IMMUNOLOGICAL EVIDENCE FOR NON-IDENTICAL SUBUNITS IN YEAST PHOSPHOFRUCTOKINASE

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1. Introduction

Proteolytically non-degraded yeast phosphofructokinase (EC 2.7.1.11) has a sedimentation constant of about 21 S and is composed of subunits having 130 000 daltons [1]. By *in vitro* incubation with a small amount of yeast extract the enzyme is proteolytically converted into another form sedimenting with 17.8 S. This form has a mol wt of 570 000 and contains subunits with 96 000 daltons [1–3]. The latter form of phosphofructokinase is also obtained by the application of purification procedures without special precautions for inhibition of contaminating intracellular proteases [3, 4]. In the course of studying the subunit structure of the non-degraded yeast phosphofructokinase regularly two distinct protein bands of about 130 000 daltons have been observed moving closely together in polyacrylamide gels containing sodium dodecyl sulphate (SDS). This finding could be caused by the following reasons: either the faster moving band might be a proteolytically modified product, which arises from the other component and which could represent an intermediate in the proteolytic conversion of the 130 000 subunit to the 96 000 one, or the enzyme could be composed of two non-identical subunits being not very different in their respective molecular weights.

In order to discriminate between these two possibilities, as a first approach the following immunological study of yeast phosphofructokinase has been undertaken.

Evidence will be presented in this contribution that yeast phosphofructokinase is composed of two kinds of subunits being non-identical in their antigenic properties and being only slightly different in their molecular weights which are in the range of about 130 000. These two subunits may be proteolytically degraded *in vitro* into a pair of fragments having about 96 000 daltons.

2. Materials and methods

Phosphofructokinase was prepared from bakers' yeast by following a purification procedure with minimum proteolytic degradation [1]. In the analytical ultracentrifuge (Phywe U 60 L) the enzyme exhibits a homogeneous sedimentation pattern. The reagents used for the electrophoretic experiments have been purchased from Serva GmbH, Heidelberg.

Antiserum against the highly purified enzyme was obtained from rabbits as described previously [1]. The analytical polyacrylamide gel electrophoresis in presence of 0.1% SDS has been carried out according to [5]. The following proteins were applied as standards for the molecular weight determination of the phosphofructokinase subunits: trypsin (23 000); aldolase (40 000); catalase (60 000); muscle phosphofructokinase (93 000) and β-galactosidase (135 000). The protein bands were stained with Coomassie brilliant blue G 250 [6].

Immunoelectrophoresis in agarose was carried out in barbital–HCl buffer (pH 8.4) at 150 V for 1 hr as described by Grabar and Williams [7]. The two dimensional immunoelectrophoretic analysis of the phosphofructokinase subunits was performed by following the principles of the Laurell technique [8] with the modification that in the first direction a polyacrylamide gel

electrophoresis in 0.1% SDS was accomplished [5]. The electrophoretic analysis in the second direction was carried out in an antibody containing agarose gel (8 hr; 100 V; 20° C). This precipitating gel contained 0.4 ml of the immune serum and 1.5% of agarose (4 ml) in 0.075 M barbital–HCl buffer (pH 8.4).

3. Results and discussion

The yeast phosphofructokinase preparations used in the following experiments contained only subunits having a molecular weight of about 130 000. By applying electrophoretic separation times of about 5 hr one single protein band is observed in the polyacrylamide gel in presence of SDS (fig. 1A). However, after prolongation of the electrophoretic separatition time from 5–15 hr a splitting of this single band into a pair of bands being distinctly separated from each other could regularly be observed (fig. 1B). These two kinds of subunits are designated as α (the upper one) and β (moving slightly faster than the α-subunit).

As it has been shown previously [1], the subunit of about 130 000 may proteolytically be converted *in vitro* into product having 96 000 daltons by incubation with a very small amount of crude yeast extract. Fig. 1C demonstrates the result of such an experiment. It may be seen, that the pair of subunits having 130 000 daltons is readily converted into a pair of fragments with molecular weights of about 96 000 (α' and β'). Fig. 1D represents an intermediate stage of this conversion process. where both pairs of subunits may be distinguished.

In order to investigate the question outlined in the Introduction, whether the β-band originates from α by partial proteolysis or whether both kinds of subunits are unrelated, the reactivity of these subunits with antibodies of yeast phosphofructokinase has been studied. For this purpose antisera have been prepared by immunization of rabbits with the native enzyme.

Fig. 2 represents the results of immunoelectrophoresis carried out by applying the native enzyme (fig. 2A), as well as the dissociated one, obtained after treatment with SDS (fig. 2B). It was observed, that not only the native enzyme but also the denatured one is precipitated by the antiserum.

For analyzing possible differences in the immunological reactivities between the α- and β-subunits, a two-

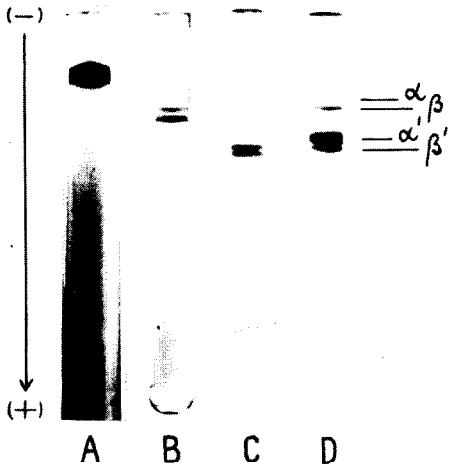

Fig. 1. Polyacrylamide gel electrophoresis of yeast phosphofructokinase in presence of sodium dodecyl sulphate: (A) Proteolytically non-degraded enzyme after electrophoresis of about 5 hr; (B) The enzyme as in (A), however after a prolongation of the migration time from 5–15 hr; (C) Enzyme, which was proteolytically readily converted to the form containing only subunits with 96 000 daltons as follows: 50 μl of phosphofructokinase (1 mg/ml) were incubated with 5 μl of yeast cell extract (diluted 1:40 referred to the intact cells) for 24 hr at 4° C, pH 7.2. The protease inhibitor present in the yeast cell extract was inhibited according to [9]; (D) An intermediate stage (12 hr incubation with the yeast extract) of the proteolytic transformation process. Electrophoretic conditions: 5 mA/tube; 25° C; 5 hr (A); 15 hr (B, C, D).

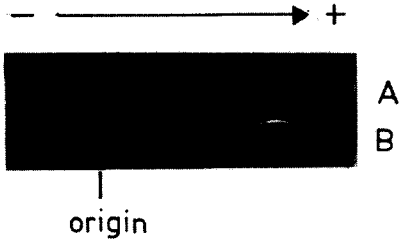

Fig. 2. Immunoelectrophoresis of native and denatured yeast phosphofructokinase. Antiserum against the native enzyme was filled into the trough. In (A) native phosphofructokinase in (B) phosphofructokinase denatured with 1% sodium dodecyl sulphate were applied. For electrophoretic conditions see Materials and methods.

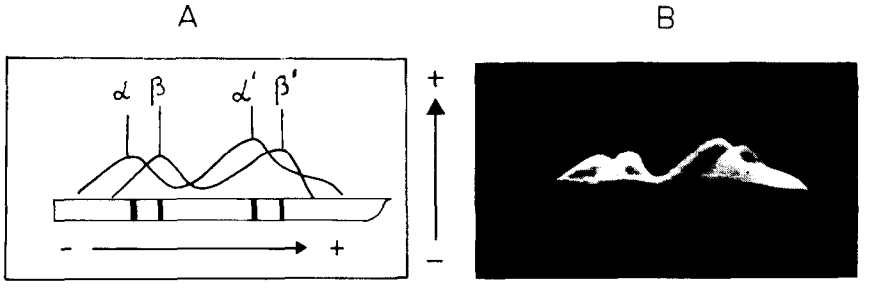

Fig. 3. Two-dimensional immunoelectrophoresis of the yeast phosphofructokinase subunits. Electrophoresis in the first direction: polyacrylamide gel electrophoresis in 0.1% sodium dodecyl sulphate. Second direction: electrophoresis of the separated subunits in an agarose gel containing antiserum. For electrophoretic conditions see Materials and methods.

dimensional electrophoresis of the SDS-treated enzyme was carried out, which combined the power of polyacrylamide electrophoresis to separate the α- and β-subunits from each other (first direction) with the immunoelectrophoresis in an antibody-containing agarose gel (second direction), where the two subunits were precipitated.

As it may be seen from fig. 3, the precipitation lines produced by the reaction of the α- and β-subunits with the antiserum cross each other. This pecularity indicates that both types of subunits are different in their antigenic properties.

In the other case, when β would originate proteolytically from α, both subunits beat at least partially the same determinant groups. This would give rise to precipitation lines merging into another without crossing.

The conclusion may therefore be drawn from this type of experiment, that the α- and β-subunits of yeast phosphofructokinase are apparently non-identical.

In addition, it may be deduced from fig. 3, that the proteolytically produced 96 000 fragments α' and β' are also antigenically different. On the other hand, the precipitation lines between α and α' as well as between β and β' are merging near the base line into another indicating an antigenic relationship between the respective members of both pairs. This of course should be expected owing to the fact that the pair of bands having 96 000 daltons originate from α and β by proteolytic degradation.

In summary, by application of this immunological procedure, especially by taking advantage of the fact that the SDS-denatured subunits are precipitated by antiserum against the native phosphofructokinase, evidence was obtained that this enzyme is composed of two non-identical subunits (α and β) with almost equal molecular weights, being in the range of 130 000.

Work is now in progress to substantiate further this result by performing a peptide mapping and an end-group-analysis of the polypeptide chains composing yeast phosphofructokinase.

Acknowledgement

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