Localization and Orientation of Subunit Delta of Spinach Chloroplast ATP-Synthase within the CF₀CF₁ Complex

1. Distinction of Shielded and Exposed Surfaces of Delta on the Thylakoid Membrane

Richard J. Berzborn and Werner Finke
Lehrstuhl für Biochemie der Pflanzen, Fakultät für Biologie, Ruhr-Universität Bochum, Postfach 102148, D-4630 Bochum 1, Bundesrepublik Deutschland
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Abbreviations: CF₀, peripheral moiety of chloroplast ATP-synthase; CF₁, membrane integral moiety of chloroplast ATP-synthase; F₁, ATPase of oxidative phosphorylation; OSCP, oligomycin sensitivity conferring protein, a coupling factor in mitochondria; α, ß, ε, subunits of CF₁; 306-0, preimmune serum of rabbit 306; 306-1,2,3...t, sucrose sulfate.

Accessibility, Antibodies, Coupling Factor, Proteolysis, Photophosphorylation

A new polyclonal antiserum against spinach CF₁ subunit delta was produced in rabbits. It decorates only one band at 21 kDa in Western immunoblots of thylakoid proteins and does not react in ELISA with δ-free four subunit CF₁(–δ); therefore it is regarded monospecific. The polypeptide used as immunogen had been purified by HPLC. Earlier antiseras against CF₁ δ cross-react with CF₁ subunit β.

The new antiserum 306 contains different antibodies; some can be absorbed with thylakoids, i.e. by δ within the assembled CF₀CF₁ complex on the membrane, others still react in ELISA with isolated CF₁. The former antibodies agglutinate thylakoids and inhibit PMS cyclic photophosphorylation. Therefore we conclude that part of the surface of CF₁ subunit δ is exposed within the quaternary structure of the ATP-synthase complex of photosynthetically active thylakoids, but part of the surface of δ is shielded.

Trypsination of isolated δ occurs at several sites, but this protease does not attack δ in situ, nor does aminopeptidase. Staphylococcus aureus protease V8 digests CF₁ δ after isolation at residues Asp₅, Glu₁₀, and Glu₁₀, but has no access to these residues of δ in situ. Thus CF₁ subunit δ seems to be shielded within the CF₀CF₁ complex to a large degree.

Direct agglutination of thylakoids by anti δ serum 306 was weak and could be improved tenfold by a Coombs serum (goat anti rabbit gammaglobulin), whereas an anti β serum agglutinated directly. From this we conclude that δ is not accessible at the top of the enzyme; the exposed part is below the large subunits α and β and oriented towards the membrane.

Introduction

Photophosphorylation in higher plant chloroplasts is catalyzed by the thylakoid embedded ATP-synthase complex CF₀CF₁, which consists of the H⁺ conducting membrane integral CF₀ moiety and the ATP synthesizing peripheral CF₁. The recognition structures and binding forces between the two parts of the ATP-synthase complex are not known in detail. Elucidation of this contact region is needed to understand how the energy of the electrochemical H⁺ gradient is transduced to the active site and where it is transformed to a conformational change in CF₁ [1, 2].

During resolution of CF₁ from CF₀ by EDTA treatment subunit δ stays bound to the other four subunits of CF₅; isolated δ can be shown to bind also to thylakoid embedded CF₅ [3]. Thus CF₁ subunit δ in situ, i.e. within the quaternary structure of the ATP-synthase complex CF₀CF₁, is both in specific contact to CF₁ and to CF₀, and could be an intermitting link in energy transduction [4].

These contact regions at the surface of the tertiary structure of subunit δ are inaccessible as long as δ is an integral part of the quaternary structure of CF₀CF₁. But there may be other regions at the surface of δ which are accessible already in situ. These regions can be distinguished using antibodies and proteases. In this publication we show that most antibodies do not react with δ in situ. Therefore nearly all immunogenic surfaces on this subunit seem to be shielded within the ATP-synthase complex. Trypsin and other proteases degrade subunit δ only after iso-
lation and not within the complex: the potential digestion sites are inaccessible in situ.

On the other hand we present evidence for limited exposure of subunit δ. We produced polyclonal antibodies which do have access to CF₁ subunit δ in situ. Also the *Staphylococcus aureus* protease V₈ has access to this part of δ exposed in situ.

### Materials and Methods

The chemicals were of the highest purity available. TPCK-treated trypsin was purchased from Sigma, *Staphylococcus aureus* V₈ and aminopeptidase from Boehringer.

Preparation of CF₁ from thylakoid membranes, isolated from market spinach, was carried out by EDTA extraction according to Lien and Racker [5], except that DEAE Sepharose CL-6B (Pharmacia) was used instead of DEAE Sephadex A 50, and the sucrose gradient centrifugation was left out.

Protein determination was done according to Lowry [6]. SDS polyacrylamide gel electrophoresis according to Lugtenberg [7]; on analytical gels (1 mm thick, 7 cm separation length, 13% acrylamide, Fluka) the samples (10 μg) were run in 5 mm slots, 3 h at room temperature with 27 mA; on preparative gels (2 mm thick, 20 cm separation length) 10–20 mg sample was run as a continuous band, 30 cm wide, 24 h at 4 °C with 35 mA; staining with Coomassie brilliant blue (Serva G 250) or in addition, after destaining with 5% methanol/7.5% acetic acid, with silver dichromate according to Merril [8].

Electroelution of subunits β and δ, and proteolytic breakdown products from CF₁, separated on SDS gels, was carried out according to Hunkapiller [9].

Immunization of rabbits was done as described [10, 4]; about 100–200 μg of electroeluted polypeptide δ after HPLC purification was used for each injection. Western immuno blot were carried out as described by Towbin et al. [11], onto nitrocellulose (Schleicher & Schuell), and using horse radish peroxidase conjugated second antibody [12]. Agglutination was observed on microscope slides [10].

The amino acid sequences of polypeptides was determined by automated gas phase Edman degradation (Applied Biosystems Sequenator) and by identification of the phenyl thiohydantoin derivatives [13].

Analytical and preparative separation of polypeptides and proteolytic peptides was done by HPLC (Waters) on reversed phase columns (C₄ and C₈, Macherey & Nagel, 300 Å, 5 μm) by acetonitrile gradient. Collected fractions were concentrated in a Speed-Vac, dissolved in buffer and tested for immunochemical reactivity in enzyme-linked immunosorbent assay (ELISA) [14].

Cyclic photophosphorylation in the presence of PMS was measured by ³²P incorporation as described [15]. Inhibition was done by incubating isolated thylakoids in sTN (100 mM sucrose, 10 mM tricine pH 8.5, 10 mM NaCl) with the sera and gammaglobulin fractions, respectively, in PBS (10 mM phosphate pH 7.4, 140 mM NaCl), 10 min at room temperature in the dark.

The gammaglobulin fraction was prepared from control- and antisera using caprylic acid and ammonium sulfate precipitation [16].

### Results

1. Experiments suggesting a high degree of inaccessibility of δ in situ

Several experiments from the literature suggest inaccessibility of CF₁ subunit δ in situ, i.e. both within the quaternary structure of the ATP-synthase complex CF₁CF₂ in the thylakoid membrane and after isolation of the complex in detergents (cp. Discussion). We present results which strengthen this point.

1.1 Antisera against δ as probes for the degree of inaccessibility

Agglutination of isolated thylakoids by a monospecific antiserum against δ can be taken as proof for accessibility of δ in situ [10, 17], inaccessibility of corresponding epitopes may be concluded, if no agglutination occurs. Antisera against spinach CF₁ δ have been produced in our laboratory by injecting into rabbits diverse preparations of δ. All these antisera agglutinated suspensions of isolated thylakoids. In Western immunoblot analysis the sera cross-react with CF₁ subunit β, however (data not shown).

Therefore one of the anti δ sera, serum 120, was absorbed with CF₁ subunit β, which had been electroeluted from CF₁ separated on preparative SDS slab gels. After removal of the antibodies cross-reacting with β residual antibodies still precipitated isolated CF₁ or dissociated δ and decorated the 21 kDa δ band in Western blot; the corresponding
epitopes on δ are inaccessible within the CF$_1$CF$_1$ complex, however, since the absorbed antiserum did not agglutinate thylakoids any more.

1.2 Proteases as probes for the degree of exposure of δ

The proteases trypsin, aminopeptidase and Staphylococcus aureus V8 were applied, to study the exposure of CF$_1$ subunit δ.

Trypsin digestion of thylakoids was performed and monoclonal antibodies against spinach CF$_1$ δ were used for specific detection of proteolysis. The production and characterization of the monoclonal antibodies will be described elsewhere (W. Finke, to be published). Trypsin had no effect on δ in situ up to 50 µg trypsin/mg chl, as analyzed in Western blots (data not shown). After isolation δ is degraded rapidly. Trypsination of δ already occurred after resolution of CF$_1$ from CF$_0$, e.g. by EDTA treatment. Since subunit δ in situ is not susceptible to trypsin digestion, no arginines and lysines are exposed, at least. CF$_1$ δ contains 6 arginines and 12 lysines in amino acid analysis (H. E. Meyer, unpublished), dispersed along the sequence deduced from cDNA [19]; we conclude that most of the protein surface is shielded inside the CF$_0$CF$_1$ complex.

Treatement of isolated thylakoids with aminopeptidase did not lead to degradation of δ, as analyzed in Western blots (data not shown). Thus the N-terminal amino acids are not susceptible to degradation by this protease and probably not exposed. Isolated δ is degraded.

Treatment of thylakoids with the protease V8 from Staphylococcus aureus decreased the apparent mol. weight of CF$_1$ δ only by about 1 kDa as analyzed in Western blot, whereas after proteolysis of isolated δ by V8 several smaller peptides were detectable: Subunit δ, isolated by electroelution of the 21 kDa band from CF$_1$, separated on preparative SDS gels, was subjected to the protease V8 (50 µg δ in 50 mm (NH$_4$)$_2$HCO$_3$ pH 7.8 plus 0.5 µg V8, 16 h at 25 °C). The incubation was stopped with DIFP (10$^{-6}$ m final conc.). The mixture was separated on SDS gels, blotted and analyzed with monoclonal antibodies. Several peptides are decorated (data not shown). In further experiments such an incubation mixture was separated on HPLC (C$_8$, 300 Å, 5 µm; Macherey & Nagel; elution with acetonitrile gradient 0–80%). Prominent well separated peaks were concentrated in a Speed-Vac and analyzed in the automated gas phase sequenator for amino acid sequence. The following peptides were obtained:

- peptide 1: NKRSVLDE
- peptide 2: FEDVFNKITGTE

The peptides sequenced were found to be identical to stretches of amino acids in the sequence of spinach CF$_1$ δ, deduced from cDNA [19]. They correspond to D$_{53}$/N$_{64}$ up to E$_{61}$ and to E$_{64}$/F$_{95}$ up to E$_{106}$. The protein data thus confirm the deduced sequence in these regions and suggest that at least the regions around residues Asp$_{53}$, Glu$_{61}$, Glu$_{64}$ and Glu$_{106}$ of CF$_1$ subunit δ are not exposed in the CF$_0$CF$_1$ complex to the aqueous environment. (The four acidic residues are not situated in the epitope of the monoclonal antibody used.)

One peptide, separated from the incubation mixture by HPLC, yielded the sequence: VIGPNNGSVP which is a stretch of amino acids from the Staph. aureus V8 protease.

2. Production of a monospecific polyclonal antiserum to CF$_1$ δ with subunit δ purified by HPLC

For topographical studies [10] as well as for quantitative determination of subunits δ [17] antisera which do not cross-react with other CF$_1$ subunits or thylakoid polypeptides are needed. We do not disregard the cross-reactivity of anti δ sera in Western blot with CF$_1$ subunit β, depicted in ref. [20] and seen in our laboratory, as “background”, but take it as a specific reaction. No sequence homologies between CF$_1$ δ [19] and CF$_1$ β [21] are apparent. In a separate publication we show that the cross-reactivity is due to a co-purification of subunit δ, used for immunization, with breakdown products of CF$_1$ β.

The electroeluted δ peptide was homogeneous on SDS gels after silver stain [8]. On overloaded SDS gels of CF$_1$ a band at 21 kDa was decorated in Western immunoblots, if an antiserum against CF$_1$ β, serum 249, was used. This cross-reaction of the anti β serum with the 21 kDa band indicates that one breakdown product of β runs exactly in the position of subunit δ on SDS gels. The earlier preparations of subunit δ had been pure enough for sequencing the 35 N-terminal amino acids of spinach CF$_1$ δ [4], but not for immunization. During isolation of CF$_1$ δ using MEGA 9 and TSK-DEAE 650 S columns [22], some proteolysis was detected and interpreted as proteolysis of subunit δ [23]. Breakdown of CF$_1$ sub-
unit $\beta$ cannot be excluded also in this case, however, since some antisera against CF$_i$ $\delta$ purified by this method still cross-react with CF$_i$ $\beta$ in Western blot (data not shown).

Therefore we applied HPLC for further purification of CF$_i$ $\delta$, electroeluted from SDS gels (Fig. 1).

Three peaks were obtained. From the apparent mol. weights and the reaction in ELISA with CF$_i$ subunit antisera it follows that peak 1 represents a trimeric form of $\delta$, peak 2 the suspected breakdown product of $\beta$, migrating at 21 kDa, and peak 3 monomeric $\delta$. The purified preparation of CF$_i$ $\delta$, peak 1, was used for immunization and yielded the anti $\delta$ serum 306.

The specificity of this anti $\delta$ serum was assessed by the following tests: The serum is positive in ELISA with isolated $\delta$, but not with $\delta$-free CF$_i$, CF$_i(-\delta)$, separated on TSK columns in the presence of Mega 9 and ATP according to ref. [22]. In Western blot analysis the serum was positive with the 21 kDa polypeptide of CF$_i$ and of thylakoids of spinach; no other polypeptide was decorated (Fig. 2). The serum is positive with 21 kDa CF$_i$ $\delta$ of pea, but not with the 25 kDa polypeptide of maize which represents CF$_i$ $\delta$ [24].

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**Fig. 1.** Purification of CF$_i$ $\delta$, electroeluted from preparative SDS gels, by HPLC. 0.2 ml of electroeluted subunit $\delta$ (100 ng, 0.05% trifluoroacetic acid) was injected onto a reversed phase column (C$_8$, 300 Å, 5 μm; Macherey & Nagel) and eluted with a acetonitrile gradient (buffer A: 0.1% TFA in water; buffer B: 80% acetonitrile in water, 0.08% TFA). All peak fractions were dried in a Speed-Vac and analyzed in ELISA (antisemum dilution 1:10,000; second antibody: goat anti rabbit gammaglobulin, Medac, 1:3000). Molecular weights of positive peaks were determined on SDS gels.

<table>
<thead>
<tr>
<th>peak</th>
<th>mol weights [KDa]</th>
<th>ELISA with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>serum 249 (anti $\beta$)</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>serum 238 (anti $\delta$)</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>serum 238 (anti $\delta$)</td>
</tr>
</tbody>
</table>

**Fig. 2.** Analysis of specificity of antiserum 306 in Western blot with thylakoids. (Isolated and washed thylakoids were separated on 13.5% SDS polyacrylamide gels as a continuous band, 56 μg Chl/cm gel, 20 cm separation length. After blotting the nitrocellulose was cut into 5 mm strips, incubated with antibodies and decorated with secondary antibody.) (1) Antiserum against spinach CF$_i$, $\delta$ 306-3, 1:200; (2) anti EDTA CF$_i$, serum 198-t, 1:500; (3) anti $\delta$, 306-4, 1:200; (4) anti spinach D$_2$ oligopeptide, 268-t, 1:200, for reference; (5) anti CF$_i$, 198-t, 1:200; (6) anti $\delta$, 306-3, 1:100; (7) anti $\delta$, 306-5, 1:200; gel with 7 cm separation length; SSU = small subunit of chloroplast ribulose bisphosphate carboxylase.)
Antiserum 306 failed to precipitate subunit δ in Ouchterlony tests and rocket immuno electrophoresis; subunit δ used as antigen had been isolated in the presence of EDTA, Mega 9 or SDS, respectively. The failure in precipitation could be due to a different conformation of δ used as immunogen after acetonitrile treatment. But because δ in the SDS conformation is positive in Western blot with the antiserum 306, we suppose that the serum contains antibodies against too few determinant groups (epitopes) on the surface of δ, and therefore does not precipitate. The serum cannot be used for quantitative determination of δ in precipitation reactions.

3. Experiments showing partial exposure of δ in situ

To show accessibility of CF₁ subunit δ within the ATP-synthase complex to antibodies, three strategies were followed: agglutination of isolated thylakoids by the anti δ serum 306, absorption of antibodies from this serum by thylakoids and analysis in Western blot, and inhibition of cyclic photophosphorylation. From the accessibility to antibodies we conclude partial exposure of CF₁ δ in situ.

3.1 Direct and indirect agglutination

The monospecific δ antiserum 306 agglutinates suspensions of isolated thylakoid systems (Table I). Thus at least one epitope of subunit δ must be accessible and exposed in situ within the CF₀CF₁ complex.

In this semiquantitative agglutination reaction the titer of the δ serum 306 was increased about 10-fold by the addition of a second antibody (goat anti rabbit gammaglobulin), whereas the titer of the β antiserum 249 was only increased about 2-fold. This is evidence that the antibodies, after having reacted with δ on one membrane surface, do not extend much and are sterically hindered to react with a second polypeptide δ anchored on another thylakoid surface, to cause agglutination [10]. After mild trypsin treatment the thylakoids are still agglutinated by this serum.

3.2 Absorption

The antiserum 306 was absorbed with thylakoid suspensions, to remove all antibodies reacting with δ in situ, and the supernatant tested in Western blot. Agglutination with the supernatants became negative, whereas the strength of the band in Western blot did nearly not decrease (blots not shown). Thus the serum contains at least two types of antibodies, one reacting with an exposed epitope of δ which agglutinates and can be absorbed, and another which cannot be absorbed with thylakoids and which is still positive with δ in Western blots. The antibodies in the absorbed serum reacted also with isolated CF₁ after EDTA treatment in ELISA; we conclude that the difference is not due to different conformations of epitopes on δ, but that most antibodies in the serum do not have access to their epitope in situ.

Table I. Direct and indirect agglutination of isolated spinach chloroplast thylakoid systems by antiserum 306 against CF₁ subunit δ.

<table>
<thead>
<tr>
<th>Serum dilution</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
<th>1:32</th>
<th>1:64</th>
<th>1:128</th>
<th>1:256</th>
<th>1:512</th>
</tr>
</thead>
</table>
| **a) Direct agglutination**
| serum         |     |     |     |      |      |      |       |       |       |
| control 306-0 | ±   | ±   | -   | -    | -    | -    | -     | -     | -     |
| anti δ 306-5  | +++ | +++ | ++  | +    | ±    | -    | -     | -     | -     |
| anti β 249-t  | +++ | +++ | +++ | +    | +    | ±    | -     | -     | -     |
| **b) Indirect agglutination**
| serum         |     |     |     |      |      |      |       |       |       |
| control 306-0 | ±   | ±   | -   | -    | -    | -    | -     | -     | -     |
| anti δ 306-5  | +++ | +++ | +++ | +++  | +++  | +++  | +     | +     | +     |
| anti β 249-t  | +++ | +++ | +++ | +    | +    | +    | ±     | -     | -     |

a) Direct agglutination: 5 µl of thylakoid suspension, 0.1 mg Chl/ml in 10 mM NaCl, were mixed on microscope slides with 5 µl of diluted serum, diluted in (PBS) 20 mM phosphate buffer pH 7.8 and 150 mM NaCl, incubated by gentle tilting for 30 sec and observed in the microscope.

b) Indirect agglutination: First incubation as above; additional second incubation with a Coombs antibody (goat anti rabbit IgG, Medac), 5 µl diluted 1:1000 with PBS.
These epitopes seem to be located on the shielded part of the surface of subunit δ within the CF₀CF₁ complex.

3.3 Inhibition of cyclic photophosphorylation by serum 306

It is not known in molecular detail, how subunit δ is involved in the function of the ATP-synthase complex. We tested the effect of antiserum 306-t and the preimmune serum of the same rabbit, 306-0, on cyclic photophosphorylation catalyzed by photosystem I and PMS. The antiserum inhibited, but also the control serum had some effect. Therefore the gammaglobulin fraction was prepared from both sera according to ref. [16], and the effect of the isolated globulins tested (Fig. 3). A clear inhibition of cyclic photophosphorylation occurred after incubation with the anti δ antibodies; the inhibitory effect of the control serum had been removed.

![Inhibition of PMS catalyzed cyclic photophosphorylation by antiserum 306 against CF₁ δ.](image)

The antibodies must have reacted with an accessible epitope on the surface of CF₁ subunit δ, exposed at the ATP-synthase complex on the active thylakoid membrane.

Discussion

The binding between the membrane integral moiety CF₀ of the photosynthetic ATP-synthase complex and the peripheral CF₁ leads to specific H⁺ conduction. The efflux from the thylakoid lumen is tightly coupled to ATP formation in CF₁ [25]. CF₁ removal causes loss of ATP formation, acceleration of H⁺ efflux and in turn an acceleration of electron transport. From hybrid reconstitution experiments it follows that the binding regions on CF₁ and CF₀ are similar among different plant species [26], but not identical (W. Finke, to be published).

It was shown that isolated CF₁ subunit δ binds to CF₀ on the thylakoid membrane [3], and depending on conditions stops H⁺ efflux and partially reconstitutes photophosphorylation [23]. The function, location and orientation of CF₁ δ within the quaternary structure of the ATP-synthase complex is not known in detail. In this publication we investigate to what extent δ is sandwiched between CF₀ and CF₁. Such a location would be required, if δ is part of the structure for H⁺ conduction through CF₀ and into CF₁ [4].

Antibodies are believed not to penetrate through biological membranes [10] or into proteins; they react with accessible epitopes at exposed surfaces. The same applies to proteolytic digestion; in the latter case the kinetics of a limited proteolysis provide additional information. In case of negative results the corresponding epitope was not accessible to the antibodies, and corresponding amino acid residues could not be reached by the specific protease, respectively.

Our antisera against CF₁ subunit δ agglutinated suspensions of isolated thylakoids. We found, however, that the earlier sera against δ cross-react in Western blots with CF₁ subunit β; the same was true for an antiserum against δ from the laboratory of N. Nelson, analyzed in ref. [20]. Therefore no conclusions could be drawn concerning the location of δ within CF₀CF₁. The cross-reaction does not hint to a sequence homology between CF₁ subunit δ [19] and CF₁ β [21], but was probably due to a co-purification of the immunogen subunit δ with a breakdown product of β of an apparent molecular weight identical to
the one of δ (Fig. 1). This peptide must be very immunogenic.

Due to the cross-reactivity of the earlier produced antiserum, quantitative determinations by rocket immunodiffusion of δ content in CF₁ preparations [17, 27] are unreliable.

In reconstitution of photophosphorylation more CF₁ or CF₁(−δ) have to be added than was removed [3, 18]. We suspected that this is due to partial proteolytic breakdown of subunit δ [24]. By use of monoclonal antibodies it can be shown that partial proteolysis of δ indeed takes place, if CF₁ is isolated by EDTA extraction of thylakoids and ion exchange chromatography at room temperature [5], but not at 4 °C. The recommendation by Lien and Racker [5] to prepare CF₁ at room temperature to prevent cold inactivation, should not be followed any more.

Hermans et al. [19] deduce from the sequence of subunit δ a high degree of “surface exposure”. The following data, however, suggest an inaccessible location of CF₁ subunit δ within the quaternary structure in situ:

- Precipitation rockets can be achieved with anti δ and CF₅CF₆ as antigen only, if the complex is dissociated, e.g. for 2 h by desoxycholate [17, 24].
- Trypsin digests δ after isolation, but not in situ; this was analyzed on Coomassie stained SDS gels in ref. [28]; in this publication, however, by specific antibodies.
- Aminopeptidase does not digest δ on the chloroplast membrane.
- The protease V8 from Staphylococcus aureus digested isolated subunit δ at four charged residues, Asp₉₃, Glu₄₀₁, Glu₄₀₄ and Glu₄₀₆₀, but not in situ. The dimensions of this hydrophilic polypeptide, determined with isolated δ in solution, do not fit, however; the calculated shape is an elongated rod of about 30 times 90—100 Å [29, 30]. Since CF₁ is depicted to be about 60—80 Å high [25], δ has to be partially exposed, even if it is sticking within the barrel of the 6 large subunits and part of the central mass, as suggested [31].

We show that subunit δ is mostly shielded, but indeed partially exposed:
- The monospecific antiserum 306 against CF₁ δ agglutinates isolated thylakoids (Table I); the agglutinating antibodies can be absorbed from the serum, whereas other antibodies after the absorption with thylakoids still react in Western blot with δ and in ELISA with CF₁.

- The earlier antisera did not contain antibodies against the exposed part of subunit δ, since they did not agglutinate thylakoid suspensions after absorption of the antibodies cross-reacting with CF₁ subunit β.
- The titer of the agglutinating antibodies 306 was rather low in direct agglutination (Table I), but could be increased tenfold by addition of a 2nd antibody, goat anti rabbit gammaglobulin (Coombs test). Each CF₁ extends for about 100 Å, but antibodies can not bridge epitopes further apart than about 120 Å; the indirect agglutination is interpreted as steric hindrance for the antibodies to bond to two exposed epitopes at the same time and thus to connect two thylakoids [32]. Therefore subunit δ seems to extend below the large CF₁ subunits α and β, and not at the top of the complex.
- Antibodies from antiserum 306 inhibit PMS mediated cyclic photophosphorylation (Fig. 3). Therefore subunit δ not only is partially accessible, but the exposed part must be involved in the function of this polypeptide in the ATP-synthase complex. Until now we had suspected that the function of δ would be located on the inaccessible part between CF₀ and CF₁.

Since E. coli F₁ δ and mitochondrial OSCP are subunits homologous to CF₁ δ as deduced from the amino acid sequence [4, 19, 24], the results on the location of CF₁ δ in the photosynthetic ATP-synthase complex suggest a similar location and orientation of the homologous polypeptides in the other systems.

In the subsequent publication we will identify the amino acid residues of δ which are exposed at the surface of the quaternary structure of CF₅CF₆ and show that the exposed part comprises not more than about 18 out of 187 residues of this CF₁ subunit.

Acknowledgements

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