1 M phosphate buffer, pH 7.5, containing 0.1 mM fructose 1,6-diphosphate, a similar effect was observed (fig. 2). In contrast to the experiments

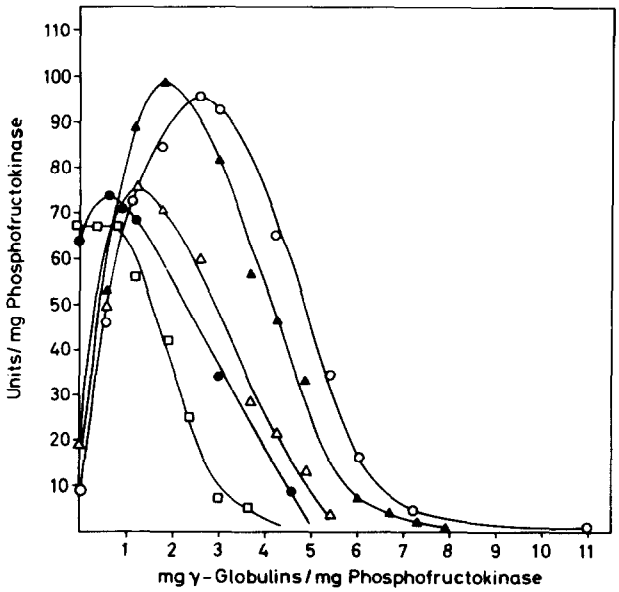

Fig. 1. Dependence of the specific activity of phosphofructokinase on the concentration of specific antibodies. Different amounts of phosphofructokinase were incubated with various concentrations of antibody-containing γ-globulins in 0.1 M imidazole-HCl and 0.2 M KCl, pH 7.5, for 15 min at 22°. Activities were measured after 4 min centrifugation at 20,000 *g* in test system A and plotted versus the ratio mg γ-globulins/mg phosphofructokinase as present in the incubation mixtures. The concentrations of phosphofructokinase were: 0.35 mg/ml (□–□), 0.14 mg/ml (●–●), 0.070 mg/ml (△–△), 0.047 mg/ml (▲–▲), and 0.035 mg/ml (○–○).

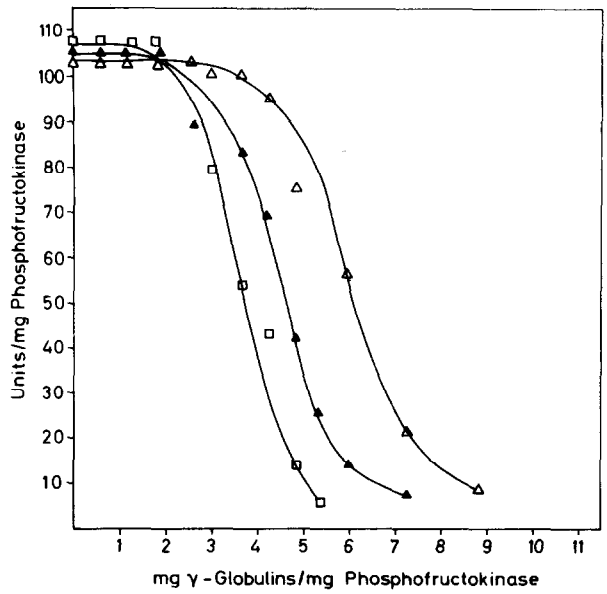

Fig. 2. Dependence of specific activity of phosphofructokinase on the concentration of specific antibodies at activating conditions. Incubation of phosphofructokinase was performed for 15 min at 22° in 0.1 M phosphate buffer, pH 7.5, containing 0.2 M KCl and 0.1 mM fructose 1,6-diphosphate. The experiments were analogous to fig. 1 and were performed with the following concentrations of phosphofructokinase: 0.070 mg/ml (□–□), 0.035 mg/ml (▲–▲), and 0.017 mg/ml (△–△).

illustrated in fig. 1, the enzyme was not activated at low concentrations of antibodies. The dependence of the formation of precipitable antigen–antibody complexes on phosphofructokinase concentration is also evident under these conditions.

The reaction of the antibodies with phosphofructokinase led to an alteration of the kinetic properties of the enzyme. The curves in fig. 3 show saturation curves for fructose 6-phosphate. The activities measured were referred to a reference activity as determined at pH 7.6 (assay system A, see Methods). Curve A shows the sigmoid saturation curve in the absence of antibodies. An analogous curve, obtained in the presence of antibodies and dispersed precipitable complexes of antigen–antibody reaction is represented by plot B. Obviously, the enzyme was highly activated by its antibodies, when assayed at pH 6.8 in the presence of inhibitory concentrations of ATP. At pH 7.6, the activity was the same as in the control.

Plot C is congruous with A and represents the saturation curve for fructose 6-phosphate as obtained in the supernatant after removal of precipitable antibody complexes by centrifugation. The activity at pH 7.6 (assay A) was about half of that in the experiment underlying curve A. The lower activity reflects the decreased phosphofructokinase concentration [1]. In comparison to the facts known on concentration dependence of phosphofructokinase saturation curves, the close correspondence of curves A and C seems remarkable, since in the absence of antibodies a decrease in phosphofructokinase concentration causes a shift of the fructose 6-phosphate saturation curve to higher substrate concentrations [1]. This effect is demonstrated by curve D, which was obtained in the absence

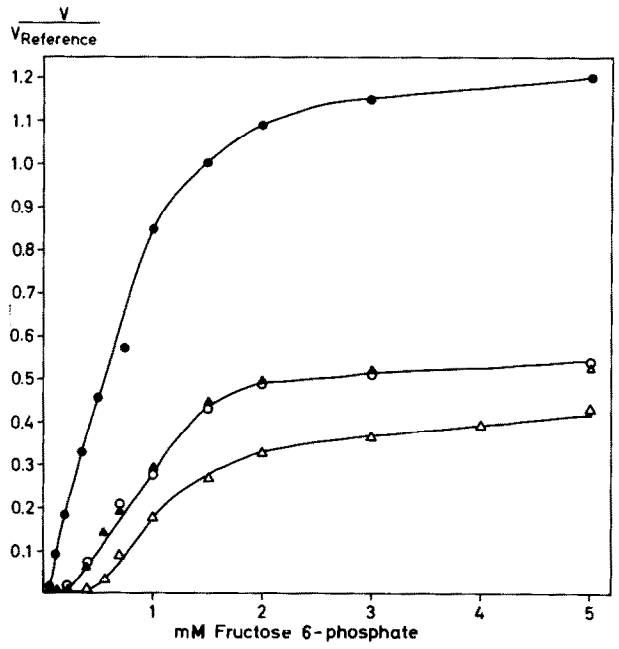

Fig. 3. Saturation of phosphofructokinase with fructose 6-phosphate in the presence of antibodies. (A–C): 0.5 mg phosphofructokinase were incubated for 15 min at 22° in 0.1 M imidazole-HCl, pH 7.3 and 0.2 M KCl with 0.86 mg antibody-containing (along with the same amount of antibody-free) γ-globulins. The activities at different fructose 6-phosphate concentrations (assay system B) were referred to reference activity as measured in assay system A. Curve A (○–○): incubation without antibodies (reference activity 65 units/mg). Curve B (●–●): incubation in the presence of antibodies (reference activity 65 units/mg). Curve C (▲–▲): same as B, but measurements were performed in the supernatant after 4 min centrifugation at 20,000 *g* (reference activity 37 units). Curve D (△–△): control experiment without antibodies in the presence of 37 units (reference activity) phosphofructokinase.

of antibodies. The enzyme concentration in the experiment was adjusted to give the same reference activity as in the experiments which are represented by curve C.

4. Discussion

The heterogeneity of rabbit muscle phosphofructokinase was first described by Ling et al. [4]. Paetkau et al. [5] suggested the different forms to be monomers, dimers and trimers. A model which is based on reversible transitions between differently aggregated forms of the enzyme was used by Hofer [2] to explain the dependence of phosphofructokinase kinetics on enzyme concentration. This phenomenon is also obvious in fig. 3, when curves A and D are compared.

Nonlinearity of precipitation curves, as visible in fig. 1 and 2, has been observed in various antigen–antibody reactions and was in most cases explained by the assumption of heterogeneous antigens and antibodies. The pronounced dependence of the precipitating effect of antibodies on phosphofructokinase concentration cannot be explained merely by heterogeneity, but demands the possibility of reversible transitions between heterogeneous forms of the enzyme. These transitions may include the dissociation of the enzymatically active monomer to inactive subunits, as well as the interconversion of monomers, dimers, trimers, etc. present in a concentration dependent association dissociation equilibrium.

It appears from curve C of fig. 3 that this equilibrium is fixed by the reaction of the antibodies with the enzyme. The same conclusion was drawn from results obtained in the preceding study [3]. Higher enzyme concentrations favour association and therefore the concentration dependence of the reaction with the antibodies is best explained assuming the higher aggregates to be more reactive with antibodies.

In the precipitable enzyme–antibody complexes, the antibodies induce a higher affinity of phosphofructokinase for its substrate fructose 6-phosphate. This effect resembles that of an allosteric activator. This does not necessarily imply an interaction of the antibody at the effector site but could be due to an induced conformational change of the enzyme. It remains unclear whether the suggested changes in conformation refer only to altered subunit interaction or increased association of the enzyme in the precipitates, which may also presuppose improved affinity for fructose 6-phosphate [2].

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