QUATERNARY STRUCTURE OF *ESCHERICHIA COLI* POLYNUCLEOTIDE PHOSPHORYLASE: NEW EVIDENCE FOR A TRIMERIC STRUCTURE

Claude PORTIER

Institut de Biologie Physico-chimique, rue P. et M.Curie, 75005 Paris, France

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1. Introduction

In the course of a recent study [1] we have shown that *E. coli* polynucleotide phosphorylase could be isolated as two forms, A and B, which are quite distinct from one another and are both catalytically active. Form A is solely composed of alpha subunits, whereas form B contains both alpha and beta chains. Form A is derived from form B according to the following reaction:

$$\underset{(B)}{\alpha_m\beta_n} \rightleftharpoons \underset{(A)}{\alpha_m + \beta_n}$$

The quaternary structure of form A, the only one so far isolated by several authors, has been the subject of several contradictory publications concerning the mass of the enzyme and of its subunits, as well as the number of these subunits [1–5]. Our latest results were strongly in favor of the existence of a trimer (mol. wt 252 000) formed by the association of three chains, each with a mol. wt of 85 000. However, our results could also be explained by the existence of a very fast dimer–tetramer equilibrium. In order to solve this point unequivocally, we employed a new technique [6] which uses a cross-linker: dimethyl suberimidate. The results obtained truly show that form A is indeed a trimer.

2. Materials and methods

2.1. *Products*

Triethanolamine was purchased from Eastman Kodak, Tris, and alcohol dehydrogenase from Sigma, sodium dodecyl sulphate from Serlabo (France) and acetone from Merck. Urea (ultrapure) was a product from Schwarz-Mann (USA), and the electrophoresis products of Canalco Industries. Methionine-tRNA-synthetase was a generous gift from Dr J. P. Waller (CNRS Lab., Gif-sur-Yvette, France). We also wish to thank A. Expert-Bezançon for the kind gift of his preparation of dimethyl suberimidate.

2.2. *Polynucleotide phosphorylase*

Form A of the enzyme was prepared as previously described [5].

2.3. *Treatment by dimethyl suberimidate*

The conditions used were those of Expert-Bezançon [7]: 100 µl of a protein solution (1 to 0.1 mg/ml) in a 10^{-2} M triethanolamine buffer at pH 7, were adjusted to pH 8.4 with a solution of triethanolamine 2 M. A solution of dimethyl suberimidate at 40 mg/ml is immediately prepared and adjusted to neutral pH with a solution of potassium 2 N; 10 µl of this solution were then added to the protein solution before incubating 90 min at 30°C. The proteins are then precipitated by 10 vol of iced acetone during 15 min.

2.4. *Electrolysis on polyacrylamide gel in the presence of sodium dodecyl sulphate (SDS)*

After centrifugation, the supernatant is eliminated and the pellet suspended in the following solution (150 µl): urea, 6 M; SDS, 1%; mercaptoethanol, 0.1 M; sodium phosphate, 0.05 M, pH 7.0. The solution is then heated during 5 sec at 100°C and cooled; 2 µl of a solution of Bromophenol Blue are then added and the solution is deposited on a 5% polyacrylamide gel

prepared according to Weber and Osborn [8]. The electrophoresis and staining of the gels were performed as previously described [5].

3. Results

3.1. *Principle of the method*

The protein amidination reaction by dimethyl suberimidate results in covalent links between various points on the polypeptide chain according to a stochastic process. When conditions are well selected, and particularly when one has an excess of amine functions, the following situation largely schematized can occur, starting from an oligomeric enzyme: some enzyme molecules do not show any covalent link between their constituting subunits, whereas other enzyme molecules show such links between two, three, ... *n* sub-units, *n* being the number of subunits per molecule of enzyme. All that needs be done then is to dissociate the enzyme and analyze it on polyacrylamide gel in the presence of sodium dodecyl sulphate in order to obtain the number of subunits. Indeed, whenever an oligomeric protein is involved the number of observed bands on the gel corresponds to the number of subunits, even though the quantitative proportions of these protein bands may vary.

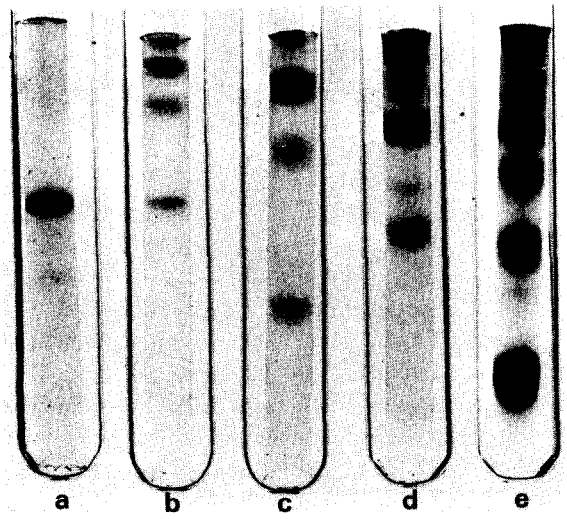

Fig.1. SDS-electrophoresis of proteins cross-linked with dimethyl suberimidate. Proteins are treated by dimethyl suberimidate at 4 mg/ml according to 'Materials and methods'. The electrophoresis is performed from top to bottom during 4 hr, except when otherwise stated. a) Untreated polynucleotide phosphorylase form A (20 μg). b) Polynucleotide phosphorylase form A (10 μg) cross-linked at 0.1 mg/ml protein. c) Same as above (b), but with a 6 hr 30 electrophoresis. d) Methionine-tRNA-synthetase (30 μg) cross-linked at 1 mg/ml protein. e) Yeast alcohol dehydrogenase (40 μg) cross-linked at 0.6 mg/ml protein.

3.2. *Applications*

a) Controls

In order to check the conditions used, we used the above method with enzymes whose quaternary structure is known. As shown in fig.1, we observed four major bands for yeast alcohol dehydrogenase which is a tetramer, and two major bands for methionine-tRNA-synthetase which is a dimer.

b) Form A of polynucleotide phosphorylase

Applying this method to form A of the enzyme, we observed three bands (fig. 1b and 1c) with molecular weights in the ratios 3:2:1. Using a 3% polyacrylamide gel yields identical results. These observations are in complete agreement with the existence of a trimer for this form of the enzyme.

4. Discussion

Under the conditions used, the three bands obtained with form A of polynucleotide phosphorylase lead us to admit the existence of a trimer which eliminates the thesis of a fast dimer–tetramer equilibrium. We must, however, strongly emphasize that it is imperative only to take into account the major species of the bands in order to avoid errors if interpretation [6,9]. It often happens that other bands, with a mass higher than that of the enzyme studied, result from parasite reactions between the oligomers. While these reactions are usually quite negligible at the concentrations of enzyme used they might sometimes prevent an absolutely clear interpretation of the results, especially when the amount of polymers formed decreases as their mass increases (fig.1, gel e); this, however, is certainly not the case of polynucleotide phosphorylase.

The results of our hydrodynamic studies by filtration on molecular sieves [1] are thus confirmed by the present work which puts an end to the uncertainty introduced in calculating the number, *n*, of subunits. Indeed, calculation of *n* yielded the following value:

$n = 2.9 \pm 0.4$. Furthermore, this result is in good agreement with observations by electron microscope since the enzyme appears as a triangle with a central hole, and was calculated to have a diameter of 85 Å [10].

Form A of polynucleotide phosphorylase therefore belongs to the rather exceptional class of enzymes of the type α_3 [11–14] which adds again to the unusual characteristics of this very peculiar enzyme.

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