Subunit δ of Chloroplast F_0F_1-ATPase and OSCP of Mitochondrial F_0F_1-ATPase: a Comparison by CD-Spectroscopy

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CD spectra have been recorded with subunit δ from chloroplast CF_0CF_1 and with OSCP from mitochondrial MF_0MF_1. These subunits are supposed to act similarly at the interface between proton transport through the F_0-portion and ATP-synthesis in the F_1-portion of their respective F_0F_1-ATPase. Evaluation of the data for both proteins revealed a very high α-helix content of ~85% and practically no β-sheets. Despite their low homology on the primary structure level (23% identity) and their different electrostatic properties (pI-values differ by 3 units), spinach δ and porcine OSCP are indistinguishable with respect to their secondary structure as measured by CD. Prediction and analysis of consensual α-helices even in poorly conserved regions indicate a high degree of structural similarity between chloroplast δ and OSCP. In view of the topology and function of δ and OSCP in intact F_0F_1 these findings are interpreted to indicate the dominance of secondary and tertiary structure over the primary structure in their supposed function between proton flow and ATP-synthesis.

Introduction

ATP synthesis at the expense of a protonmotive force is catalyzed by F_0F_1-ATPases in thylakoids, mitochondria and many microorganisms. F_0F_1-ATPases consist of a membrane-embedded proton channel F_0 and a water-soluble F_1-portion, which carries the active sites. Upon removal from F_0, F_1 catalyses ATP hydrolysis. F_1 consists of five polypeptides named α, β, γ, δ, and ε in order of decreasing molecular mass. Subunit δ of chloroplast and *E. coli* F_1 and their mitochondrial counterpart OSCP are small proteins of ~21 kDa mass. They are located between the F_1 and F_0 parts and indispensable for functional F_0F_1. The position of the 'small' subunits (γ, δ/OSCP, ε) at the interface between F_0 and F_1 points to a special role of these subunits with respect to the link – whatever its nature – between H^+-translocation and ATP synthesis/liberation. For general reviews on F_0F_1-ATPases see ref. [1] to [3], the role of subunits δ and OSCP has been reviewed in ref. [4].

In view of their location and supposedly similar function in the F_0F_1 complex it is surprising that subunit δ is an acidic protein (pI = 5.7, S. Engelbrecht, unpublished data) whereas OSCP is basic (pI = 8.5, F. Penin, unpublished data). Based on similar quaternary structure of F-type ATPases from different sources, a common mechanism is expected and also similar structures of the protein subunits. On the basis of sequence homologies alone this expectation is not met. Of the five CF_1-subunits only the sequences of α and β are highly homologous to other species, while γ, δ and ε show only weak similarities. This trend is even more pronounced for the subunits of the F_0-portion.

Polarity inversions within homologous protein complexes isolated from different sources are not uncommon, *e.g.* cytochrome *c*-oxidase/cytochrome *c* [5]. It is also not uncommon that different primary structures result in very similar folding patterns and domain structure, *e.g.* the two domains of the bifunctional enzyme *N*-5′-phosphoribosylanthranilate isomerase/indole-3-glycerol-

Abbreviations: CF_0CF_1, chloroplast F_0F_1-ATPase; CF_0, chloroplast proton channel (membrane-embedded); CF_1, chloroplast ATPase (soluble part); MF_0, MF_1, EF_0 and EF_1 are the respective terms for the mitochondrial and *E. coli* proteins; OSCP, mitochondrial oligomycin sensitivity-conferring protein; CD, circular dichroism.

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phosphate synthase [6]. This prompted us to study the secondary structure of chloroplast δ and mitochondrial OSCP.

CD spectroscopy offers a sensitive method for determining the proportion of certain secondary structure elements. Dupuis, Zaccai, and Satre have previously measured the CD spectrum of beef heart OSCP [7] and calculated 43% α-helical structure. With improved instrumentation and data evaluation we studied porcine OSCP for comparison with spinach chloroplast δ.

Materials and Methods

Spinach δ was prepared from CF_1 by anion exchange chromatography in the presence of detergent, followed either by rechromatography or by hydrophobic interaction chromatography as described [8, 9]. Porcine OSCP was prepared by selective extraction of preextracted mitochondrial membranes, followed by cation exchange chromatography as described [10]. All preparations were SDS-electrophoretically homogeneous (data not shown).

Protein determinations were performed according to Sedmak and Grossberg [11]. Alternatively, the Pierce bicinchoninic acid version of the Lowry procedure was used (Pierce Europe B.V., POB 1512, NL-BA Oud Beijerland, The Netherlands). Lysozyme, bovine serum albumin and ovalbumin were used as standards. Whereas spinach δ gave the same response with both assays, porcine OSCP was grossly underestimated by the Sedmak and Grossberg procedure. Data were therefore cross-checked by amino acid analysis.

Circular dichroism spectra were measured with a Jasco J-500 automatic recording spectropolarimeter coupled to a Jasco J-DPY data processor. Curves were recorded digitally and fed through the data processor for signal averaging and baseline subtraction. Samples at a concentration of 50–100 μg protein/ml in 10 mM Tris/HCl pH 7.5 were scanned from 190–240 nm in a dichroically neutral quartz cuvette with a path length of 1.0 mm. The sensitivity was 2.0 m°/cm, time constant 2 s, scanning speed 5 nm/min. Spectra were averaged over four scans. A signal-averaged baseline was subtracted.

For estimation of secondary structure content, points taken at 0.5 nm intervals were processed using the CD application package of CONTIN [12] run on a VAX 11/780 computer. This program analyses a given CD spectrum as a linear combination of the CD spectra of 16 proteins whose secondary structure content is known, and gives the result as percent α-helix, β-sheet and 'remainder' (a mixture of extended coils and reverse turns). A total of four different preparations was measured both with spinach chloroplast δ and pig heart mitochondrial OSCP. Secondary structure predictions and predicted pI's were calculated with the University of Geneva PcGene program.

Results and Discussion

Fig. 1 shows representative CD spectra for spinach δ (sample 2) and porcine OSCP (sample 1). Measurements are indicated by points, the line represents a fit as calculated according to ref. [12]. Fit parameters are given in Table I. The relative proportions of secondary structure elements are summarized in Table II. Both proteins are highly α-helical to about 85%. Whereas OSCP seems to be virtually devoid of any β-sheet structure, the

Table I. Summary of possible solutions for fits of measured CD spectra. All data are in %.

		δ_1			δ_2		δ_3	δ_4
δ	α-Helix:	85	82	86	89	91	71	81
	β-Sheet:	3	4	6	9	0	23	16
	Remainder:	12	15	8	3	9	6	3
		$OSCP_1$			$OSCP_2$	$OSCP_3$		$OSCP_4$
OSCP	α-Helix:	85	79	93	81	89	85	89 85
	β-Sheet:	0	0	0	0	0	2	0 0
	Remainder:	15	21	4	19	11	13	11 15

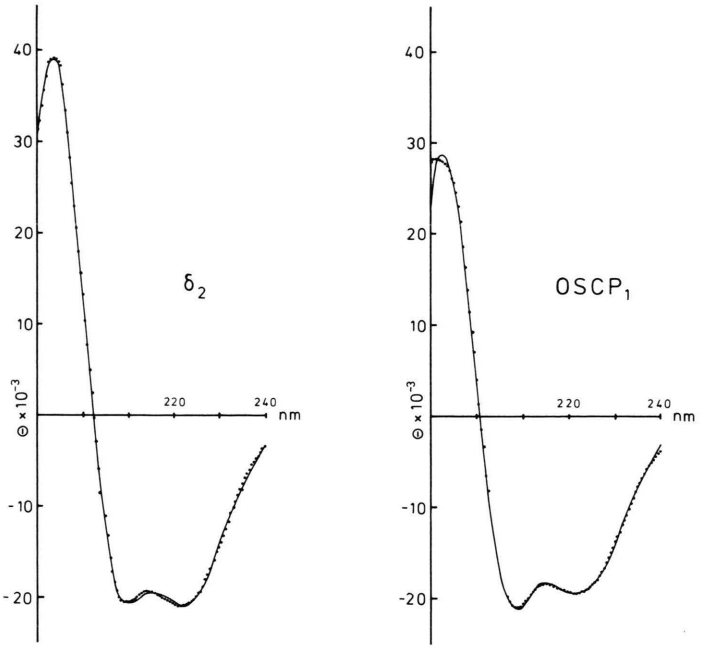

Fig. 1. CD spectra of spinach chloroplast δ (sample 2, left part of the figure) and porcine heart mitochondrial OSCP (sample 1, right part of the figure). Points are experimental, curves are theoretical fits.

Table II. Summary of secondary structure composition as evaluated from CD spectra ($n = 4$).

	δ	OSCP
α-Helix [%]	83.6 ± 6.1	85.8 ± 4.2
β-Sheet [%]	8.7 ± 7.5	0.3 ± 0.7
Remainder [%]	8.0 ± 4.1	13.6 ± 4.9

figures for δ lie between 0 and 23%. δ_3 seems to be somewhat anomalous. On the average, OSCP shows about 14% of 'remainder' and δ 8%.

Of the 187 amino acids of spinach δ, 156 thus are in helical conformation and about 16 in β-sheets. For OSCP, 163 amino acids out of 190 are in helical conformation. Although CD data do not yield information concerning the alignment of secondary structure elements along the primary structure, the sheer amount of α-helix and the near identity of this amount for both δ and OSCP suggest that these two proteins share a similar three-dimensional structure.

Dupuis *et al.* have previously calculated an amount of 43% α-helical structure for bovine OSCP [7]. Although the primary structure of porcine OSCP is not known, porcine and bovine OSCP are highly similar: the amino acid analyses are very close, the 18 N-terminal residues are the same and an epitope for monoclonal antibodies is shared by both proteins [13]. Therefore the discrepancy between Dupuis *et al.* evaluation and the one presented here is likely due to the improved evaluation method and also to the extended range of measurement (190–240 nm *vs.* 200–240 nm). Commonly used prediction programs for secondary structure [14, 15] grossly underestimate the α-helix content both of spinach δ (39–50%) and bovine OSCP (49–62%). Only the use of the Double-Prediction method [16], which takes into account the predicted structural class of the protein, improves the prediction of secondary structure elements. Indeed, with this method OSCP is predicted as an all α-protein with 79% α-helix and almost no β-sheet (3%) and chloroplast δ is predicted as an α + β protein in very good agreement with the CD data.

Since CD data do not yield tertiary structure information, prediction methods have been used here for this purpose. It is possible to constrain the prediction program to fit the secondary structure content as measured by CD, using optimized decision constants [14]. Fig. 2 shows the alignment and predicted structures after application of the Double Prediction program [17]. *E. coli* δ is included in

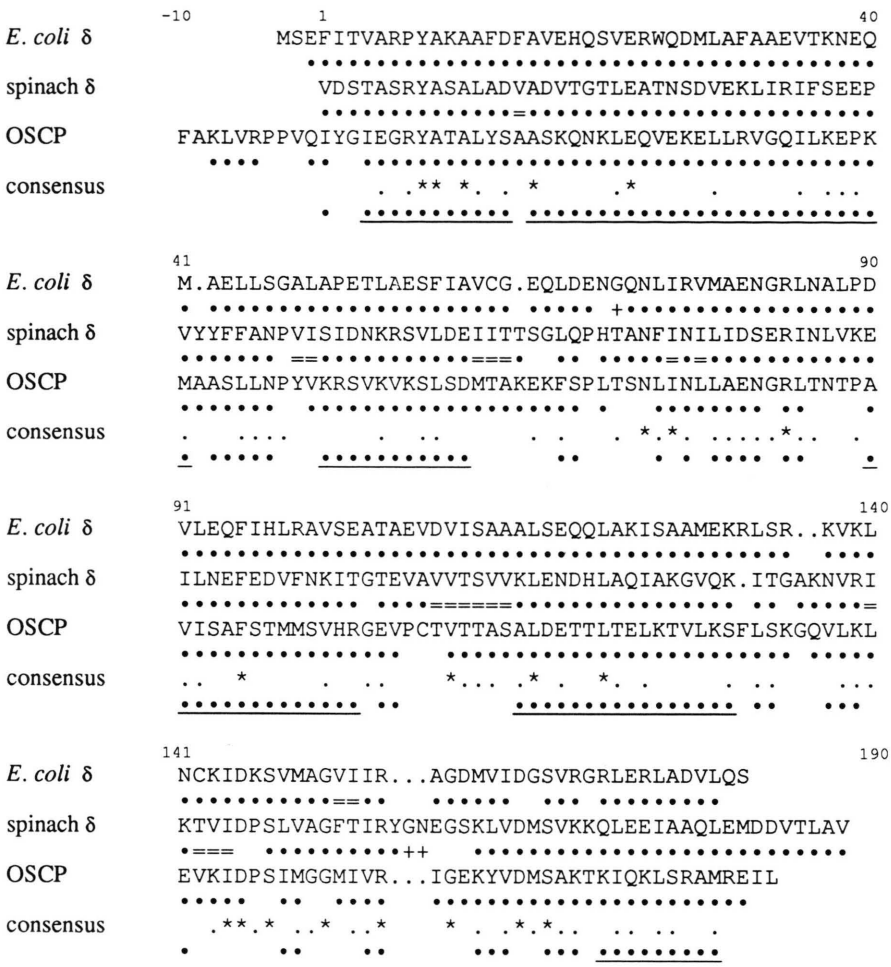

Fig. 2. Comparison between predicted secondary structure of *E. coli* δ, spinach chloroplast δ and bovine OSCP. For spinach δ and OSCP the prediction method DOUBLE PREDICTION [16] was constrained to fit the content of secondary structure obtained by CD (Table II), using optimized decision constants [14]. The decision constants chosen for spinach δ were −0.17 for α-helix, +0.07 for β-sheet, and 0 for turn and coil. For OSCP, the respective values were −0.03, +0.05, 0, −0.03. For *E. coli* δ, all constants were set to 0. Alignment of the three sequences was done with the program PARSIMONY [26]. Identical alignments were obtained with CLUSTAL [27] and MULTALIN [28]. Stars indicate perfect matches between all three sequences, small points indicate identical residues in two sequences. Symbols of predicted secondary structure: α-helix: ●, β-sheet: =, turn: +, coil: blank. Consensual α-helical segments are underlined. All calculations were performed by using the software package ANTHEPROT [29].

the figure because the high mutational frequency of this organism serves as a convenient means to sort out 'essential' amino acids. Although *E. coli* δ has not been measured here by CD spectroscopy, the functional equivalence of *E. coli* δ and spinach δ as observed in hybrid-reconstitution experiments [17] leaves little doubt about the structural similarity of these two proteins. OSCP appears to be more closely related to *E. coli* δ (25% homology) than to chloroplast δ (22%). The degree of similarity of the three sequences is especially impressive in the range of Pro 70 to Leu 87 and Pro 146 to Met 181. The presence of six consensual α-helices clearly appears in Fig. 2. Helical wheel projection of these consensual α-helices revealed a comparative distribution of hydrophobic, hydrophilic, and charged residues between the three proteins (not shown). However, the well conserved regions do not necessarily belong to the consensual α-helices but might be very important in maintaining a similar three-dimensional structure. The results leave little doubt that chloroplast δ, *E. coli* δ, and mitochondrial OSCP share a similar three-dimensional structure.

Based on small-angle neutron scattering Dupuis *et al.* calculated molecular dimensions of about 9 × 3 × 3 nm for bovine OSCP [7]. Based on rotational diffusion in solution Wagner *et al.* calculated dimensions of about 10 × 2.8 nm for spinach δ [18]. How do these findings relate to the high α-helix content of δ and OSCP? With a rise per residue of 0.15 nm 160 amino acids would give a sin-

gle stretched-out α-helix of 24 nm length. The above dimensions then allow for only two parallel helices spanning the entire length of the molecule. Such an arrangement also fits into the model of F_0F_1-ATPases as proposed by Gogol *et al.* [19]. These workers observed a narrow stalk between EF_0 and EF_1 (using electron microscopy). The diameter of the stalk would allow for four to tive closely packed α-helices. Subunit *b* of EF_0 (I in CF_0) is predicted to consist of only two α-helices outside the membrane [20, 21].

E. coli δ contains proline residues in positions 7, 52, and 89, spinach δ contains prolines in positions 40, 48, 70, and 146, and OSCP contains prolines in positions −4, −3, 39, 48, 70, 89, 107, and 146. It remains to be seen in what manner these proline residues, especially those located in the center of the molecules, are accommodated in the tertiary structure. Proline, if not starting/breaking helices, is known to introduce kinks which loosen up the packing density.

The present study was triggered by the observation that OSCP does not 'fit the picture' because it has a basic pI as compared to the acidic spinach and *E. coli* δ subunits. In view of the similarity on the secondary structure level reported here, the failure of OSCP to substitute for spinach δ in hybrid reconstitution experiments with CF_1(-δ) (S. Engelbrecht, unpublished data) is in contrast to the behaviour of *E. coli* δ [17] and may be explained by the reversal in charge.

Does this reversal allow for identification of contact sites for δ and OSCP on neighbouring subunits? Spinach δ contains 13 aspartic acids and 12 lysines, whereas (bovine) OSCP contains 4 aspartic acids and 20 lysines. Numbers for the other charged residues are somewhat balanced. In order to shift the pI of OSCP into the acidic range and likewise the pI of δ into the basic range, one would have to exchange about 7 aspartic acids for lysines and *vice versa,* or twice this amount of each amino acid alone. The sequence alignment of spinach and *E. coli* δ and bovine OSCP (ref. [4], Fig. 2) reveals that the (spinach δ) aspartic acids, if not conserved, are mostly substituted for by serines and threonines in OSCP. Roughly the same is true for the substitution of OSCP-lysines in spinach δ. The 'additional' lysines in OSCP are scattered throughout the sequence in a way which completely obscures possible counterparts.

Subunits *b* of EF_0 and MF_0 and subunit I of spinach CF_0 are considered to be the main binding partners of δ and OSCP [1−4]. Based on the amino acid sequences [21−23], the predicted pI's of these proteins, δ and OSCP are summarized in Table III. It is evident that a charge reversal like the one between δ and OSCP has not occurred with F_0-subunits I and *b*. Furthermore, helical wheel plots (not shown) reveal that any 'pairing' of subunits is possible at least theoretically: Rotational and translational shifts neither make salt bridges nor regions of electrostatic repulsion obvious. The complex F_0F_1-ATPase eludes further structural conclusions at this level. Its variability of primary structure remains enigmatic.

Table III. Nomenclature and theoretical isoelectric points of some F_0F_1 subunits. Please note the difference between experimentally determined pI's and predicted values (5.7 *vs.* 4.41 for spinach δ and 8.5 *vs.* 10.66 for OSCP).

Subunit (*E. coli* and chloroplast)	Counterpart in mitochondria	Predicted pI
EF_0-*b*	MF_0-*b*	5.9
CF_0-I	MF_0-*b*	8.6
MF_0-*b*		9.7
EF_1-δ	OSCP	4.71
CF_1-δ	OSCP	4.41
OSCP		10.66

The combined evidence presented here and formerly [17] favors the view that spinach and *E. coli* δ and OSCP are very similar proteins not on the primary, but on the secondary and most likely also on the tertiary structure level. It has been reported that the N-terminal half of OSCP shows some sequence homology with EF_0-*b* [24]. In view of the high predicted content of α-helices in EF_0- and MF_0-*b* and CF_0-I [21−23] and the observed highly α-helical structure of δ and OSCP this finding should not be interpreted to indicate variations in small subunit arrangements between F_0 and F_1 from various sources (OSCP being a 'mixture' of subunits δ and *b*/I of bacteria or chloroplasts). A mutant *E. coli* strain which carries

CF_0-I instead of EF_0-*b* grows similar to the wild type [25] although CF_0-I and EF_0-*b* are even less homologous than δ/OSCP. The molecular mechanism of coupling proton movement to ATP liberation most probably has been conserved, despite the variability of primary structure. This points to the dominance of secondary to quaternary structure in the function of F_0F_1-ATPases.

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[1] E. Schneider and K. Altendorf, Microbiol. Rev. **51,** 477–497 (1987).
[2] A. E. Senior, Physiol. Rev. **68,** 177–231 (1988).
[3] R. H. Fillingame, The Bacteria **XII,** 345–391 (1990).
[4] S. Engelbrecht and W. Junge, Biochim. Biophys. Acta **1015,** 379–390 (1990).
[5] E. A. E. Garber, T. L. Luntz, and E. Margoliash, Prog. Clin. Biol. Res. **274,** 749–769 (1988).
[6] J. P. Priestle, M. G. Grütter, J. L. White, M. G. Vincent, M. Kania, E. Wilson, T. S. Jardetzky, K. Kirschner, and J. N. Jansonius, Proc. Natl. Acad. Sci. U.S.A. **84,** 5690–5694 (1987).
[7] A. Dupuis, G. Zaccai, and M. Satre, Biochemistry **22,** 5951–5956 (1983).
[8] S. Engelbrecht and W. Junge, FEBS Lett. **219,** 321–325 (1987).
[9] S. Engelbrecht, G. Althoff, and W. Junge, Eur. J. Biochem. **189,** 193–197 (1990).
[10] P. Archinard, F. Penin, C. Godinot, and D. C. Gautheron, Methods Enzymol. **126,** 455–460 (1986).
[11] J. J. Sedmak and S. E. Grossberg, Anal. Biochem. **79,** 544–552 (1977).
[12] S. W. Provencher and J. Glöckner, Biochemistry **20,** 33–37 (1981).
[13] C. Godinot, S. Colorio, F. Cretin, Inçaurgarat, G. Deléage, and B. Roux, Biochimie **71,** 917–929 (1989).
[14] J. Garnier, D. J. Osguthorpe, and B. Robson, J. Mol. Biol. **120,** 97–120 (1978).
[15] O. Gascuel and J. L. Golmard, CABIOS **4,** 357–365 (1988).
[16] G. Deléage and B. Roux, Protein Engineering **1,** 289–294 (1987).
[17] S. Engelbrecht, G. Deckers-Hebestreit, K. Altendorf, and W. Junge, Eur. J. Biochem. **181,** 485–491 (1989).
[18] R. Wagner, E. C. Apley, S. Engelbrecht, and W. Junge, FEBS Lett. **230,** 109–115 (1988).
[19] E. P. Gogol, U. Lücken, and R. A. Capaldi, FEBS Lett. **219,** 274–278 (1987).
[20] J. E. Walker, M. Saraste, and N. J. Gay, Nature **298,** 867–869 (1982).
[21] C. R. Bird, B. Koller, A. D. Auffret, A. K. Huttly, C. J. Howe, T. A. Dyer, and J. C. Gray, EMBO J. **4,** 1381–1388 (1985).
[22] J. E. Walker, M. Saraste, and N. J. Gay, Biochim. Biophys. Acta **768,** 164–200 (1984).
[23] J. E. Walker, M. J. Runswick, and L. Poulter, J. Mol. Biol. **197,** 89–100 (1987).
[24] Y. A. Ovchinnikov, N. N. Modyanov, V. A. Grinkevich, N. A. Aldanova, P. V. Kostetsky, O. E. Trubetskaya, T. Hundal, and L. Ernster, FEBS Lett. **175,** 109–112 (1984).
[25] G. Schmidt, A. J. W. Rodgers, S. M. Howitt, A. L. Munn, G. S. Hudson, T. A. Holten, P. R. Whitfeld, W. Bottomley, F. Gibson, and G. B. Cox, Biochim. Biophys. Acta **1015,** 195–199 (1990).
[26] D. F. Feng and R. F. Doolittle, J. Mol. Evol. **25,** 351–360 (1987).
[27] D. G. Higgins and P. M. Sharp, Gene **73,** 237–244 (1988).
[28] F. Corpet, Nucl. Acids Res. **16,** 10881–10890 (1988).
[29] G. Deléage, F. F. Clerc, B. Roux, and D. C. Gautheron, CABIOS **4,** 351–356 (1988).