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# PURIFICATION AND SUBUNIT STRUCTURE OF PHENYLALANYL-tRNA SYNTHETASE FROM HEN LIVER MITOCHONDRIA

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Hen liver mitochondrial phenylalanyl-tRNA synthetase is purified to homogeneity by a series of steps including salting-out chromatography, salting-out affinity chromatography in the presence of $tRNA^{Phe}$, dissociation of the enzyme-tRNA complex on DEAE-cellulose, chromatography on DEAE-Sepharose CL-6B and Sepharose 6B. The enzyme appears to be a tetrameric enzyme with a molecular weight of 255 000, as determined by gel filtration, with a subunit structure of $\alpha_2\beta_2$ ($\alpha$ = 57 000, $\beta$ = 66 000), as determined by sodium dodecyl sulfate gel electrophoresis.

## INTRODUCTION

In eucaryotic cells, mitochondria possess their own protein synthesizing apparatus, including specific aminoacyl-tRNA synthetases (E.C. 6.1.1.-). Up to now, studies have mainly been devoted to mitochondrial aminoacyl-tRNA synthetases from yeast and Tetrahymena (1-4). Only very few studies have been performed with animal mitochondrial aminoacyl-tRNA synthetases, using crude extracts or partially purified enzymes (5-7).

Structural characterisation of a mitochondrial aminoacyl-tRNA synthetase and comparison of its structural and catalytic properties with those of its cytoplasmic counterpart require the purification of both enzymes of the two compartments within the cell. As a preliminary step toward the elucidation of the physiological significance and evolutionary relationship we reported the purification and properties of the turkey liver cytoplasmic phenylalanyl-tRNA synthetase elsewhere (8). In this paper we present an isolation and purification procedure for hen liver mitochondrial phenylalanyl-tRNA synthetase (E.C. 6.1.1.20) and evidence for its subunit structure. To our knowledge this is the first report on an animal mitochondrial aminoacyl-tRNA synthetase purified to homogeneity.

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## MATERIALS AND METHODS

Fresh hen liver was obtained from a local butcher. Aminoacylation tests were performed routinely as described (9). The enzymatic unit is defined as formation of 1 nmol of Phe-$tRNA^{Phe}$ per minute at 37°C. Protein concentration was determined according to Warburg and Christian (10). Gel electrophoresis for molecular weight determination of the subunits was carried out according to Fairbanks et al. with 0.1 % sodium dodecyl sulfate and 7 % acrylamide (11). $tRNA^{Phe}$ was isolated from unfractionated baker's yeast (Boehringer, Mannheim, FRG) as described (12).

Hen liver mitochondria from 2 kg fresh hen liver were isolated according to Bustamante et al. (13) with slight modifications, the purity being checked ultrastructurally and biochemically by De Duve's plot (14). The final yield was 35-40 mg mitochondrial protein per gram wet liver. Purified mitochondria were frozen in liquid nitrogen, pulverized with a mortar and pestle and homogenized in 30 mM Tris-HCl (pH 8.2), 50 mM KCl, 20 mM $MgCl_2$, 10 % glycerol, 0.1 mM phenylmethylsulfonylfluoride (PMSF), 0.1 mM diisopropylfluorophosphate, 5 mM 2-mercaptoethanol, 1 mM EDTA and 1 mM dithiothreitol (DTT) with an electric mincing machine with 6 strokes for 15 seconds. The crude extract was centrifuged for 1 hr at 20 000 rpm in the SS34 rotor and re-extracted. The combined supernatants were brought to 75 % ammonium sulfate saturation at 4°C. The precipitate was collected by centrifugation, dissolved in buffer A (50 mM Tris-HCl pH 7.8, 5 mM $MgCl_2$, 1 mM EDTA, 2 mM 2-mercaptoethanol, 1 mM DTT, 0.1 mM PMSF and 20 % glycerol),adjusted to an ammonium saturation, where first turbidity occurs, and applied to a (6 x 35 cm) Sepharose 4B column (Pharmacia Fine Chemicals, Freiburg, FRG), equilibrated with starting buffer at 60 % ammonium sulfate saturation. The column was developed by running a 2 x 1.5 l gradient from 48 % to 15 % ammonium sulfate saturation at a flow rate of 90 ml/hr with 19 ml fractions (Fig. 1). The pooled active fractions were applied to salting-out chromatography at pH 8.2 on a Sepharose 4B column (3.5 x 30 cm), developed with a 2 x 600 ml gradient from 48 % to 15 % ammonium sulfate saturation at a flow rate of 65 ml/hr with 10 ml fractions (Fig. 2). The pooled fractions were brought to 85 % ammonium sulfate saturation, the protein was collected by centrifugation and dissolved in buffer A. 50 $A_{260}$--units of $tRNA^{Phe}$ were added to the clear solution for induction of a ligand--induced solubility shift in salting-out chromatography (15). This solution was brought to approximately 30 % ammonium sulfate saturation, applied to a Sepharose 4B column (3.5 x 30 cm) equilibrated with buffer A + 60 % ammonium sulfate saturation and the protein eluted by running a 2 x 600 ml gradient from 45 % to 15 % ammonium sulfate saturation at a flow rate of 60 ml/hr with 10 ml fractions. Again protein from the active fractions was collected by precipitation with ammonium sulfate and centrifugation, dissolved in buffer B (30 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 1 mM EDTA, 2 mM 2-mercaptoethanol, 1 mM DTT and 0.1 mM PMSF), dialysed against this buffer + 50 % glycerol, brought to 0.2 M KCl and run through a 50 ml column of DE-52 (Whatman, IC Chemie, Munich, FRG) to cleave the complex between the enzyme and $tRNA^{Phe}$. The eluant was dialysed against buffer B + 40 % glycerol and then applied to a column (1.6 x 6 cm) of DEAE-Sepharose CL-6B (Pharmacia Fine Chemicals, Freiburg, FRG). Elution was performed with a linear gradient (2 x 60 ml) from 0 to 300 mM KCl at a flow rate of 10 ml/hr with 1 ml fractions (Fig. 4). The pooled fractions were treated as before. To avoid loss of enzymatic activity upon gel diffusion and to gain optimal resolution, only a quarter of this material at one time was run through a Sepharose 6B column (1.2 x 44 cm) with buffer B + 25 % glycerol at a flow rate of 3 ml/hr with 0.5 ml fractions (Fig. 5). This column was also used for analytical gel filtration after calibration. The active fractions were pooled, dialysed against buffer B + 50 % glycerol and kept at -20°C.

## RESULTS

The detailed results of the purification procedure, starting with 2 kg hen liver and 7700 mg mitochondrial protein, are summarized in Table 1.

Table 1. Summary of the purification of phenylalanyl-tRNA synthetase from hen liver mitochondria.

| | total activity (units) | total protein (mg) | specific activity[a] (units/mg) | purification (fold) | yield (%) |
|---|---|---|---|---|---|
| extract | 1000 | 7700 | 0.13 | 1 | 100 |
| 1st salting-out chromatography | 760 | 1260 | 0.6 | 4.6 | 75 |
| 2nd salting-out chromatography | 640 | 740 | 0.86 | 6.6 | 63 |
| salting-out affinity chromatography | 530 | 65 | 8.1 | 63 | 52 |
| DEAE-Sepharose CL-6B | 200 | 6 | 33 | 257 | 20 |
| Sepharose 6B | 100 | 0.8 | 124 | 954 | 10 |

[a]measured with yeast cytoplasmic tRNA.

As we described for the cytoplasmic turkey liver phenylalanyl-tRNA synthetase (8), the mitochondrial enzyme is well stabilized under the conditions of salting-out chromatography. Mitochondrial phenylalanyl-tRNA synthetase activity elutes at 33 % ammonium sulfate saturation from the first column (Fig. 1). The cytoplasmic enzyme appears at 35 % saturation under these conditions (Sternbach et al., this laboratory, unpublished). Rechromatography at a different pH results in a nearly twofold reduction of protein content (Fig. 2). On complexation with $tRNA^{Phe}$ a slight solubility shift occurs, in

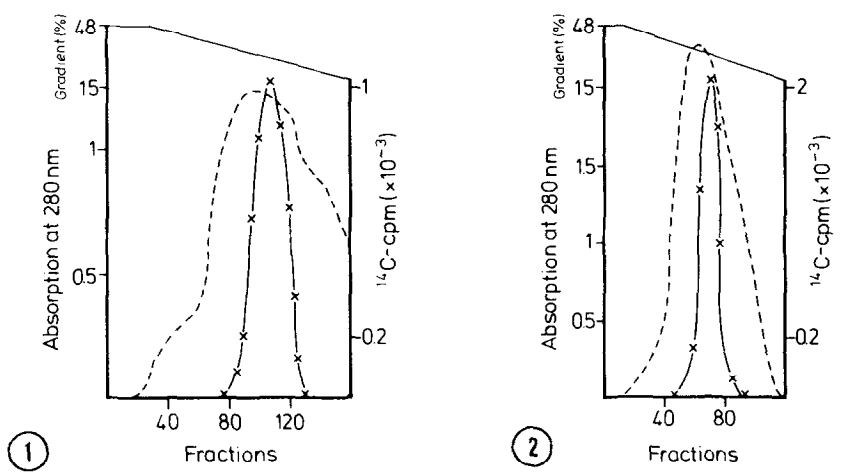
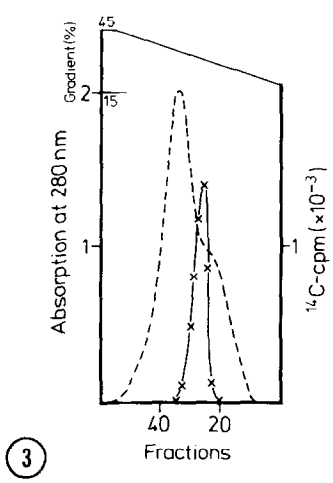



Figure 1: First salting-out chromatography at pH 7.8. ---, Absorption at 280 nm; x——x mitochondrial phenylalanyl-tRNA synthetase activity.
Figure 2: Second salting-out chromatography at pH 8.2. Legend as in Fig. 1.



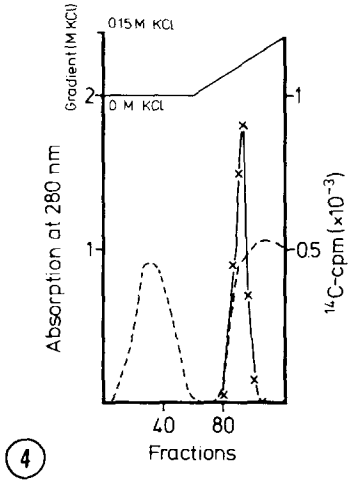



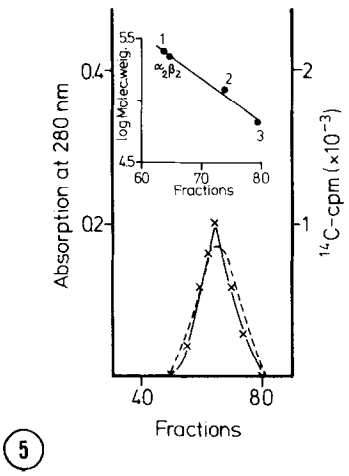

Figure 3: Salting-out affinity chromatography with $tRNA^{Phe}$ at pH 7.8.
Legend as in Fig. 1.

Figure 4: DEAE-Sepharose CL-6B chromatography at pH 7.5.
Legend as in Fig. 1.

this case 3 %, which is within the resolving power of the gradient (Fig. 3). A similar shift is noticed for the cytoplasmic enzyme of turkey liver (8). After cleavage of the complex the enzyme is eluted with 70 mM KCl at pH 7.5 from DEAE-Sepharose CL-6B (Fig. 4). Again there is a difference in chromatographic behaviour compared with the cytoplasmic enzyme which is eluted with 120 mM KCl (Sternbach et al., this laboratory, unpublished). The gel filtration on Sepharose 6B improves the purity of the enzyme, which elutes from the column as a single peak coinciding with the 280 nm absorption. This step is also used for analytical gel filtration after calibration of the column (Fig. 5). The molecular weight is estimated to be 255 000. Sodium dodecyl sulfate gel electrophoresis shows two bands (α and ß) of molecular weights 57 000 and 66 000 respectively (Fig. 6). These results suggest a subunit structure of $\alpha_2\beta_2$.

## DISCUSSION

The hen liver mitochondrial phenylalanyl-tRNA synthetase resembles in its molecular weight and quaternary structure the phenylalanyl-tRNA synthetases already studied in various organisms (8,16). Hen liver mitochondrial and cytoplasmic phenylalanyl-tRNA synthetase can be distinguished by their



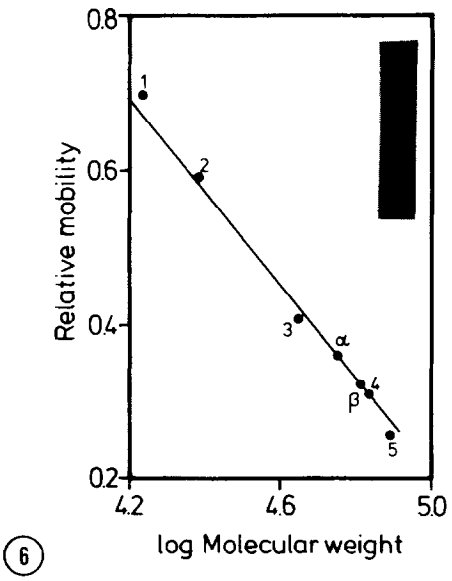




Figure 5: Sepharose 6B chromatography. Legend as in Fig. 1. Standards for calibration: yeast cytoplasmic phenylalanyl-tRNA synthetase [1], yeast cytoplasmic valyl-tRNA synthetase [2] and bovine serum albumin [3].

Figure 6: Polyacrylamide gel electrophoresis of the subunits (α, β) of mitochondrial hen liver phenylalanyl-tRNA synthetase in 0.1% sodium dodecyl sulfate. The standards are: myoglobin [1], chymotrypsinogen A [2], ovalbumin [3], bovine serum albumin [4] and glycerin-3-phosphate dehydrogenase [5].

chromatographic behaviour. With the purified mitochondrial enzyme more detailed comparisons are now possible, including substrate specificity, mechanism of providing sufficient accuracy, and antigenic determinants, since the significance of the emergence of these heterotopic isoenzymes and their relationship is still open to conjecture. In the case of the two aspartate aminotransferase isoenzymes amino acid sequences indicate that they are homologous proteins and that the duplication of the gene occurred approximately $10^9$ years ago, i.e. at the time of the emergence of eucaryotic cells, the time of the appearance of intracellular heterotopism of the two isoenzymes being unknown (17-20). On the other hand, a high degree of homology among the amino terminal sequences of chicken liver mitochondrial superoxide dismutase and the E.coli enzyme with no significant homology to the bovine erythrocyte enzyme has been reported (21). A comparison of the proofreading mechanism may give hints to a possible common origin, because they have been shown to differ significantly among the well-characterized E.coli, yeast, Neurospora

and cytoplasmic turkey liver phenylalanyl-tRNA synthetases (22,23). Further studies in these respects are currently in progress in our laboratory.

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REFERENCES

1. Chiu, A.O.S., and Suyama, Y. (1975) Arch. Biochem. Biophys. 171, 43-54.
2. Schneller, J.M., Schneller, C., Martin, R., and Stahl, A.J.C. (1976) Nucleic Acids Res. 3, 1151-1165.
3. Diatewa, M., and Stahl, A.J.C. (1980) Biochem. Biophys. Res. Commun. 94, 189-198.
4. Felter, S., Diatewa, M., Schneider, C., and Stahl, A.J.C. (1981) Biochem. Biophys. Res. Commun. 98, 727-734.
5. Buck, C.A., and Nass, M.K. (1969) J. Mol. Biol. 41, 67-82.
6. Lynch, D.C., and Attardi, G. (1976) J. Mol. Biol. 102, 125-141.
7. Nielsen, J.B., and Haschemeyer, A.E.V. (1976) Biochemistry 15, 348-355.
8. Gabius, H.J., von der Haar, F., and Cramer, F. (1982) submitted to Biochemistry.
9. Igloi, G.L., von der Haar, F., and Cramer, F. (1979) Meth. Enzymol. 59, 282-291.
10. Warburg, O., and Christian, W. (1942) Biochem. Z. 310, 384-421.
11. Fairbanks, G., Steck, T.L., and Wallach, D.F.H. (1971) Biochemistry 10, 2606-2617.
12. Schneider, D., Solfert, R., and von der Haar, F. (1972) Hoppe-Seyler's Z. Physiol. Chem. 353, 1330-1336.
13. Bustamante, E., Soper, J.W., and Pedersen, P.L. (1977) Anal. Biochem. 80, 401-408.
14. De Duve, C., Pressman, B.C., Gianetto, R., Wattiaux, R., and Appelmans, F. (1955) Biochem. J. 60, 604-617.
15. von der Haar, F. (1978) FEBS Letters 94, 371-374.
16. Joachimiak, A., and Barciszewski, J. (1980) FEBS Letters 119, 201-211.
17. Ovchinnikov, Y.A., Egorov, C.A., Aldanova, N.A., Feigina, M.Y., Lipkin, V.M., Abdulaev, N.G., Grishin, E.V., Kiselev, A.P., Modyanov, N.N., Braunstein, A.E., Polyanovky, O.L., and Nosikov, V.V. (1973) FEBS Letters 29, 31-34.
18. Doonan, S., Doonan, H.J., Hanford, R., Vernon, C.A., Walker, J.M., Bossa, F., Barra, D., Carloni, M., Fasella, P., Riva, F., and Walton, P.L. (1973) FEBS Letters 38, 229-233.
19. Kagamiyama, H., Sakakibara, R., Wada, H., Tanase, S., and Morino, Y. (1977) J. Biochem. 82, 291-294.
20. Shlyapnikov, S.V., Myasnikov, A.N., Severin, E.S., Myagkova, M.A., Torchinsky, Y.M., and Braunstein, A.E. (1979) FEBS Letters 106, 385-388.
21. Steinman, H.M., and Hill, R.L. (1973) Proc. Natl. Acad. Sci. USA 70, 3725-3729.
22. von der Haar, F., Gabius, H.J., and Cramer, F. (1981) Angew. Chem. 93, 250-256; Angew. Chem. Int. Ed. Engl. 20, 217-223.
23. Gabius, H.J., von der Haar, F., and Cramer, F. (1982) submitted to Biochemistry.