Structural and functional characterization of FoF1-ATP synthase on the extracellular surface of rat hepatocytes

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Abstract

Extracellular ATP formation from ADP and inorganic phosphate, attributed to the activity of a cell surface ATP synthase, has so far only been reported in cultures of some proliferating and tumoral cell lines. We now provide evidence showing the presence of a functionally active ecto-FoF1-ATP synthase on the plasma membrane of normal tissue cells, i.e. isolated rat hepatocytes. Both confocal microscopy and flow cytometry analysis show the presence of subunits of F1 (αβγδε), and Fo (FoI-PVP(b) and OSCP) moieties of ATP synthase at the surface of rat hepatocytes. This finding is confirmed by immunoblotting analysis of the hepatocyte plasma membrane fraction. The presence of the inhibitor protein IF1 is also detected on the hepatocyte surface. Activity assays show that the ectopic-ATP synthase can work both in the direction of ATP synthesis and hydrolysis. A proton translocation assay shows that both these mechanisms are accompanied by a transient flux of H+ and are inhibited by F1 and Fo-targeting inhibitors. We hypothesise that ecto-FoF1-ATP synthase may control the extracellular ADP/ATP ratio, thus contributing to intracellular pH homeostasis.

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1. Introduction

The FoF1-ATP synthase complex is a mitochondrial inner membrane-bound enzyme responsible for ATP synthesis from ADP and inorganic phosphate (Pi) driven by a transmembrane electrochemical proton gradient. The complex is structurally and functionally composed of three parts: the catalytic sector F1, consisting of five subunits with a stoichiometry of 3α,3β,γ,ε,δ, the proton translocating, or membrane integral sector Fo, and a stalk connecting F1 with Fo, both consisting of a variable number of subunits [1–5]. In mitochondria ATP synthesis is coupled to proton translocation from the cytosol to the matrix space. ATP hydrolysis drives proton translocation in the reverse direction and is selectively inhibited by the intrinsic inhibitor protein (IF1) [6].

Recent reports show that some structural subunits of the mitochondrial FoF1-ATP synthase are located on the outside of the plasma membrane of different cell lines, such as human umbilical vein endothelial cells (HUVEC) and human hepatocarcinoma cells (HepG2) [7–9]. In these and other proliferating cell lines an active extracellular ATP synthesis has been reported, which is attributed to an ecto-ATP synthase [8–10]. An ecto-adenylate kinase (AK) and ecto-nucleoside diphosphokinase (NDPK) activity have also been detected in these cells [9–11].

To date, the role of the ecto-FoF1-ATP synthase is not yet clear. It has been shown that angiostatin, a potent inhibitor of angiogenesis [12,13], reduces cellular proliferation by inhibiting the ATP synthase activity at the surface of HUVEC [7,8,14]. Martinez et al. [9] reported that, in HepG2 cells, the high-density lipoprotein (HDL) receptor, with high-affinity for apolipoprotein A-1 (ApoA-1), is identical to the β-chain of ATP synthase. In differentiating mouse 3T3-L1 preadipocytes, Arakaki et al. [15] found that ApoA-1 can bind to the ectopic β-chain of H+–ATP synthase, with consequent inhibition of the activity of cell surface ATP synthase and alteration of lipid metabolism in these cells. However, even in the same cell line, differences have been reported regarding the expression level and activity of ecto-FoF1-ATP synthase [10]. It is important to underline that all the above-mentioned studies were conducted on cultured cells, from tumoral and/or proliferating cell lines, and some discrepancies in results have been often attributed to the different culture conditions used, such as the commercial medium employed, the presence or absence of fetal bovine serum and growth factors in the culture medium [10].

The aim of this study was to investigate the possible presence and, if so, the activity and subunit composition of an ecto-FoF1-ATP synthase
in isolated normal tissue cells in the absence of added serum and growth factors. To this end, we choose freshly isolated hepatocytes which, unlike cells in culture, do not need any addition to the medium, and represent an appropriate experimental tool, the closest to in vivo conditions. Isolated hepatocytes in suspension are able to retain, at least for some time, the characteristics of living cells. Hepatocytes were also chosen in the light of the central role of the liver in intermediary and energy metabolism.

The present study represents the first indication of structural and functional connection between the catalytic and proton translocation sectors of the F_{0}F_{1}-ATP synthase complex at the hepatocyte surface. ATP synthesis and hydrolysis values are also reported.

2. Materials and methods

2.1. Chemicals

Oligomycin, CCCP, p,p’-di (Adenosin-5-) pentaphosphate (Ap5A), collagenase Type I, BSA (fraction V fatty acid-free), antimycin A, and ATP bioluminescence assay kit were from Sigma-Aldrich; NADH, pyruvate kinase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, hexokinase, ATP, ADP and phosphoenolpyruvate (PEP) were from Roche; acrylamide, N,N’-methylenebisacrylamide, SDS and Bradford protein assay were from Bio-Rad; ACMA, Alexa Fluor 488-conjugated goat anti-rabbit and (HRP)-conjugated goat anti-rabbit antibody, FM 4-64 FX and horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody as from Molecular Probes; monoclonal antibody against mitochondrial COX IV was from Santa Cruz. All other chemical reagents were of analytical grade.

2.2. Preparation of isolated hepatocytes

Male Wistar rats (200–250 g) were kept in a temperature-controlled room under an artificial light regime of 12 h light, 12 h darkness. A commercial mash was fed for ad libitum consumption and the animals had free access to water. All experiments were conducted according to local and national guidelines for animal experimentation.

Hepatocytes were isolated and purified essentially according to Seglen [16], as modified [17]. Hepatocytes were separated from other cell types by centrifuging at 100 g for 2 min and then washed twice in Krebs–Henseleit buffer (25 mM NaHCO_{3}, 120 mM NaCl, 5 mM KCl, 1 mM KH_{2}PO_{4}, 2 mM MgSO_{4}, 2.5 mM CaCl_{2}, 20 mM glucose, 1% BSA) to completely remove collagenase. Finally, hepatocytes were filtered through a 60 μm nylon filter. The integrity and viability of the cells were confirmed with a trypan blue-exclusion test and at least 95% of the cells remained viable at the end of the preparation.

Freshly isolated hepatocyte suspensions were used in all the experiments.

2.3. Confocal microscopy

Hepatocytes (about 2 × 10^{6}) were kept at 4 °C, washed with cold PBS and then incubated for 15 min with 2% goat serum in PBS, pH 7.4. Cells were washed three times and incubated for 1 h at 4 °C with rabbit polyclonal antibodies against each of the F_{0}F_{1}-ATP synthase subunits: anti-F_{1-}α/β, anti-F_{1-}PVP(b), anti-F_{1-}γ or anti-OSCP (final volume 500 μl). All antibodies were diluted 1:200 in 2% goat serum in PBS. An aliquot of the cells (2 × 10^{6}; positive control) was permeabilized with 100% methanol at 4 °C for 10 min, washed twice in PBS and labelled with antibody as described above. Hepatocytes were then washed in PBS and incubated with goat anti-rabbit IgG conjugated with Alexa Fluor 488 (diluted 1:100 in 2% goat serum in PBS, final volume 500 μl) for 1 h at 4 °C in the dark; finally cells were washed three times with PBS and fixed in 4% formaldehyde in PBS. To selectively label plasma membranes, before formaldehyde fixation, hepatocytes were incubated with a fluorescent lipophilic probe (FM 4-64 FX) by the procedure described by the manufacturer. Appropriate controls, with cells incubated either with the primary or the secondary antibody alone, were performed. Immunolabelled hepatocytes were visualized under a laser scanning confocal microscope system (LSM Pascal, Zeiss) equipped with He–Ne and Ar lasers and coupled to an Axiovirt 200 inverted microscope (Zeiss). Confocal images were recorded using LSM 5 Pascal software.

2.4. Flow cytometry

Hepatocytes were immunostained by the same procedure and antibodies used for confocal microscopy. After the final wash, cells were fixed overnight at 4 °C with PBS containing 2% formaldehyde. Control experiments were performed by cell permeabilization with cold methanol for 10 min before incubation with primary antibodies. After excitation at a wavelength of 488 nm, median fluorescence intensity (MFI) was determined for each sample on a FACScan flow cytometer (Becton-Dickinson) and analyzed by CELLQUEST software (Becton-Dickinson).

2.5. Plasma membrane isolation

Plasma membranes were isolated from rat hepatocytes following the protocol reported in [18]. Briefly, hepatocytes were resuspended in 4 volumes of 0.25 M STM buffer (0.25 M sucrose, 5 mM Tris/HCl pH 7.4, 1 mM MgCl_{2}) and homogenized in a 10 ml Potter–Elvejem homogenizer using a tight-fitting teflon-coated pestle. The homogenate was centrifuged at 280 g × 5 min. The supernatant was saved and the pellet was resuspended in 0.25 M STM buffer and again centrifuged as above. The resulting supernatant was added to the first one and centrifuged at 1500 g × 10 min. The pellet was resuspended in 0.25 M STM buffer and 2 M STM buffer was added to obtain a final sucrose concentration of 1.42 M. Four milliliters of the sample was added to poly-allomer tubes and overlaid with 0.8 ml of 0.25 M STM buffer. After ultracentrifugation at 82,000 g × 60 min, the pellicle at the interface was collected, resuspended in 0.25 M STM buffer and centrifuged at 12,000 g × 10 min. The final pellet was used as the plasma membrane fraction.

2.6. Preparations

Mitochondria were obtained from liver homogenate as in [19]. Submitochondrial particles (SMP) were obtained by sonication of isolated rat liver mitochondria in the presence of 4 mM EDTA (ESMP) at pH 8.5 or in the presence of 1 mM MgATP (MgATP-SMP) [20]. The percent of inversion of SMP was found to range from 96 to 100% [21]. Native IF_{1} was prepared from MgATP-SMP as in [22]. The subunits F_{1-}α/β, F_{1-}γ, F_{1-}PVP(b), and OSCP were collected by electro-elution from SDS-PAGE in 10 mM Tris/HCl, pH 7.4, 0.01% (w/v) SDS, 50% (v/v) glycerol [23] and used to produce specific polyclonal antibodies. The IgG fraction was purified by a combination of precipitation with caprylic acid and ammonium sulfate as in [24].

2.7. Electrophoresis and Western blot procedure

SDS-PAGE was performed on slab gel with linear gradient of polyacrylamide 12–20% [25]. 30 μg proteins of ESMP or hepatocyte plasma membrane were subjected to SDS/PAGE and electrototransferred to nitrocellulose in 125 mM Tris/HCl pH 8.6, 192 mM glycine, 2% methanol (v/v) at about 100 mA for 1 h at room temperature in a semidyry apparatus. Nitrocellulose sheet were blocked with 3% BSA in 50 mM Tris/HCl pH 7.4, 0.9% NaCl (w/v) for 1 h at 37 °C and incubated overnight at 4 °C with antibodies against rabbit anti-F_{1-}F_{1}-ATP synthase subunits diluted in the same buffer [5]. Moreover, to exclude any mitochondrial contamination, nitrocellulose sheet was also incubated in the same conditions with antibody anti-COX IV.
Immunodecoration was performed with HRP conjugated goat anti-rabbit and visualized using an enhanced chemiluminescence substrate kit. Densitometric analysis was performed with a Camag TLC Scanner II at 590 nm. The quantity of antigen detected was evaluated from the computed peak area from which the relative background was subtracted.

2.8. ATP synthesis assay

2.8.1. Bioluminescent luciferase assay

Hepatocytes (about 1×10⁶) were incubated at 37 °C with 200 μl of HEPES buffer (150 mM NaCl, 2 mM MgCl₂, 10 mM HEPES at the indicated pH) with or without mitochondrial ATP synthase inhibitors. After 4 min, 100 μM ADP alone or with 5 mM Pi, at the same pH of the HEPES buffer, was added. At the time indicated, the reaction was stopped by centrifugation (15 s at 300 g). If necessary, the resulting supernatant was neutralized with 0.01 N NaOH or 0.01 N HCl and used for determination of the extracellular ATP content by the bioluminescence assay kit according to the manufacturer’s instructions. The pellet containing hepatocytes was lysed with 0.1 N NaOH for 10 min at room temperature. The supernatant obtained after centrifugation (15,000 g×4 min) was neutralized with 0.1 N HCl and used for determination of intracellular ATP as described above.

2.8.2. Spectrophotometric determination

Hepatocytes (about 1×10⁶) were incubated at 37 °C in a mixture containing 10 mM HEPES at the indicated pH, 150 mM NaCl, 2 mM MgCl₂, 1 mM EDTA, 20 mM glucose, 4 U/ml hexokinase and 300 μM of the adenylate kinase inhibitor Ap5A. After 4 min, 100 μM ADP alone or with 5 mM Pi, at the same pH of the mixture, was added. The reaction was stopped by centrifugation (as above). The supernatant was added to a mixture containing 1 mM MgCl₂, 150 mM Tris/HCl pH 7.4 and 7 U/ml of glucose-6-phosphate dehydrogenase. NADP (1 mM) was added and glucose-6-phosphate was determined following NADP reduction using a dual-wavelength spectrophotometer (Beckman Coulter DU 800, wavelength couple 360/374 nm with an ε = 2.01 mM⁻¹).

2.9. ATP hydrolysis assay

Extracellular ATP hydrolyase activity was measured by an ATP-regenerating system [6]. Hepatocyte suspensions were incubated in a reaction mixture containing 100 mM NaCl, 20 mM KCl, 5 mM MgCl₂, 0.5 mM EGTA, 40 mM Tris/HCl pH 7.4, 1 mM PEP, 10 U/ml lactate dehydrogenase, 4 U/ml pyruvate kinase, 0.1 mM NADH. The reaction was started by ATP addition and followed by monitoring the oxidation of NADH at 25 °C, by dual-wavelength spectrophotometer (Beckman Coulter DU 800) at the wavelength couple 360/374 nm with an ε = 2.01 mM⁻¹.

2.10. Measurement of proton conduction

Proton conduction across the plasma membrane was monitored following ACMA fluorescence quenching (excitation 410 nm, emission 490 nm) [26] using a Jasco FP6200 spectrofluorimeter. The reaction mixture was the same as used for ATP assays (ATP synthesis or hydrolysis). Hepatocytes (0.5 mg) were incubated in the ATP synthesizing or hydrolysing buffer with or without FoF1-ATP synthase inhibitors. After 4 min, 2 μM ACMA was added and proton conduction was monitored by the addition of ADP+Pi or ATP for determination of proton conduction during ATP synthesis or hydrolysis, respectively.

2.11. Protein determination

Protein concentration was determined by using Bradford Bio-Rad protein assay with BSA as a standard.

2.12. Statistical analysis

Results were computed with Microsoft Excel. Comparison was made using one-way analysis of variance (ANOVA) followed by a post-hoc Tukey’s-B test. All statistical analyses were performed using an SPSS/PC computer program. Differences were considered statistically significant at P<0.05.

3. Results

3.1. Identification of the ATP synthase subunits at the hepatocyte surface

Isolated hepatocytes obtained after in situ liver perfusion with a collagenase solution represent the most appropriate procedure to obtain excellent yields (≈95%) of viable cells, with 70–90% in the form of single cells [16,27]. Therefore, hepatocyte suspensions were used throughout this study. Following isolation, hepatocytes were immediately incubated with polyclonal antibodies raised against subunits

![Fig. 1. Surface localization of FoI-PVP(b) subunit of ATP synthase in non-permeabilized hepatocytes. Rat hepatocytes were incubated with the antibody against the FoI-PVP(b) subunit. Pictures show confocal optical sections taken along the z-axis, starting with the external surface of hepatocytes in (a), and ending with confocal plane inside the cells in (h). Magnification ×40. Representative images of three separate experiments.]
Fig. 2. Co-localization of surface FoF₁-ATP synthase subunits with FM 4–64 FX in freshly isolated hepatocytes. Non-permeabilized hepatocytes were immunostained with specific antibodies raised against F₁-α/β (section a), FoI-PVP/b (section b), F₁-γ (section c) and OSCP (section d) subunits of the F,F₁-ATP synthase (green fluorescence, panels 1), washed with cold PBS and incubated with the specific plasma membrane dye FM 4–64 FX (red fluorescence, panels 2). Extensive co-localization is apparent in the merged images (yellow fluorescence, panels 3). In the panels d′.1,2,3 a detail of co-localization on the hepatocyte membrane surface, using the antibody against OSCP subunit, is shown. In e.1, intact hepatocytes were incubated with a polyclonal rabbit antibody against acetyl-CoA carboxylase. In e.2 the immunofluorescence obtained with the antibody against F₁-α/β subunits in permeabilized cells is shown. Magnification ×40. Representative images of three separate experiments.
(F1-α/β and F1-γ) of the catalytic F1 sector and two subunits of the stalk sector i.e. F0-I-PVP(b) and OSCP [13]. After incubation with fluorochrome-conjugated secondary antibody, hepatocytes were visualized under confocal microscopy. Fig. 1 shows images obtained with the F0-I-PVP(b) antibody; the same pattern was obtained with all the antibodies used (data not shown). Confocal optical sectioning through the z-axis, taken every 1.3 μm, showed extensive fluorescence at the surface of cells; no fluorescence was present in confocal sections inside the cells. It should be noted that cells were not permeabilized and only the cell surface is exposed to antibodies.

To rule out the possibility that the rabbit antibodies bound specifically to the cell surface, hepatocytes were also incubated with polyclonal rabbit antibody against acetyl-CoA carboxylase, a cytosolic enzyme highly expressed in these cells [28]. Interestingly, no fluorescence was visible outside or inside the hepatocytes (Fig. 2, panel e1). To confirm surface localization of the antibody-linked fluorescence in intact hepatocytes, a lipophilic styryl compound (FM 4–64 FX) which inserts into the outer leaflet of the surface membrane was used. An extensive co-localization of the green fluorescence of the antibodies against F0-F1-ATP synthase subunits (Fig. 2, F1-α/β panel a, F0-I-PVP panel b, F1-γ panel c, and OSCP panel d) with the red fluorescence of FM 4–64 FX was observed (Fig. 2). The patchy and punctuate staining pattern, typically observed in raft proteins [18,29], suggests that subunits of the F0-F1-ATP synthase (Fig. 2, panels d1, d2 and d3 represent magnification of d.1, d.2 and d.3 respectively) are in the plasma membrane rafts. A strong fluorescence pattern, characteristic of mitochondrial staining, was produced when permeabilized cells were exposed to the F1-α/β antibody (Fig. 2, e2).

Confocal microscopy observations were then confirmed by cytofluorimetric analysis of freshly isolated hepatocytes incubated with antibodies. The distribution of fluorescence intensities (Fig. 3, panels a, b and c) obtained after incubation with F1-α/β, F0-I-PVP, and OSCP antibodies (Fig. 3, plots 2), showed a noticeable fluorescence increase relative to that observed when only the secondary antibody was used (Fig. 3, plots 1). In permeabilized cells (Fig. 3, plots 3) the intensity of fluorescence was further increased. Like in confocal microscopy, hepatocytes were incubated with antibody against acetyl-CoA carboxylase obtaining a MFI similar to that obtained incubating hepatocytes with only secondary antibody (data not shown). It is worth underlining that there was a specific saturable binding of antibodies, directed against the different subunits of the F1 and stalk sectors, on the hepatocyte surface (Fig. 3, panel d).

![Fig. 3. Flow cytometry analysis of the level of F0-F1-ATP synthase subunits localized at the hepatocyte surface. Hepatocytes were incubated with primary specific antibodies raised against F1-α/β (panel a and circles in panel d), F0-I-PVP(b) (panel b and triangles in panel d), OSCP (panel c and square in panel d) subunits of FoF1-ATP synthase. The samples were analyzed by FACScan flow cytometry. Histogram plots: trace 1, non-permeabilized cells incubated with secondary antibody only; trace 2, non-permeabilized cells incubated with secondary and primary antibodies; trace 3, permeabilized cells incubated with both antibodies. Representative histograms of three separate experiments. (Panel d) The MFI of hepatocytes incubated with only the secondary antibody was subtracted from each trace. Values reported in the figure are means of three separate experiments performed with duplicate samples ± standard error (SE).]
3.2. Comparative content of the ectopic-ATP synthase subunits

Western blot analysis, carried out with antibodies against mitochondrial subunits of the F₁ and “stalk” sectors of the F₉F₁-ATP synthase complex, in rat liver ESMP and isolated plasma membrane, confirmed the presence of the enzyme subunits on the hepatocytes plasma membrane (Fig. 4). Interestingly, a constant ratio of the content of the immunodetected subunits between plasma membrane and ESMP proteins was observed (insert to Fig. 4), thus indicating that the ecto-F₉F₁-ATP synthase subunits are present in the same stoichiometry as in the mitochondrial complex. Using a monoclonal antibody against COX IV (a subunit of the cytochrome oxidase complex) as a marker of the mitochondrial inner membrane, no immunoreaction in the plasma membrane lane was observed (Fig. 4, lane 1) thus showing the absence of mitochondrial contamination in the plasma membrane fraction.

3.3. Extracellular ATP synthesis

Intra- and extracellular ATP contents were determined after incubation of isolated hepatocytes in the presence of ADP or ADP+Pi. The measurements were performed by using a dual-wavelength spectrophotometer and a bioluminescent assay, both yielding similar results.

The intracellular ATP content did not change significantly during incubation with ADP or ADP+Pi (Fig. 5, panel a). Under the same experimental conditions the extracellular ATP level was higher in the presence of both ADP and Pi as compared to that obtained in the presence of ADP alone (Fig. 5, panel b). The trypan blue-exclusion assay showed that cells were still intact at the end of the incubation time (data not shown). These results indicate that the intracellular ATP pool was not contributing to the extracellular ATP content and that at the membrane surface of hepatocytes an ATP synthesizing enzyme is really located. Extracellular ATP produced in the presence of ADP alone was not influenced by oligomycin (known inhibitor of mitochondrial F₉F₁-ATP synthase). This ATP formation was likely due to ecto-nucleotide kinase activities. Antimycin A, a known inhibitor of mitochondrial complex III, had no effect on extra and intracellular oligomycin-sensitive ATP synthesis (Fig. 5, panels c and d). These findings indicate that mitochondria don’t contribute to the measured extracellular ATP synthesis.

The oligomycin-sensitive extracellular ATP, produced in the presence of both ADP and Pi, was depressed by the uncoupler CCCP, or by F₁-α/β antibody (Fig. 5, panel d column 4 vs 2). Extracellular ATP synthesis was also depressed by aurovertin, a specific inhibitor of the F₁ sector of the mitochondrial ATPase [30] (data not shown). It is worth underlining that, under the same experimental conditions, these inhibitors had no effect on the intracellular ATP level (Fig. 5, panel c). Thus, the ecto-F₉F₁-ATP synthase complex produced ATP at the outer surface of the hepatocyte plasma membrane.

3.4. Relationship between synthesis of ATP and transmembrane ΔμH⁺

A transmembrane proton gradient should be used to produce ATP by the ecto-F₉F₁-ATP synthase complex. To investigate this aspect, hepatocytes were incubated in acid, neutral, or basic extracellular...
These measurements, carried out by dual-wavelength spectrophotometry, showed a direct relationship between increase in the pH of the extracellular medium and increase in extracellular oligomycin-sensitive ATP synthesis (Fig. 6, panel a).

The relationship between ATP synthesis and transmembrane proton gradient was further analyzed by direct measurement of the intracellular pH with the fluorescent probe ACMA. Acidification of the intracellular space, produced by activating glycolysis with glucose addition to the hepatocyte suspension, resulted in quenching of the ACMA fluorescence due to accumulation of the probe inside the hepatocytes (insert to Fig. 6, panel b). Addition of ADP plus Pi to the hepatocyte suspension incubated with glucose resulted in a significant increase of the ACMA fluorescence. This was prevented by omission of Pi or addition of oligomycin and reversed by the addition of the proton conducting uncoupler CCCP (Fig. 6, panel b). Thus extracellular synthesis of ATP is coupled to intracellular H⁺ consumption.

3.5. Extracellular ATP hydrolysis

ATP hydrolase activity was performed in an ATP-regenerating system. More than 30% of the hydrolase activity was sensitive to oligomycin (Fig. 7, column 2 vs 1). The data presented in Fig. 7 show that the oligomycin-sensitive ecto-ATP hydrolase activity was markedly inhibited by an antibody against mitochondrial F₁ sector. Addition of isolated F₁ or of an antibody against IF₁ produced inhibition or activation of ATP hydrolysis, respectively.
Hydrolysis of externally added ATP to isolated hepatocytes resulted in intracellular acidification as shown by quenching of the ACMA fluorescence. This was prevented by inhibiting ATP hydrolysis with oligomycin or IF1 and reversed by CCCP (Fig. 8).

4. Discussion

In the last few years the dogma that the F_{o}F_{1}-ATP synthase complex is strictly a component of the mitochondrial inner membrane has been abandoned. Moser et al. [7,8] first presented data on the presence of some mitochondrial-like proteins such as α/β subunits of the F_{1} catalytic sector of the F_{o}F_{1}-ATP synthase complex on the surface of plasma membrane of endothelial cell cultures. Subsequent reports indicated the presence of other subunits (γ, CF6) of the F_{1} sector of the ATP synthase in the plasma membrane of various proliferating cell lines [9,10,15,31]. Moreover, it has been indicated that also IF1 appears to be associated with the cell surface F_{o}F_{1}-ATP synthase [29,32,33], operating as a specific inhibitor of the complex. Recent studies have proposed an involvement of the complex in adipocyte differentiation [11,15] as well as in hepatic high-density lipoprotein (HDL)
endocytosis in HepG2 cells [9]. It has been suggested that in the latter cells ectopic-ATP synthase β-chain, which is identical to the HDL receptor, can play an important role in HDL endocytosis and that the membrane-bound H⁺-ATP synthase activity is regulated by ApoA-1, a principal plasma apolipoprotein [9,15].

In the above-mentioned studies, depending on cell type and culture conditions, different results in extracellular ATP synthesis rate (ranging from picomoles to nanomoles) were reported [10]. In studies on primary human hepatocyte cultures, Fabre et al. [34] reported only ATP hydrolysis by ecto-F₁-Fₐ-ATPase. The presence of an ecto-ATP synthase has been also questioned by Quillen et al. [35] who in HUVEC did not find any F₃-F₄-ATP synthase activity.

To the best of our knowledge no study has been performed to date on the ectopic H⁺-ATP synthase in isolated normal tissue cells and in the absence of factors normally present in culturing conditions. As experimental tool closest to the in vivo situation, hepatocyte suspensions were used.

By using different techniques like confocal microscopy, flow cytometry as well as immunoblot analysis, here we show the presence of the whole F₁-OₐF₁-ATP synthase complex localized on the cell surface as well as immunoblotting analysis, here we show the presence of the whole FoF₁-ATP synthase complex localized on the cell surface in human endothelial cells, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 2811–2816.


[9] L.O. Martinez, S. Jacquet, J.P. Esteve, C. Rolland, C. Cabezon, E. Champagne, T. Sainz, R. Díaz, E. Jirillo, J. Jirillo, M. Zanotti, Oligomycin-sensitive reaction (Fig. 5). The synthesis of ATP was synthesized, in the presence of ADP and Pi, extracellular ATP by an oligomycin-sensitive extracellular ATP synthesis. This shows that the cytoplasmic proton gradient induced by CCCP depressed the synthesis and hydrolysis and their sensitivity to specific inhibitors of the F₃-F₄-ATP synthase indicate that the ectopic enzyme corresponds to cell surface ATP synthase. The membrane surface FoF₁-ATP synthase may play a role in the extracellular ATP synthase and proliferation of human umbilical vein endothelial cells, Mol. Cancer Res. 1 (2003) 931–939.


