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Characterization of the α and β-subunits of the F_0F_1-ATPase from the alga *Polytomella* spp., a colorless relative of *Chlamydomonas reinhardtii*

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Abstract

The isolation and partial characterization of the oligomycin-sensitive F_0F_1-ATP synthase/ATPase from the colorless alga *Polytomella* spp. is described. Purification was performed by solubilization with dodecyl-β-D-maltoside followed by Sepharose Hexyl ammonium chromatography, a matrix that interacts with the F_1 sector of mitochondrial ATPases. The α-subunit, which migrates on SDS-polyacrylamide gels with an apparent molecular mass of 55 kDa, was identified by the N-terminal sequencing of 47 residues. This subunit exhibited a short extension at its N-terminus highly similar to the one described for the unicellular alga *Chlamydomonas reinhardtii* (Nurani, G. and Franzén L.-G. (1996) Plant Mol. Biol. 31, 1105–1116). In whole mitochondria, the α-subunit was susceptible to limited proteolytic digestion induced by heat. An endogenous protease removed the first 22 residues of the mature α-subunit. Subunit β was also identified by N-terminal sequencing of 31 residues. This subunit of 63 kDa exhibited a higher apparent molecular mass than α, as judged by its mobility on denaturing polyacrylamide gel electrophoresis. This β-subunit is 7–8 kDa larger than the β-subunits of other mitochondrial ATPases. It is suggested that the β-subunit from *Polytomella* spp. may have a C-terminal extension similar to that described for the green alga *C. reinhardtii* (Franzén, L.-G. and Falk, G. (1992) Plant Mol. Biol. 19, 771–780). In addition, it was found that the C-terminal extension of the β-subunit of *C. reinhardtii* showed homology with the endogenous ATPase inhibitors from various sources and with the ϵ-subunit from the F_0F_1-ATP synthase from *Escherichia coli*, which is considered to be a functional homolog of the inhibitor proteins. The data reported here provide the first biochemical evidence for a close relationship between the colorless alga *Polytomella* spp. and its photosynthetic counterpart *C. reinhardtii*. It is also suggested that the C-terminal extensions of the β-subunits of the ATP synthases from these algae, may play a regulatory role in these enzymes.

Keywords: Colorless alga; Mitochondrion; ATPase, F_0F_1; (*Polytomella*); (*Chlamydomonas reinhardtii*)

Abbreviations: EDC, 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide; EDTA, ethylenediaminetetraacetic acid; HA, hexyl ammonium; M_r, apparent relative molecular mass; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethanesulfonylfluoride; PVDF, poly(vinylidene difluoride)

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1. Introduction

The genus *Polytomella* is believed to have evolved from a *Chlamydomonas*-like ancestor by loosing its cell wall and functional chloroplasts [1–3]. Morphologically and structurally the colorless alga of the genus *Polytomella* resemble the photosynthetic algae of the genus *Chlamydomonas* [4]. Both genus exhibit similar nuclear encoded beta-tubulin genes [5], closely related 18S rDNA sequences [3], homologous mitochondrial *cox1* genes [6] and similar nuclear [5] and mitochondrial [6] codon usage bias. Nevertheless, no biochemical evidence that shows that these two algae are related has been provided so far.

The F_0F_1-ATP synthase/ATPase (EC 3.6.1.3) plays a central role in energy conservation in mitochondria, chloroplasts and bacteria. This membrane-bound complex catalyzes the phosphorylation of ADP by inorganic phosphate using the protonmotive force generated by the light-driven or substrate-driven electron transfer chains [7–9]. The protein complex consists of two main domains, one is the membrane-bound sector F_0, involved in proton translocation. The other is F_1, the extrinsic domain that catalyses the synthesis of ATP; when F_1 is dissociated from F_0, it catalyses ATP hydrolysis [10]. The F_1-ATPase contains five subunits in a $3\alpha/3\beta/1\gamma/1\delta/1\epsilon$ stoichiometry [11]. The three dimensional structure of the mitochondrial bovine heart F_1-ATPase determined by Abrahams et al. [12] shows that β-subunits alternate with α-subunits in a sphere-like structure, building a catalytic core that rotates during catalysis [13,14]. From prokaryotes to eukaryotes, including higher plants, the structure of the α and β-subunits appears to be fairly well conserved between organisms [11]. However, in the green alga *C. reinhardtii* the α and β-subunits of the mitochondrial ATPase exhibit particular features. On SDS-PAGE, these two subunits migrate as proteins larger than those from mitochondria of other species, with apparent molecular masses of 55 kDa and 63 kDa for the α and β-subunits, respectively [15,16]. The primary structures of these subunits derived from the cDNA sequences indicate that the mature α-subunit is longer due to an extension of 15–18 residues at the N-terminus [16], while the β-subunit exhibits an extension of 60–70 residues at its C-terminal end [17]. Therefore, the characterization of the two largest subunits of the ATPase appeared of interest to gain insight into the evolutionary relationship between the colorless alga *Polytomella* spp. and its green counterpart *C. reinhardtii*.

In this study we report on the isolation of the oligomycin-sensitive F_0F_1-ATP synthase from *Polytomella* spp., some of the structural characteristics of the α and β-subunits are described; the data provides evidence that indicates that there is a close relationship between the colorless alga *Polytomella* spp. and its photosynthetic counterpart *C. reinhardtii*. The effect of heat treatment on whole mitochondria and isolated F_0F_1-ATPase is also described. In the former, heat treatment induces cleavage of 22 residues at the N-terminal region of the α-subunit.

2. Materials and methods

2.1. Biological materials

Polytomella spp. (198.80, E.G. Pringsheim) was obtained from the algae collection at the University of Gottingen (Germany), and maintained by Dr. Jovita Martínez at the Microbiological Collection of the Department of Biotechnology (CINVESTAV del I.P.N., Mexico) with register CDBB-951. The algae were grown in the medium described by Wise [18] supplemented with vitamins B_1 (0.06 mg/l) and B_{12} (0.08 mg/l) [19].

2.2. Preparation and fractionation of mitochondria

Mitochondria from *Polytomella* spp. were prepared as described by Gutiérrez-Cirlos et al. [20]. Whole mitochondria (30 mg of protein/ml) were washed twice with 2 vols. of 20 mM Tris-Cl (pH 7.6) containing 2 mM EDTA (buffer A) in presence of the following protease inhibitors: 1 mM PMSF, 5 mM ϵ-amino caproic acid, and 1 mM benzamidine. The resulting pellet of mitochondrial membranes was resuspended at a protein concentration of 20 mg/ml in buffer A containing 0.2 mM PMSF. This preparation was used for the purification of the F_0F_1-ATP synthase.

2.3. Purification of the F_0F_1-ATP synthase

All steps were carried out at 4°C. The resuspended pellet of washed mitochondrial membranes was solu-

bilized in the presence of 25 mM dodecyl maltoside. The suspension was incubated on ice for 30 min and centrifuged for 30 min at maximum speed in a benchtop Eppendorf centrifuge. The large yellowish pellet was discarded, and the supernatant (1–2 ml) was applied to a Sepharose HA (Pharmacia) column (1 × 20 cm) [21,22] equilibrated with buffer A containing 10 mM cholate, 1 mg/ml of phospholipids (purified soybean asolectin), 2 mM ATP, and the mixture of protease inhibitors. The ATP synthase was eluted with 1 M KCl as described by Dreyfus et al. [21]. The active fractions were pooled and precipitated by addition of one volume of saturated ammonium sulfate, and stored at −70°C until used.

2.4. Activity measurements

ATPase hydrolytic activity of mitochondria or of the isolated complex was followed spectrophotometrically at room temperature using an ATP regenerating system that follows NADH oxidation at 340 nm described by Pullman et al. [23]. Activities are expressed as μmol ATP hydrolyzed/min/mg of protein. Protein concentrations were determined according to Lowry et al. [24] with the modifications of Markwell et al. [25].

2.5. Protein analysis

SDS-PAGE was performed according to Laemmli [26] using 1.2 mm thick slab gels with 9% or 12% acrylamide. Gels were stained with Coomassie Blue. The molecular mass markers used were phosphorylase *b* (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21 kDa) and lysozyme (14.4 kDa). For N-terminal sequencing, the polypeptides were electrotransferred onto a PVDF ProBlott membrane (Applied Biosystems) at 40 V for 2.5 h (4°C) in 50 mM boric acid, 50 mM Tris-base buffer, without adjusting pH. The membranes were stained with Coomassie Blue R, destained, and air dried. N-terminal sequence analysis of the polypeptide bands of 63, 55 and 53 kDa was carried out by J. d'Alayer at the Laboratoire de Microséquençage des Protéines (Institut Pasteur, Paris, France) on an 473A Sequencer (Applied Biosystems). In an alternative protocol, the 63 kDa polypeptide was also transferred to a ProBlott membrane following the procedure of Matsudaira [27], and sequenced on a 470 Microsequencer with on-line PTH analysis (Applied Biosystems) at the W.M. Keck Foundation Biotechnology Resource Laboratory, Yale University, USA.

2.6. Limited proteolysis of the α-subunit from the F_0F_1-ATPase

For experiments with mitochondrial membranes, incubations at 50°C for the indicated periods of time were carried out. For experiments with solubilized membranes, mitochondria were treated with 25 mM dodecyl maltoside in 20 mM Tris-Cl (pH 7.6). The effects of heat treatment on the polypeptide composition were analyzed by SDS-PAGE (12% acrylamide).

2.7. Sequence analysis

Sequences were analyzed using the GCG Sequence Analysis Software Package (Genetics Computer Group, Madison, WI). Alignments were carried out with the Pileup program [28] using sequences in the SwissProt data bank. The terms similarity and homology are used as suggested by Reeck et al. [29]. Accepted amino acid substitutions were K = R, D = E, S = T, I = L = M = V and F = Y.

3. Results

3.1. Purification of the F_0F_1-ATPase from Polytomella spp.

We developed a two-step protocol to purify the mitochondrial F_0F_1-ATP synthase from the colorless alga *Polytomella* spp. Table 1 summarizes the recovery of protein and ATPase activity during the purification. Mitochondria were solubilized in the presence of the non-ionic detergent dodecyl maltoside at low ionic strength. In the presence of 25 mM dodecyl maltoside, approximately 40% of the total protein was solubilized, but a preferential extraction of the F_0F_1-ATP synthase was achieved. At higher ionic strength (100 mM NaCl), all the respiratory complexes are extracted at lower detergent concentrations [20]. Upon solubilization the total activity of the sample increased from 0.16 to 0.64 μmol ATP hy-

Table 1
Purification of the F_1F_0-ATPase by Sepharose HA chromatography

Fraction	Total protein (mg)	Total activity [a]	Specific activity [b]
Washed mitochondria	11.40	1.8	0.16
Dodecyl maltoside extract	4.50	3.0	0.64
SepHA chromatography	0.72	1.1	1.60

Activity was measured as described in Section 2.
[a] Total activity expressed in μmol of ATP hydrolyzed/minute.
[b] Specific activity expressed in μmol of ATP hydrolyzed/min/mg of protein.

drolyzed/min/mg of protein; activations of 30–50% were obtained depending on the mitochondrial preparation, probably due to the presence of detergent. The solubilized ATPase activity was more than 80% oligomycin-sensitive, at concentrations of 1–2 μg/ml of the inhibitor.

The solubilized material was then subjected to a chromatographic step on a Sepharose HA column as described previously [21], and the oligomycin-sensitive ATP synthase was released from the column by the addition of 1 M KCl. The isolated complex exhibited a specific hydrolytic activity that ranged from 1 to 5 μmol ATP hydrolyzed/min/mg of protein at 25°C. This value is comparable to that measured for the ATP synthase purified from *C. reinhardtii* mitochondria (2.2 μmol ATP hydrolyzed/min per mg of protein)[16]. With respect to the activity in washed mitochondria from *Polytomella* spp., the Sepharose HA chromatography step of the dodecyl maltoside extract increased the specific ATPase activity 10-fold. The F_0F_1-ATPase recovered from Sepharose HA exhibited 10 polypeptides with apparent molecular masses that ranged from 6 to 63 kDa. Fig. 1 shows the large polypeptides of this preparation.

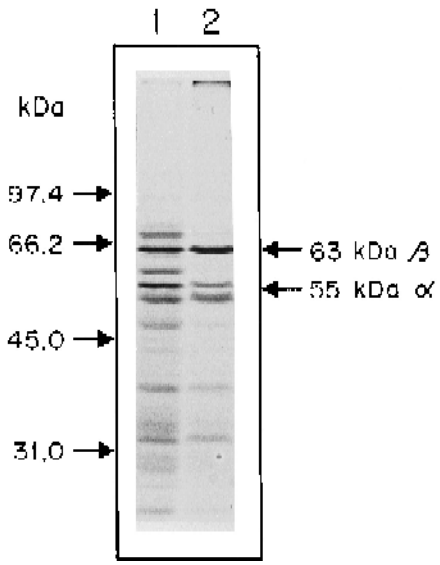

Fig. 1. The mitochondrial F_0F_1-ATPase complex from *Polytomella* spp. The F_0F_1-ATPase preparation was analyzed on a 12% acrylamide gel stained with Coomassie brilliant blue. Lane 1, dodecyl maltoside solubilization supernatant obtained from 100 μg of washed mitochondria. Lane 2, F_0F_1-ATPase fraction eluted from the Sepharose HA column and precipitated with ammonium sulfate. Molecular mass markers are indicated on the left hand side of the figure.

3.2. Identification of the α and β-subunits of the mitochondrial F_0F_1-ATP synthase from Polytomella spp.

The two largest and most abundant polypeptides of the mitochondrial ATP synthase of *Polytomella* spp. (63 and 55 kDa) were subjected to N-terminal sequence analysis. The sequence of 31 residues of the 63 kDa polypeptide and of 47 residues of the 55 kDa polypeptide were obtained (Fig. 2).

The N-terminal sequence of the 55 kDa polypeptide from *Polytomella* spp. ATPase exhibited a high similarity with the α-subunit of the ATP synthase from *C. reinhardtii* [16], 35 out of 40 residues were identical (Fig. 3A). It is relevant that the α-subunits of both *Polytomella* spp. and *C. reinhardtii* have N-terminal domains that are 15–18 residues longer than those from other species, these extensions show hydrophilic properties and a positive net charge.

The N-terminal portion of the 63 kDa polypeptide of the ATP synthase from *Polytomella* spp. was sequenced. Its sequence is markedly similar to that of β-subunits of F_1 from other species (Fig. 3B). Thus

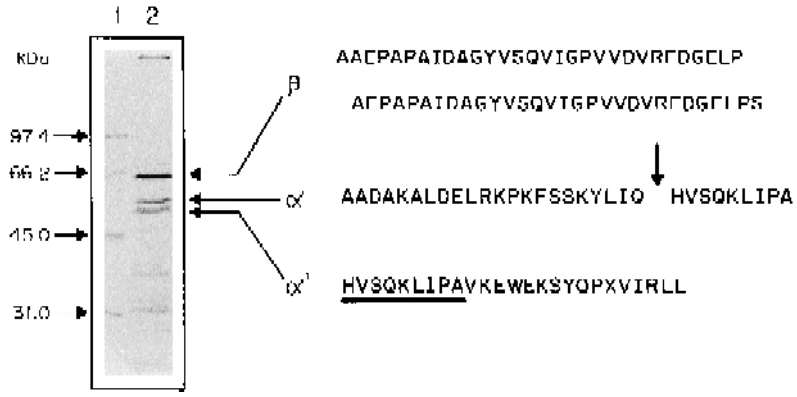

Fig. 2. N-terminal sequences of the β and α-subunits of the mitochondrial F_0F_1-ATPase from *Polytomella* spp. N-terminal sequences of the 63 kDa, 55 kDa and 53 kDa polypeptides, identified as β and α-subunits, and as the α' fragment respectively. The arrow shows the cleavage site of the α-subunit by the soluble protease. The underlined sequence allowed the identification of the α' polypeptide as the limited proteolytic fragment derived from the α-subunit.

the 63 kDa polypeptide was identified as the β-subunit of the ATP synthase, albeit on SDS-PAGE, it appears significantly larger than the β-subunits from mammals, higher plants, fungi and bacteria. In this latter context, it is relevant that the β-subunit of *C. reinhardtii* also migrates as a protein with a M_r of 63 kDa [17], suggesting that the enzymes from *C. reinhardtii* and *Polytomella* spp. are closely related. Furthermore, the comparison of the N-terminal sequences of the β-subunits of the two algae show that out of 30 residues, 22 are identical. The nucleotide sequence of the *C. reinhardtii* cDNA encodes a unique C-terminal extension of 60–70 residues in the β-subunit [17] which explains its lower mobility on SDS-PAGE. Based on the similar M_r of the β-subunit from *Polytomella* spp., it is likely that this-sub-

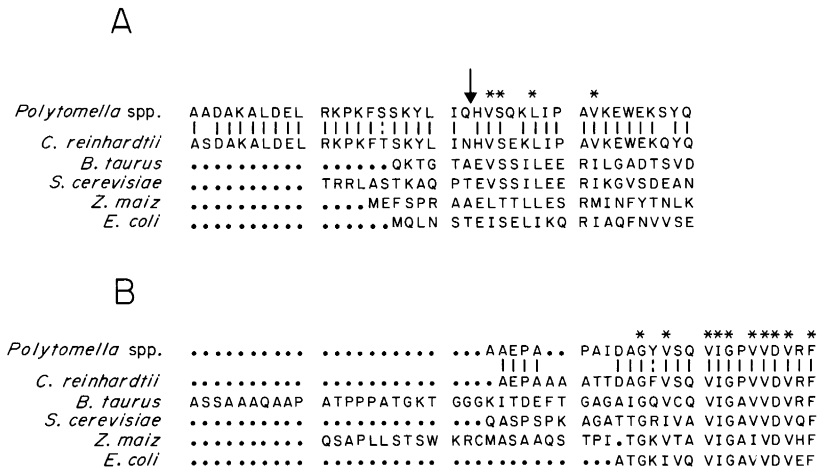

Fig. 3. Sequence alignment of the N-terminal regions from the α and β-subunits of the F_0F_1-ATP synthase from Polytomella spp. with those from various sources. Asterisks denote conserved and semi-conserved residues in the aligned sequences. Panel A, sequences of α-subunits: *Polytomella* spp. (this work), *C. reinhardtii* [16], bovine (*Bos taurus*) [38], yeast (*Saccharomyces cerevisiae*) [39], maize (*Zea mais*) [40], and *E. coli* [41]. The arrow shows the cleavage site of the α-subunit by the putative protease. Panel B, sequences of β-subunits: *Polytomella* spp. (this work), *C. reinhardtii* [17], bovine [42], yeast [43], maize [44], and *E. coli* [41].

unit also contains a similar C-terminal extension of 60–70 residues.

The sequence analysis of the β-subunit of the ATP synthase from *Polytomella* spp. showed two overlapping sequences with a major and a minor component. The major sequence (which represents 70% of the protein, as calculated from the integration of the sequencing peaks) is shown in Fig. 2. The minor sequence (30% of the protein) is identical to the principal component, except that it starts at the second residue (Fig. 2). The amino acid sequence of the same subunit, was determined by different protocols (see Section 2); the results with the two protocols showed the same two overlapping sequences.

3.3. Proteolytic cleavage of the α-subunit of the F_0F_1-ATPase from Polytomella spp.

Incubation of the solubilized mitochondria at 50°C for 15 min was found not to affect the activity of the oligomycin-sensitive ATPase. While the major part of the solubilized proteins were denatured and further pelleted, the ATPase remained soluble and active. Approximately 60% of the proteins could be pelleted after the heating step, including the largely hydrophobic proteins such as the bc_1 complex and cytochrome *c* oxidase (not shown). In contrast, the supernatant contained approximately 60% of the oligomycin-sensitive ATPase activity.

In whole mitochondria, heat treatment led to the simultaneous decrease in the 55 kDa α-subunit and the appearance of the 53 kDa polypeptide. This degradation was not complete, even after 1 h of incubation (Fig. 4). The N-terminal sequence analysis of the 53 kDa polypeptide matched with the sequence of the α-subunit, starting at residue 23 (Fig. 2). Since an unique sequence was determined, the heat-induced proteolytic step appears to be specific, yielding an α-subunit without the extension (fragment α'). The M_r of this fragment is comparable to that of the α-subunits of other species. Therefore, our results suggest that it exists a protease which removes specifically the N-terminal extension of the α-subunit in *Polytomella* spp.

The ATPase purified from heated whole mitochondria still absorbed to the Sepharose HA column, and was released by 1 M KCl, indicating that the action of the soluble protease did not have any effect on the sequence of the β-subunit that interacts with the chromatographic matrix. In addition, when the purified F_0F_1-ATPase obtained from non-heated mitochondria was heated at 50°C, no proteolytic degradation of the α-subunit was observed, indicating that the putative protease was completely removed in the chromatographic step.

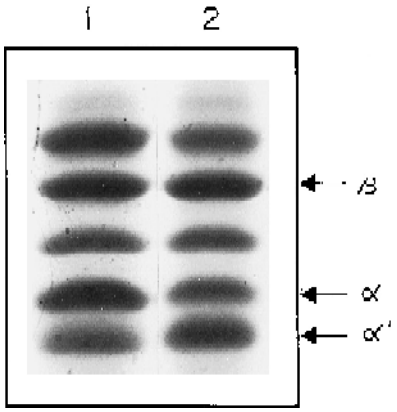

Fig. 4. Induction of a specific proteolysis of the α subunit of the mitochondrial F_0F_1-ATPase. Analysis on 12% SDS-PAGE stained with Coomassie blue. Whole mitochondria (50 μg of protein, lane 1) were incubated at 50°C for 1 h (lane 2).

When the same experiment was performed with two washes of the mitochondria in the presence of protease inhibitors (see Section 2), no proteolysis of the 55 kDa polypeptide was observed. To confirm this result, the mitochondria were incubated with the supernatant derived from the mitochondrial washes. Under these conditions, limited proteolysis of the α-subunit was observed, giving rise again to the 53 kDa cleavage product (data not shown). We conclude that the protease is soluble, active in the presence of protease inhibitors, and easily removed from the mitochondria.

4. Discussion

4.1. The mitochondrial F_0F_1-ATP synthase from Polytomella spp.

This work describes the purification of an oligomycin-sensitive F_0F_1-ATP synthase from *Polytomella* spp. By N-terminal amino acid sequence analysis, the two high molecular mass subunits of 63

and 55 kDa were identified as the β and α-subunits, respectively. The rate of the oligomycin-sensitive ATPase activity of the purified F_0F_1-ATP synthase complex is comparable to that of other organisms, regardless of the notable structural differences between the α and β-subunits.

4.2. The α-subunit of the mitochondrial F_0F_1-ATP synthase from Polytomella spp.

The α-subunit was susceptible to limited proteolytic digestion induced by heating, probably due to the unmasking of a soluble mitochondrial protease activity. This putative endopeptidase removes specifically the first 22 residues from the α-subunit. This N-terminal extension exhibits a high similarity with its counterpart in *C. reinhardtii* [16], but not with that of other α-subunits sequences reported in databases (Fig. 3A). These findings suggest that the N-terminal portion of the α-subunit is unique to members of the family Chlamydomonadaceae. The structural or functional role of this N-terminal extension remains to be established.

4.3. The β-subunit of the mitochondrial F_0F_1-ATP synthase from Polytomella spp.

The 63 kDa polypeptide was also subjected to N-terminal sequence analysis, which allowed its identification as the β-subunit. The N-terminal sequence of the β-subunit from *Polytomella* spp. was found to be highly similar to the sequence of *C. reinhardtii*, i.e. of 31 residues, 20 were identical. Two overlapping sequences of this subunit were determined during the N-terminal sequence analysis. This 'ragged sequence' was confirmed by sequencing the protein prepared by a different protocol. The β-subunit of *C. reinhardtii* is encoded in the nucleus and imported into mitochondria. It contains a presequence that directs the protein into the organelle which is removed by a specific protease in the mitochondrial matrix [15,17]; a consensus sequence AXRXF/A has been suggested to be the recognition site for this proteolytic step [16]. The presence of two overlapping N-terminal sequences in the mature protein may reflect an imperfect precision of the matrix protease. The two alanine residues at the start of the sequence may give rise to two different proteolytic sites separated by a single residue. Nevertheless, the 'mitochondrial processing peptidase' seems to work preferentially (70%) on site +1 rather than on site +2 of the β-subunit from *Polytomella* spp.

4.4. The carboxy-terminal extension of the β-subunit in the algae Chlamydomonadaceae

The β-subunit from the F_0F_1-ATPase of *Polytomella* spp. migrates with a M_r comparable with that of *C. reinhardtii*, significantly different from that of other organisms (7–8 kDa larger). Since the β-subunit of the F_0F_1-ATP synthase complexes from the algae Chlamydomonadaceae share highly homologous N-terminal sequences with various other species, it is likely that the difference in electrophoretic mobility of the β-subunit of *Polytomella* spp. is due to the presence of a C-terminal extension similar to that of the β-subunit of *C. reinhardtii* [17]. The sequencing of the gene encoding the β-subunit in *Polytomella* spp. and/or the C-terminus sequence of the the β-subunit polypeptide should confirm this suggestion. Nurani and Franzén [16] have suggested that in *C. reinhardtii*, the N-terminal extension of the α-subunit (positively charged) may interact electrostatically with the C-terminal extension of the β-subunit (negatively charged). Nevertheless, its function remains to be determined. When the sequence of the C-terminal extension from *C. reinhardtii* reported by Franzén and Falk [17] was compared to that of the endogenous inhibitor protein (IF_1) of the F_0F_1-ATPase [30,31], a significant sequence similarity became apparent (Fig. 5 and Table 2). The C-terminal extension of the β-subunit showed homology with the IF_1 from various sources and with the ϵ-subunit from the F_0F_1-ATP synthase from *Escherichia coli*, which is considered to be a functional homolog of the inhibitor protein from bovine heart [32,33]. Secondary structure analysis of the 60–70 residues C-terminal region of the β-subunit of *C. reinhardtii* predicted the presence of an α-helix structure, characteristic of the C-terminal portions of the inhibitor proteins [34] (data not shown). Therefore, we hypothesize that the C-terminal extension of the β-subunit from the F_0F_1-ATP synthase from *Polytomella* and *Chlamydomonas*, may be the equivalent of IF_1 of mitochondria or of the ϵ-subunit of *E. coli*. It is also noted that the crystallographic structure of the F_1 portion of the ATP synthase determined by Abrahams et al.

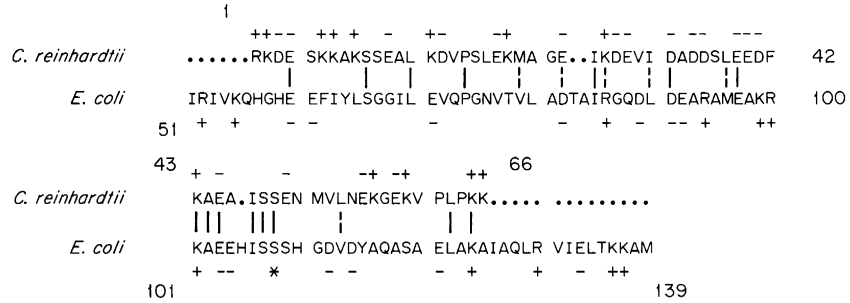

Fig. 5. Sequence alignment of the C-terminal region from the β-subunit of the F_0F_1-ATP synthase from *C. reinhardtii* with the ε-subunit from the F_0F_1-ATP synthase from *E. coli*. The sequences compared are the C-terminal extension of the β-subunit from the ATPase of *C. reinhardtii* [17]; and residues 51–139 from the ε-subunit from the F_0F_1-ATP synthase from *E. coli* [41]. Charged residues are indicated. The asterisk indicates serine 108 in the ε-subunit of *E. coli* (see text and reference [36]).

[12], suggests that this highly charged extension may be in close proximity to the γ-subunit and to the α–β interface. In addition, it is pointed out that the region KAEXXISS, which corresponds to residues 101–108 in the ε-subunit from *E. coli*, is conserved in residues 565–571 of the β-subunit of *C. reinhardtii* (Fig. 5). The ε-subunit from *E. coli* can be cross-linked to the β-subunit by the water-soluble reagent EDC [35]. Furthermore, site directed substitution of serine 108 to a cysteine residue in the ε-subunit of *E. coli* leads to an azide-maleimide-catalyzed crosslinking between the ε and the α-subunit [36] (Fig. 5), suggesting a nucleotide-dependent movement of the ε-subunit between α and β-subunits [37]. Furthermore, when the C-terminal extension of the β-subunit of *C. reinhardtii* was compared in a pileup analysis with IF_1 sequences from different sources (data not shown), the region KAEXXISS aligned with residues 39–47 of the bovine IF_1 sequence, a region that is included between residues 14–47, which constitutes the minimal inhibitory sequence of this IF_1 polypeptide [51]. If the hypothesis here proposed is correct, the functional role of this C-terminal extension in *C. reinhardtii* and in *Polytomella* spp. merits further studies, since there must be three copies per complex (one for each β-subunit), in contrast to the inhibitor protein or the ε-subunit from other systems in which there is only a single copy per ATP synthase. *Polytomella* spp. may be the organism of choice to study the role of this extension, since mitochondria are obtained in a high yield, and the F_0F_1-ATPase may be purified in a simple, two-step procedure.

Round has proposed that the colorless algae arose from a green ancestor by a secondary evolutionary event that involved the loss of the photosynthetic apparatus [1]. The finding that the F_0F_1-ATP synthase of *Polytomella* spp. and *C. reinhardtii* have significant similarities in their polypeptide composition,

Table 2
Comparison matrix of the C-terminal sequence from the β-subunit of the F_0F_1-ATP synthase from *Polytomella* spp. with IF_1 sequences and with the ε-subunit from *E. coli*

		I	II	III	IV	V	VI	VII
I	β extension from *C. reinhardtii*	100						
II	IF_1 from *C. elegans*	51	100					
III	ε-subunit from *E. coli*	47	38	100				
IV	IF_1 from bovine	44	56	38	100			
V	IF_1 from *P. jadinii*	42	65	56	62	100		
VI	IF_1 from *S. cerevisiae*	41	52	38	60	71	100	
VII	IF_1 from rat	41	56	48	76	59	61	100

The sequences compared are IF_1 from *Pichia jadinii* [45]; IF_1 from *Saccharomyces cerevisiae* [46]; IF_1 from bovine heart (*Bos taurus*) [47]; IF_1 from rat (*Rattus norvegicus*) [48,49]; IF_1 from *Caenorhabditis elegans* [50]; the C-terminal extension of the β-subunit from the ATPase of *C. reinhardtii* [17]; and the ε-subunit from the F_0F_1-ATP synthase from *E. coli* [41]. Numbers indicate % similarity.

molecular masses of their subunits, and in the extensions of their α and β-subunits, indicates that indeed there is a close relationship between this two algae. The particular features of the two main subunits which comprise the core of the F_1 domain in the mitochondrial ATP synthase from the algae Chlamydomonadaceae may reflect peculiar functional characteristics of this complex and/or novel pathways for its biogenesis and assembly.

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