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Comparative study of subunits of phenylalanyl-tRNA synthetase from *Escherichia coli* and *Thermus thermophilus*

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FPLC separation of α- and β-subunits of phenylalanyl-tRNA synthetases from *E. coli* MRE-600 and *Thermus thermophilus* HB8 has been carried out in the presence of urea. Native α-subunits of both enzymes were primarily α_2-dimers and tended to aggregate. Most *E. coli* enzyme β-subunits were monomeric and only a small fraction was represented by β_2-dimers. All thermophilic β-subunits were β_2-dimers. It was shown that monomers and all forms of homologous subunits had no catalytic activity in $tRNA^{Phe}$ aminoacylation. For the enzymes and their subunits, titration curves were obtained and isoelectric points were determined. The comparison of the relative surface charges indicated similarity of the surfaces of entire enzymes and the corresponding β-subunits. α-Subunits displayed a distinctly different pH dependence of the surface charge. A spatial model of the oligomeric structure and a putative mechanism for its formation are discussed.

Thermophilic phenylalanyl-tRNA synthetase; Oligomeric structure; Isoelectric point

1. INTRODUCTION

PheRS (EC 6.1.1.20), one of the key enzymes of protein biosynthesis, has a complex quarternary structure of $\alpha_2\beta_2$-type. Isolated *E. coli* enzyme subunits were shown to have no catalytic activity [1–3]. As for the PheRS from the extreme thermophile *Thermus thermophilus*, it has been purified and characterized only recently [4] and no data on its catalytic activity are yet available.

Here we present a rapid and efficient technique for separation of subunits of phenylalanyl-tRNA synthetases from *E. coli* and *Th. th.* as well as some observations related to the behavior of the subunits in solution and possible mechanism of enzyme oligomerization.

2. MATERIALS AND METHODS

Preparation of 90–97% pure PheRS species from *E. coli* MRE-600 and *Th. th.* HB8 purified according to [4,5] and total *E. coli* MRE-600 tRNA containing 3% $tRNA^{Phe}$ were used in this study.

Dissociation was achieved by incubation in 6 M urea at 25°C for 1.5 h for the mesophilic enzyme and in 8 M urea for 3 h for the thermophilic enzyme.

The subunits were separated on a FPLC Mono Q HR 5/5 column (Pharmacia, Bromma, Sweden) equilibrated with 0.02 M Tris-HCl buffer (pH 7.5) containing 6 M and 8 M urea for the mesophilic and thermophilic enzymes, respectively. Elution was with a 0–0.3 M NaCl

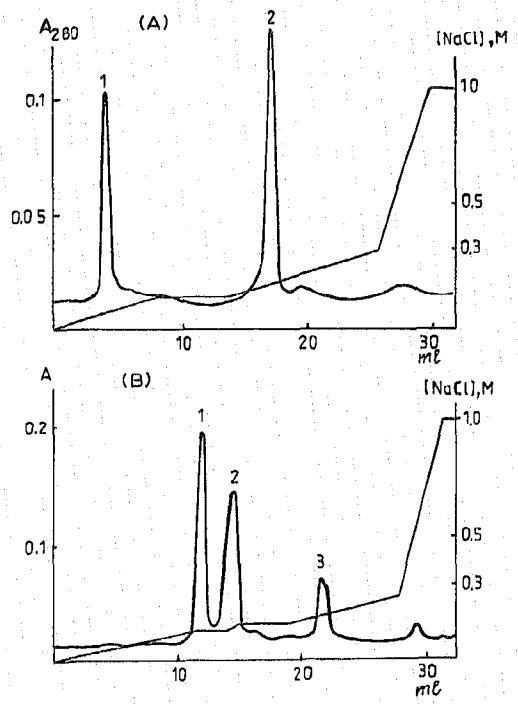

Fig. 1. (A) Separation of subunits of PheRS from *E. coli*: 1 = α-subunit, 2 = β-subunit. (B) Separation of subunits of PheRS from *Thermus thermophilus*: 1 = α-subunit, 2 = β-subunit, 3 = mixture of α- and β-subunits.

Abbreviations: PheRS, phenylalanyl-tRNA synthetase; *Th. th.*, *Thermus thermophilus*.

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linear gradient in 0.02 M Tris-HCl (pH 7.5) containing 6 M and 8 M urea. The full gradient volume was 30 ml.

The Phast system (Pharmacia, Bromma, Sweden) was used to assign peaks and the purity of the isolated subunits. SDS-electrophoresis was performed in polyacrylamide gels 'Phast Gel Gradient 10–15'. For native electrophoresis, gels 'Phast Gel Gradient 8–25' were used (with 10–15% and 8–25% acrylamide, respectively).

To determine pI by the titration curves [6] at pH 4–6.5, gels 'Phast Gel IEF 4–6.5' were used. The curves at pH from 3 to 9 were obtained on gels 'Phast Gel IEF 3–9'. The above gels contained 5% acrylamide and 3% bisacrylamide. Two-dimensional electrofocusing was employed. When the required pH gradient was achieved marker proteins with known pI were applied to the first and last paths of the gel, then the gel was turned by 90°, the protein under study was spread in the middle lane of the gel and subjected to electrophoresis in another direction. The gels were first Coomassie and then silver-stained according to [7] to reveal minor lanes.

The isolated subunits were dialyzed against 3 changes of 50 mM Tris-HCl buffer, pH 7.5, then against the same buffer with 50% glycerol, and stored at −20°C. The concentration of subunits (usually 0.1–0.3 mg/ml) was determined spectrophotometrically according to [8].

The subunits were reassociated by incubation of the predialyzed samples for 1 h at 25°C.

3. RESULTS AND DISCUSSION

3.1. *The properties of the individual subunits under native conditions*

Typical isolation of subunits is shown in Fig. 1 (A and B). The process was evidently more efficient in the case of the *E. coli* enzyme, which completely dissociated in the presence of 6 M urea. On the contrary, the complete dissociation of the thermophilic enzyme could never be achieved (Fig. 1B) although it was performed in 8 M urea for at least 2 h. This means that the α-β contacts in this enzyme are much stronger than in the mesophilic

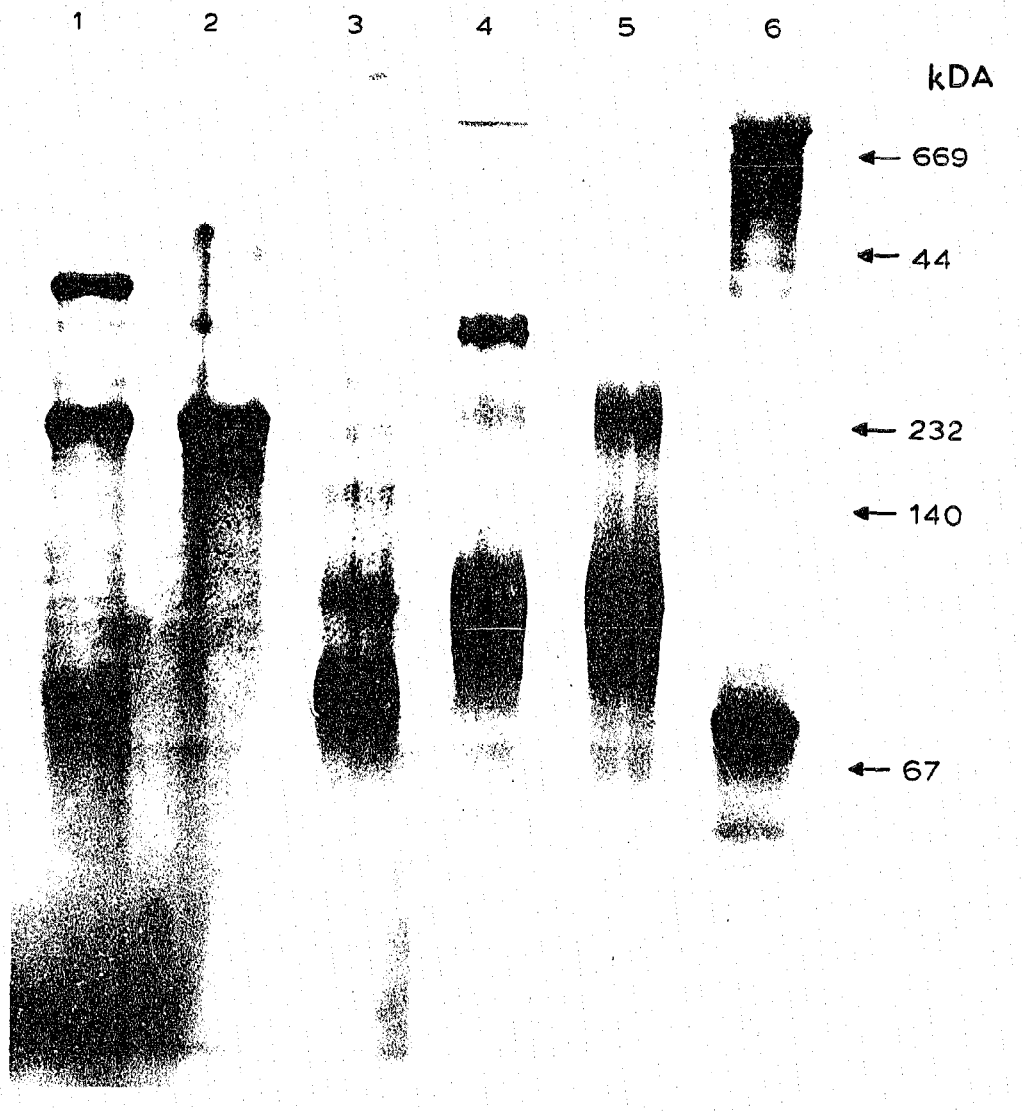

Fig. 2. Gradient native electrophoresis of phenylalanyl-tRNA synthetases and their subunits: 1 = mixture of $\alpha_{Th.\ th.}$ and $\beta_{Th.\ th.}$, 2 = $\beta_{Th.\ th.}$, 3 = $\alpha_{Th.\ th.}$, 4 = mixture of $\alpha_{E.\ coli}$ and $\beta_{E.\ coli}$, 5 = $\beta_{E.\ coli}$, 6 = $\alpha_{E.\ coli}$.

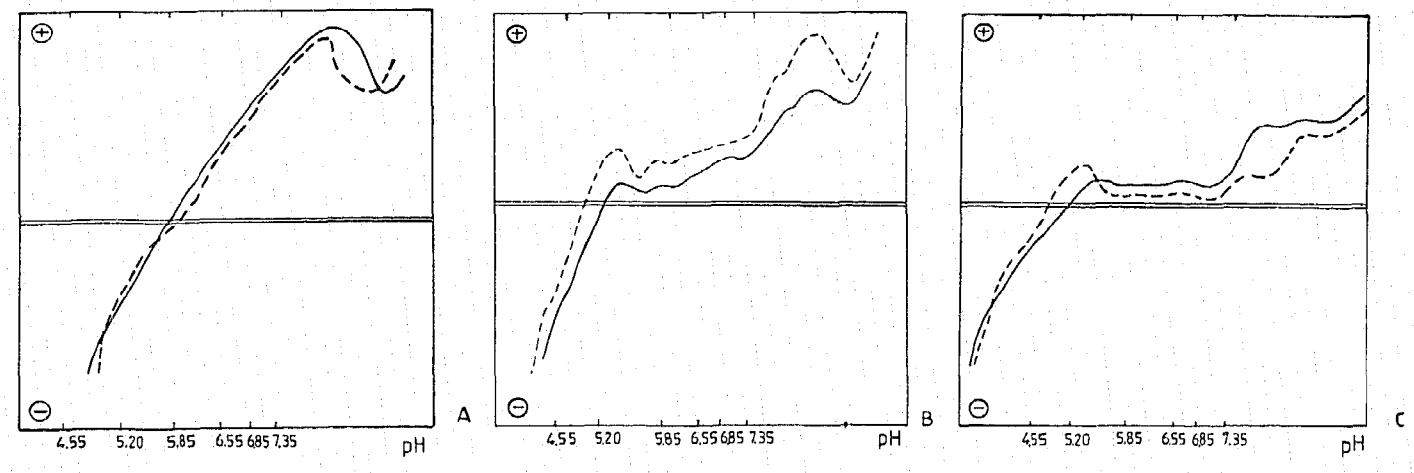

Fig. 3. Titration curves of phenylalanyl-tRNA synthetases and their subunits: (A) $\alpha_{E.\ coli}$ (----) and $\alpha_{Th.\ th.}$ (—); (B) $\beta_{E.\ coli}$ (----) and $\beta_{Th.\ th.}$ (—); (C) $PheRS_{E.\ coli}$ (----) and $PheRS_{Th.\ th.}$ (—).

one. SDS-electrophoresis revealed no impurities in the subunit preparations.

According to native gradient electrophoresis (Fig. 2) the *E. coli* enzyme β-subunits are mostly monomers (100 kDa) and only a small fraction of them are β_2-dimers (230 kDa). Unlike β-subunits, α-subunits have a tendency to aggregation (a number of lanes with the molecular weight difference of about 40 kDa) and exist in the form of α_2-dimers (75 kDa). The thermophilic PheRS revealed α_2- and β_2-dimers (80 kDa and 190 kDa) and no monomeric forms. Like their mesophilic counterparts, the α-subunits from *Th. th.* tended to aggregate (a set of lanes with approximately 40 kDa difference of molecular weight).

It was found that individual subunits of neither of the enzymes catalyzed aminoacylation of $tRNA^{Phe}$. However their reassociation led to the 50% reactivation. Therefore, taking into account the results of native electrophoresis, we concluded that neither α- and β-monomers nor α_2- and β_2-dimers are catalytically active during aminoacylation.

3.2. *Charge surface properties and pI of enzymes and their subunits*

The method of titration curves at pH from 4 to 6.5 was used to find isoelectric points. We preferred the titration curve technique to the usual isoelectrofocusing because the enzymes as well as their subunits are poor solutes and precipitate before reaching pI. We obtained the following pI values: $pI(\alpha_{E.\ coli}) = 6.05\pm0.03$; $pI(\alpha_{Th.\ th.}) = 5.20\pm0.03$; $pI(\beta_{E.\ coli}) = 4.85\pm0.03$; $pI(\beta_{Th.\ th.}) = 5.13\pm0.03$; $pI(PheRS_{E.\ coli}) = 4.75\pm0.03$; $pI(PheRS_{Th.\ th.}) = 5.00\pm0.03$. While the *E. coli* enzyme and its β-subunit gave similar values, the pI of the α-subunit was radically different. The pI values of the thermophilic enzyme and of its α-subunit as well as of the β-subunit were close.

Also we have obtained titration curves at pH between 3 and 9 showing pH dependence of the surface charge. It is interesting that the curves obtained for α-subunits of both enzymes are very similar (Fig. 3A). Both displayed noticeable aggregation especially near the pI value at which several parallel minor curves appeared with $\Delta pI=0.15$. As to the titration curves corresponding to β-subunits (Fig. 3B) they differed considerably, which may be due to the fact that the thermophilic β-subunits are mostly β_2-dimers while the mesophilic ones are predominantly monomers in solution. Therefore the most intense curve in the mesophilic subunit gel is likely to belong to the monomers while that in the thermophilic one, to β_2-dimers. The shapes of the titration curves of the phenylalanyl-tRNA synthetases are very similar (Fig. 3C) as well as being qualitatively similar to those of the corresponding β-subunits. On the contrary, the observed properties of α-subunits are different from those of both β-subunits and full oligomers. The above fact and the data regarding the tendency of α-subunits to form aggregates allow the suggestion that

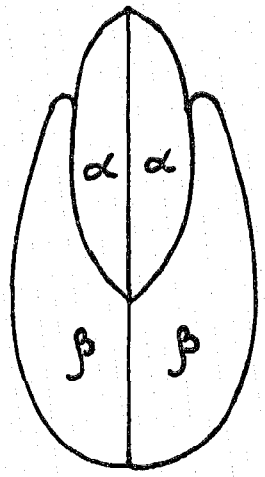

Fig. 4. Spatial model of subunit organization of procaryotic phenylalanyl-tRNA synthetase.

during association, α-subunits locate in the oligomer in such a manner that most of their surface is screened by β-subunits.

3.3. *Mechanism of oligomerization*

In order to follow the intermediate stages of oligomerization, the mixtures of α- and β-subunits of both enzymes were subjected to native electrophoresis (Fig. 2, lanes 1 and 4). The pictures obtained did not reveal any new intermediates besides those mentioned above, so self-assembly of the two given enzymes was assumed to follow the mechanism:

(1)	$\alpha + \alpha \rightarrow \alpha_2$ $\beta + \beta \rightarrow \beta_2$	(α-subunits make α_2 dimers, the same for β-subunits)
(2)	$\alpha_2 + \beta_2 \rightarrow \alpha_2\beta_2$	(α_2-dimers associate with β_2-dimers)

3.4. *Spatial model of subunit organization*

PheRS is known to be a functional dimer [9] with active centers, according to the affinity modification data, located in the area of α–β-subunit contact [10]. There must therefore be two such areas. As follows from the results of small-angle X-ray scattering [11] the enzyme macromolecule is spatially an ellipsoid, with axes ratio ≈ 2.2. We have detected the contacts of α–α and β–β-types (the subunits of both enzyme can exist in the form of dimers). Based on the totality of the above mentioned data, the spatial model shown in Fig. 4 was constructed. Recently Fasiolo et al. [12] have modeled the spatial oligomeric structure of the PheRS from yeast. They based this on the reported α–α contacts (native electrophoresis revealed α_2- and α_4-forms). The suggested model is linear, as β–β contacts were not detected possibly due to the insufficient sensitivity of the employed gel staining. It is not excluded, however, that eukaryotic PheRS have different oligomeric structure.

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