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A YEAST MITOCHONDRIAL INNER MEMBRANE 30K HYDROPHOBIC PROTEIN : COMPARISON WITH SUBUNIT 32K OF THE CYTOCHROME BC 1 COMPLEX

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SUMMARY: A yeast mitochondrial inner membrane hydrophobic protein 30K has been isolated and compared to subunit 32K of the yeast cytochrome bc 1 complex. Both proteins are translated on mitochondrial ribosomes, have nearly the same molecular weight and similar aminoacid compositions. Comparison was carried out by immunological techniques with specific antibodies, and by studying 3 yeast strains having mutations in the COB region of the mitochondrial DNA. Results show that the two proteins are not identical.

INTRODUCTION : A 30 kilodalton (30K) hydrophobic protein was extracted from the yeast mitochondrial inner membrane. Aminoacid composition, apparent molecular weight, molar ratio of acidic to basic aminoacids, and polarity were previously determined (1). Interaction between this protein and the other inner membrane constituents, especially phospholipids was studied (2). No information is available concerning the function of this protein.
Among the other known yeast mitochondrial inner membrane proteins synthesized on mitoribosomes, only subunit 32K of the cytochrome bc 1 complex (3, 4) has similar properties to the 30K hydrophobic protein. Therefore we compare the two proteins using three immunological procedures : ELISA (Enzyme-linked immunosorbent assay), Ouchterlony gel immunodiffusion and precipitation of protein-antibody complexes by Staphylococcus aureus.
On the other hand, 3 yeast strains bearing mutations in the COB region of the mitochondrial DNA are used to study the influence of the mutation on the 30K hydrophobic protein synthesis.

MATERIALS AND METHODS

Saccharomyces cerevisiae strain ρ^+ IL 8-8C is grown as described in (5). Preparation of mitochondria is performed according to (6) except that cytohelicase (I.B.F., 92390 VILLENEUVE LA GARENNE) is used (7). Mitochondria are further purified on a sucrose gradient (8). Separation, delipidation and purification of the inner membrane 30K hydrophobic protein is performed as described in (1).
Antibodies against the hydrophobic proteins are produced in rabbits by means of four consecutive injections of the hydrophobic protein in a 8 week period : the first injection in the muscle comprised 0.6 mg of protein in 1‰ (w/v) SDS solution supplemented with complete Freund's adjuvant ; the

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second is 1 mg in 1 ‰ SDS intravenously, the third is 0.6 mg in 1 ‰ SDS subcutaneously supplemented with complete Freund's adjuvant and the last is 1 mg in 1 ‰ SDS intravenously.
Antibodies against the yeast apocytochrome b were kindly provided by G. SCHATZ and are described in (9).
The immunological analysis of the hydrophobic protein is performed as in (10) for ELISA and as in (11, 12) for the Ouchterlony immunodiffusion.
Precipitation of antigens complexed with antibodies by the Staphylococcus aureus protein A (13) is realized as follows : the 30K hydrophobic protein is mixed with antibodies against the cytochrome b ; then S. aureus bearing protein A is added to precipitate the complex ; the antigen-antibodies complexes are eluted with a tris-buffer 10 mmol/l, 2-mercaptoethanol 0.15 mol/l, EDTA 1 mmol/l, glycerol 10 % (v/v) containing 1 % SDS. Eluted products are deposited on 9 per cent polyacrylamide gel. The same procedure is realized with a total inner membrane proteins solution containing the cytochrome bc 1 complex, as a positif control and with the 30K hydrophobic protein mixed with non immunized rabbit serum as a negatif control.
Gel electrophoresis is carried out in presence of 1 % (w/v) SDS according to (14) with 4 mol/l urea added. Protein is estimated by the method of Lowry (15) with bovine serum albumin as a standard.
The mutant yeasts used are : mit$^-$ cyt b$^-$226, a pin point mutation in COB region (16) ; G171, a box (4-2) mutation leading to synthesis of 14 K cytochrome b (17), instead of normal 32 K cytochrome b as in the wild strain; G 625 a large deletion, with no cytochrome b synthesis at all (18). They are grown anaerobically as described in (5), except that the concentration of the galactose in the growth medium is increased to 10 % and glucose is omitted. Preparation of promitochondria is performed as in (19).

RESULTS

1. The 30K hydrophobic protein tested by ELISA against specific 30K hydrophobic protein antibodies shows a maximal colour intensity (p. nitroaniline liberated) at 400 nm when the antigen-antibody ratio (w/v) is 1/10 ; it does not improve with higher amounts of the antibodies (Figure 1). Tested against cytochrome b antibodies, the hydrophobic protein does not react.

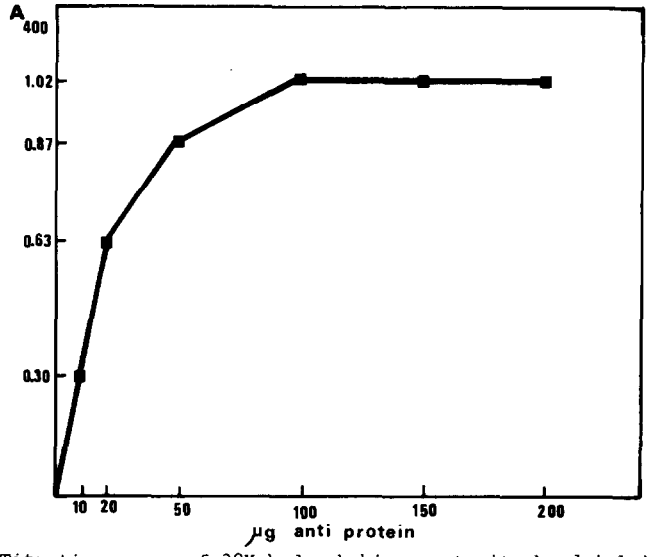

Figure 1 : Titration curve of 30K hydrophobic yeast mitochondrial inner membrane protein (10 µg) with specific antibodies, by ELISA . Secondary antibodies are labeled with E. coli alcaline phosphatase.

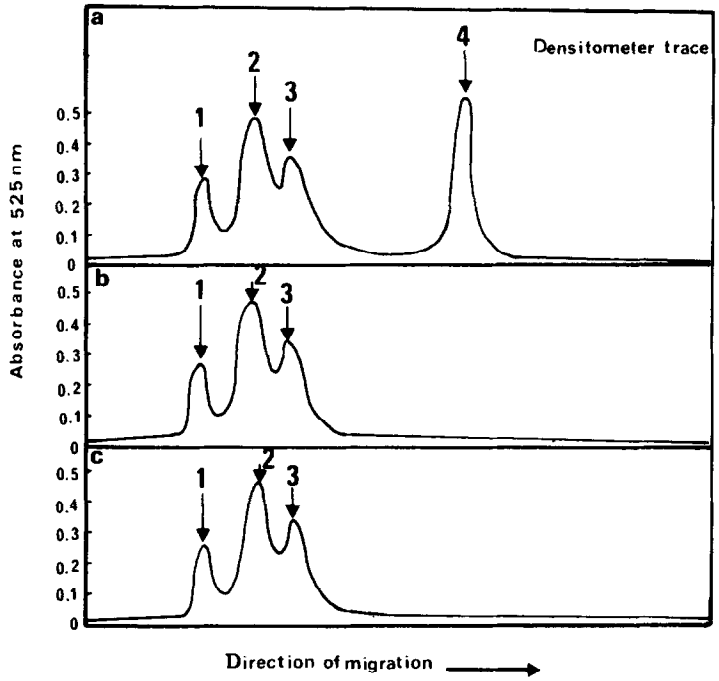

Figure 2 : 9 % polyacrylamide gel electrophoresis of immune complexes obtained with yeast cytochrome b antibodies, precipitated with S. aureus protein A and eluted with tris-buffer.

a) Total mitochondrial inner membrane proteins (containing cytochrome b).
b) 30K hydrophobic protein.
c) 30K hydrophobic protein with non immunized rabbit serum (negatif control).

1,2,3 : immunoglobuline fraction ; 4 : cytochrome b 32K subunit.

2. Complex precipitation with protein A : Figure 2 shows gel electrophoresis densitometer trace of the three complexes obtained with 30K hydrophobic protein and total inner membrane proteins against cytochrome b antibodies, and 30K hydrophobic protein against non immunized rabbit serum ; peaks 1, 2, 3 correspond to immunoglobulines, 4 to subunit 32K of cytochrome b. No precipitation could be obtained when the 30K hydrophobic protein is treated with the cytochrome b antibodies (Fig. 2b). Subunit 32K of cytochrome b present in the total inner membrane proteins solution is shown to be precipitated by cytochrome b antibodies (Fig. 2a).

3. Ouchterlony immunodiffusion : The center well contains 20 µl of the cytochrome b antibodies, the peripheral well 10 µl of 30K hydrophobic protein ; no detectable precipitation lines could be obtained after diffusion and staining. But on the other hand precipitation lines are obtained either with the cytochrome b antibodies tested against the inner membrane fraction, or with the hydrophobic protein tested against its specific antibodies (Results not shown).

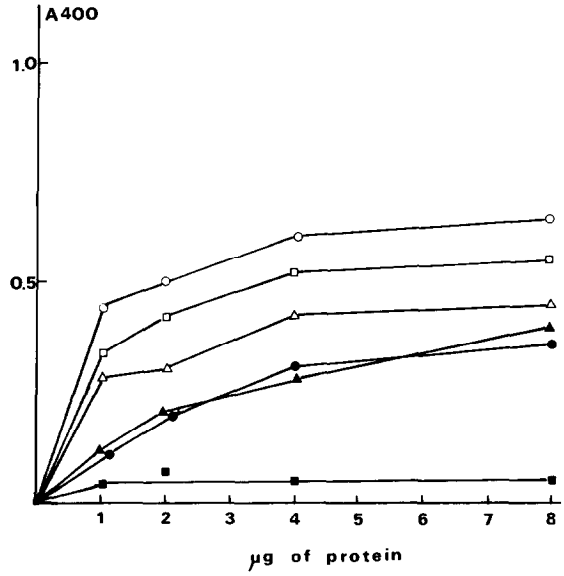

Figure 3 : Immuno reactivity measured by ELISA mitochondrial inner membrane proteins from ρ^+ yeast (O,●), from mutants G171 with cytochrome b 14 000 (Δ,▲) and G625 without cytochrome b (□,■) against 30K hydrophobic protein antibodies (O,Δ,□) or against cytochrome b antibodies (●,▲,■).

4. Search for the 30K hydrophobic protein in 3 mitochondrial DNA mutants : The hydrophobic protein is extracted in each case from the log phase cell promitochondria. Assayed by the method of Lowry, only a very small amount of protein can be found as compared with the hydrophobic protein extracted from ρ^+ yeast strain. However SDS-polyacrylamide gel electrophoresis of the three mutants total inner membrane proteins show a 30K band with the same mobility as that of the hydrophobic protein extracted from the ρ^+ strain. Inner membranes of the yeast wild strain and the two mitochondrial mutants G171 and G625 are analyzed for the presence of the 30K hydrophobic protein by ELISA with antibodies against 30K hydrophobic protein and for cytochrome b, with antibodies against cytochrome b. Results in Figure 3 show that the two mutants still contain 30K hydrophobic protein when compared to the wild strain. The G625 mutant does not contain any cytochrome b, as expected ; the G171 mutant does react with the cytochrome b antibodies (9), even it contains only 14 K instead of 32 K cytochrome b, as in the wild yeast.

DISCUSSION

Yeast mitochondrial inner membrane proteins may be of either nuclear or mitochondrial origin. The hydrophobic protein studied in this work is synthetized on mitochondrial ribosomes (1). We compare this protein with another known inner membrane protein, taking into account the following

properties : aminoacid composition, molar ratio of acidic to basic aminoacids, polarity and apparent molecular weight. A satisfactory analogy can be noticed between the hydrophobic protein and subunit 32K of the cytochrome bc 1 complex described by Katan and Groot (3, 4) but the results obtained here, show antigenic differences between both.

There is no cross reaction between the 30K hydrophobic protein we extracted from the inner membrane of yeast mitochondria and the 32K subunit of the cytochrome bc 1 complex present in the same membrane. The different immunochemical approaches of the problem gaves us the same results : immunoprecipitation with the aid of protein A, immunoprecipitation in gel, or enzyme-linked immunosorbent assay. Purification of the 30K hydrophobic protein involves the use of organic solvents. The procedure might induce conformational changes of the hydrophobic protein as compared to the native protein present in the mitochondrial inner membrane, and the antibodies raised might have good specificity toward extracted 30K hydrophobic protein, but poor affinity toward the native protein. This is apparently not the case, because figure 3 shows that the specific antibodies react well with the native 30K hydrophobic protein. On the other hand yeast strains having mutations in the COB region of the mitochondrial DNA do not respire but are still able to synthetize 30K hydrophobic protein with identical solubility, electrophoretical and immunological properties as the one found in the ρ^+ strain, though in smaller amounts. It must be noticed that the mutants synthetize only promitochondria (20), in place of entire mitochondria. Mutation in the COB region of the mitochondrial DNA does impair respiratory function, but does not hinder synthesis of the 30K hydrophobic protein. Our results show that a mitochondrial mutant synthetizing a 14 K cytochrome b (G171) and an other mitochondrial mutant which does not synthetize cytochrome b at all (G625), are still able to synthetize the 30K hydrophobic protein of the mitochondrial inner membrane. Our results suggest that the 30K hydrophobic protein is not identical with the 32K subunit of the cytochrome bc 1 complex. The amino acid composition of yeast cytochrome b, derived from DNA sequence analysis (21) is in accordance with our results. The precise localization and the function of the hydrophobic protein is now under investigation in our laboratory.

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