Subunit δ of Chloroplast F\(_8\)F\(_1\)-ATPase and OSCP of Mitochondrial F\(_8\)F\(_1\)-ATPase: a Comparison by CD-Spectroscopy

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CF\(_1\), MF\(_1\), OSCP, CD-Spectroscopy, Subunit δ

CD spectra have been recorded with subunit δ from chloroplast CF\(_8\)CF\(_1\) and with OSCP from mitochondrial MF\(_8\)MF\(_1\). These subunits are supposed to act similarly at the interface between proton transport through the F\(_1\)-portion and ATP-synthesis in the F\(_1\)-portion of their respective F\(_8\)F\(_1\)-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 (pl-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\(_8\)F\(_1\), 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\(_8\)F\(_1\)-ATPases in thylakoids, mitochondria and many microorganisms. F\(_8\)F\(_1\)-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\(_8\)F\(_1\). 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\(_8\)F\(_1\)-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\(_8\)F\(_1\) complex it is surprising that subunit δ is an acidic protein (pl = 5.7, S. Engelbrecht, unpublished data) whereas OSCP is basic (pl = 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-S'-phosphoribosylanthranilate isomerase/indole-3-glycerol-
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 CF1 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 bicinechoninic 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 PeGene 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>
<thead>
<tr>
<th>Sample</th>
<th>Oscp1</th>
<th>Oscp2</th>
<th>Oscp3</th>
<th>Oscp4</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Helix</td>
<td>85</td>
<td>79</td>
<td>81</td>
<td>89</td>
</tr>
<tr>
<td>β-Sheet</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Remainder</td>
<td>15</td>
<td>21</td>
<td>4</td>
<td>19</td>
</tr>
</tbody>
</table>

Table I. Summary of possible solutions for fits of measured CD spectra. All data are in %.
Table II. Summary of secondary structure composition as evaluated from CD spectra ($n = 4$).

<table>
<thead>
<tr>
<th></th>
<th>$\delta$</th>
<th>OSCP</th>
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<tbody>
<tr>
<td>$\alpha$-Helix [%]</td>
<td>83.6 ± 6.1</td>
<td>85.8 ± 4.2</td>
</tr>
<tr>
<td>$\beta$-Sheet [%]</td>
<td>8.7 ± 7.5</td>
<td>0.3 ± 0.7</td>
</tr>
<tr>
<td>Remainder [%]</td>
<td>8.0 ± 4.1</td>
<td>13.6 ± 4.9</td>
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</table>

The figures for $\delta$ lie between 0 and 23%. $\delta_3$ seems to be somewhat anomalous. On the average, OSCP shows about 14% of 'remainder' and $\delta$ 8%.

Of the 187 amino acids of spinach $\delta$, 156 thus are in helical conformation and about 16 in $\beta$-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 $\alpha$-helix and the near identity of this amount for both $\delta$ and OSCP suggest that these two proteins share a similar three-dimensional structure.

Dupuis et al. have previously calculated an amount of 43% $\alpha$-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 $\alpha$-helix content both of spinach $\delta$ (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 $\alpha$-protein with 79% $\alpha$-helix and almost no $\beta$-sheet (3%) and chloroplast $\delta$ is predicted as an $\alpha + \beta$ 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* $\delta$ is included in
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 $9 \times 3 \times 3$ nm for bovine OSCP [7]. Based on rotational diffusion in solution Wagner et al. calculated dimensions of about $10 \times 2.8$ 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-
Subunit δ of Chloroplast F₀F₅-ATPase
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
F₀F₅-ATPases as proposed by Gogol et al. [19].
These workers observed a narrow stalk between
EF₀ and EF₁ (using electron microscopy). The
meter of the stalk would allow for four to tive
closely packed α-helices. Subunit b of EF₀ (I in
CF₀) 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 observa-
tion 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 hy-
brid reconstitution experiments with CF₀-(δ) (S.
Engelbrecht, unpublished data) is in contrast to the
behaviour of E. coli δ [17] and may be ex-
plained by the reversal in charge.

Does this reversal allow for identification of
contact sites for δ and OSCP on neighbouring sub-
units? Spinach δ contains 13 aspartic acids and 12
lysines, whereas (bovine) OSCP contains 4 aspar-
tic 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 con-
served, 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 through-
out the sequence in a way which completely ob-
scures possible counterparts.

Subunits b of EF₀ and MF₀ and subunit I of
spinach CF₀ are considered to be the main binding
partners of δ and OSCP [1–4]. Based on the amino
acid sequences [21–23], the predicted pl’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 F₀-subunits I and b. Furthermore, helical
wheel plots (not shown) reveal that any ‘pairing’ of
subunits is possible at least theoretically: Rota-
tional and translational shifts neither make salt
bridges nor regions of electrostatic repulsion ob-
vious. The complex F₀F₅-ATPase eludes further
structural conclusions at this level. Its variability
of primary structure remains enigmatic.

Table III. Nomenclature and theoretical isoelectric
points of some F₀F₅ subunits. Please note the difference
between experimentally determined pl’s and predicted
values (5.7 vs. 4.41 for spinach δ and 8.5 vs. 10.66 for
OSCP).

<table>
<thead>
<tr>
<th>Subunit (E. coli and chloroplast)</th>
<th>Counterpart in mitochondria</th>
<th>Predicted pl</th>
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<tbody>
<tr>
<td>EF₀-b</td>
<td>MF₀-b</td>
<td>5.9</td>
</tr>
<tr>
<td>CF₀-I</td>
<td>MF₀-b</td>
<td>8.6</td>
</tr>
<tr>
<td>MF₀-b</td>
<td>OSCP</td>
<td>9.7</td>
</tr>
<tr>
<td>EF₀-δ</td>
<td>OSCP</td>
<td>4.71</td>
</tr>
<tr>
<td>CF₀-δ</td>
<td>OSCP</td>
<td>4.41</td>
</tr>
<tr>
<td>OSCP</td>
<td></td>
<td>10.66</td>
</tr>
</tbody>
</table>

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 EF₀-b [24].
In view of the high predicted content of α-helices
in EF₀-δ and MF₀-b and CF₀-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
F₀ and F₁ from various sources (OSCP being a
‘mixture’ of subunits δ and b/l of bacteria or chlo-
roplasts). A mutant E. coli strain which carries
CF₀-I instead of EF₀-b grows similar to the wild type [25] although CF₀-I and EF₀-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 F₀F₁-ATPases.

Acknowledgements

Expert technical assistance of Martina Blomeier and preparation of Fig.1 by Hella Kenneweg are gratefully acknowledged. This work was supported by a grant of the Deutsche Forschungsgemeinschaft (SFB 171/B3). Thanks are also due to Dr. Gilbert Deléage for his expert contribution to the prediction analysis.