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Amino acid sequence of a new mitochondrially synthesized proteolipid of the ATP synthase of *Saccharomyces cerevisiae*

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The purification and the amino acid sequence of a proteolipid translated on ribosomes in yeast mitochondria is reported. This protein, which is a subunit of the ATP synthase, was purified by extraction with chloroform/methanol (2/1) and subsequent chromatography on phosphocellulose and reverse phase h.p.l.c. A mol. wt. of 5500 was estimated by chromatography on Bio-Gel P-30 in 80% formic acid. The complete amino acid sequence of this protein was determined by automated solid phase Edman degradation of the whole protein and of fragments obtained after cleavage with cyanogen bromide. The sequence analysis indicates a length of 48 amino acid residues. The calculated mol. wt. of 5870 corresponds to the value found by gel chromatography. This polypeptide contains three basic residues and no negatively charged side chain. The three basic residues are clustered at the C terminus. The primary structure of this protein is in full agreement with the predicted amino acid sequence of the putative polypeptide encoded by the mitochondrial *aap*1 gene recently discovered in *Saccharomyces cerevisiae*. Moreover, this protein shows 50% homology with the amino acid sequence of a putative polypeptide encoded by an unidentified reading frame also discovered near the mitochondrial ATPase subunit 6 gene in *Aspergillus nidulans*.
Key words: ATP synthase/mitochondrially translated/proteolipid/sequence/subunit

Introduction

A small number of polypeptides is synthesized by mitochondria. In yeast the first product clearly identified was a proteolipid of mol. wt. 8000 (Sierra and Tzagoloff, 1973). This protein, called subunit 9 of the ATP synthase was identified as the dicyclohexylcarbodiimide (DCCD)-binding protein (Sebald *et al.*, 1979a) and it is involved in proton translocation through the F_O part of the ATP synthase (Fillingame, 1980). The *Oli* 1 gene has been shown to code for the DCCD-binding protein (Hensgens *et al.*, 1979; Macino and Tzagoloff, 1979).

A second subunit implicated in oxidative phosphorylation (Murphy *et al.*, 1980) is also translated on mitochondrial ribosomes. This protein of mol. wt. 21 500 is the product of the *Oli* 2 locus (Macino and Tzagoloff, 1980). There is evidence that a third subunit, translated on mitochondrial ribosomes also belongs to the ATP synthase complex (Esparza *et al.*, 1981; Macreadie *et al.*, 1982). This subunit has an apparent size of 10 kd based on its mobility in SDS-polyacrylamide gels (Orian *et al.*, 1981; Esparza *et al.*, 1981). We have previously shown that this protein is soluble in organic solvents and can bind inorganic phosphate *in vitro* (Guérin and Napias, 1978; Velours *et al.*, 1980).

Macreadie *et al.* (1983) proposed that the gene *aap1* located between the *Oli* 2 and *Oxi* 3 loci in *Saccharomyces cerevisiae* codes for the 10 kd polypeptide for the following reasons: (i) mutations in this region cause the loss of the 10-kd polypeptide; (ii) the amino acid composition of this protein (Velours *et al.*, 1982) fits very well with the amino acid composition of the predicted *aap1* gene product.

Here we report the primary structure of this hydrophobic protein which is in fact 48 residues long. Its amino acid sequence was identical with the predicted gene product (Macreadie *et al.*, 1983) and was 50% homologous with the predicted *Aspergillus nidulans* URF-x gene product (Grisi *et al.*, 1982).

Results

Purification of the proteolipid

We modified the extraction procedure of Velours *et al.* (1980) since the amount of pure protein recovered was not sufficient for sequencing. As described in Materials and methods, we used the extraction procedure of the DCCD-binding protein proposed by Cattell *et al.* (1971). The diethyl ether proteolipid

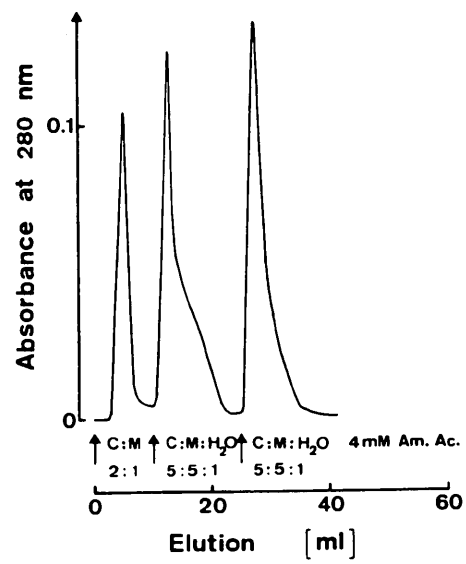

Fig. 1. Chromatography of proteolipids on phosphocellulose. Phosphocellulose powder was successively washed with water, glacial acetic acid, water and the pH of the suspension was brought to 9 with ammonia. The slurry was poured into a column (1 x 6 cm). The column was washed with methanol and chloroform/methanol (2/1). 20 mg of proteolipids in chloroform/methanol (2/1) were loaded. The separation was performed at a flow rate of 0.5 ml/min with (C:M) chloroform/methanol (2/1), (C:M:H_2O) chloroform/methanol/ water (5/5/1); finally the ionic strength was increased by addition of 4 mM of ammonium acetate (Am.Ac) to the last solvent mixture. Elution was followed at 280 nm.

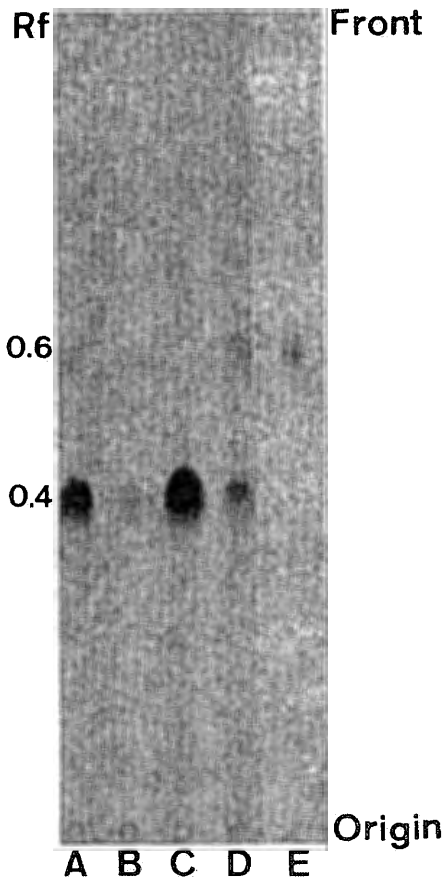

Fig. 2. T.l.c. of proteolipids. Mitochondria were labelled *in vivo* by $^{35}SO_4^{2-}$ in the presence of cycloheximide (Velours *et al.*, 1980). The proteolipids were extracted and purified. Aliquots were analysed by t.l.c. on reverse phase plates, and autoradiography of the plates were performed. **(A)** Dialyzed diethyl ether proteolipid pellet; **(B)** diethyl ether proteolipid supernatant; **(C)** phosphocellulose chromatography:chloroform/methanol/water (5/5/1) fraction; **(D)** phosphocellulose chromatography: 4 mM ammonium acetate fraction; **(E)** reverse phase h.p.l.c.: fraction chloroform/methanol (1/2), 10% water and 0.24 M of ammonium acetate.

pellet was dialyzed to remove phospholipids. The subsequent purification steps were essentially the same as described before (Velours *et al.*, 1982). However, this change in the extraction procedure resulted in the presence of high amounts of DCCD-binding protein in the extracts. This problem was solved by using phosphocellulose chromatography (Figure 1). Non-protein material including phospholipids was eluted with chloroform/methanol, 2/1. The major part of the DCCD binding protein (*Rf* = 0.4 in the t.l.c. system in Figure 2C) was eluted with chloroform/methanol/water, 5/5/1. Increasing the ionic strength by adding 4 mM ammonium acetate led to the elution of a third fraction. The DCCD-binding protein was also present in this fraction, but a considerable enrichment of the other mitoribosomically translated proteolipid (*Rf* = 0.6) was achieved (Figure 2D). Analysis of this last fraction by reverse phase h.p.l.c. gave results identical to Figure 2 of Velours *et al.* (1982). The first eluted peak contained a pure protein showing an electrophoretic mobility corresponding to an apparent mol. wt. of 10 000 (Velours *et al.*, 1982). This fraction was totally free of DCCD-binding protein as shown in Figure 2E, and was used for sequencing.

The amount of protein recovered after each purification step is compiled in Table I. The pure proteolipid was submitted to gel filtration on a Bio-Gel P-30 column equilibrated in 80% formic acid. From mol. wt. standards a 5500 mol. wt. value was calculated for the proteolipid (Figure 3).

Table I. Protein recovery during the purification of the proteolipid

Fractions	Protein (mg)[a]
Mitochondria	1000
Diethyl ether proteolipid pellet[b]	6.5
Phosphocellulose chromatography (4 mM ammonium acetate fraction)	0.5
Reverse phase h.p.l.c. (chloroform/methanol 1/2, 10% water fraction)	0.08

[a]The protein samples were dissolved in 5% SDS, then submitted to the method of Lowry *et al.* (1951).
[b]The amount of protein was determined after dialysis.

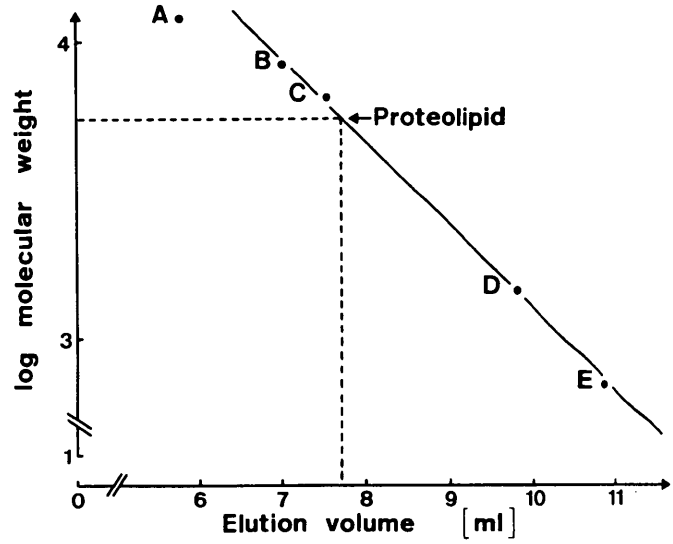

Fig. 3. Gel chromatography of the purified proteolipid. The protein (50 μg) was precipitated at −20°C upon diethyl ether addition. After centrifugation, the pellet was solubilized in 100% formic acid. Chromatography was performed on a Bio-Gel P-30 (minus 400 mesh) column (0.4 x 150 cm). Elution was performed in 80% formic acid at a flow rate of 0.7 ml/h. Aliquots were withdrawn from the collected tubes and analysed by t.l.c. on silica plates (HPTLC Fertigplatten Kieselgel 60 F 254 mit Konzentrierungszone; Merck). The plates were developed in n-butanol/acetic acid/water/pyridine (68/14/25/40). The proteins were visualized by u.v. light at 366 nm. Protein standards were: A: cytochrome *c*; B: DCCD-binding protein from *E. coli*; C: aprotinin; D: bacitracin; E: Leu-Trp-Met-Arg-Phe. The dotted lines intercept the abscissa at 7.7 ml and the log mol. wt. scale at 3.74.

Sequence analysis

Analysis of the whole protein. The amino acid composition of the purified proteolipid (Table II) shows an abundance of hydrophobic residues except for glycine and alanine which differentiates this protein from the DCCD-binding protein of yeast mitochondria. Alanine, cysteine, histidine and tryptophan are absent. The percentage amino acid composition was in agreement with the one previously published (Velours *et al.*, 1982). However, a slight deviation occurred in the amounts of aspartic acid, glutamic acid, and alanine residues, probably due to the increased purity of the proteolipid.

The whole protein was immobilized on diisothiocyanate-activated glass since the amino acid composition revealed a

Table II. Amino acid analysis of the whole protein and the peptides after cyanogen bromide cleavage

Amino acid	Whole protein		Amount in CNBr fragments			
	mol/mol	mol/100	CB 2[b]	CB 3[b]	CB 4[b]	CB 5[c]
			mol/mol			
Tryptophan	n.d. (0)	0				
Cysteine	0 (0)	0				
Aspartic acid	1.1 (1)	2.1		1.1 (1)		
Methionine sulfone	3.9 (4)	8.3				
Threonine	2.0 (2)	4.2		0.9 (1)	0.9 (1)	
Serine	3.0 (3)	6.2			0.9 (1)	1.9 (2)
Homoserine	0 (0)	0	0.5 (1)	traces(1)	0.6 (1)	
Glutamic acid	3.0 (3)	6.2	0.9 (1)	0.9 (1)	0.9 (1)	
Proline	3.2 (3)	6.2	n.d.(2)	n.d. (0)	n.d.(1)	n.d.(0)
Glycine	1.1 (1)	2.1		1.3 (1)		
Alanine	0 (0)	0				
Valine	2.1 (2)	4.2	0.6 (1)			1.0 (1)
Isoleucine	3.4[a] (4)	8.3			1.9 (2)	1.9 (2)
Leucine	10.2[a] (12)	25	1.3 (1)	3.1 (3)	4.2 (4)	4.0 (4)
Tyrosine	2.7 (3)	6.2	0.4 (1)	0.4 (1)		0.5 (1)
Phenylalanine	6.8 (7)	14.6	2.0 (2)	1.0 (1)	3.0 (3)	1.1 (1)
Histidine	0 (0)	0				
Lysine	1.1 (1)	2.1				1.1 (1)
Arginine	2.1 (2)	4.2				2.0 (2)
	48	99.9	9	10	14	14

[a]Despite prolonged hydrolysis times (72 h), the molar amounts of isoleucine and leucine were smaller than expected.
[b]Cyanogen bromide peptides were isolated by reverse phase h.p.l.c. with formic acid, water and propanol as solvents (Figure 5).
[c]Cyanogen bromide peptides were isolated by gel chromatography on Bio-Gel P-30 in 80% formic acid (Figure 4).
The values in parenthesis represent the numbers of amino acid residues obtained after sequence analysis. n.d.: not determined.

single lysine residue. The Edman degradation started with methionine only when the glass-bound protein had been treated with methanolic HCl. This suggests that formyl-methionine is the amino-terminal residue. In one sequencer run employing 60 nmol of protein, the first 46 residues of the amino acid sequence were established (Figure 6). After residue 46 which is a serine, no amino acid residue was detected by reverse phase h.p.l.c. of phenylthiohydantoin derivatives. Because of the coupling procedure used, position 47 was most likely occupied by the single lysine residue.

Cyanogen bromide fragments. The proteolipid contains four methionines at position 1, 10, 20 and 34 which were discovered by the sequence analysis of the whole protein. Cyanogen bromide cleavage after these residues yields four fragments which were isolated by gel chromatography (Figure 4) or by reverse phase h.p.l.c. (Figure 5). Fragment CB 5 was isolated by gel chromatography. This peptide which contains 14 amino acid residues is devoid of homoserine. It was immobilized to the aminated glass via the C terminus. Amino acid analysis and sequencing revealed this peptide to cover the C-terminal sequence of the protein, beginning with isoleucine 35. Lysine was found to be the residue 47. A leucine was identified at position 48. There were no amino acid derivatives

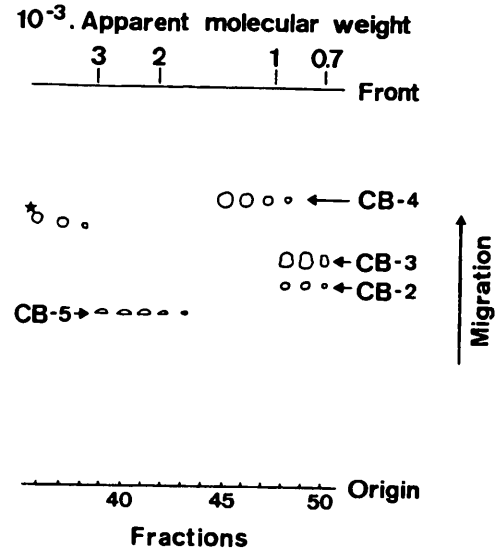

Fig. 4. Gel chromatography of cyanogen bromide fragments of the proteolipid. 350 μg of protein were cleaved with cyanogen bromide. The fragments were dissolved in 100% formic acid and chromatographed on a Bio-Gel P-30 (minus 400 mesh) column (0.4 x 150 cm). Elution of the column and analysis of each tube content were performed as described in the legend of Figure 3. Only the part of the t.l.c. containing the cyanogen bromide fragments is shown. ★ uncleaved protein.

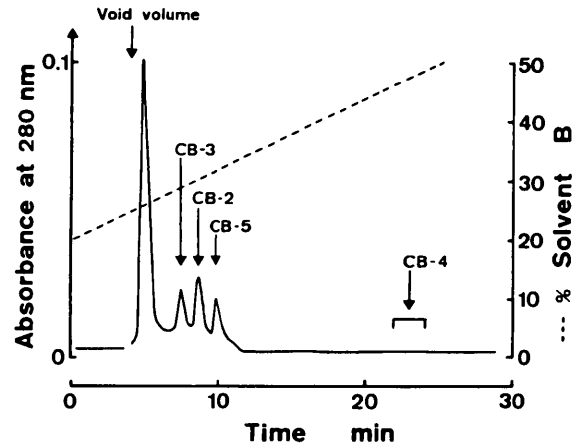

Fig. 5. Reverse phase h.p.l.c. of the cyanogen bromide fragments. 25 μg of cyanogen bromide fragments were separated on a Lichrosorb RP 18−5 μ column eluted for 1 h with 60% formic acid in water (solvent A) and a linear gradient of 60% formic acid in 2-propanol (solvent B). Elution (0.6 ml/min) was followed at 280 nm and by analysis of each tube by t.l.c. as in Figure 3. Only half of the elution time is reported on the figure. The large first peak did not contain peptide material as judged from 6 N HCl hydrolysis followed by amino acid analysis.

found after position 48. Thus, the C-terminal amino acid residue of the protein is a leucine.

While only fragments CB 5 and CB 4 were obtained in pure form after Bio-Gel P-30 chromatography, a complete separation of the four peptides was achieved by reverse phase h.p.l.c. using formic acid and 2-propanol (Figure 5). The fragments CB 3 and CB 4 were sequenced in order to confirm the sequence obtained from the whole protein. Figure 6 compiles all the data establishing the sequencing.

Secondary structure and membrane integration. Using the method of Von Heijne (1981) which considers the free energy gain upon transition of a given amino acid residue from a coil

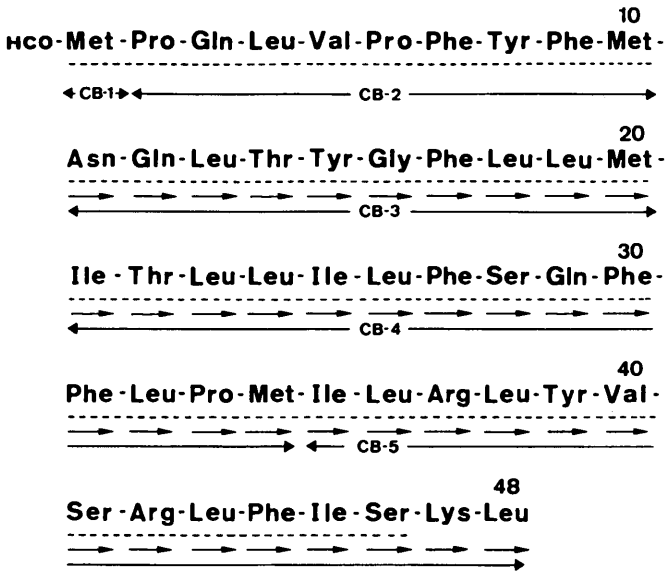

Fig. 6. Amino acid sequence of the proteolipid isolated from yeast mitochondria. - - - the dotted line indicates residues identified by automatic solid phase Edman degradation of the whole protein. → short arrows indicate residues identified from the purified peptides.

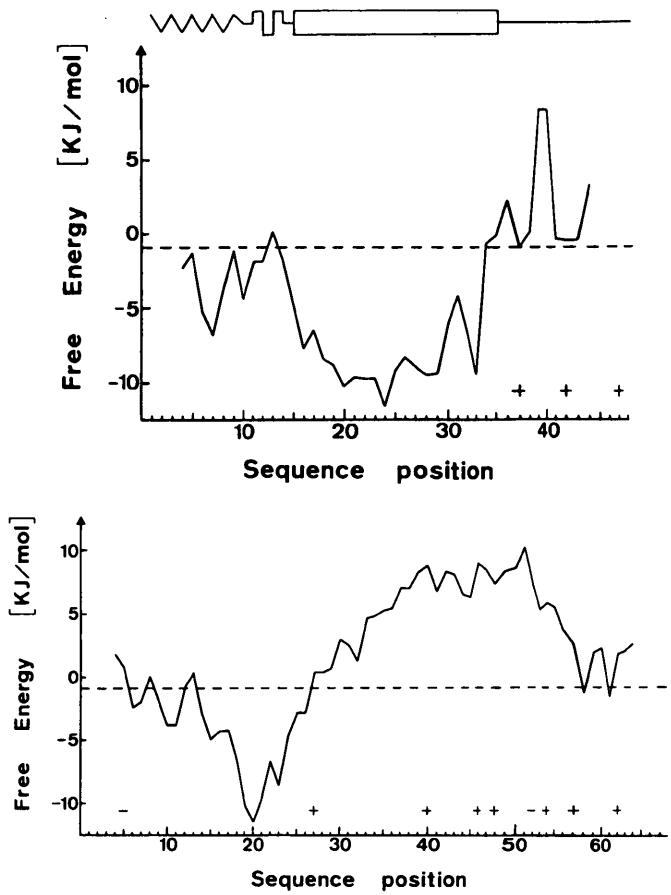

Fig. 7. Prediction of secondary structure and membrane permeating segment into the proteolipid. α-helical segments (▭); β-structure (⋀⋁); β-turn (⌐⌐); random coil (———) were calculated applying four different prediction methods. The free energy gains during a transition from water to the hydrophobic medium of the membrane, of seven consecutive amino acids was calculated for all amino acid sequence positions. The area below the dotted line indicates increased probability for a location in the lipid bilayer. Positive and negative charges are indicated. A: proteolipid of yeast mitochondria. B: product of URFA6L of human mitochondria.

structure in water into an helical structure in the membrane, residues 15–35 are predicted to be located in the membrane (Figure 7A). The secondary structure prediction methods (Maxfield and Scheraga, 1976; Robson and Suzuki, 1976; Nogano, 1977; Chou and Fasman, 1978) consistently indicated an α-helix in this range. Thus, the protein very likely traverses the membrane once by this α-helical segment. Stretches of ~20 hydrophobic residues are found in almost all membrane proteins analysed so far or in leader sequences of secretory proteins and it is now generally accepted that an α-helix is the thermodynamically optimal conformation for a peptide chain integrated in the membrane. Around position 14 a β-turn is indicated. Due to the hydrophilicity of the involved residues this segment is probably located outside the membrane. For the N terminus from residue 1 to 9 the predictions gave ambiguous results slightly favoring a β-sheet structure. In this segment are located two proline residues which prevent the formation of a continuous α-helix. This results in several unpaired backbone amide and carbonyl groups providing a large hydrophilic area which most likely is not integrated in the membrane.

Clearly the C-terminal part from residue 36 is predicted to be outside the membrane. In this segment are located the three basic residues. The secondary structure might be a random coil.

Discussion

Main features of this proteolipid

This polypeptide contains only 48 residues. Methionine is the N-terminal residue and leucine is the C-terminal residue. The abundance of hydrophobic amino acid residues as well as the small size may explain its solubility in chloroform/methanol (2/1). As in the DCCD-binding proteolipids, tryptophan, histidine, cysteine are missing (Fillingame, 1976; Graf and Sebald, 1978; Sebald *et al.*, 1979b; Hoppe and Sebald, 1980). A difference between this protein and the DCCD-binding proteolipids sequenced so far is the low amount of glycine and the absence of alanine. Furthermore, this protein does not contain glutamic or aspartic acid residues, and thus no negatively charged side chains. This proteolipid is a basic protein since two arginine, one lysine and only one carboxyl group (C-terminal residue) are present. Such behaviour may explain the order of elution during phosphocellulose chromatography (Figure 1) since the DCCD-binding protein contains no net charge (Sebald *et al.*, 1979b) (three positives and three negatives). The basic property may also account for the binding of phosphate (Guérin and Napias, 1978; Velours, 1982).

A mol. wt. of 5870 daltons was calculated. This result agrees very well with the 5500 mol. wt. value obtained by gel chromatography. Furthermore, only four peptidic fragments were recovered after cyanogen bromide cleavage of the whole protein, which is in agreement with the amount of four methionines (one is N-formyl-methionine, and three methionines are located inside the polypeptide chain). However, this protein migrated with an apparent mol. wt. of 10 000 during polyacrylamide electrophoresis containing SDS (Velours *et al.*, 1982). This result means either the protein is still an aggregate (dimer) in SDS-gel electrophoresis, or the high hydrophobicity and the basic behaviour of this protein

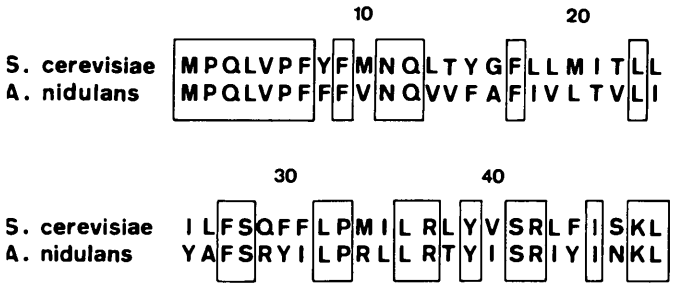

Fig. 8. Conserved residues in proteolipids of two fungi. The amino acid sequence of the proteolipid isolated from yeast mitocondria was compared with the predicted amino acid sequence encoded by the URF-x of *A. nidulans* mitochondrial DNA. ▭ area shows homologous amino acids.

cause an abnormal migration. Intrinsic charges of polypeptides become relatively important at low mol. wts. as shown by Swank and Munkres (1971).

Evidence that this proteolipid is encoded by the mitochondrial DNA

It was previously reported that this protein was translated on mitochondrial ribosomes (Velours *et al.*, 1980, 1982), since it was labelled *in vivo* in the presence of cycloheximide. Macreadie *et al.* (1982) reported that two *mit*$^-$ mutations cause the loss of the 10 000 mol. wt. polypeptide in the ATP synthase of *S. cerevisiae*. These mutations appeared to map outside known mitochondrial genes, between the *Oxi* 3 and *Oli* 2 genes (Macreadie *et al.*, 1983). DNA sequencing in this region revealed two open reading frames potentially coding for polypeptides 48 and 53 amino acids in length. However, correlation with amino acid composition of the gene product (Velours *et al.*, 1982) and considerations of codon usage favored the polypeptide 48 amino acids long, denoted subunit 8 of the ATP synthase, as the product of this new gene called *aap1* (ATPase-associated protein). Our data confirm the assumption that the polypeptide 48 amino acids long is translated. The predicted sequence and the primary structure described in this paper are identical despite the fact that the *S. cerevisiae* strains studied were unrelated.

There is evidence that this protein is encoded in mitochondrial DNA of another fungus. An unidentified reading frame (URF-x) was located near the ATPase subunit 6 gene of *A. nidulans* (Grisi *et al.*, 1982). This URF could code for a polypeptide 48 amino acids long. Comparison between the predicted sequence and the primary structure of the proteolipid described here revealed 50% homology (Figure 8). An analogous gene found in mammalian mitochondrial DNA could be URF A6L which overlaps the ATPase subunit 6 gene (Anderson *et al.*, 1981, 1982; Bibb *et al.*, 1981). Despite a low homology between the predicted amino acid sequences and the polypeptides of fungi, the following analogies could be found between URF A6L product and the proteolipid of yeast mitochondria (Figure 7A and B). (i) These proteins are basic; (ii) the positively charged side chains are located on the C-terminal part of the protein outside the membrane; (iii) a common hydrophobic region most likely embedded in the lipidic bilayer is observed; (iv) a more striking similarity occurs at the N-terminal portions of the proteins which exhibit the common sequence Met-Pro-Gln-Leu-.

The high conservation of the N-terminal part of the protein may indicate an important role of the N terminus either in the assembly of the complex or in the relationships with other subunits.

The proteolipid described here is a membranous protein which appears to be a subunit of F_O (Esparza *et al.*, 1981; Macreadie *et al.*, 1982) in eucaryotic cells. No analogous polypeptide occurs in preparations of F_O from *Escherichia coli* (Negrin *et al.*, 1980; Friedl and Schairer, 1981). On the other hand, an open reading frame (gene 1, preceding the subunit a gene) was found in the gene cluster coding for the ATP synthase (Gay and Walker, 1981; Kanazawa *et al.*, 1981; Nielsen *et al.*, 1981). The gene 1 could code for a polypeptide of 127 amino acids. This putative product would be a basic protein, probably also membrane embedded, but there are no homologous sequences between the gene 1 product and the proteolipid described here.

The function of the proteolipid is not clear at present. A role in phosphate binding at the ATP synthase level or in phosphate translocation was postulated since a phosphate binding activity was observed (Guérin and Napias, 1978; Velours, 1982), for the polypeptide isolated either from whole mitochondria or from the ATP synthase.

It is noteworthy that a proteolipid capable of phosphate binding (Blondin, 1979) has been isolated from beef heart mitochondria; its amino acid composition fits well with the predicted amino acid composition of the putative product of URF A6L (Anderson *et al.*, 1982). Thus it appears that the phosphate-binding protein is the product of the *aap1* gene in yeast and of the URF A6L in bovine heart mitochondria. It will be interesting to test a possible ionophoric property of the proteolipid in reconstituted systems.

Materials and methods

Cell culture and preparation of mitochondria

Cells of the diploid yeast strain *Yeast Foam* were grown aerobically, as previously described, in a medium containing 0.8% glucose (Arselin de Chateaubodeau *et al.*, 1976). The preparation of ^{35}S-labelled or unlabelled mitochondria has been described by Velours *et al.* (1980).

Preparation of the proteolipid

Proteolipids were extracted from yeast mitochondria (30 mg protein/ml) by 25 volumes of chloroform/methanol (2/1). The suspension was stirred at room temperature for 18 h. After filtration, the crude extract was washed by 1/5 volume of water. The emulsion was centrifuged at 2000 g for 30 min at room temperature. The organic phase was concentrated to dryness. The lipidic residue was dissolved in a mixture of chloroform/methanol (2/1) (6 ml per g of extract). Then 4 volumes of pre-cooled diethyl ether were added to the mixture which was left at −20°C for 18 h. The precipitated proteolipids were recovered by centrifugation at 2000 *g* for 30 min at −20°C. The pellet was dissolved in a few ml of chloroform/methanol (2/1). This solution was dialyzed for 3 days against a chloroform/methanol (2/1) mixture in order to remove most of the phospholipids. Dialysis tubings retaining 12 000 − 14 000 daltons were previously acetylated according to Craig (1967). Aggregates were then removed by centrifugation. The supernatant was fractionated by ion exchange chromatography as described earlier (Velours *et al.*, 1982). The fraction containing the proteolipid was finally purified by reverse phase h.p.l.c. The proteolipid was kept in chloroform/methanol 2/1 at −20°C under nitrogen. The purity of the proteolipid was checked by reverse phase t.l.c. Plates (Whatman KC 18 F) were developed in the following mixture: chloroform/methanol (1/2) containing 0.24 M of ammonium acetate and 6% of water. After drying the spots were visualized by iodine vapours and by autoradiography when the cells were previously *in vivo* labelled.

Purification of cyanogen bromide fragments

120 μg of proteolipid were dried, and dissolved in 400 μl of formic acid containing 100 mg/ml of CNBr. After solubilization of the sample, 100 μl of water were added and the mixture was left at room temperature for 24 h. The solvents were removed in a flash evaporator at 50°C. The products were dissolved in 100% formic acid.

The peptide fragments were fractionated on a Bio-Gel P-30 column (minus 400 mesh, 0.4 x 150 cm) in 80% formic acid. Flow rate was 0.7 ml/h. Fractions of 0.23 ml were collected. Aliquots were taken for t.l.c. amino acid analysis and sequencing (Hoppe and Sebald, 1980).

The peptide fragments were also separated by reverse phase h.p.l.c. with a Lichrosorb RP 18 column (5 μm, Merck). Separation was achieved by a gradient from 60% formic acid in water (solvent A) to 60% formic acid in 2-propanol (solvent B). Flow rate was 0.6 ml/min. Gradient duration was 1 h. Elution was monitored at 280 nm. Fractions of 0.6 ml were collected. Aliquots were taken for t.l.c. amino acid analysis and sequencing.

Sequencing methods

300 μg of whole protein were precipitated at −20°C with 4 volumes of diethyl ether. After centrifugation, the pellet was dissolved in 500 μl of chloroform/methanol (2/1). 50 μl of triethylamine were added to the solution. 100 mg of phenylenediisothiocyanate-activated porous glass beads (230 Å) were then added and incubated for 16 h at room temperature. 100 μl of ethanolamine were then added and the incubation proceeded again for 1 h. The glass was washed with chloroform/methanol (2/1), methanol, trifluoroacetic acid. Deformylation was carried out in 1 M HCl dissolved in dry methanol for 4 h at room temperature. The glass was washed with methanol, diethylether and then dried.

Peptides obtained after cyanogen bromide cleavage and containing a C-terminal homoserine were coupled in dimethyl-formamide to the aminopropyl-glass by the homoserine lactone procedure (Horn and Larusen, 1973). Immobilization of the C-terminal containing peptide was achieved after protection of the ε amine group of the lysine residue by phenylisothiocyanate (Beyreuther *et al.*, 1977). Coupling to the aminated glass was performed at 37°C for 16 h in trifluoroethanol/dimethylformamide (1/1) in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole (Laursen, 1977). The amino acid sequences were determined with a solid-phase sequencer (model 12, Sequemat, Watertown, USA). Identification of the phenylthiohydantoin amino acids was performed by t.l.c. (Hoppe and Sebald, 1980) or by reverse phase h.p.l.c. (Lottspeich, 1980). Amino acid analyses were performed with an automated Biotronic (LC 2000) amino acid analyser.

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