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Seven mammalian aminoacyl-tRNA synthetases co-purified as high molecular weight entities are associated within the same complex

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Seven aminoacyl-tRNA synthetases from sheep liver were co-purified as high mol. wt. entities to constant specific activities. The purified multienzyme preparation displayed an apparent mol. wt. of $\sim 10^6$ and was composed of 11 distinct polypeptides, as revealed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). To test the assumption that all of these components were physically associated within the same complex, the purified preparation was subjected to immunoprecipitation by antibodies raised against its lysyl- or methionyl-tRNA synthetase component. Depending on the limiting concentrations of the specific antibodies used, from 5 to 40% of the input protein was recovered in the immunoprecipitate. Its polypeptide composition, as revealed by SDS-PAGE, was indistinguishable from that of the original material. The immunoprecipitation reaction was highly specific, as attested by the observation that IgG from non-immunized rabbit failed to precipitate any of the 11 polypeptides, even when used in 30-fold molar excess over input protein. We conclude that co-precipitation of all of these polypeptides by antibodies directed against a single component of the purified preparation is a consequence of their physical association within the same multienzyme complex.
Key words: sheep liver/aminoacyl-tRNA synthetase complex/monospecific antibodies/immunoprecipitation

Introduction

A distinctive feature of aminoacyl-tRNA synthetases from higher eukaryotes, compared with those from lower eukaryotes and prokaryotes, is their occurrence as high mol. wt. complexes. The complexes described range from fragile supra-molecular assemblies comprising all or most aminoacyl-tRNA synthetases (Bandyopadhyay and Deutscher, 1971; Saxholm and Pittot, 1979; Vadeboncoeur and Lapointe, 1980; Charezinski and Borkowski, 1981), to those involving only a limited yet variable number of these enzymes, in sufficiently stable association to withstand purification by conventional procedures (Vennegoor and Bloemendal, 1972; Som and Hardesty, 1975; Denney, 1977, Hele and Hebert, 1977; Dang and Yang, 1979; Kellermann *et al.*, 1979; Johnson and Yang, 1981). We have recently reported the extensive purification, from sheep liver and spleen, as well as from rabbit liver and reticulocytes, of high mol. wt. complexes (mol. wt. 10^6) containing the same seven aminoacyl-tRNA synthetases specific for isoleucine, leucine, methionine, glutamine, glutamic acid, lysine, and arginine (Kellermann *et al.*, 1982). The purified complexes originating from sheep and rabbit displayed 11 and 10 polypeptide components, respectively, as revealed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Moreover, it was shown that their polypeptide composition was species specific. The evidence available (Kellermann *et al.*, 1982; Mirande *et al.*., 1982a) favours the view that these purified complexes represent discrete entities comprising each of the polypeptide components identified by SDS-PAGE. However, an alternative possibility could be envisaged, according to which these components may be unevenly distributed within distinct classes of complexes of similar size and chromatographic properties, hence accounting for their extensive co-purification.

We show here that mono-specific antibodies directed against lysyl-tRNA synthetase or methionyl-tRNA synthetase from sheep liver, used in conjunction with protein A-Sepharose, lead to specific immunoprecipitation of each of the 11 polypeptides that characterize the purified aminoacyl-tRNA synthetase complex from sheep liver. This result demonstrates that the 11 polypeptide components that co-purify as high mol. wt. entities are physically associated within the same multienzyme complex.

Results

Immunoprecipitation of the complex by anti-lysyl-tRNA synthetase antibodies

The characteristic polypeptide composition of the purified aminoacyl-tRNA synthetase complex from sheep liver, as revealed by SDS-PAGE, is shown in Figure 1, lane A. The assignment of subunit mol. wts. and aminoacyl-tRNA synthetase activities to individual polypeptide components is based on previous studies (Mirande *et al.*, 1982a, 1982b).

The lysyl-tRNA synthetase-specific antibodies (anti-LRS_{79K}) were raised against the polypeptide of mol. wt. 79 000 separated from the other components of the complex by SDS-PAGE (Mirande *et al.*, 1982b) and were purified by immunoadsorption to the purified complex immobilized on CNBr-activated Sepharose, as described in Materials and methods. The correspondence between the polypeptide of mol. wt. 79 000 and lysyl-tRNA synthetase activity was established previously by two independent procedures (Mirande *et al.*, 1982b).

Immunoprecipitation was achieved by incubating the native complex with anti-LRS_{79K} antibodies, followed by precipitation with protein A-Sepharose. After centrifugation, the polypeptide composition of the immunoprecipitate was compared with that of the supernatant by SDS-PAGE. As shown in Figure 1, the immunoprecipitates obtained after incubation of 0.3 μM complex with 0.3 μM (lane C) or 1.2 μM antibodies (lane E) contained each of the 11 polypeptides representative of the native complex (lane A). Furthermore, the relative proportions of these components did not differ significantly from those present in the non-precipitated fractions (lanes B and D, respectively). The fraction of the complex precipitated by equimolar or 4-fold higher concentrations of anti-LRS_{79K} antibodies was estimated at 5% or 25%, respectively, based on the relative intensities of the gel patterns stained with Coo-

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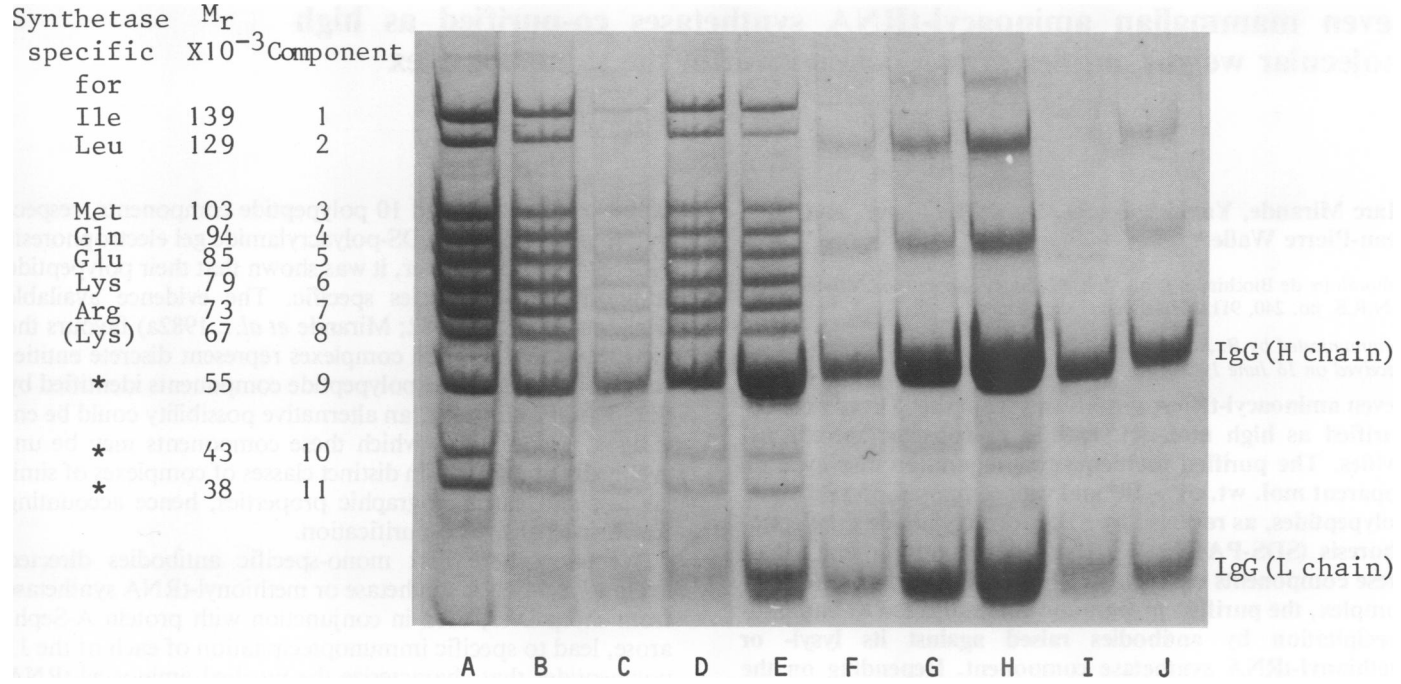

Fig. 1. Immunoprecipitation of the purified aminoacyl-tRNA synthetase complex from sheep liver by anti-lysyl-tRNA synthetase antibodies. The following protein samples were subjected to SDS-PAGE followed by staining with Coomassie brilliant blue R 250: **A**: Purified aminoacyl-tRNA synthetase complex from sheep liver alone (22 μg protein). The numbering system adopted to designate the 11 polypeptide components, as well as the subunit mol. wt. and aminoacyl-tRNA synthetase activity assignments are those described earlier (Mirande *et al.*, 1982a, 1982b). Unassigned polypeptide components are designated by asterisks. **B** and **C**: Immunoprecipitation of the purified complex (0.3 μM) by anti-LRS_{79K} IgG (0.3 μM) and protein A-Sepharose. After centrifugation, 20% (v/v) of the supernatant (**B**) and 100% of the washed immunoprecipitate (**C**) were analysed. **D** and **E**: Immunoprecipitation of the purified complex (0.3 μM) by anti-LRS_{79K} IgG (1.2 μM) and protein A-Sepharose. After centrifugation, 20% of the supernatant (**D**) and 100% of the precipitate (**E**) were analysed. **F, G,** and **H**: Mock immunoprecipitation of 0.3 μM purified complex by 0.3 μM (**F**), 1.2 μM (**G**), and 3 μM (**H**) control IgG from non-immunized rabbit and protein A-Sepharose. After centrifugation, 100% of each precipitate was analysed. **I** and **J**: anti-LRS_{79K} antibodies (5 μg) and control IgG (5 μg) alone, respectively.

massie brilliant blue R250 and on the amounts of the supernatant and immunoprecipitate subjected to electrophoresis, as specified in the legend of Figure 1.

The specificity of the immunoprecipitation was verified by replacing the mono-specific antibodies by various concentrations of purified antibodies originating from non-immunized rabbits (control IgG). As shown in Figure 1, lanes F, G, and H, the fractions sedimenting with protein A-Sepharose after pre-incubation of the complex (0.3 μM) with 0.3 μM, 1.2 μM, or 3 μM control IgG, respectively, were devoid of the polypeptide components characteristic of the complex. The same result was obtained using 10 μM control IgG (result not shown). It was previously verified by the blotting procedure that these control IgG failed to interact with any of the 11 components of the purified complex (Figure 3, lane c).

Immunoprecipitation of the complex by anti-methionyl-tRNA synthetase antibodies

The methionyl-tRNA synthetase-specific antibodies (anti-MRS_{103K}) were directed against the polypeptide of mol. wt. 103 000 separated from the other components of the complex by SDS-PAGE (Mirande *et al.*, 1982b). They were purified by immunoadsorption as described in Materials and methods. The correspondence between the polypeptide of mol. wt. 103 000 and methionyl-tRNA synthetase activity was established previously by three independent procedures (Mirande *et al.*, 1982a,1982b; Brevet *et al.*, 1982). As shown by the blotting procedure in Figure 3, lane b, these antibodies interacted exclusively with the polypeptide of mol. wt. 103 000 out of the 11 components that characterize the purified complex from sheep liver. In contrast, the antibodies raised against the native complex and purified by immunoadsorption to immobilized complex, recognized nine of the 11 polypeptide components (Figure 3, lane a). It is noteworthy that antibodies against component 4 were present in the anti-complex IgG fraction prior to purification by immunoadsorption, thus leaving component 9 as the only polypeptide from the native complex which failed to elicit production of antibodies.

Immunoprecipitation of the native complex was achieved by incubation with an equimolar concentration (0.3 μM) of anti-MRS_{103K} antibodies, followed by precipitation with protein A-Sepharose. After centrifugation, the polypeptide composition of the immunoprecipitate was compared with that of the supernatant by SDS-PAGE. As shown in Figure 2, lane C, the immunoprecipitate contained each of the 11 polypeptides representative of the native complex (lane A), in relative proportions that did not differ significantly from those present in the non-precipitated fraction (lane B). The fraction of the complex recovered in the immunoprecipitate was estimated as 15%, based on the criteria specified in the preceding section. Decreasing the concentration of the native complex to 0.06 μM under otherwise identical incubation conditions, resulted in recovery of close to 40% of the total protein in the immunoprecipitate, the polypeptide composition of which was again representative of that of the original complex (result not shown).

Control of the selectivity of the immunoprecipitating reaction

The selectivity of the immunoprecipitation by anti-MRS_{103K} antibodies was verified by analysing the immuno-

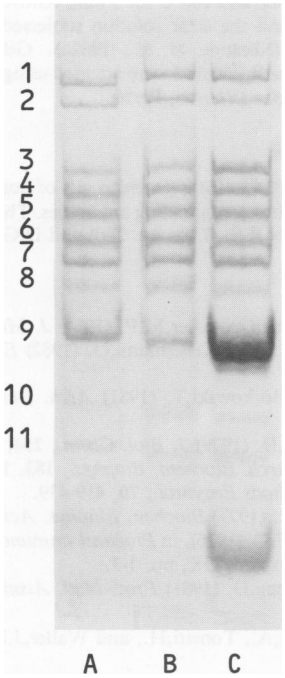

Fig. 2. Immunoprecipitation of the purified aminoacyl-tRNA synthetase complex from sheep liver by anti-methionyl-tRNA synthetase antibodies. The following protein samples were subjected to SDS-PAGE followed by staining with Coomassie-brilliant blue R 250: **A**: Purified complex alone (10 μg). Bands are numbered according to Figure 1. **B** and **C**: Immunoprecipitation of the purified complex (0.3 μM) by anti-MRS_{103K} IgG (0.3 μM) and protein A-Sepharose. After centrifugation, 10% (v/v) of the supernatant (**B**) and 100% of the washed immunoprecipitate (**C**) were analysed.

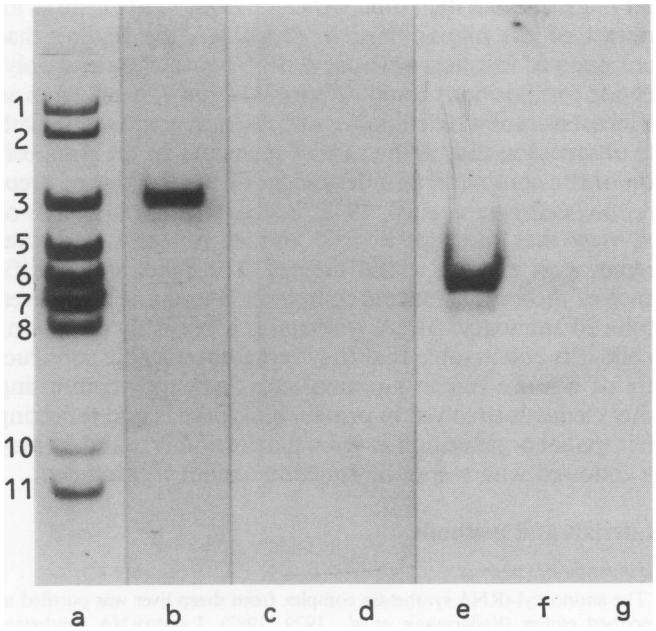

Fig. 3. Specificity of the antibodies and selectivity of the immunoprecipitation verified by the protein blotting procedure. Protein samples were subjected to SDS-PAGE, transferred to nitrocellulose, incubated successively with the specified IgGs, and ^{125}I-labeled protein A, followed by autoradiography. **a, b, c,** and **g**: The polypeptide components of the purified complex from sheep liver exposed to anti-complex IgG (**a**), anti-MRS_{103K} IgG (**b**), control IgG (**c**) and anti-yeast lysyl-tRNA synthetase IgG (**g**). Bands in (**a**) are numbered according to Figure 1. **d**: Lysyl-tRNA synthetase from yeast exposed to anti-MRS_{103K} IgG. **e** and **f**: Analysis of the immunoprecipitate obtained after incubation of equimolar concentrations (0.3 μM) of purified complex from sheep liver, lysyl-tRNA synthetase from yeast and anti-MRS_{103K} IgG, followed by precipitation with protein A-Sepharose, as described in Materials and methods. After centrifugation, 10% (v/v) of the supernatant (**e**) and 100% of the washed immunoprecipitate (**f**) were fractionated by SDS-PAGE, transferred to nitrocellulose, exposed to anti-yeast lysyl-tRNA synthetase IgG, and ^{15}I-labelled protein A, followed by autoradiography.

precipitate obtained after incubation of equimolar concentrations (0.3 μM) of purified complex from sheep liver, lysyl-tRNA synthetase from yeast, and anti-MRS_{103K} IgG, followed by precipitation with protein A-Sepharose and centrifugation. The proteins from the supernatant and the washed immunoprecipitate were fractionated by SDS-PAGE, transferred to nitrocellulose, and exposed to anti-yeast lysyl-tRNA synthetase IgG. The results shown in Figure 3 (lanes e and f) clearly indicate that no trace of yeast lysyl-tRNA synthetase was associated with the immunoprecipitate. It was previously verified by the blotting procedure that no interaction occurred between yeast lysyl-tRNA synthetase and anti-MRS_{103K} IgG (Figure 3, lane d) as well as between the purified complex from sheep liver and anti-yeast lysyl-tRNA synthetase IgG (Figure 3, lane g). The choice of lysyl-tRNA synthetase to test the selectivity of the immunoprecipitation by anti-MRS_{103K} antibodies was dictated by its close structural similarity to the corresponding enzyme from the complex of sheep liver. The enzyme from yeast is a dimer possessing a subunit mol. wt. of 72 000 (Rymo *et al.*, 1972), while the enzyme from sheep liver has a subunit mol. wt. of 79 000 (Mirande *et al.*, 1982b) and is likely to occur as a dimer within the complex (Mirande *et al.*, 1982a; Johnson and Yang, 1981).

Discussion

In the present study, the highly purified multi-component aminoacyl-tRNA synthetase complex from sheep liver was immunoprecipitated using limiting concentrations of specific antibodies directed to a single component of the complex. Using anti-lysyl-tRNA synthetase antibodies, ~5% or 25% of the protein was immunoprecipitated by an equimolar concentration or a 4-fold molar excess of the antibody, respectively. Similar results were obtained using anti-methionyl-tRNA synthetase antibodies. In each of these cases, the SDS-PAGE patterns afforded by the immunoprecipitate and the soluble fraction were virtually indistinguishable from that of the original material, both in terms of the number of components present and their relative intensities. These results, obtained with antibodies directed to two distinct components of the purified preparation, are not compatible with the existence of a heterogenous population of complexes. Two kinds of control experiments attest to the specificity of the immunoprecipitation reaction. Firstly, IgG from non-immunized rabbit, even when used in 30-fold molar excess, did not lead to precipitation of any of the components of the complex. Secondly, when equimolar concentrations of the complex and yeast lysyl-tRNA synthetase were exposed to antibodies directed against the methionyl-tRNA synthetase component of the complex, no trace of non-specifically adsorbed yeast lysyl-tRNA synthetase was detected in the immunoprecipitate. Taken together, these results support the conclusion that co-precipitation of each of the 11 polypeptides by antibodies directed against a single component is a consequence of their physical association within the same complex.

We have recently presented evidence in support of the view

that these purified high mol. wt. complexes do not arise as an artefact of the preparation. In particular, the finding that complexes of identical aminoacyl-tRNA synthetase and polypeptide compositions could be purified from two cell types as different as rabbit reticulocytes and liver, in conjunction with the observation that in the case of reticulocytes the composition of the complex was independent of the method of preparation (Kellermann *et al.*, 1982), argues strongly in favour of the view that these particulate entities reflect a structural organization existing within the cell. The functional significance of these multienzyme complexes containing only seven of the 20 aminoacyl-tRNA synthetases is presently unknown. While it is conceivable that they represent a stable substructure of a more fragile supramolecular assembly comprising other elements involved in protein biosynthesis and reflecting their spatial organisation *in vivo*, the possibility that they may be endowed with a specific function cannot be excluded.

Materials and methods

Purification of enzymes

The aminoacyl-tRNA synthetase complex from sheep liver was purified as described earlier (Kellermann *et al.*, 1979, 1982). Lysyl-tRNA synthetase purified to homogeneity from *Saccharomyces cerevisiae*, as well as the corresponding antibodies raised in rabbit and purified by ammonium sulphate precipitation, were gifts from B. Çırakoğlu.

Preparation and purification of antibodies

Mono-specific antibodies against lysyl-tRNA synthetase (anti-LRS_{79K} antibodies) or methionyl-tRNA synthetase (anti-MRS_{103K} antibodies) were raised in rabbit against the polypeptides of mol. wts. 79 000 and 103 000, respectively, separated from the other nine components of the purified complex from sheep liver by SDS-PAGE (Mirande *et al.*, 1982b). The corresponding IgG fractions, obtained by ammonium sulphate precipitation (Hudson and Hay, 1976), were further purified by immunoadsorption. The immunoadsorbant was prepared by covalent coupling of the purified complex (20 mg) to CNBr-activated Sepharose 4B (8 ml packed gel), following a general procedure (Livingston, 1974). Each IgG fraction (100 mg protein), dialysed against phosphate buffered saline (PBS) (137 mM NaCl, 2.5 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2), was incubated batch-wise with a separate lot of the immunoadsorbant for 1 h at 20°C, followed by transfer of the slurry into a column and washing with PBS at 4°C until the absorbance of the eluate at 280 nm was <0.05. Stepwise elution of IgG was achieved with 0.2 M glycine-HCl at pH 2.8 and 2.2. The two fractions were immediately neutralized with 1 M K_2HPO_4, pooled, concentrated by ultra-filtration, dialysed against PBS containing 50% glycerol and stored at −20°C at a concentration of 0.2 mg/ml.

Control IgG, isolated from serum of non-immunized rabbits by ammonium sulphate precipitation, were purified by adsorption on protein A-Sepharose CL-4B (Pharmacia) (Engvall, 1981) in PBS buffer, followed by elution with 0.2 M glycine-HCl at pH 2.8 and further processing as above.

Anti-complex antibodies directed against the purified aminoacyl-tRNA synthetase complex from sheep liver were raised in rabbit by repeated injections of 0.7 mg native complex. The corresponding IgG-fraction, obtained by ammonium sulphate precipitation (Hudson and Hay, 1976), was further purified by immunoadsorption to the purified complex covalently linked to Sepharose 4B, by the procedure described above.

Specificity of the purified antibodies verified by the protein blotting procedure

Protein samples were subjected to SDS-PAGE, transferred to cellulose, successively incubated with purified IgG and ^{125}I-labelled protein A, followed by autoradiography, as previously described (Mirande *et al.*, 1982a).

Immunoprecipitation

Specified amounts of the purified complex (apparent mol. wt. 10^6) (Mirande *et al.*, 1982a) and antibodies (anti LRS_{79K}, anti-MRS_{103K}, or control IgG) were incubated in 1.5 ml conical polypropylene tubes (Eppendorf) at 4°C for 30 min in PBS-NP40 (PBS buffer containing 0.5% Nonidet P40 and 20% glycerol), in a final volume of 0.2 ml. Precipitation was achieved by addition of 0.04 ml of a 1:1 suspension of protein A-Sepharose gel in PBS-NP40, incubation at 4°C for 30 min, followed by centrifugation for 2 min at 10 000 *g*. The supernatant was recovered for analysis by SDS-PAGE. The precipitate was washed four times with 0.1 ml PBS-NP40, suspended in 0.05 ml SDS solution (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 40 mM dithioerythritol, 1% SDS, 50 mg/ml sucrose, and 0.05 mg/ml bromophenol blue), heated at 50°C for 30 min and 100°C for 1 min, centrifuged 5 min at 10 000 *g* to eliminate the gel, and the clear solution subjected to electrophoresis as described previously (Mirande *et al.*, 1982a). Gels were stained with Coomassie brilliant blue R 250 and were scanned using a Vernon Model PHI 5 integrating densitometer (Vernon, Paris).

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