YEAST PHENYLALANYL-tRNA SYNTHETASE: ISOLATION OF SUBUNITS ON ORGANOMERCURIAL-SEPHAROSE COLUMNS

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1. Introduction

Among the aminoacyl-tRNA synthetases built up of different types of subunits, only the *E. coli* glutamyl- [1] and glycyl- [2] enzymes were until now fractionated in their constitutive subunits. This fractionation allowed the study of the biological activity of the isolated subunits of these enzymes. Such a study was up to now not possible in the case of yeast phenylalanyl-tRNA synthetase (PRS) which has an $\alpha_2\beta_2$ structure [3,4], since the separation of the 2 subunits was only performed on an analytical scale. Taking advantage of the presence of highly reactive thiol groups in this enzyme (see accompanying paper), we were able to separate both types of subunits on organomercurial-Sepharose columns. This paper will describe the purification of the constitutive subunits of PRS.

2. Materials and methods

Yeast phenylalanyl-tRNA synthetase, free of 2-mercaptoethanol, was prepared as described in the accompanying paper [5]. *p*-Aminophenylmercuriacetate was synthetised as previously described [5]. Sepharose 4B was purchased from Pharmacia (Uppsala). Cyanogen bromide was obtained from Merck-Schuchardt. All other chemicals used were analytical grade reagents. Radiochemicals were purchased from the Commissariat à l'Energie Atomique (Saclay France).

2.1. *Preparation of the organomercurial-Sepharose*

p-Aminophenylmercuriacetate was covalently bound to cyanogen bromide activated Sepharose, according to the technique described by Cuatrecases [6]: 50 ml of Sepharose 4B, in suspension in 50 ml of water were treated with 12.5 g of cyanogen bromide at 20°C. The pH was maintained at 11 by the addition of 8 M NaOH. After 12 min, ice was added in the suspension, to cool it rapidly and the activated Sepharose was immediately filtered and rinsed with 600 ml of ice cold water. The gel was then introduced in 50 ml of 0.1 M phosphate buffer pH 8.5 containing 8.8 mg (25 μmol) of *p*-aminophenylmercuriacetate and the suspension was gently shaken overnight at 4°C. The gel was then recovered, and extensively rinsed with phosphate buffer. The amount of bound mercurial was estimated by reacting an aliquot of the gel suspension with an excess of 2-mercaptoethanol and back-titrating the excess of 2-mercaptoethanol with DTNB [7]. All the organomercurial was found to have reacted with the activated Sepharose. Thus, the gel contained 500 nmol of *p*-aminophenylmercuriacetate per ml.

2.2. *Incubation of the phenylalanyl-tRNA synthetase with the organomercurial-Sepharose*

4 mg of PRS (15 nmol) freed of 2-mercaptoethanol in solution in 0.8 ml of 50 mM Tris–HCl buffer pH 8.0, 10 mM $MgCl_2$, 10% glycerol (v/v) were treated with 1.2 ml of organomercurial-Sepharose, corresponding to 600 nmoles of *p*-aminophenylmercuriacetate (40-fold molar excess with respect to the enzyme). The suspension was kept for 1 hr at 37°C

with gentle intermittent shaking. After that time, the gel was filtered and extensively rinsed with the incubation buffer, until the optical density at 280 nm was zero. No enzymatic activity was found in the filtrate.

2.3. *Elution of the subunits*

A first elution was performed in the following conditions: the gel was suspended in 2 ml of incubation buffer containing 6 M guanidine hydrochloride and kept at room temperature for 1 hr. The gel was then filtered in a small column and the elution was continued with the same buffer. 1 ml fractions were collected. Usually, only the first two or three fractions, containing high concentrations of protein were recovered, but the gel was washed with at least 30 ml of the elution buffer, until no optical density emerged from the column.

A second elution was then performed in the following conditions: gel was withdrawn from the column and suspended in 2 ml of incubation buffer, containing 1% (v/v) 2-mercaptoethanol. After 1 hr at room temperature, the gel was filtered on a small column and the elution was continued with the same buffer. 1 ml fractions were collected. Usually, only the two or three first fractions contained protein and were pooled.

2.4. *Renaturation of the separated subunits*

$tRNA^{Phe}$ was added to the protein solutions recovered from the first and second elutions, to a final concentration of 1 μM. Phenylalanine and ATP were also added to final concentrations of 1 mM and the solutions were dialysed overnight at 4°C against 50 mM Tris–HCl buffer pH 7.4, 0.1 mM EDTA, 10 mM $MgCl_2$. 1 mM 2-mercaptoethanol, 1 mM Phe, 1 mM ATP, 50% (v/v) glycerol. A control experiment was performed by mixing aliquots of the proteins recovered in the first and second elution and submitting the mixture to the same renaturation treatment as for the separated subunits.

2.5. *Electrophoretic control of separated subunits*

Polyacrylamide electrophoreses, either in non-dissociating conditions or in the presence of sodium dodecylsulphate, were carried out as described in the accompanying paper [5].

3. Results and discussion

Fig.1 shows the patterns obtained by SDS-polyacrylamide electrophoresis of the proteins recovered either during the elution with guanidine hydrochloride (gel C) or with 2-mercaptoethanol (gel A). The protein eluted by guanidine hydrochloride mainly consists of the β subunit mol. wt = 63 000 (90%). A small contamination with non reacted α subunit can be detected ($\simeq$ 10%). Small amounts of degradation compounds are also visualised. The protein eluted by 2-mercaptoethanol is composed of pure α subunit (mol. wt = 73 000). No contamination by the β subunit could be detected by gel electrophoresis, even when loading the gel with a very large amount of

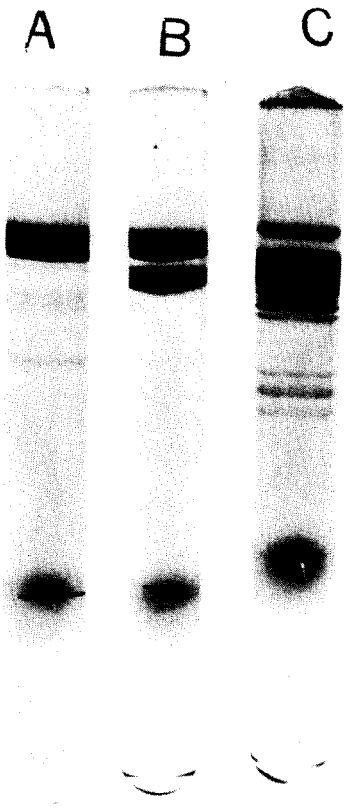

Fig.1. Electrophoretic patterns on SDS-polyacrylamide gels of the separated subunits: gel A: fraction eluted from the organomercurial-Sepharose by 2-mercaptoethanol. gel B: native phenylalanyl-tRNA synthetase. gel C: fraction eluted from the organomercurial-Sepharose by 6 M guanidine-hydrochloride.

Fig. 2. Polyacrylamide gel electrophoresis in non-dissociating conditions. gel 1: native PRS. gel 2: mixture of the isolated α and β subunits submitted to the renaturation process. gel 3: isolated α subunit after renaturation. gel 4: isolated β subunit after renaturation.

protein (around 100 μg). Some very faint bands, corresponding to degradation compounds are seen. It must be noticed that a heavier degradation takes place on the β subunit than on α subunit. This degradation might be due to some contamination of PRS by proteases, which would partly split the enzyme during the incubation with the organomercurial-Sepharose at 37°C. Fasiolo et al. [8] already observed a higher sensitivity of β subunit to proteolytic cleavage

Fig. 2 shows the patterns observed by polyacrylamide gel electrophoresis in non dissociating conditions: a mixture of separated α and β subunits, submitted to the renaturation process, affords (gel 2) native enzyme with a rather good yield (60 to 70%). This renaturation is confirmed by activity measurements, since the specific activity of the renatured enzyme is around 70% of that of the native PRS. It can be seen from gel 3, that pure α subunit, when submitted to the renaturation process has a tendency to polymerize, ginving rise to higher molecular weight compounds: two bands are clearly visible, which should correspond to α_2 and α_4 structures, according to their relative mobilities. Some aggregation compounds also remain at the top of the gel. On the contrary, it can be seen from gel 4 that most of the separated β subunit is in the monomeric form; the faint bands which correspond to dimer and tetramer mobilities might be due to the slight contamination by α subunit, which would give rise to α–β and α_2–β_2 associations.

The study of the biological properties and of the binding sites of separated subunits is under progress.

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