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AFFINITY CHROMATOGRAPHY OF RAT LIVER AMINOACYL-tRNA SYNTHETASE COMPLEX

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Summary

The affinity column lysyldiaminohexyl-Sepharose 4B has been synthesized for the purification of aminoacyl-tRNA synthetase complexes. Lysyl-tRNA synthetase (EC 6.1.1.6) bound specifically to the Sepharose-bound lysine. The purified lysyl-tRNA synthetase was associated with arginyl-tRNA synthetase (EC 6.1.1.16) and sedimented at 18S and 12S. A 24S lysyl-tRNA synthetase bound specifically to the affinity column and also found associated with arginyl-tRNA synthetase. The results favor the model of a heterotypic multienzyme complex of mammalian aminoacyl-tRNA synthetases.

Introduction

The high molecular weight complexes of mammalian aminoacyl-tRNA synthetase complexes have been documented (1-9). We have reported a brief account of the isolation of 24S, 18S, and 12S complexes containing at least lysyl- and arginyl-tRNA synthetases from rat liver and the possible structural relationship of the complexes (10). We report here a rapid purification of the aminoacyl-tRNA synthetase complexes by affinity column chromatography on lysyldiaminohexyl Sepharose 4B. Taking advantage of the specific interaction of Sepharose-bound lysine and lysyl-tRNA synthetase, we also attempt to differentiate three possible structural models of aminoacyl-tRNA synthetase complexes. The complexes may be a heterotypic multi-enzyme complex or a homotypic enzyme complex with or without carrier molecule. The results of affinity chromatography favor the heterotypic multienzyme complex model.

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Materials and Methods

Radioactive [U-^{14}C]-L-arginine (290 mCi/mmol) and [U-^{14}C]-L-lysine (282 mCi/mmol) were purchased from New England Nuclear, Inc. Rat liver transfer RNA was purchased from Biogenics Research Corporation (Chagrin Falls, Ohio). Catalase, β-galactosidase, orthonitrophenylsufenyl chloride, lysine-HCl, and diaminohexyl-Sepharose 4B (AH Sepharose 4B, 6-10 μmoles/ml capacity) were obtained from Sigma Chemical Co. All other chemicals were analytical grade or the purest form available.

Buffer A contains 50 mM Tris-HCl (pH 7.5), 5 mM Mg acetate, and 2 mM dithioerythreitol (DTE). Aminoacylation assays were performed according to Som and Hardesty (5) with modifications. The enzyme reaction was quenched after a 10-minute incubation at 37°C by spotting 50 μl aliquots from a total reaction volume of 125 μl on a 3 MM filter paper pad according to Yang and Söll (11).

All of the following procedures were performed at 0-4°C unless otherwise indicated. Fresh rat livers dissected from 6-9 month cervically dislocated WF male rats (Microbiological Associates) were homogenized, subjected to differential centrifugation, and subsequently chromatographed on a Sephadex G-200 column according to Vennegoor and Bloemendal (4). The excluded fractions from gel filtration were collected and concentrated with a PM30 membrane in an Amicon concentrator to the material designated as G200C (ca. 40 mg protein/ml). The G200C was stored in liquid nitrogen until used.

Linear sucrose gradients (20-40%) were made in Buffer A with 25 mM KCl. Samples (0.2 ml) and standards (catalase, 11.3S, β-galactosidase, 16S) were loaded on identical gradients and centrifuged simultaneously for 14 hrs at 4°C and 40,000 rpm in an SW50L rotor using a Spinco Model L ultracentrifuge. The 24S lysyl-tRNA synthetase was obtained by sedimenting G200C on a 5-20% sucrose gradient for 6 hrs as described previously (10).

The diorthonitrophenylsulfenyl lysine (di-ONPS-Lys) derivative was prepared according to Zervas et al (12). Coupling of 4 gm of AH Sepharose 4B (in 16 ml buffer) to 0.5 gm of di-ONPS-LYS and subsequent workup was done according to Robert-Gero and Waller (13). The affinity column was equilibrated with either Buffer B (Buffer A plus 200 mM KCl and 1 mM ATP) or Buffer C (0.2 M potassium phosphate (pH 7.5), 25 mMKCl, 5 mM $MgAc_2$, 2 mM DTE, and 1 mM ATP) and was used as the starting buffer; the high salt concentrations were used to eliminate nonspecific ionic interactions (13). After sufficient elution, a second buffer (pH 7.5) identical to the starting buffer plus 20 mM lysine was used to elute off the bound enzyme.

Results

A typical affinity column chromatogram is shown in Figure 1. Lysyl-tRNA synthetase was enriched about 5000-fold with 40% yield by affinity chromatography. When 20 mM arginine instead of lysine in Buffer B was used as the second buffer no detectable lysyl-tRNA synthetase or arginyl-tRNA synthetase was eluted as shown in Table I,

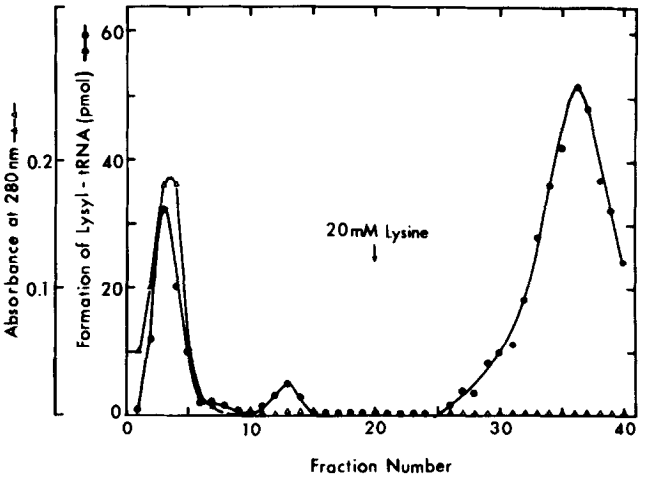

Fig. 1 Chromatogram of G200C on lysyl-AH Sepharose 4B. Approximately 1.2 mg G200C was loaded to a 3 ml column (0.7 x 8 cm) pre-equilibrated with Buffer B. The column was first eluted with Buffer B then with 20 mM lysine in Buffer B at 10 ml/hr, and fractions of 1 ml were collected. 10 µl was assayed.

TABLE I. Recovery of Lysyl-tRNA Synthetase on Lysyl-AH Sepharose 4B

Experiment	Total Protein (mg)[a]	Second Buffer	Affinity Gel	% Lysyl-tRNA Synthetase[b]
1. G200C	1.2	Buffer B + 20 mM Lysine	Fresh	85
2. G200C	4.3	Buffer B + 20 mM Lysine	Fresh	50
3. G200C	4.3	Buffer B + 20 mM Lysine	1st reuse	30
4. G200C	4.3	Buffer B + 20 mM Lysine	2nd reuse	0
5. G200C	4.3	Buffer B + 20 mM Arginine	Fresh	0
6. 24S	0.1	Buffer C + 20 mM Lysine	Fresh	>90

a. Amount of protein loaded was estimated by the ratio of A_{280} and A_{260} (14).

b. Percentage of purified lysyl-tRNA synthetase activity was based on the total recovered lysyl-tRNA synthetase activities. Approximately 60% of the lysyl-tRNA synthetase activity loaded on the affinity column was recovered in all cases.

indicating that the elution by lysine was indeed specific. The recovery of lysyl-tRNA synthetase eluted by 20 mM lysine depends heavily on the amount of total protein loaded as shown in Table I. Fresh lysyl-AH Sepharose 4B should be used since repeated use of the gel reduced the capacity and resolution of the column drastically (see Table I).

The purified lysyl-tRNA synthetase was associated with tRNA synthetases determined by aminoacylation. As shown in Figure 2, the lysyl- and arginyl-tRNA synthetase activities were found cosedimented at 18S and 12S in sucrose gradient suggesting that the two enzymes are physically associated in these complexes.

We have previously reported that the lysyl-tRNA synthetase in G200C cosedimented at 24S with at least the activities of arginyl-, isoleucyl-, leucyl- and methionyl-tRNA synthetase (10). The 24S lysyl-tRNA synthetase (0.1 mg) was further purified by a lysyl-AH Sepharose 4B column as shown in Figure 3. The lysyl-tRNA synthetase activity was retained on the column and can be specifically eluted with 20 mM lysine in Buffer C. The purified lysyl-tRNA synthetase was again associated with arginyl-tRNA synthetase activity.

Discussion

We report here the purification and structural elucidation of lysyl-tRNA synthetase complexes by affinity column chromatography.

The multiple forms of rat liver aminoacyl-tRNA synthetase have been documented (3,4,8). We have previously proposed a working hypothesis on the structural relationship of various aminoacyl-tRNA synthetase forms (10). However, the physical association of lysyl-tRNA synthetase with other aminoacyl-tRNA synthetase activities was never demonstrated beyond codesimenta-

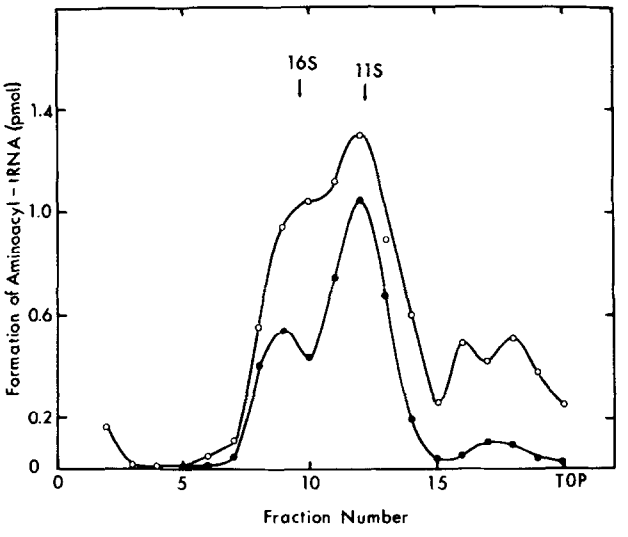

Fig. 2 Cosedimentation of purified lysyl-tRNA synthetase (—●—●—) and arginyl-tRNA synthetase (—o—o—) on a 20-40% sucrose gradient (see material and methods). 75 µl was assayed.

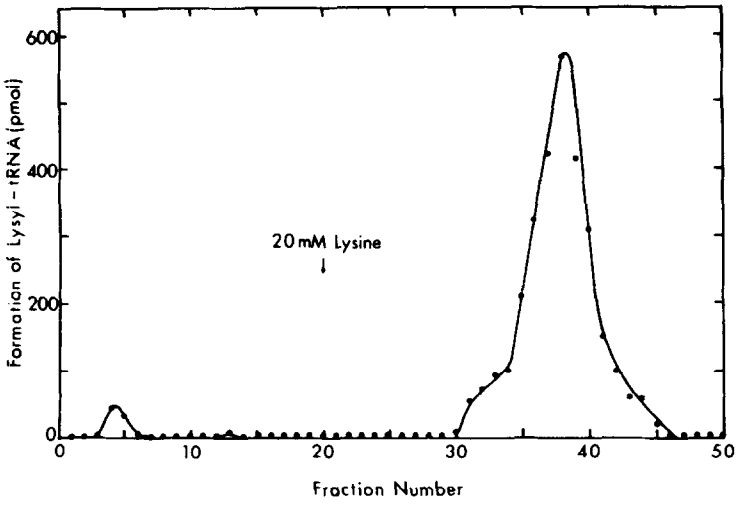

Fig. 3 Chromatogram of 24S lysyl-tRNA synthetase complex on lysyl-AH Sepharose 4B. (cf. Table I. Experiment 6). 50 µl was assayed.

tion and coelution from various chromatographic columns. It is to our knowledge that this is the first time that mammalian amino-

acyl-tRNA synthetases are observed to coelute on abiospecific affinity column in contrast to prokaryotic aminoacyl-tRNA synthetase (13). Reconstitution of the large complexes from smaller complexes has been difficult. The specific interaction of enzymes with the affinity column can provide an alternative to delineate the structure of multienzyme complexes.

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