Localization and Orientation of Subunit Delta of Spinach Chloroplast ATP-Synthase within the CF_0CF_1 Complex
1. Distinction of Shielded and Exposed Surfaces of Delta on the Thylakoid Membrane

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Z. Naturforsch. **44c,** 153–160 (1989); received June 24/October 7, 1988

Accessibility, Antibodies, Coupling Factor, Proteolysis, Photophosphorylation

A new polyclonal antiserum against spinach CF_1 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 $CF_1(-\delta)$; therefore it is regarded monospecific. The polypeptide used as immunogen had been purified by HPLC. Earlier antisera against CF_1 δ cross-react with CF_1 subunit β.

The new antiserum 306 contains different antibodies; some can be absorbed with thylakoids, *i.e.* by δ within the assembled CF_0CF_1 complex on the membrane, others still react in ELISA with isolated CF_1. The former antibodies agglutinate thylakoids and inhibit PMS cyclic photophosphorylation. Therefore we conclude that part of the surface of CF_1 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 CF_1 δ after isolation at residues Asp_{53}, Glu_{61}, Glu_{95} and Glu_{106}, but has no access to these residues of δ *in situ.* Thus CF_1 subunit δ seems to be shielded within the CF_0CF_1 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 extending 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 CF_0CF_1 which consists of the H^+ conducting membrane integral CF_0 moiety and the ATP synthesizing peripheral CF_1. 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 CF_1 [1, 2].

During resolution of CF_1 from CF_0 by EDTA treatment subunit δ stays bound to the other four subunits of CF_1; isolated δ can be shown to bind also to thylakoid embedded CF_0 [3]. Thus CF_1 subunit δ *in situ, i.e.* within the quaternary structure of the ATP-synthase complex CF_0CF_1, is both in specific contact to CF_1 and to CF_0, 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 CF_0CF_1. 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-

Abbreviations: CF_1, peripheral moiety of chloroplast ATP-synthase; CF_0, membrane integral moiety of chloroplast ATP-synthase; F_1, ATPase of oxidative phosphorylation; OSCP, oligomycin sensitivity conferring protein, a coupling factor in mitochondria; α, β, γ, δ, ε, subunits of CF_1; 306-0, preimmune serum of rabbit 306; 306-1,2,3..t, successive bleedings of *anti* δ serum; Chl, chlorophyll; DIFP, diisopropyl fluorophosphate; PMS, phenazine methosulfate.

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/89/0100–0153 $ 01.30/0

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 CF_1 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 CF_1 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 CF_1, 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 blots* 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 (C_4 and C_8, 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 ^{32}P 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 CF_1 subunit δ *in situ, i.e.* both within the quaternary structure of the ATP-synthase complex CF_0CF_1 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 CF_1 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 CF_1 subunit β, however (data not shown).

Therefore one of the *anti* δ sera, serum 120, was absorbed with CF_1 subunit β, which had been electroeluted from CF_1 separated on preparative SDS slab gels. After removal of the antibodies cross-reacting with β residual antibodies still precipitated isolated CF_1 or dissociated δ and decorated the 21 kDa δ band in Western blot; the corresponding

epitopes on δ are inaccessible within the CF_0CF_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.

Trypsination 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_0CF_1 complex.

Treatment 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 $(NH_4)_2HCO_3$ 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: N K R S V L D E
peptide 2: F E D V F N K I T G T E

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_{54} up to E_{61} and to E_{94}/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_{94} and Glu_{106} of CF_1 subunit δ are not exposed in the CF_0CF_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: V I G P N N G S V P which is a stretch of amino acids from the *Staph. aureus* V8 protease.

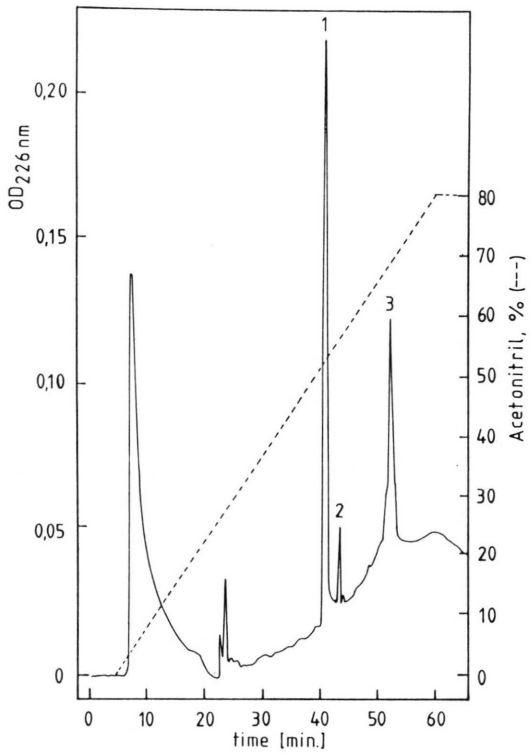
2. Production of a monospecific polyclonal antiserum to CF_1 δ with subunit δ purified by HPLC

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 β cannot be excluded also in this case, however, since some antisera against CF_1 δ purified by this method still cross-react with CF_1 β in Western blot (data not shown).

Therefore we applied HPLC for further purification of CF_1 δ, electroeluted from SDS gels (Fig. 1).



peak	1	2	3
mol.weights [KDa]	60	?	21
ELISA with:			
serum 249 (anti β)	-	++	-
serum 238 (anti δ)	++	±	++

Fig. 1. Purification of CF_1 δ, electroeluted from preparative SDS gels, by HPLC. 0.2 ml of electroeluted subunit δ (100 µg, 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 (antiserum dilution 1:10,000; second antibody: goat *anti* rabbit gammaglobulin, Medac, 1:3000). Molecular weights of positive peaks were determined on SDS gels.

Three peaks were obtained. From the apparent mol. weights and the reaction in ELISA with CF_1 subunit antisera it follows that peak 1 represents a trimeric form of δ, peak 2 the suspected breakdown product of β, migrating at 21 kDa, and peak 3 monomeric δ. The purified preparation of CF_1 δ, peak 1, was used for immunization and yielded the *anti* δ serum 306.

The specificity of this *anti* δ serum was assessed by the following tests: The serum is positive in ELISA with isolated δ, but not with δ-free CF_1, $CF_1(-\delta)$, 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_1 and of thylakoids of spinach; no other polypeptide was decorated (Fig. 2). The serum is positive with 21 kDa CF_1 δ of pea, but not with the 25 kDa polypeptide of maize which represents CF_1 δ [24].

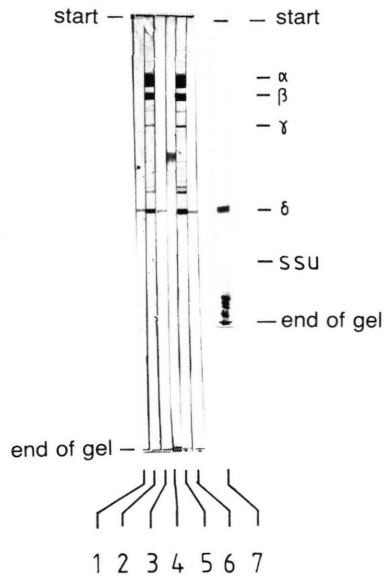


Fig. 2. Analysis of specificity of antiserum 306 in Western blot with thylakoids. (Isolated and washed thylakoids were separated on 13.5% SDS polyacrylamide gels as a continuous band, 56 µg Chl/cm gel, 20 cm separation length. After blotting the nitrocellulose was cut into 5 mm strips, incubated with antibodies and decorated with second antibody.) (1) Antiserum against spinach CF_1 δ 306-3, 1:200; (2) *anti* EDTA CF_1, serum 198-t, 1:500; (3) *anti* δ, 306-4, 1:200; (4) *anti* spinach D_2 oligopeptide, 268-t, 1:200, for reference; (5) *anti* CF_1, 198-t, 1:200; (6) *anti* δ, 306-3, 1:100; (7) *anti* δ, 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 CF_1 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 CF_1 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 CF_0CF_1 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 CF_1 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 CF_1 subunit δ.

	Serum dilution								
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
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 CF_0CF_1 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.

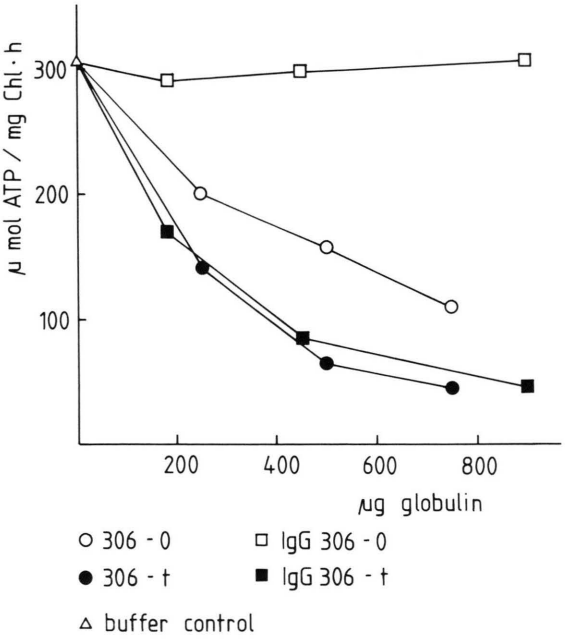


Fig. 3. Inhibition of PMS catalyzed cyclic photophosphorylation by antiserum 306 against CF_1 δ. (Isolated spinach thylakoid systems with 10 µg Chl were incubated as described under "Materials and Methods", the reaction mixture added and the sample illuminated with saturating red light for 1 min; the conc. of gammaglobulin had been determined according to Lowry with BSA as standard [6], concentration of globulin in complete sera was assumed to be 10 mg/ml.)

The antibodies must have reacted with an accessible epitope on the surface of CF_1 subunit δ, exposed at the ATP-synthase complex on the active thylakoid membrane.

Discussion

The binding between the membrane integral moiety CF_0 of the photosynthetic ATP-synthase complex and the peripheral CF_1 leads to specific H^+ conduction. The efflux from the thylakoid lumen is tightly coupled to ATP formation in CF_1 [25]. CF_1 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 CF_1 and CF_0 are similar among different plant species [26], but not identical (W. Finke, to be published).

It was shown that isolated CF_1 subunit δ binds to CF_0 on the thylakoid membrane [3], and depending on conditions stops H^+ efflux and partially reconstitutes photophosphorylation [23]. The function, location and orientation of CF_1 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 CF_0 and CF_1. Such a location would be required, if δ is part of the structure for H^+ conduction through CF_0 and into CF_1 [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 CF_1 subunit δ agglutinated suspensions of isolated thylakoids. We found, however, that the earlier sera against δ cross-react in Western blots with CF_1 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 CF_0CF_1. The cross-reaction does not hint to a sequence homology between CF_1 subunit δ [19] and CF_1 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 antisera, quantitative determinations by rocket immunodiffusion of δ content in CF_1 preparations [17, 27] are unreliable.

In reconstitution of photophosphorylation more CF_1 or $CF_1(-\delta)$ 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 CF_1 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 CF_1 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 CF_1 subunit δ within the quaternary structure *in situ:*

- Precipitation rockets can be achieved with *anti* δ and CF_0CF_1 as antigene 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, Asp_{53}, Glu_{61}, Glu_{94} and Glu_{106}, 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 CF_1 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 CF_1 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 CF_1.
- 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 CF_1 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 CF_1 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 bind to two exposed epitopes at the same time and thus to connect two thylakoids [32]. Therefore subunit δ seems to extend below the large CF_1 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 CF_0 and CF_1.

Since *E. coli* F_1 δ and mitochondrial OSCP are subunits homologous to CF_1 δ as deduced from the amino acid sequence [4, 19, 24], the results on the location of CF_1 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 CF_0CF_1 and show that the exposed part comprises not more than about 18 out of 187 residues of this CF_1 subunit.

Acknowledgements

The antisera against CF_1 subunit δ, 120, and β, 249, have been produced by G. Bonnekamp and W. Nier in our laboratory. The protein sequence analysis was carried out by Dr. H. E. Meyer in the Fakultät für Medizin, Ruhr-Universität Bochum. We appreciate skilful technical assistance of Mrs. R. Oworah-Nkruma and Mr. H. Korte. The investigations have been supported by grants from the Deutsche Forschungsgemeinschaft (Be 664 and SFB 168) to R. J. Berzborn.

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