ARGINYL-tRNA SYNTHETASE FROM *BACILLUS STEAROTHERMOPHILUS*: SUBUNIT STRUCTURE OF ENZYME

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Received 21 November 1975

1. Introduction

In previous reports, Parfait [1,2] has described arginyl-tRNA synthetase from *Bacillus stearothermophilus* as a monomeric protein of 78 000 daltons which forms an intermediary complex composed of the enzyme dimer in presence of $tRNA^{Arg}$, prior to the aminoacylation reaction. The dimerisation was deduced from results obtained by sucrose gradient centrifugation. Our purpose was to check that hypothesis by physico-chemical studies. It was first necessary to obtain a highly purified enzyme. Two purification pathways have been described [1,2]: in the first procedure, the crude extract was submitted to ammonium sulphate precipitation, with heavy loss of enzyme activity, before adsorption on alumina gel and chromatography on DEAE-cellulose and hydroxyapatite. In the second, ammonium sulphate precipitation and adsorption on alumina were replaced by partition in a biphasic aqueous system used for purification of glutamyl-tRNA synthetase from *E. coli* [3].

Our purification shows that two subunits, α and β (mol. wt. 43 000 ± 3000 and 78 000 ± 2000 respectively) are associated in a complex having arginyl-tRNA synthetase activity. But the presence of both subunits is not required for $tRNA^{Arg}$ acylation: α alone is the catalytic subunit while β alone exhibits no significant activity.

2. Materials, methods and results

Our purification pathway included all the steps

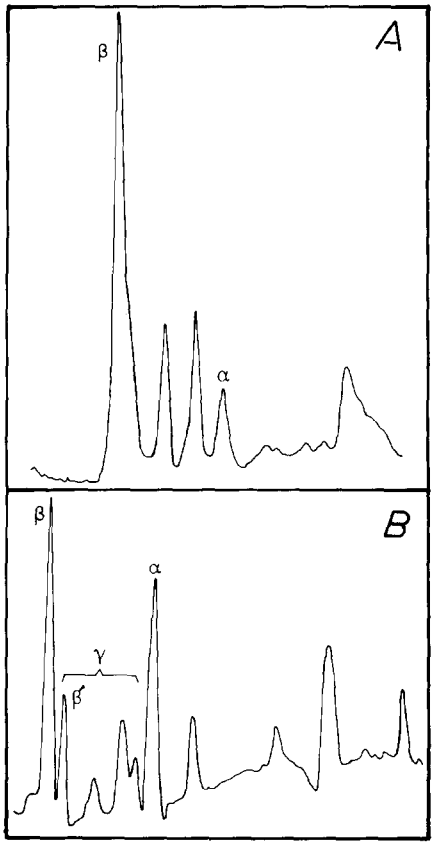

Fig.1. Electrophoresis of arginyl-tRNA synthetase in sodium dodecyl sulphate as described by Laemmli [4]. The gels were stained with Coomassie brilliant blue and scanned at 550 nm with a Gilford spectrophotometer. (A) Enzyme purified with ammonium sulphate precipitation and used by Parfait [1]. It contains much β and γ proteins but little α component. (B) Fraction I (7.5 μg) purified as described above and containing equal amounts of α and β proteins which means about 2 equivalents of α per equivalent of β.

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described earlier [1,2] except the ammonium sulphate precipitation step. This led to an enzyme preparation (Fraction I) which is more active but less homogeneous on dodecylsulphate electrophoresis [4]. Fig.1 A and B, compare the protein composition of enzyme prepared by both methods. A protein with a mol. wt. of 78 000, called by us β, was the major component of the purification from first procedure of Parfait [1] but no longer in our preparation; the most significant difference occurs at the level of a protein of 43 000 daltons that we called α.

Fraction I is free of all other aminoacyl-tRNA synthetases and shows after electrophoresis [5] at pH 7.0 to 8.7 (Tris-HCl buffer) a main peak (about 80–85% of the material) and some minor contaminants. The enzyme activity resides in the main peak as found by extraction of gel slices.

Fraction I was then fractionated by preparative electrophoresis (fig.2) under other conditions [6] with the device of P. Nokin [7]. When the fractions were submitted to dodecylsulphate electrophoresis [4], they were shown to contain several components in many fractions but only two components, α and β proteins, in the fractions with the highest activity. In spite of the large difference of molecular weight, α and β were not separated by non-denaturing gel electrophoresis. Attempts were made to separate them by affinity chromatography [8] on a column of Bio-gel (P200) to which *E. coli* tRNA had been coupled according to Grosjean et al. [9]. Both components, α and β, were retained on the tRNA column and eluted together indicating that they remain tightly associated or that both are able to bind tRNA.

Partial separation of native α and β was achieved by ammonium sulphate precipitation of Fraction I. At 45% saturation [10], the precipitate is poor in α and enriched in β (fig.3) while the supernatant is enriched in α. At 52% saturation, the supernatant is much enriched in α. Complete separation was not achieved, but in comparing the activity of the various fractions with their content in α and β as determined by dodecylsulphate electrophoresis [11], it was clear that the enzyme activity is related to the α content.

Gel electrophoresis of the supernatant at 45% saturation under the conditions described earlier [6] made it possible to obtain α free from β and to demonstrate that α alone was fully active (table 1) proportionally to its content.

Until now, we conclude that the arginyl-tRNA synthetase activity is due to a protein α of 43 000 daltons and is not related to the 78 000 mol. wt. polypeptide (β) as Parfait believed. It would seem that the activity found by Parfait in association with the large polypeptide was due to a contamination of

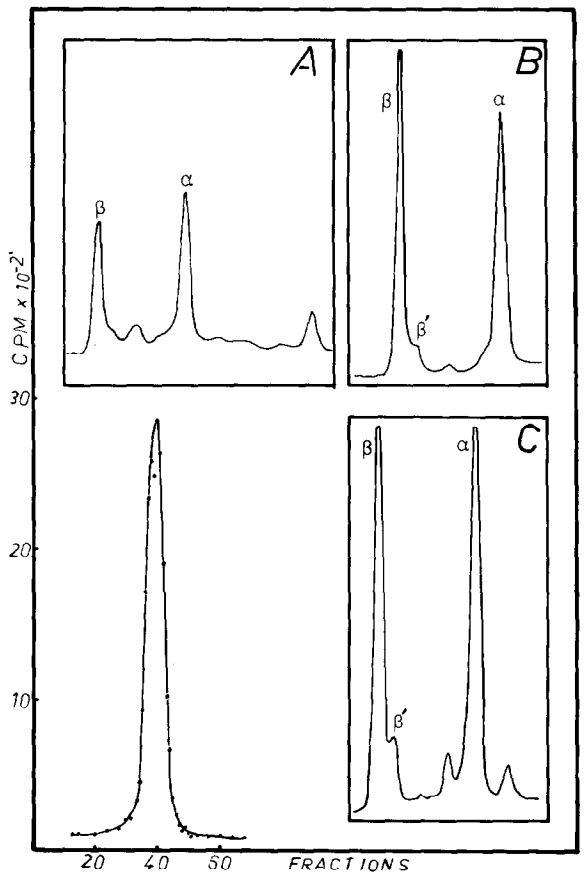

Fig.2. Electroelution of Fraction I. 75 μg were submitted to electrophoresis with gel polymerized in the device described by Nokin [7]. Continuous electrophoresis was carried out while a pump (3 ml/h) removed the lower buffer from the small chamber in contact with the bottom of the gel: fractions (280 μl) were collected in separate tubes and assayed for activity (10 μl-aliquots were incubated for 20 min at 37°C in 60 μl of the standard solution) (•—•). Tests of purity in denaturing electrophoresis were also done with these fractions: 300 μl from fractions number 37 and 38 (A), 39 to 42 (B) and 44 and 45 (C) were loaded on the gels after heat denaturation.

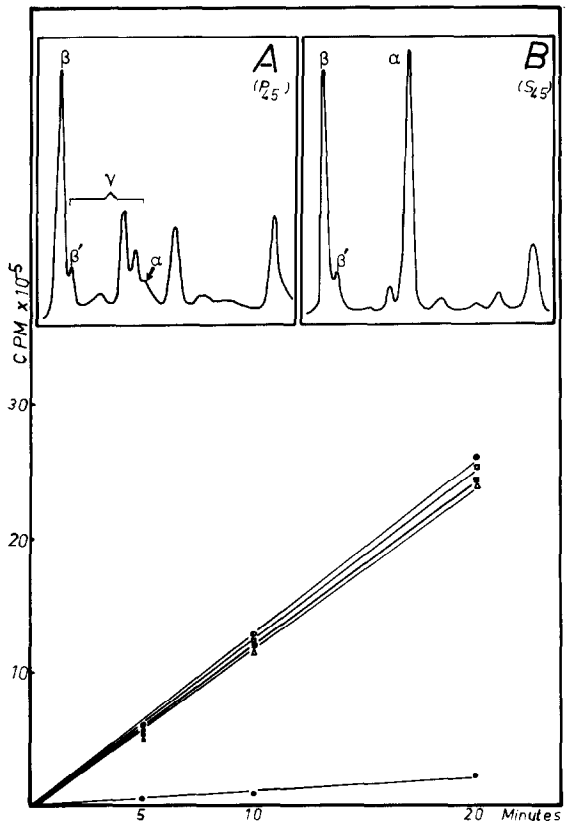

Fig.3. Ammonium sulphate precipitation at 45% saturation of Fraction I. Solid ammonium sulphate was added to 300 μl enzyme (500 μg/ml of Tris-HCl buffer pH 8.0, 1 mM dithioerythritol and 30% glycerol) to give 45% saturation. The supernatant (340 μl) was removed and the precipitate dissolved in 340 μl of 10 mM Tris-HCl buffer pH 7.4 containing 1 mM dithioerythritol and 20% glycerol. Aliquots of S45 (supernatant) and P45 (precipitate) diluted for enzyme assays were incubated at 37°C with the reaction mixture. Samples were removed after 5, 10 and 20 min to determine the quantity of [^{14}C]arginine bound to tRNA^{Arg} (cpm) and extrapolated to the total volume of P45 (●) and S45 (△). Addition of S + P (■) was assayed for recovered initial activity (I.A.: ⊙) before ammonium sulphate precipitation (□: S+P calculated from results of P and S alone). Denaturing electrophoresis were performed with precipitate (A) and supernatant (B) dialysed against 10 mM Tris-HCl buffer pH 7.4 containing 10 mM 2-mercaptoethanol and 20% glycerol.

β by a small amount of the active polypeptide α in his preparation.

The subunit character of β and the true structure of arginyl-tRNA synthetase from *Bacillus stearothermophilus* is still under study.

Acknowledgements

We wish to thank Professor H. Chantrenne for permission to work in his laboratory, for his helpful suggestions and his contribution to elaboration of our text.

Table 1
Preparative electrophoresis was carried out with Fraction I (23 μg) and S45 (20 μg estimated)

Sample		Number of gel slice									
		20	21	22	23	24	25	26	27	28	29
Fraction I	β %	100	100	54	19	14	20	17	–	25	0
	α %	0	0	46	81	86	80	83	–	75	100
Activity (cpm)		0	0	10	2050	2060	380	100	130	100	60
S45	β %	100	68	20	0	0	0	0	–	–	–
	α %	0	32	80	100	100	100	100	–	–	–
β' detected		+	–	–	–	–	–	–			
Activity (cpm)		0	0–10	100	1800	210	130	50			

S45 is the supernatant obtained by ammonium sulphate precipitation of Fraction I (45% saturation). The gels are sliced and each piece is eluting during 15 h with 0.15 ml buffer as described by Ostrem and Berg [12]. Each fraction was assayed for arginyl-tRNA synthetase activity [1] (5 μl-aliquots in 50 μl of reaction mixture incubated for 20 min at 37°C) expressed as cpm and for protein distribution (considering α + β = 100%) by gels electrophoresis in denaturing conditions [4].

We also wish to express our gratitude to Professor A. Lousse from 'Faculté de Médecine Vétérinaire. Service de Physiologie. Université de Liège' for granting special leave of absence to undertake this project.

We wish to extend our thanks to Drs H. Grosjean, C. Takada and J. Charlier for helpful discussions and suggestions, and particularly to Mrs. S. De Henau who supplied most of the enzyme (Fraction I) used for experiments.

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