ACTIVATION OF RABBIT MUSCLE PHOSPHOFRUCTOKINASE BY SPECIFIC ANTIBODIES

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1. Introduction

Phosphofructokinase from rabbit skeletal muscle exists in differently aggregated molecular forms [1]. Its smallest enzymatically active unit has a molecular weight of about 370,000 and is able to dissociate into inactive subunits [2, 3]. The inactivation of phosphofructokinase is prevented by its substrate fructose 6-phosphate and other metabolites, which are known as positive effectors of the enzyme (e.g. inorganic phosphate, fructose 1,6-diphosphate, glucose 1,6-diphosphate, AMP, etc.). Reactivation of the inactivated enzyme has also been observed [3, 4]. The present study demonstrates that inactivation of phosphofructokinase may also be inhibited by specific antibodies. Furthermore, it will be shown that activation of phosphofructokinase by its antibodies may be observed under certain conditions.

2. Materials and methods

Crystalline phosphofructokinase of about 230 units/mg specific activity was prepared from rabbit skeletal muscle according to [5]. This enzyme lost about half of its specific activity during 12 mon storage.

Antibodies against phosphofructokinase were obtained by immunizing a 3 mon old wether. A total of 25 mg crystalline enzyme was thoroughly emulsified in complete Freund's adjuvant (Behringwerke, Marburg) and injected i.m. within 2 weeks. The antibody-containing serum was fractionated with Na_2SO_4 [6] and the precipitated γ-globulin fraction was redissolved in 0.9% NaCl. Before use, the antibodies were exhaustively dialyzed against incubation buffer.

All experiments were controlled using the correspondingly isolated γ-globulin fraction of a non-immunized wether of the same strain and age. Additional controls were performed also in a globulin-free system. No difference was, however, observed between these 2 types of control experiments.

The assay for phosphofructokinase contained in 1 ml volume: 50 μmole triethanolamine-HCl, 5 μmole EDTA, 8 μmole $MgSO_4$, 0.4 μmole NADH, 1 μmole ATP, 3 μmole fructose 6-phosphate, 100 μg aldolase, 30 μg triosephosphate isomerase, and 80 μg glycerolphosphate dehydrogenase; pH 7.6. Protein concentrations were determined according to [7].

3. Results

The influence of antibodies on the specific activity of rabbit muscle phosphofructokinase is shown in fig. 1 and 2. Curve A in fig. 1 represents specific activities of phosphofructokinase samples which were incubated with increasing amounts of antibodies. In this series of experiments, antibody–antigen complexes were not removed. The incubation system contained 0.1 M imidazole and 0.2 M KCl, pH 7.5. Rabbit muscle phosphofructokinase does not exhibit full specific activity under the conditions of this preincubation. It may be seen that the activity of the enzyme (19 units/mg at zero antibody concentration) was increased by low concentrations of antibodies and reached a maximum of 99 units/mg in the presence of 12.7 μg γ-globulins. At higher antibody concentrations, however, the activity of the enzyme was reduced and finally reached the level of the control.

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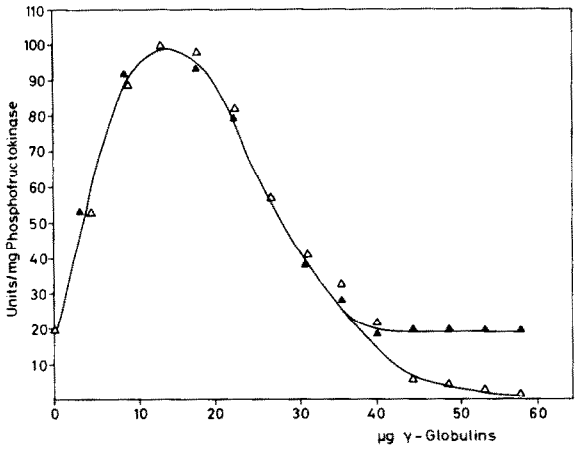

Fig. 1. Specific activities of phosphofructokinase in the presence of antibodies. 0.047 mg crystalline phosphofructokinase, dissolved in 1 ml 0.1 M imidazole-HCl and 0.2 M KCl, pH 7.5, were incubated with increasing amounts of antibody-containing γ-globulins, as indicated on the abscissa. Curve A (▲—▲) represents specific activities as measured after 15 min incubation at 22° without centrifugation. Curve B (△—△): activities in the supernatants after centrifugation (4 min, 20,000 *g*) as referred to the initial phosphofructokinase concentrations.

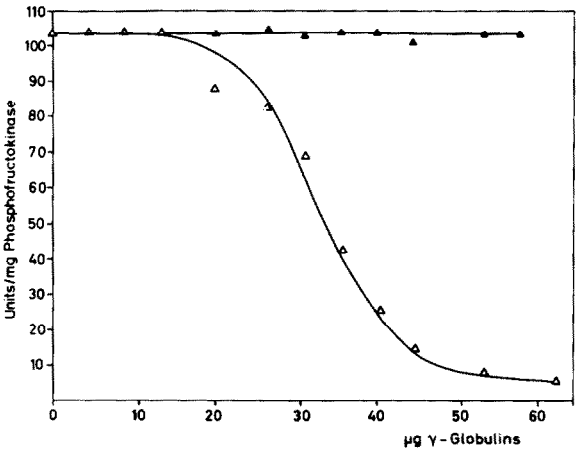

Fig. 2. Specific activities of 0.035 mg/ml phosphofructokinase, which was incubated with increasing amounts of antibodies in 0.1 M phosphate buffer, containing 0.2 M KCl and 0.1 mM fructose 1,6-diphosphate, pH 7.5. Curve A (▲—▲): without centrifugation, Curve B (△—△): activity in the supernatants after centrifugation (4 min, 20,000 *g*).

Curve A of fig. 2 shows a parallel experiment, which was performed in a different buffer system: 0.1 M potassium phosphate, pH 7.5, containing 0.2 M KCl and 0.1 mM fructose 1,6-diphosphate. As is illustrated by Curve A in fig. 2, the activity of the enzyme amounted to 103.5 units/mg in the antibody-free control assay and no change in enzyme activity was detected in the presence of increasing amounts of antibodies.

Curves B of fig. 1 and 2 refer to experiments in which precipitable enzyme–antibody complexes were removed by centrifugation. Activity determinations were performed in the remaining supernatants. (Protein concentrations in fig. 2 were not corrected for precipitated enzyme.) There is no linear relationship between the concentration of antibodies and the amount of enzyme precipitated.

Another series of experiments is summarized in fig. 3. In this graph specific activities of phosphofructokinase were plotted against enzyme concentration in the test. As was confirmed by previous studies [3], a distinct decrease in the specific activity of phosphofructokinase was observed when the enzyme was diluted in the absence of stabilizing substances, such as the substrate fructose 6-phosphate or positive effectors of the enzyme. This type of inactivation is also visible in curve B of fig. 3. Curve A refers to experiments in which the enzyme was preincubated with its antibodies before dilution. It is obvious that in the presence of antibodies, the inactivation of phosphofructokinase was largely prevented.

4. Discussion

Inhibitory effects of antibodies on enzymes were reported in numerous cases. So far, however, only few examples are known in which enzymes are activated by their antibodies [7, 8]. Under the conditions of this study, there is no inhibitory effect of antibodies on phosphofructokinase, thus suggesting that the active center was not a determinant for antigenic activity. On the other hand, the antibodies protected phosphofructokinase from inactivation by dilution [3]. The loss in activity at low enzyme concentrations is most probably caused by dissociation into inactive subunits, most probably with a molecular weight of 180,000. The antibodies seem to stabilize the active

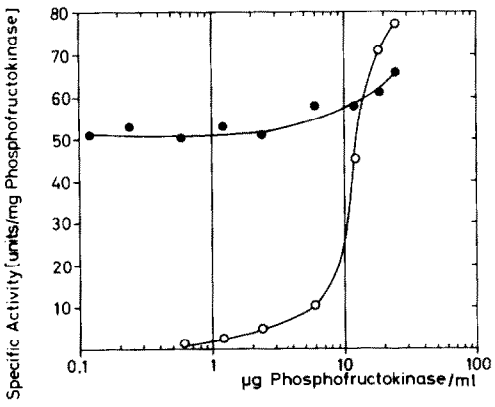

Fig. 3. Specific activities of phosphofructokinase after dilution in 0.1 M imidazole buffer, pH 7.3. Different amounts of phosphofructokinase were incubated for 15 min in the presence (Curve A, •–•) of 4.3 mg/ml antibody-containing γ-globulins or the same concentration of antibody-free γ-globulins (Curve B, ○–○). The abscissa refers to the final concentrations in the assays, for which the enzyme was diluted 25-fold after incubation.

conformation of the enzyme and to prevent dissociation into subunits.

A similar effect is known from low molecular weight activators, such as fructose 1,6-diphosphate or other positive effectors [3]. It is not clear, of course, whether the protective effect is exerted by binding the antibodies to the effector site or by stronger binding of the subunits in the presence of antibodies. The latter possibility would explain why at high concentrations of antibodies the same state is conserved as present before addition of antibodies. Low molecular weight activators would rather lead to an increase in specific activity.

When phosphofructokinase was preincubated in an activator-free buffer system, low concentrations of antibodies led to an increase to almost the same specific activity as measured in a system which contained optimal concentrations of the low molecular weight activators inorganic phosphate and fructose 1,6-diphosphate. At higher antibody concentrations, this effect was reversed. Parallel experiments showed that the addition of antibodies had no effect on phosphofructokinase activity, when the enzyme was already activated by fructose 1,6-diphosphate. The activation by antibodies could be due to hindered dissociation and/or a conformational change of the enzyme, caused by a special combination of enzyme and antibodies, which was not present at higher concentrations of antibodies. From this point of view, the effects demonstrated in fig. 1 and 3 would be generated by different mechanisms and would be possibly due to heterogeneous types of antibodies or to different states of the antigen–antibody complexes.

References

[1] V.H. Paetkau, H.A. Lardy, J. Biol. Chem. 242 (1967) 2035.
[2] V.H. Paetkau, E.S. Younathan and H.A. Lardy, J. Molecular Biol. 33 (1968) 721.
[3] H.W. Hofer and D. Pette, Hoppe-Seyler's Z. Physiol. Chem. 349 (1968) 1105.
[4] D. Pette and H.W. Hofer, in: Control of Energy Metabolism, eds. B. Chance, R.W. Estabrook, J.R. Williamson (Academic Press, New York, 1965) p. 71.
[5] H.W. Hofer and D. Pette, Hoppe-Seyler's Z. Physiol. Chem. 349 (1968) 995.
[6] R.A. Kekwick, Biochem. J. 34 (1940) 1248.
[7] G. Beisenherz, H.J. Boltze, Th. Bücher, R. Czok, K.H. Garbade, E. Meyer-Arendt and G. Pfleiderer, Z. Naturforsch. 8 b (1953) 555.
[8] F.G. Lehmann, Biochim. Biophys. Acta 235 (1971) 259.
[9] M.R. Pollock, Ann. N.Y. Acad. Sci. 103 (1963) 989.