# Amino acid sequence of the oligomycin sensitivity-conferring protein (OSCP) of beef-heart mitochondria and its homology with the $\delta$-subunit of the $F_1$-ATPase of *Escherichia coli*

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The complete amino acid sequence of the oligomycin sensitivity-conferring protein (OSCP) of beef-heart mitochondria is reported. The protein contains 190 amino acids and has a molecular mass of 20967. Its structure is characterized by a concentration of charged amino acids in the two terminal segments (N 1–77 and C 128–190) of the protein, whereas its central region is more hydrophobic. The earlier reported homology of the protein with the $\delta$-subunit of *E. coli* $F_1$, based on the terminal amino acid sequences of OSCP, is further substantiated.

*Amino acid sequence* *Oligomycin sensitivity-conferring protein* *Homology* *Mitochondrial $H^+$-ATPase* *Amino acid residue*

## 1. INTRODUCTION

Oligomycin sensitivity-conferring protein (OSCP) is a component of the mitochondrial $H^+$-ATPase [1] required for the interaction of the catalytic ($F_1$) and $H^+$-translocating ($F_0$) moieties of the enzyme [2]. For a number of years it was generally believed that OSCP has no counterpart in chloroplasts and bacteria. Recently, however, determination of the N- and C-terminal sequences of beef-heart OSCP [3] has revealed a homology [4] between this protein and the $\delta$-subunit of *E. coli* $F_1$ [5]. These findings were paralleled by observations [6,7] suggesting a functional similarity between the two proteins. Here we report the complete aminoacid sequence of OSCP from beef-heart mitochondria, which further substantiates the homology between this molecule and the $\delta$-subunit of $F_1$ from *E. coli*.

*Abbreviations:* SDS, sodium dodecyl sulphate; PTH, phenylthiohydantoin; OSCP, oligomycin sensitivity-conferring protein

## 2. MATERIALS AND METHODS

OSCP was isolated from beef-heart mitochondria as in [8]. The homogeneity of the protein samples was tested by SDS-electrophoresis in polyacrylamide gel [9] and by the analysis of the N-terminal amino acid. The hydrolyses of carboxymethylated (CM)-protein by trypsin and *Staphylococcus aureus* proteinase were carried out under standard conditions. The tryptic hydrolysate was separated by ion-exchange chromatography on an AG-50 W × 4 cation exchanger in pyridine–acetate buffers with concentration- and pH-gradients. Subsequent purification of the fractions was achieved by paper chromatography in a butanol–pyridine–acetic acid–water (15:10:3:12) system and by high performance liquid chromatography

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(HPLC) on Silasorb $C_{18}$ (Lachema) ($CH_3CN$ gradient 0–35% in 0.01 M $CH_3COONH_4$, pH 5.6). The cyanogen bromide cleavage of the protein was carried out in 70% HCOOH at the 500-fold reagent excess. Cyanogen bromide peptides and the peptides of the *S. aureus* proteinase hydrolysis were fractionated on BioGel P-10 in 30% HCOOH followed by HPLC on Silasorb $C_8$ (Lachema), Nucleosil $C_8$ and Nucleosil $C_{18}$ (Macherey-Nagel) ($CH_3CN$ gradient 0–70% in 0.1% $CF_3COOH$).

The amino acid sequence of the peptides was determined as in [10] and by automatic degradation on a Beckman 890C sequencer (102974 program). PTH amino acids were identified by HPLC [11] and mass-spectrometry [12]. The amino acid composition of the protein and the peptides was determined using a Durrum D-500 analyzer.

## 3. RESULTS AND DISCUSSION

OSCP has been reported to consist of a single polypeptide chain ($M_r$ 18000) [1] . The structural investigation started with an exhaustive tryptic hydrolysis of CM-protein, which yielded 28 peptides and free arginine and lysine. The complete amino acid sequences were established for all the tryptic peptides except for peptide T-15 (fig.1). This peptide was isolated in negligible yield due to practically complete non-specific hydrolysis at the Phe-Ser bond.

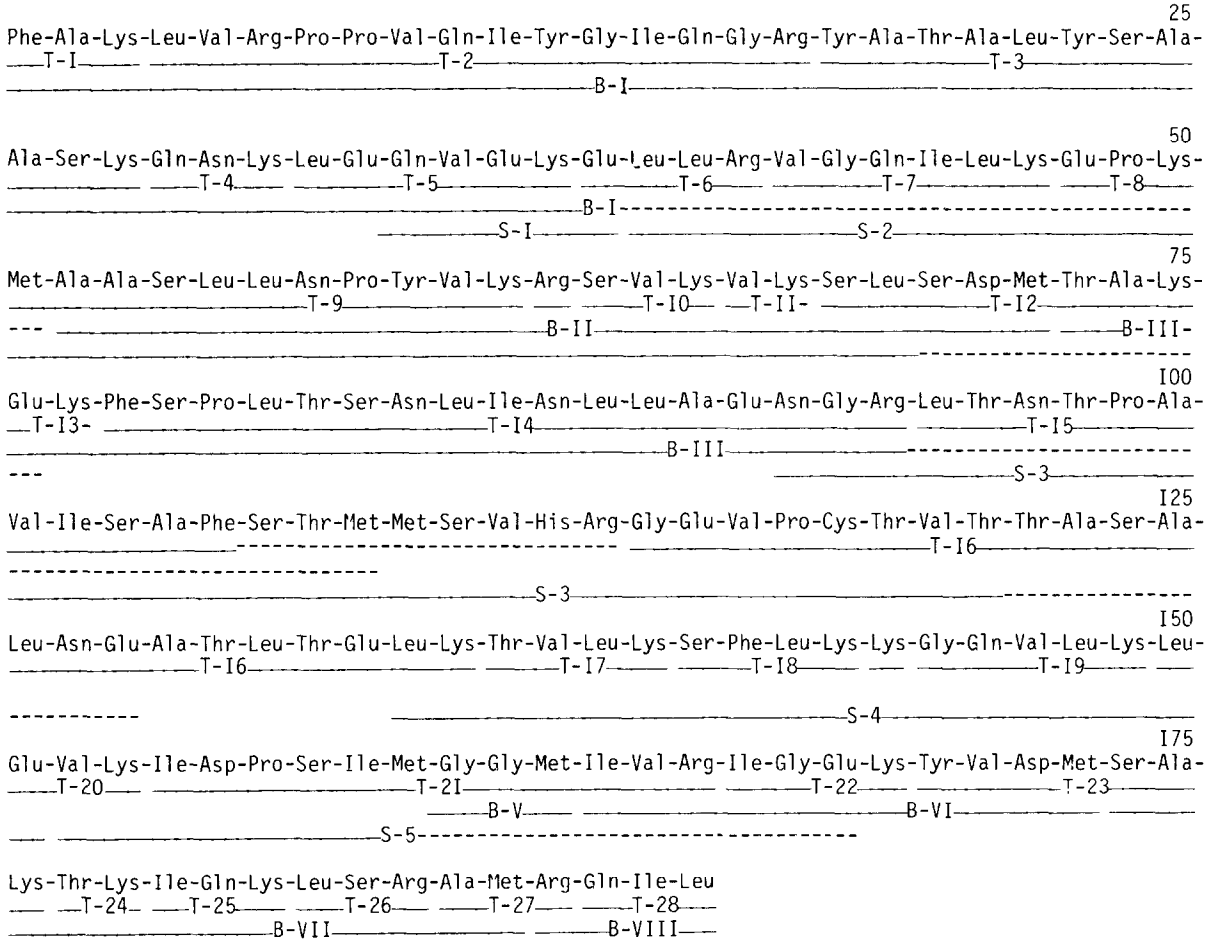


Fig.1. Primary structure of oligomycin sensitivity-conferring protein (OSCP) from beef-heart mitochondria. (T) Tryptic peptides. (B) Cyanogen bromide peptides. (S) Peptides from *Staphylococcus aureus* proteinase hydrolysate. Solid lines indicate sequences determined by automatic or manual methods. Residues which were not identified are indicated by broken lines.

In order to obtain large fragments of the polypeptide chain, the cyanogen bromide cleavage of OSCP was performed. Incomplete splitting of the peptide bonds Met-Thr (72–73), Met-Met-Ser (108–110), Met-Ser (173–174) drastically decreased the yield of individual peptides and complicated the separation procedure. Moreover, heterogeneity of the N-terminal sequence of peptide B-IV (109, 110–159) prevented its isolation in a homogeneous state.

The complete primary structure of OSCP is given in fig.1. The N-terminal sequence of the protein (37 amino acid residues) was established by automatic degradation. The structural data on corresponding methionine-containing tryptic peptides allowed to combine in one block the cyanogen bromide peptides B-V-B-VIII (154–190) and peptides B-II and B-III.

The additional information on the protein structure was obtained upon the analysis of peptides of the *S. aureus* proteinase hydrolysis of CM-protein. Only the peptides necessary for protein molecule reconstruction are indicated in fig.1. Thus data on the amino acid sequence of peptide S-1 and those obtained by automatic degradation of peptide S-2 made possible the determination of the complete structure of peptide B-I and its overlapping with peptide B-II. The structural analysis of peptides S-3, S-4, S-5 provided the information on the structure of the central part of the protein molecule (the C-terminal region of fragment B-III, fragment B-IV). Finally the N-terminal sequence determination of peptide S-5 resulted in an overlapping of the fragments B-IV and B-V.

The OSCP molecule consists of 190 amino acid residues ($M_r$ 20967) and has the following amino acid composition: Asp-3, Asn-7, Thr-13, Ser-16, Glu-11, Gln-8, Pro-8, Gly-9, Ala-16, Met-8, Val-16, Cys-1, Ile-11, Leu-23, Tyr-5, Phe-4, His-1, Lys-21, Arg-9.

A characteristic feature of the structure is the location of the charged amino residues in N (1–77)- and C (128–190)-terminal parts of the molecule whereas its central part is of more hydrophobic character. It probably plays a role in the interaction of OSCP with the $F_1$ and $F_0$ moieties of the ATPase complex.

Comparative analysis of the complete amino acid sequences of OSCP and $\delta$-subunit of $F_1$ of *E. coli* ATPase (fig.2) reveals a considerable structural homology not only in C- and N-terminal regions of their molecules [3,4] but also in the central part. These data and available information on a functional similarity between OSCP and $\delta$-subunit [6,7] suggest that OSCP is a real counterpart of the *E. coli* $\delta$-subunit.

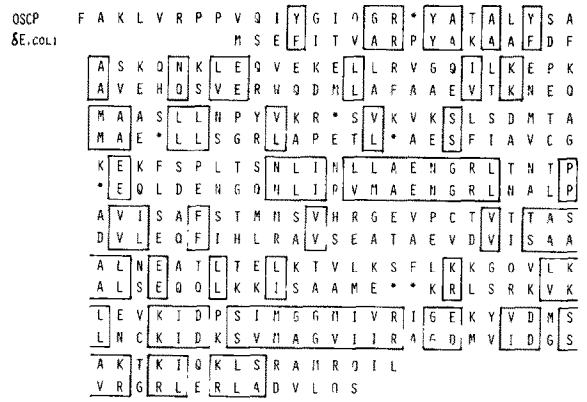


Fig.2. Comparison of amino acid sequences of mitochondrial oligomycin sensitivity-conferring protein (OSCP) and *E. coli* $\delta$-subunit of $H^+$-ATPase. Homologous sequences are boxed, deletions are indicated by asterisks. Sequence of *E. coli* $\delta$-subunit is from [5].

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