Oligomycin sensitivity-conferring protein (OSCP) of beef heart mitochondria

Internal sequence homology and structural relationship with other proteins

Yu.A. Ovchinnikov, N.N. Modyanov, V.A. Grinkevich, N.A. Aldanova, P.V. Kostetsky, O.E. Trubetskaya, T. Hundal* and L. Ernster*

Shemyakin Institute of Biorganic Chemistry, USSR Academy of Sciences, Moscow, 117312 USSR and **Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden*

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Structural analysis of oligomycin sensitivity-conferring protein (OSCP) revealed repeating sequences (residues 1–89, 105–190) suggesting an evolution of the protein by gene duplication. In addition to the reported homology with the δ-subunit of *Escherichia coli* F_1ATPase, OSCP also shows a certain homology with the b-subunit of *E. coli* F_0 and the ADP/ATP carrier of mitochondria.

F_0F_1 Oligomycin sensitivity-conferring protein Subunit Sequence homology Structural relationship Comparative analysis

1. INTRODUCTION

Proton-translocating ATPases isolated from mitochondria and bacteria have similar structural organizations [1–4]. These multi-subunit enzyme complexes are composed of two main components: a soluble catalytic moiety, F_1, projecting into the water phase; and a membrane sector, F_0, forming a proton-conducting channel. Bacterial F_0 includes 3 different subunits, a, b and c. The mitochondrial F_0 is more complex and contains at least two additional polypeptides, oligomycin sensitivity-conferring protein (OSCP) [5] and F_6 [6]. In the case of the *Escherichia coli* ATPase the b-subunit is proposed to be important for the interaction of F_1 and F_0 and to extend about 80 Å from the membrane surface, forming a stalk around which F_1-subunits are associated [7]. The δ- and ε-subunits of bacterial ATPase are required for the F_1–F_0 interaction [8–11] and, apparently, both of them are in contact with the b-subunit [12].

The complete amino acid sequence of OSCP has been determined [13]. Comparative analysis of the complete amino acid sequences of OSCP and the δ-subunit of F_1 from *E. coli* ATPase revealed a considerable structural homology, not only in the C- and N-terminal regions of the molecules [14,15], but also in their central parts [13]. These data, and available information on a functional similarity between OSCP and the *E. coli* δ-subunit [16,17], suggest that OSCP is a real counterpart of the *E. coli* δ-subunit. The mitochondrial δ-subunit seems to be a counterpart of the bacterial ε-subunit [15]. No protein homologous to the b-subunit of *E. coli* ATPase has so far been found in the mitochondrial ATPase.

We report a comparative analysis of the amino acid sequences of OSCP with those of other proteins. The results reveal an internal homology in the OSCP molecule itself and a certain structural homology of OSCP with both the b-subunit of *E. coli* ATPase and the mitochondrial ADP/ATP carrier.

2. MATERIALS AND METHODS

A modified 'Diagon' program was used [18,19]

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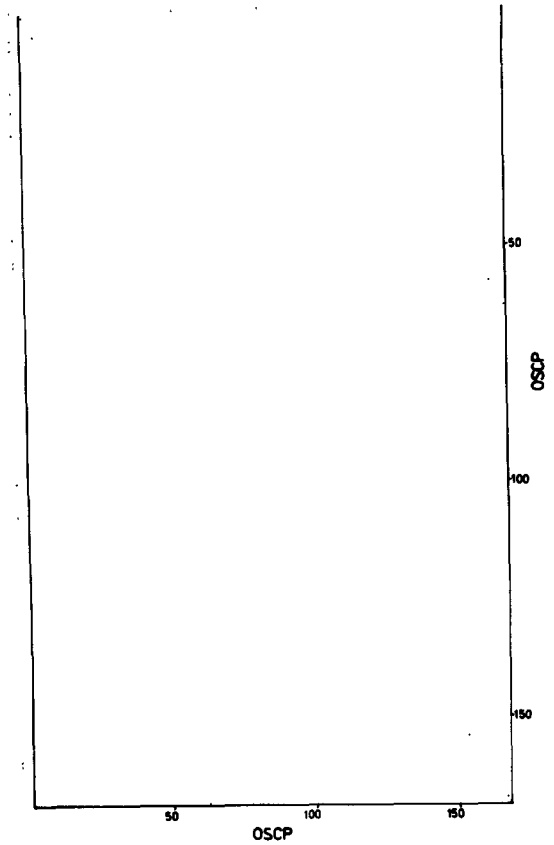

Fig.1. Final comparison matrix of OSCP with itself. Deletions are introduced for maximum internal homology. Length of the comparison segment, 28 residues, and comparison result is marked if the number of coincidences is 8 residues or more. Number of points at matrix, covering regions 19–54 and 123–157, corresponds to a similarity of 11 residues, which exceeds the 1% level of random similarity.

to find segments of homology in the comparable amino acid sequences. The similarity between peptide fragments of equal length was evaluated by the number of coinciding amino-acid residues.

Probability of the random appearance of homologous domains was estimated by computer modeling [20]. A great number (500) of artificial amino acid sequences, the length and composition of which were identical to those of comparable proteins, was obtained and analyzed. Deletion was also taken into account.

Computation of the results was performed by means of FORTRAN programs adopted for a Hewlett-Packard 3000 computer.

3. RESULTS AND DISCUSSION

The comparison matrixes of OSCP with itself are very specific, containing two repeating sequences. The internal homology was most clearly revealed at a comparison segment length of 15 amino acid residues and the details manifest themselves at a segment length of 7 residues (not shown). These matrixes allow the identification of 3 deletions located in the C-terminal region of the protein molecule: one after Val-171 and two after Lys-135. The final comparison matrix, including two additional deletions (one after Lys-47 and the other after Gly-168) which were introduced for reaching maximal internal homology, is shown in fig.1, and the repeating sequences of OSCP are aligned in fig.2; the latter are linked by a segment of 15 amino acids (90–104). Of 90 amino acids, 40 residues are identical or conservatively substituted in these two sequences. Thus OSCP appears to have evolved by a process of gene duplication.

It is noteworthy that the δ-subunit of *E. coli* ATPase, which is a counterpart of mitochondrial OSCP, has no such clearly manifested internal homology. This indicates that, during evolution, the mitochondrial protein was more stable than the bacterial one.

Another striking feature of the OSCP structure is the concentration of hydrophobic amino acids in the N-terminal region of the molecule. Of 20 amino acid residues (7–26) 75% are hydrophobic. This hydrophobic domain may serve to anchor OSCP in the membrane and may also be involved in the interaction of OSCP with other F_0 subunit(s). Comparison of the hydrophobic domain of OSCP with the homologous region in the polypeptide chain of the *E. coli* δ-subunit [21–23]:

OSCP	(7–26)	P	P	V	Q	I	Y	G	I	E	G	R	Y	A	T	A	L	Y	S	A	A
δ-subunit	(1–18)			M	S	E	I	T	V	A	R	P	Y	A	K	A	A	F	D	F	A

shows that the latter is more hydrophilic (60% hydrophobicity, 5 charged residues instead of 2 in OSCP). (More recent analysis shows that a glutamic acid residue is located in position 15 of the OSCP sequence and not glutamine as reported earlier [13].) The hydrophobic domain in the N-terminal sequence of OSCP may explain why this polypeptide remains bound to the membrane sector, while the corresponding subunit in the *E. coli*

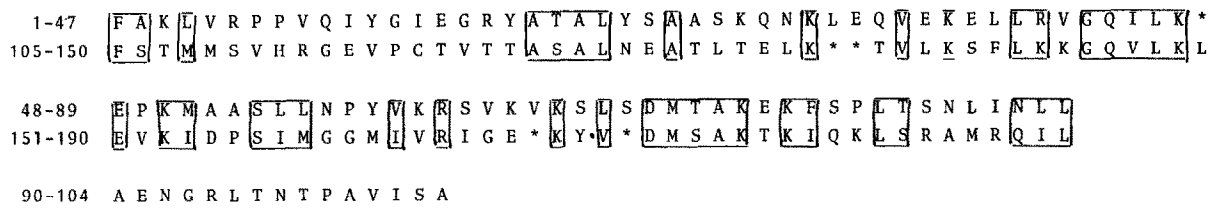

Fig.2. Alignment of repeating sequences in OSCP. Identical residues and conserved substitutions are boxed, deletions are indicated by asterisks.

ATPase, i.e., the δ-subunit, accompanies F_1 when the latter is removed from the membrane.

Analysis of the hydrophobicity profile of OSCP (not shown) shows a similarity to that of the *E. coli* b-subunit [24]. The b-subunit has a hydrophobic segment at the N-terminus which is embedded in the membrane, while the rest of the molecule is extremely hydrophilic and protrudes from the lipid bilayer. This part is in contact with the δ- and ϵ-subunits, thus binding F_1 to F_0 [7,12]. Comparative analysis of OSCP and the b-subunit [21,23,25] revealed a considerable homology between the repeating sequences of OSCP and the central part of the b-subunit (fig.3). The latter has been shown to contain repeated sequences [12]. Evidently, OSCP includes structural elements of both the δ- and b-subunits of the *E. coli* F_0F_1 ATPase. Whether OSCP and the b-subunit have any similar functions is a subject of current study.

A sequence homology was unexpectedly found between the repeating regions of OSCP (residues 1–84) and the N-terminal part of the ADP/ATP carrier (residues 15–105) (fig.4) [25]. The ADP/ATP carrier is an integral protein of the inner mitochondrial membrane which exchanges cytoplasmic ADP for ATP synthesized inside the mitochondrion. The sequence homology between the two proteins is difficult to explain since they have very different functions in the mitochondria. The roots of the structural similarity presumably lie in the common origin of evolutionary ways for the two molecules.

A comparative analysis of OSCP with other membrane proteins is currently in progress.

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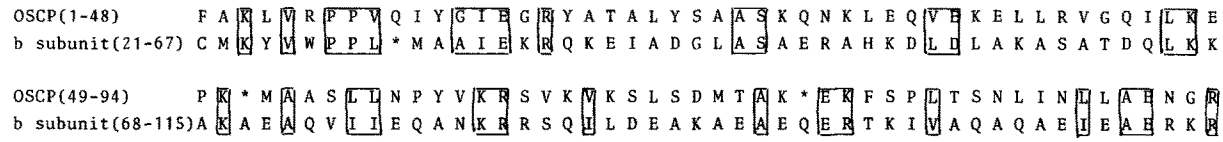

Fig.3. Comparison sequences of mitochondrial OSCP and *E. coli* b-subunit. Identical residues and conserved substitutions are boxed, deletions are indicated by asterisks. The sequence of the b-subunit is taken from [21,23,25].

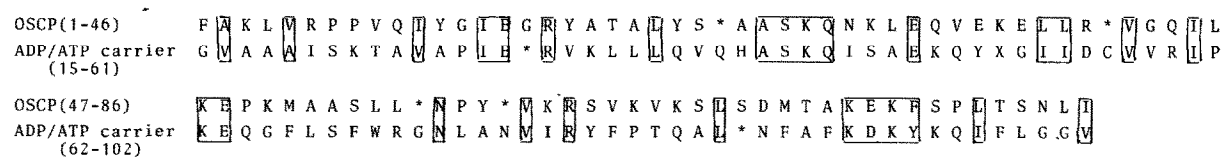

Fig.4. Comparison of amino-acid sequence of beef-heart OSCP and mitochondrial ADP/ATP carrier. Homologous sequences are boxed, deletions are indicated by asterisks. The sequence of the ADP/ATP carrier is taken from [26].

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