STUDIES ON THE OLIGOMERIC STRUCTURE OF YEAST PHOSPHOFRUCTOKINASE BY MEANS OF CROSS-LINKING DIIMIDOESTERS

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SUMMARY

Intracellular cross-linking of yeast phosphofructokinase with a series of diimidoesters of different chain length resulted in the appearance of tetramers as largest cross-linked product of the enzyme subunits. The native enzyme is evidently composed of eight subunits being arranged in two tetramers $\alpha_4\beta_4$. In the tetramers the monomers are probably assembled in tetrahedral geometry.

INTRODUCTION

Phosphofructokinase (EC 2.7.1.11) from baker's yeast (Saccharomyces cerevisiae) has a molecular weight of 750 000 to 800 000 (1). It is composed of two types of subunits (α and β)differing slightly in their molecular weights and occurring in one to one ratio (2, 3). On the basis of a subunit molecular weight of approximately 120 000 (1, 3) as determined by polyacrylamide gel electrophoresis in sodium dodecylsulfate (SDS) a hexameric structure of the enzyme was proposed (1). A hexameric composition of this enzyme was also suggested by Wilgus et al. (4).

Abbreviation: SDS, sodium dodecyl sulfate.

From a thorough analysis of SDS-binding to yeast phosphofructokinase and an investigation of the hydrodynamic behaviour of the SDS-enzyme complex studied by means of sedimentation equilibrium measurements an apparent subunit molecular weight of about 100 000 could be evaluated (G. Kopperschläger, J. Bär and K. Nissler, manuscript in preparation). These results point to an octameric, rather than a hexameric structure of the enzyme. For phosphofructokinase from brewer's yeast an octameric structure was recently proposed (5).

In order to distinguish between the two possible structures the application of intramolecular cross-linking appeared to be a useful way of approach. By variation of the chain length of the bifunctional reagents and of the time of reaction information could be expected about the number of the composing subunits and their spatial arrangement (6).
The results of the respective study will be communicated in this paper.

MATERIALS AND METHODS

Proteolytically non-modified phosphofructokinase was isolated in homogeneous form from baker's yeast (Stellhefe I, VEB Backhefe Leipzig) as described previously (1, 3). The diimidoesters were synthesized according to Davies andStark (7) from the corresponding dinitriles. The latter were products of Fluka (Buchs, Switzerland) and Merck (Darmstadt, W.-Germany). Cross-linking was carried out at 4 °C in 0.25 M triethanolamine/HCl buffer, pH 8.0,containing 4 mM fructose 6-phosphate or 4 mM ATP, respectively. The diimidates were dissolved in this medium immediately before addition of the protein. The enzyme was dialysed against 0.1 M phosphate buffer, pH 7.0 for 15 h and then added to the medium giving a final concentration of 0.5 mg of protein/ml. The proportion of the amounts of the respective diimidates to the enzyme protein was varied from 1 mg/mg to 6 mg/mg of enzyme.
Denaturation of the cross-linked products was performed in 1 % (w/v) SDS and 1 % (v/v) 2-mercaptoethanol at 100 °C for 5 min after concentrating the samples to approximately 3 mg of protein/ml by means of Amicon Centriflo Membrane Cones 26-CF-50.

SDS-polyacrylamide gel electrophoresis was performed at 8 mA/tube for 3 h according to Davies and Stark (7), with an acrylamide concentration of 3.5 %. The gels were stained and destained as described by Taucher et al. (3). Sedimentation velocity experiments were carried out at 50 000 rev/min at 20 °C in a Phywe analytical ultracentrifuge U 60L using the UV optics at 280 nm.
The obtained resolution patterns of the cross-linked products have been compared with the model distribution as derived from the theoretical approach of Hucho et al. (6) using a Hewlett Packard Calculator model 9821 A.

RESULTS

Cross-linking of native yeast phosphofructokinase with dimethyl suberimidate (1 mg/mg of enzyme) and subsequent separation of the products by means of SDS-polyacrylamide gel electrophoresis results in the appearance of four protein bands representing the monomer, dimer, trimer, and tetramer of the subunits (Figures 1 and 2). In dependence on the time allowing the cross-linking reaction to proceed the fraction of the monomer decreases and that of the tetramer increases continuously. When higher concentrations of dimethyl suberimidate (6 mg/mg of enzyme) are applied no larger integers than the tetramer can be detected apart from a diffuse region in the neighbourhood of the starting point of electrophoresis.

The distribution of the various cross-linked species depends on the length of the carbon chain of the bifunctional reagent (Fig. 3). With increasing chain length the amount of the tetramer increases whereas the portions of the monomer and of the dimer decrease inversely.

To exclude alterations in the oligomeric structure of phosphofructokinase like dissociation or association in the course of the cross-linking reaction, which might be of in-

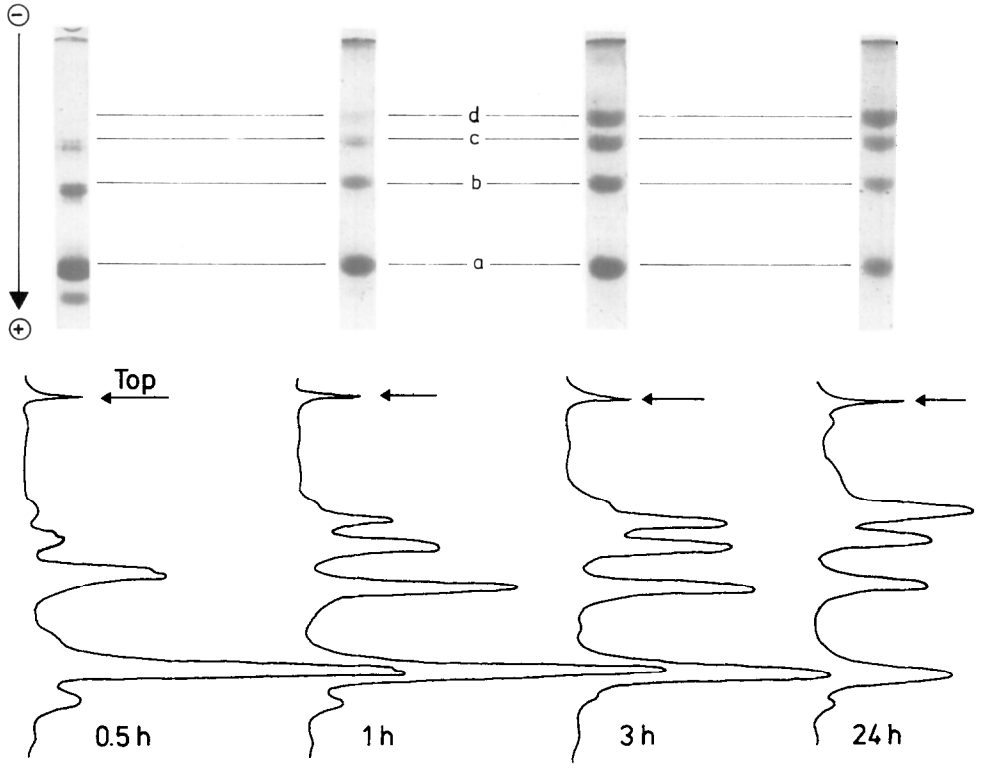

Fig. 1: Time course of intramolecular cross-linking of yeast phosphofructokinase by application of dimethyl suberimidate as ascertained by SDS-polyacrylamide gel electrophoresis.

Cross-linking was performed with 1 mg of dimethyl suberimidate/mg of enzyme at 4 °C as described in the experimental section. After electrophoretic separation the gels were scanned at 570 nm. The bands represent the monomer (a), dimer (b), trimer (c), and tetramer (d) of phosphofructokinase subunits, respectively. The applied enzyme developed only one band in SDS-electrophoresis with the same mobility as band (a) (not shown in this Figure). An incidentally observed faint band moving faster than band (a) could be identified as transitorily appearing proteolytic cleavage product of the enzyme being formed by traces of endogenous proteases during cross-linking (1, 4). Analysis was carried out after 0.5, 1, 3, and 24 hours after starting the reaction.

fluence on the number and the proportion of the cross-linked products, sedimentation velocity measurements of the enzyme before and after the reaction with dimethyl suberimidate

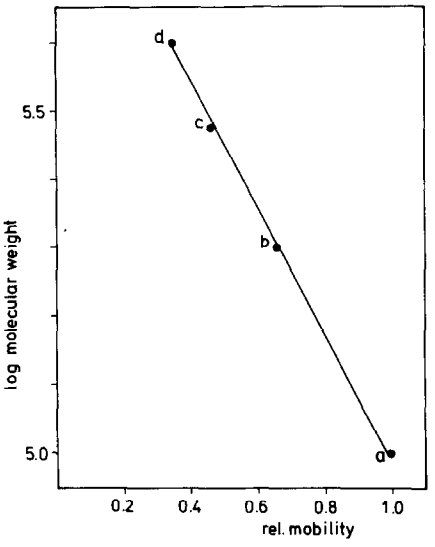

Fig. 2: Half-logarithmic plot of the molecular weights of the cross-linked phosphofructokinase subunits versus their relative mobilities.

Cross-linking was performed with dimethyl dodecylimidate (0.5 mg/mg of enzyme) at 4 °C for 24 h. The relative mobilities were calculated from the densitometric recordings; (a) represents the phosphofructokinase subunit monomer, (b) the dimer, (c) the trimer, (d) the tetramer.

have been carried out. The cross-linked phosphofructokinase was found to have an apparent sedimentation coefficient of 19.6 S (protein concentration 0.3 mg/ml) correlating well with the sedimentation constant of the unmodified enzyme with 20.6 S (1). Hence, the oligomeric composition of the enzyme does not change under the conditions of cross-linking.

In order to get an idea about the possible arrangement of the subunits within the native phosphofructokinase molecule twelve electrophoretic resolution patterns obtained by variation of the reaction time and of the carbon chain length of the bifunctional reagent have been compared with

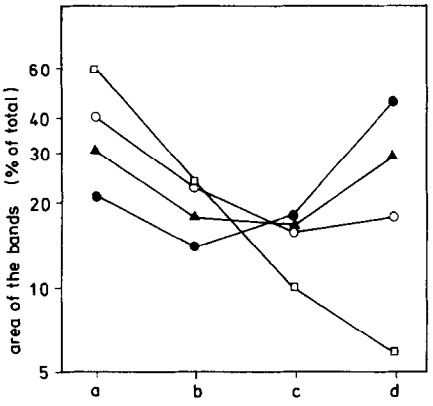

Fig. 3: Dependence of the relative amounts of the various cross-linked products of yeast phosphofructokinase on the chain length of the bifunctional reagent.

Cross-linking was carried out with 1 mg of bifunctional reagent/mg of enzyme for 24 h at 4 °C.
(□) dimethyl adipinimidate; (o) dimethyl pimelinimidate; (▲) dimethyl suberimidate; (●) dimethyl dodecylimidate. The proportions of the individual species were obtained by densitometric integration.

model calculations for tetrahedral and planar geometries, as described by Hucho et al. (6, see Appendix by Pohl).

By using the following equation:

$$\sum_{i=1}^{4} (c_i^{exp} - c_i^{theo})^2 \rightarrow \text{minimum}$$

c_i^{exp} ≙ fractions of monomers, dimers, trimers, and tetramers as found experimentally;

c_i^{theo} ≙ computed fractions of the oligomers on the basis of the respective model, the tetrahedral model was found to give the best fit to the experimental results.

DISCUSSION

Intramolecular cross-linking of native yeast phosphofructokinase gives rise to the appearance of dimers, trimers, and

tetramers of the composing subunits. By variation of the reaction time and of the length of the carbon chain of the bifunctional reagent the tetrameric species has been found to be the predominating cross-linked product. Evidently, this cannot be brought into accordance with the original concept of a hexameric structure of yeast phosphofructokinase (1, 4). The predominance of tetramers in connection with the molecular weights of the undissociated enzyme (750 000 - 800 000) and of the monomers (100 000) as determined after dissociation with SDS by ultracentrifugation points to an octameric composition of phosphofructokinase from yeast.

In an octameric molecule containing equal amounts of two types of different subunits theoretically the following simple cases of interactions may be conceived:

1) all of the contact domains α-α, β-β, and α-β are equally reactive towards the cross-linking reagents; in this case all species from monomers up to octamers should be expected;

2) cross-linking proceeds only between the α- and β-subunits; in this case dimers should become dominant in the reaction products;

3) cross-linking occurs only in the contact domains between either α and α or β and β respectively, but not between α and β; in this case the tetrameric form should be the largest observable polymerization product of the enzyme. Because α and β differ slightly in their electrophoretic mobility giving rise to distinct bands moving closely together, the cross-linked species should ideally produce double bands when case three is followed.

From the three models only the third one correlates to the experimental resolution patterns. Tetramers were really found as largest cross-linked products and double bands were occasionally distinguishable (Fig. 1, left gel). Hence, the native enzyme is most probably composed of two tetramers $\alpha_4\beta_4$. A comparison of the experimental results with the model computations made a tetrahedral symmetry of each of the tetramers likely. The octameric assembly is rather stable and does not show any association or dissociation under a broad range of conditions. In this respect, the yeast enzyme differs significantly from the tetrameric mammalian phosphofructokinase (8).

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