THE MOLECULAR WEIGHT OF THE POLYPEPTIDE CHAINS OF YEAST PHOSPHOFRUCTOKINASE

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SUMMARY

When proteolytic artifacts were prevented, yeast phosphofructokinase was found to contain six polypeptide chains each having a molecular weight of 10^5.

INTRODUCTION

Preliminary measurements have been reported which indicate that yeast phosphofructokinase contains multiple polypeptide chains each having a molecular weight of 4.4×10^4 (1) or of 6.0×10^4 (2). Since many enzymes purified from yeast have been found (3-8) to contain active proteases which fragment the purified enzymes, the molecular weight of the subunits of yeast phosphofructokinase was reinvestigated using conditions which minimize proteolysis.

MATERIALS AND METHODS

Phosphofructokinase was purified from bakers yeast by the procedure of Atzpodien and Bode (9) with two modifications: the cells were ruptured in the presence of a 1mM solution of the proteolytic inhibitor phenylmethanesulfonyl fluoride (Sigma) and exclusion chromatography was done with Biogel 1.5m (Biorad) instead of Sepharose 4B. The purification procedure described by Lindell and Stellwagen (1) was discontinued since it involves incubation

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of the enzyme preparation in 8% ethanol at 42°, a condition that is likely to enhance proteolysis.

SDS-polyacrylamide gel electrophoresis (10) was performed, and polypeptide chain molecular weights were determined, as described by Weber and Osborn (11) except in regard to the preparation of samples for electrophoresis (see below). The molecular weight standards used were myosin (220,000) (11), phosphorylase a (100,000) (12), bovine serum albumin (68,000) (11) and catalase (60,000) (11).

RESULTS AND DISCUSSION

Phosphofructokinase purified by the modified Atzpodien and Bode procedure had a specific activity of 200 $\pm$ 10 units/mg when assayed as described previously (1). As shown in Figure 1, gel 1, the purified enzyme migrated as a single component when subjected to gel electrophoresis in triethanolamine buffer at pH 6.8 and 4°. A molecular weight of 5.9×10^5 was calculated for the enzyme from equilibrium sedimentation measurements at 5°, using 0.2 mM ATP, 2 mM 2-mercaptoethanol, and 50 mM phosphate buffer, pH 6.8, as the solvent. At 25°, both gel electrophoresis and equilibrium sedimentation experiments indicated the presence of aggregated species.

When phosphofructokinase from such a preparation was dissociated and subjected to electrophoresis in gels containing either 0.1% SDS or 6.25 M urea at pH 3.2, two kinds of polypeptide chains were observed, as shown in Figure 1, gels 2 and 4. Upon storage of the purified active enzyme at 4° either in solution or as an ammonium sulfate precipitate, the relative amounts of the two electrophoretic species changed, as seen in Figure 1 by comparing gel 2 with gel 3, and gel 4 with gel 5. The predominant electrophoretic species present after enzyme storage is designated "polypeptide B" and the other species "polypeptide A". The mobilities of polypeptides A and B in gels containing detergent correspond to molecular weights of about 10×10^4 and 8.8×10^4, respectively. These results suggest that one or more proteolytic enzymes present as contaminants in the purified phosphofructo-

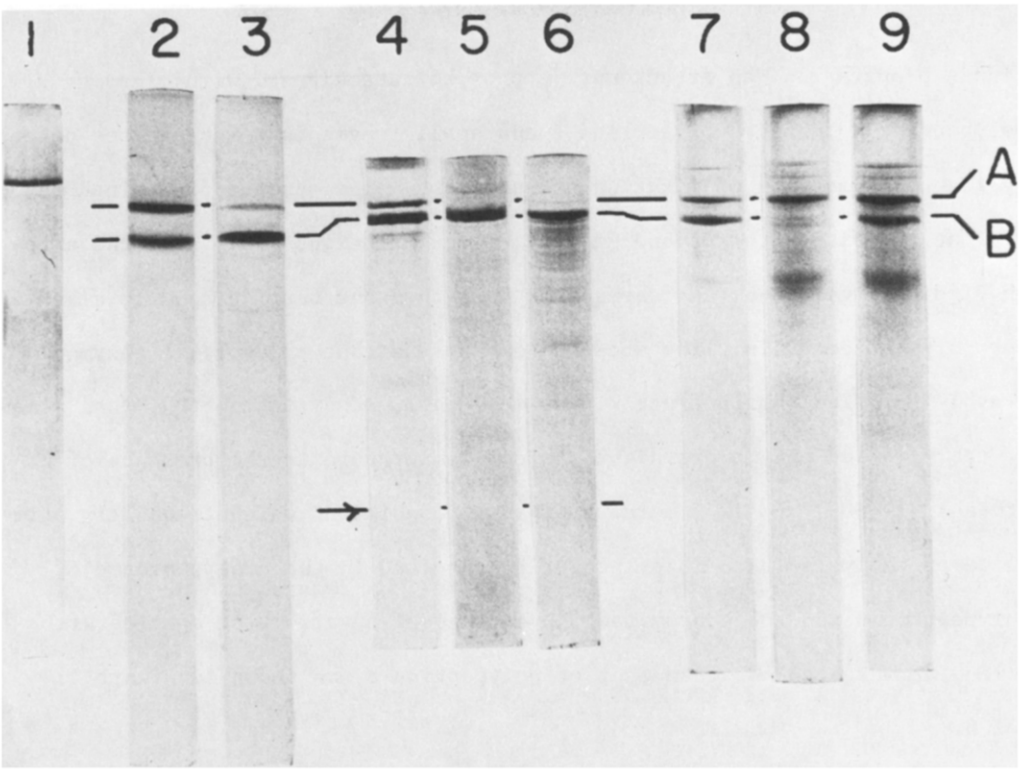

Figure 1. Polyacrylamide gel electrophoresis of yeast phosphofructokinase. Samples were applied to the tops of the gels as pictured. The anode was at the bottom for gels 1 and 4-9, and at the top for gels 2 and 3. Gel 1, freshly prepared (modified Atzpodien and Bode procedure) native enzyme was subjected to electrophoresis on 5% acrylamide in triethanolamine buffer, pH 6.8 (16), for 2.5 hours at 200 V. Gel 2, freshly prepared enzyme dissociated in 6.25 M urea and 0.9 M acetic acid was subjected to electrophoresis on 15% acrylamide in the same solution (17) for 12 hours at 120 V. Gel 3, identical to gel 2 except that the native enzyme had been stored for 12 days at 4° in a solution containing 0.2 mM ATP, 2 mM 2-mercaptoethanol, and 50 mM phosphate, pH 7.0, prior to dissociation with the acidified urea. Gel 4, freshly prepared enzyme (a different preparation than that used in gel 2) treated with 1% SDS and 1% 2-mercaptoethanol for 5 minutes at 100° (7) and then subjected to electrophoresis on 10% gels as described by Weber and Osborn (11). Gel 5, identical to gel 4 except that the native enzyme had been stored at 4° for 3 weeks either as an ammonium sulfate precipitate or in a solution containing 0.2 mM ATP, 2 mM 2-mercaptoethanol, and 50 mM phosphate buffer, pH 6.8, prior to dissociation in detergent. Gel 6, identical to gel 4 except that the enzyme was incubated overnight at room temperature in 5% ethanol, 1% SDS, and 1% 2-mercaptoethanol prior to electrophoresis (the 100° treatment was omitted). Gel 7, freshly prepared enzyme (as for gel 1) was boiled for 3 minutes, mixed with 5 volumes of hot 7M guanidinium chloride and boiled again for 3 minutes, reduced with 0.2M 2-mercaptoethanol at 100°, incubated for several hours at 37°, alkylated by reaction with excess iodoacetate at pH 10.5 for 10 minutes, and dialyzed against water prior to electrophoresis on 10% SDS-gels containing half the normal amount of cross-linking reagent (11). Gel 8, identical to gel 7 except that the enzyme preparation used was partly purified by the accelerated method described in the text. Gel 9, identical to gels 7 and 8 except that the sample applied was an equivolume mixture of the samples applied to each of those gels.

kinase preparation can attack native phosphofructokinase, with cleavage of polypeptide A to form polypeptide B and small fragments which are not detected by the usual methods of fixation and staining. The absence of stained material at a position corresponding to a molecular weight of 13,000 (the arrow in Figure 1, gels 4-6) indicates that more than one peptide bond is cleaved per polypeptide chain. The presence of at least one proteolytic enzyme in freshly purified phosphofructokinase was confirmed by incubation of an aliquot of the purified enzyme overnight at room temperature in the presence of 5% ethanol, 1% SDS, and 1% 2-mercaptoethanol, conditions which frequently stimulate proteolysis (8). This incubation resulted in the disappearance of polypeptide A and the concomitant appearance of a variety of species with molecular weights less than that of polypeptide B, as shown in Figure 1, gel 6.

The decrease in the ratio of polypeptide A: polypeptide B observed during storage of the purified enzyme suggested that a similar change may occur during the several days required for purification. In order to establish the A/B polypeptide ratio in the native enzyme, an accelerated procedure for the partial purification of the enzyme was developed. A suspension of yeast cells was ruptured by sonication in the presence of phenylmethanesulfonylfluoride, fractionated with protamine sulfate and ammonium sulfate as described by Atzpodien and Bode (9), and then subjected to exclusion chromatography using Biogel 1.5m. The eluate fraction possessing the highest specific activity (about 30 units/mg) was obtained within 3 hours after rupture of the cells. This fraction was boiled in the absence and then in the presence of guanidine hydrochloride, reduced, and then alkylated to prevent proteolysis prior to or during SDS-gel electrophoresis (7). As shown in Figure 1, gel 8, an electrophoretic species appeared at the position corresponding to polypeptide A. This species accounted for 17% of the stained material on the gel. The specific activity of the sample applied to the gel indicated that 15% of the protein in the

sample was phosphofructokinase. No significant amount of stained material appeared at the position corresponding to polypeptide B. As shown in Figure 1, gel 9, the species identified as polypeptide A in the partially purified sample co-electrophoreses with authentic polypeptide A.

These results indicate that native yeast phosphofructokinase, at neutral pH at 4°, contains six polypeptide chains each having a molecular weight of 1.0×10^5. Polypeptide chains derived from yeast phosphofructokinase which have molecular weights of 8.8×10^4, 6.0×10^4, and 4.4×10^4 are presumed to be proteolytic fragments of the 10×10^4 Dalton polypeptides. The observed variations in the relative amounts of these four species are probably due to differences in the procedures employed for purification and dissociation of the enzyme, to the length of storage of the purified enzyme, and to differences in specificity among the particular proteolytic enzymes (13-15) contaminating various preparations of phosphofructokinase.

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