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COMPARISON OF YEAST MITOCHONDRIAL Phe-tRNA SYNTHETASE SUBUNITS TO THEIR CYTOPLASMIC COUNTERPARTS : ISOLATION AND DETERMINATION OF AMINO ACID COMPOSITIONS.

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SUMMARY : The α and β subunits of yeast mitochondrial Phe-tRNA synthetase are separated and isolated by means of chromatography on DEAE-cellulose, after enzyme alkylation with iodoacetate. The comparison of amino acid compositions of yeast mitochondrial and cytoplasmic native Phe-tRNA synthetases and their components shows significant differences. Results indicate that the two enzymes are coded for by different nuclear genes.

INTRODUCTION : Yeast mitochondrial and cytoplasmic Phe-tRNA synthetases have the same $\alpha_2\beta_2$ structure (1,2) and are coded for by nuclear DNA (3). Although the test of immuno-inactivation showed that there is no cross-reactivity between antibodies of the two enzymes (4), we could not answer the question relative to the genetic origin of the different subunits building up these enzymes. In this respect, we have isolated the α and β subunits of yeast mitochondrial Phe-tRNA synthetase and compared their amino acid compositions with those of cytoplasmic homologous proteins.

MATERIAL AND METHODS

Yeast mitochondrial Phe-tRNA synthetase was purified according to (1).

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Abbreviations used : mt, mitochondrial ; ct, cytoplasmic ; PheRS, phenylalanyl-tRNA synthetase.

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Isolation of α and β subunits of yeast mitochondrial Phe-tRNA synthetase :

Reduction and carboxymethylation :

The modified procedure of Crestfield and coll. was used (5). Purified mitochondrial Phe-tRNA synthetase was dialyzed against 20 mM NH_4HCO_3 and lyophilized. The powder (3 mg) was dissolved in 3 ml of 0.5 M Tris-HCl buffer, pH 8.4, containing 2 mM EDTA, 7 M guanidine hydrochloride. 2-mercaptoethanol was added at a final concentration of 0.175 M. The reduction was performed under nitrogen for 2 h at + 50°C. The alkylation was carried out, under nitrogen, by adding iodoacetate in 0.5 M Tris-HCl buffer, pH 8.4, at a final concentration of 0.2 M. The alkylation reaction was proceeded for 40 min at + 25°C in the dark, and terminated by the addition of 0.175 M 2-mercaptoethanol. The sample was dialyzed against 10 mM potassium phosphate buffer, pH 7.4, containing 20 mM 2-mercaptoethanol 1 mM EDTA, 6 M urea (dialysis buffer) at + 4°C in the dark.

DEAE-cellulose chromatography

The dialyzed sample was applied to a column (1 x 8 cm) of DEAE-cellulose (DE-52, Whatman, Balston), previously equilibrated with dialysis buffer. Elution was performed with a linear gradient (2 x 15 ml) from 0 to 400 mM KCl in the same buffer at a flow rate of 12 ml/h. Fractions of 1 ml were collected.

The purity and the size of the subunits were examined by SDS-7.5 % acrylamide gel electrophoresis according to (6).

Amino acid analysis :

Fractions A and B of DEAE-cellulose chromatography were extensively dialyzed against 0.5 % (v/v) acetic acid and lyophilized. The samples were hydrolysed under nitrogen in 6 M HCl containing 0.5 % thiodiglycol (v/v) and a crystal of phenol for 24 h at + 110°C (7). The hydrolysates were analyzed on a Durrum D500 amino acid analyser. The cysteine and methionine contents were determined after performic acid oxydation (8). Tryptophan was estimated according to (9). The results correspond to the means of two experiments.

Identification of N-terminal residues :

The dansyl chloride method was used as described in (10,11).

RESULTS

Preparation of α and β subunits of yeast mitochondrial Phe-tRNA-synthetase

In order to study the structural similarities between mitochondrial and cytoplasmic subunits of yeast Phe-tRNA synthetases, we have developed an isolation procedure for pure individual subunits of yeast mitochondrial Phe-tRNA synthetase. The elution pattern of S-alkylated mitochondrial Phe-tRNA synthetase of yeast is shown in figure 1.

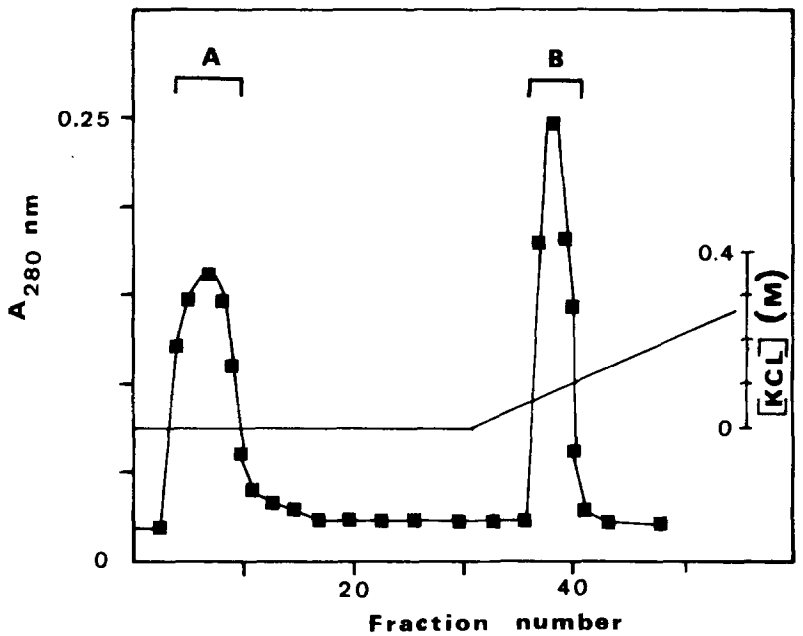

Figure 1 : Yeast mitochondrial Phe-tRNA synthetase α and β subunits chromatographic separation on the DEAE-cellulose column.

As revealed by SDS-7.5 % acrylamide gel, the unbound fraction A corresponds to the β subunit and bound fraction B eluted by 100 mM KCl contains the α subunit. No cross contamination could be detected in the different electrophoregrams. Faint bands, corresponding to the β_2 and α_2 structures as identified from their respective molecular weights and the N-terminal amino acids of fractions A and B, could be seen in the electrophoregrams corresponding to fractions A and B, respectively. These bands disappear in the presence of 2-mercaptoethanol.

Search for structural similarities

To settle the question whether structural similarities between the subunits of mitochondrial and cytoplasmic Phe-tRNA synthetases exist, we have compared their amino acid compositions determined in the same conditions. The results are presented in table I. The content of some amino acid residues is similar in the various subunits : Ile and Asx in the β subunits, Pro in the α subunits, and Thr, Arg in the α and β subunits. On the contrary, significant differences for all other amino acid residues appear between mitochondrial and cytoplasmic (7) subunits. The subunits of the two enzymes also exhibit differences in N-terminal residues. Using the dansyl chloride method

Table I : Amino acid compositions of yeast mitochondrial and cytoplasmic native Phe-tRNA synthetase and their subunits.

Amino acid	mt-α	mt-β	mt-2(α+β)	Native mt-PheRS	Mean for mt-PheRS	mt-Δ (%)	ct-α	ct-β	ct-2(α+β)	Native ct-PheRS
Cys	7	4	22	22	22	0.00	11	2	26	25
Asx	81	66	294	294	294	0.00	73	62	270	284
Thr	32	30	124	120	122	1.63	30	29	118	128
Ser	29	35	128	122	125	2.40	39	29	136	136
Glx	87	56	286	242	264	8.33	77	70	294	292
Pro	32	29	122	122	122	0.00	30	22	104	100
Gly	71	39	220	200	210	4.76	34	30	128	134
Ala	54	46	200	212	206	2.90	41	28	138	142
Val	38	35	146	170	158	7,30	34	25	118	120
Met	13	4	34	34	34	0.00	10	12	44	45
Ile	29	29	116	134	125	6.96	36	27	126	126
Leu	51	51	206	222	214	3.67	60	62	244	236
Tyr	14	11	50	54	52	3.77	20	16	72	68
Phe	19	19	76	80	78	2.53	30	28	116	114
Trp	8	1	18	16	17	5.71	5	7	24	24
His	10	9	38	32	35	8.23	14	13	54	56
Lys	61	48	218	226	222	1.78	47	40	174	176
Arg	24	21	90	90	90	0.00	27	20	94	90
Total	618	533	2388	2392	2390		617	522	2278	2296
Molecular Weight	72000	58000	260000	260000	260000		70000	60000	260000	260000
Reference							(7)	(7)	(7)	(7)

Values are expressed as amino acid residues per mole.
mt - Δ : experimental error calculated from the amino acid compositions of mt Phe RS and mt - 2 (α+ β).

we have only found one amino acid, Glu, for the mitochondrial native enzyme and its β subunit, whereas for the α subunit, no DNS - amino acid can be detected. As reported in (7), the cytoplasmic native enzyme and its α subunit show Pro residue in the NH_2-terminal position, and no NH_2-terminal residue is dectected with the β subunit. These results clearly demonstrate that there is no similarity in the compositions of the subunits of the two homologous enzymes within the limits of experimental error.

The data of amino acid analysis show that the smaller mitochondrial β subunit is not a proteolysed form of the larger mitochondrial α subunit. There are more serine residues in the β subunit than in the α subunit. The composition of the mitochondrial native enzyme is in good agreement with that of the $\alpha_2\beta_2$ tetramer calculated on the basis of the compositions of isolated mitochondrial subunits.

DISCUSSION

Our results demonstrate that mitochondrial subunits of yeast Phe-tRNA synthetase can be considered as pure, according to different analytical tests used : polyacrylamide gel electrophoresis, NH_2-terminal amino acid analysis, amino acid compositions.

In a recent work (12), we demonstrated by means of enzyme-linked immunosorbent assay (ELISA) that the native mitochondrial Phe-tRNA synthetase and the isolated mitochondrial enzyme subunits have a good specificity towards antibodies against pure mitochondrial Phe-tRNA synthetase. In contrast, no cross-reactivity could be observed with native cytoplasmic Phe-tRNA synthetase (12). The differences in the amino acid compositions between mitochondrial and cytoplasmic subunits show that the immunological behaviour may be due to difference in the nature of the antigenic sites. Immunological results and amino acid analysis firmly enable us to conclude that the mitochondrial and cytoplasmic α or β subunits are coded for by different nuclear genes.

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