CONCERNING THE SUBUNIT STRUCTURE OF POLYNUCLEOTIDE PHOSPHORYLASE FROM *E. COLI*

H. LEHRACH, K. SCHÄFER and K.H. SCHEIT
Max-Planck-Institut für experimentelle Medizin, Abteilung Chemie, Göttingen, Germany

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1. Introduction

At the present time it is generally believed that polynucleotide phosphorylase from *E. coli* with a MW of about 200,000 is composed of 6 identical subunits, each having a molecular weight of 30,000, which are arranged in two layers thus forming a dihedral enzyme molecule. Experimental evidence for this structure is based on density gradient centrifugation in 8 M urea [1], polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) [2] and electron microscopy [3] of highly purified enzyme. It was quoted by Thang [1] that sodium dodecyl sulfate treatment of polynucleotide phosphorylase and subsequent polyacrylamide gel electrophoresis leads to the formation of three species having molecular weights of 30,000, 55,000 and 100,000 respectively. The heterogeneity of the dissociation products was explained as the result of incomplete dissociation. Since no experimental details have been reported so far, we reinvestigated the dissociation of polynucleotide phosphorylase by sodium dodecyl sulfate.

2. Materials and methods

E. coli polynucleotide phosphorylase was isolated as described by Williams et al. [4]. The preparation of core and holo *E. coli* RNA polymerase followed the procedure of Burgess [5].

The enzymatic activity of polynucleotide phosphorylase in disc polyacrylamide gels was assayed as described by Thang [6].

3. Results and discussion

The enzyme preparation of polynucleotide phosphorylase used in the following experiments showed two enzymatically active protein bands when subjected to disc polyacrylamide gel electrophoresis (fig. 1). A similar phenomenon was previously reported by Thang and interpreted as a proteolytic degradation of native polynucleotide phosphorylase [1]. Treatment of this enzyme preparation with SDS and 2-mercaptoethanol leads to a single protein band in SDS-polyacrylamide gel electrophoresis (fig. 2b). Preincubation in 8 M urea for 30 min at 25°, conditions which according to Klee [7] and Thang [1] are sufficient to dissociate polynucleotide phosphorylase from either *Micrococcus luteus* or *E. coli*, followed by SDS treatment and SDS-polyacrylamide gel electrophoresis again revealed the existence of only one protein species (fig. 2a). The experimental conditions of SDS treatment employed here have been found optimal for dissociation of a variety of enzymes into their subunits without leading to extensive breakdown of polypeptide chains. The dissociation of RNA polymerase of *E. coli* into β, β', σ and α subunits under identical conditions is given as an example (fig. 2e). From fig. 2c and d it can be seen that polynucleotide phosphorylase from *E. coli* after treatment with SDS shows the same mobility as the σ subunit of RNA polymerase from *E. coli* in SDS-polyacrylamide gel electrophoresis. The molecular weight of the dissociated polynucleotide phosphorylase was determined as 95,000 Daltons from a semilogarithmic plot of electrophoretic mobilities versus molecular weight (fig. 3).

Although the polynucleotide phosphorylase preparation contained two active protein species as

Fig. 1. Disc polyacrylamide gel electrophoresis of polynucleotide phosphorylase. Analytical disc polyacrylamide gel electrophoresis was performed using the following system: separation gel containing 7.5% acrylamide and 0.1% methylene bisacrylamide in tris-HCl 8.9 and concentration gel containing 4% acrylamide and 0.1% methylene bisacrylamide in tris-H_3PO_4 pH 7.2; upper buffer: tris-glycine pH 8.9; lower buffer: tris-HCl pH 8.1. Protein was stained with Amido Black.

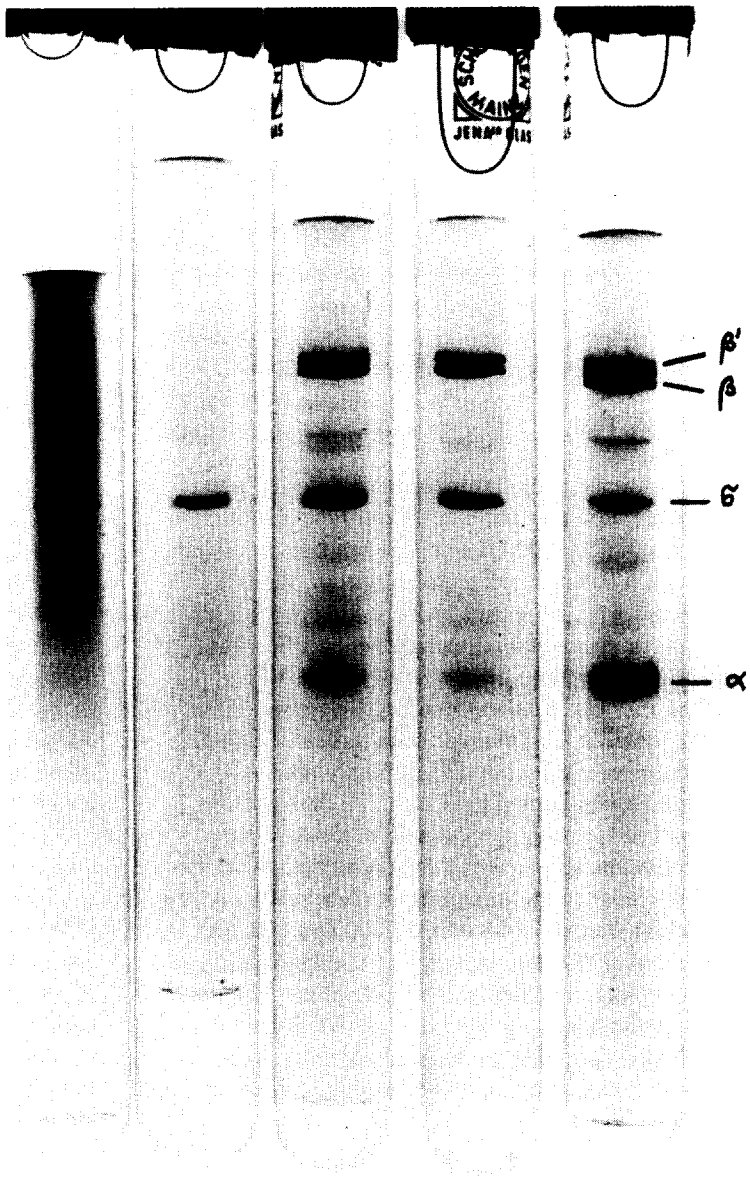

Fig. 2. SDS gel electrophoresis of polynucleotide phosphorylase from *E. coli* [8]. SDS gels contained 0.1% SDS, 0.1 M sodium phosphate, pH 7.2, 5% acrylamide and 0.135% methylene bisacrylamide. Electrophoresis was carried out for 3.5 hr (gel *b*: 4 hr) at 8.2 mA per gel. Polynucleotide phosphorylase and RNA polymerase from *E. coli* were preincubated in 0.1 M sodium phosphate, pH 7.2, containing 0.1% SDS and 1% β-mercaptoethanol for 1 min at 100°. An additional treatment with iodoacetamide in accordance with Shapiro et al. lead to similar results [9]. 20 μl samples containing 6–10 μg protein were applied after mixing with 7 μl glycerol. In gel *a* polynucleotide phosphorylase was incubated in 8 M urea 30 min at 25° and then treated with SDS and β-mercaptoethanol.

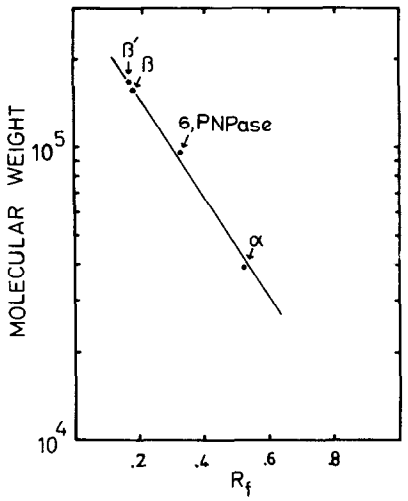

Fig. 3. Molecular weight estimation from SDS polyacrylamide gels. The subunits of *E. coli* RNA polymerase were used as markers [9]: β' (165,000), β (155,000), σ (195,000) and α (39,000). Polynucleotide phosphorylase protein (PNPase) could not be separated from the σ subunit.

shown by disc polyacrylamide gel electrophoresis no separation occurred during SDS-polyacrylamide gel electrophoresis. Since the minimal molecular weight difference which can be resolved by this technique is about 3,000 Daltons the degradation product differs by no more than 3,000 Daltons or about 30 amino acid residues from the native enzyme. This result may explain why this limited proteolysis leads to a still active protein. The above investigation gave the fol-

The above investigation gave the following results:

1) No evidence for an incomplete dissociation of polynucleotide phosphorylase was obtained under the conditions employed.

2) Dissociation products showing molecular weights of ca. 30,000 Daltons or 55,000 Daltons could not be observed.

3) The dissociation of polynucleotide phosphorylase from *E. coli* leads to a single species with a molecular weight of 95,000 Daltons.

4) The contaminating enzymatic active degradation product of polynucleotide phosphorylase differs from the native enzyme by the loss of no more than 30 amino acid residues.

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