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Interaction of bacterial F_1-ATPase with octyl glucoside and deoxycholate

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Purified F_1-ATPase from *Micrococcus lysodeikticus (Micrococcus luteus)* contains extensive and easily accessible areas capable of hydrophobic interaction. These hydrophobic areas were demonstrated by the binding of a non-ionic and a mild anionic detergent to this protein, evidenced by charge shift electrophoresis and measured by equilibrium gel chromatography with labelled detergents. F_1-ATPase bound 0.06 ± 0.01 g octyl glucoside per g protein and 0.12 ± 0.01 g deoxycholate per g protein, which amount to 81 and 119 amphiphile molecules per protein molecule, respectively. Deoxycholate and octyl glucoside inhibited the Ca^{2+}- and Mg^{2+}-dependent ATP hydrolytic activity of the enzyme. The inhibition by octyl glucoside was moderately cooperative. Binding of these detergents to the enzyme did not seem to induce any disruption of its quaternary structure, although the spontaneous dissociation of the δ subunit, which is not essential for the enzyme activity, increased during deoxycholate treatment. These results suggest that hydrophobic domains play a role in the enzymatic activity of this coupling factor and/or in its interaction with the membrane.

Introduction

The energy-transducing adenosine triphosphatases, designated as F_0F_1-ATPases or H^+-ATPases, are complex systems responsible for the synthesis of ATP during oxidative phosphorylation and photophosphorylation. These enzyme complexes are made up of two sectors. One of them, named as F_0, constitutes a hydrophobic sector which is embedded in the lipid bilayer of the membrane and acts as a proton channel. The other, known as F_1-ATPase or F_1 factor, contains the catalytic site(s) for ATP synthesis or hydrolysis and can be released from the membrane in a water-soluble form by mild procedures. In this soluble state the F_1 factor is only able to catalyze the hydrolysis of ATP. This F_1-ATPase has been the subject of intensive studies during recent years [1–4]; however, the molecular mechanisms of ATP synthesis and hydrolysis remain to be elucidated. In this regard, current ideas suggest the requirement of energy for the release of tightly bound ATP to the enzyme into the solution and/or for the binding of P_i and ADP rather than for the synthesis of ATP from bound ADP and P_i [5,6]. A hydrophobic environment in the F_1-ATPase catalytic site might facilitate the bound ATP synthesis without an appreciable energy consumption. In this context, a suggestive mechanism for ATP synthesis by the F_1 factor considering a hydrophobic environment of the enzyme has been proposed [7].

However, little attention has been paid to the putative hydrophobic properties of the F_1 factors. The F_1-ATPase isolated from *Micrococcus lysodeikticus (Micrococcus luteus)* constitutes one of

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Abbreviations: ANS, anilinonaphthalene sulfonate; P_i, inorganic phosphate.

the very few exceptions. This bacterial F_1-ATPase (named in general as BF_1 factors) contains four different subunits designated as α, β, γ and δ [8], with an apparent stoichiometry of $\alpha_3\beta_3\gamma_{1-2}\delta_{1-2}$ [2,9–13]. This enzyme requires the presence of divalent cations to hydrolyze ATP, showing a different cation specificity depending on its physical state. Ca^{2+} is a better activator than Mg^{2+} in the purified BF_1 factor from *M. lysodeikticus*, whereas the opposite occurs in the membrane-bound BF_1 factor [14].

It has been shown by solubilization and reconstitution experiments that detergents interact reversibly with eukaryotic and prokaryotic F_0F_1-ATPase complexes [15,16]. Nevertheless, the binding of detergents to the F_1 factor has not been documented.

Previous work reported the interaction of the two major polypeptides (α and β) isolated from the *M. lysodeikticus* F_1-ATPase with mild detergents [9], suggesting that both subunits possess hydrophobic domains. The putative existence of apolar or hydrophobic domains in the surface of the BF_1 factor from *M. lysodeikticus* was also inferred by hydrophobic interaction chromatography [17]. Furthermore, previous studies [18] indicated that the *Escherichia coli* F_1-ATPase was able to interact with phospholipid vesicles and that the δ subunit of the enzyme was not involved in the association of the F_1 factor with phospholipid. In the present work we show conclusive evidence for the presence of hydrophobic domains in the F_1-ATPase from *M. lysodeikticus* by studying the interaction of mild detergents with this BF_1 factor. Furthermore, we discuss the putative role of these hydrophobic regions in the functional and structural properties of the enzyme.

Materials and Methods

Chemicals

ATP was purchased from Pabst Laboratories Biochemicals (Milwaukee, WI, U.S.A.) and [γ-^{32}P]ATP from Amersham International (Amersham, U.K.). Deoxycholic acid, octyl-β-D-glucopyranoside (octyl glucoside) and Triton X-100 were from Calbiochem (La Jolla, CA, U.S.A.). [^{3}H]Deoxycholic acid and glucose [^{14}C]octyl-β-D-glucopyranoside were from New England Nuclear (Dreieich, F.R.G.). Sephadex G-25 and G-50 and Sephacryl S-300 were from Pharmacia (Uppsala, Sweden). Acrylamide and *N,N'*-methylene-bisacrylamide were obtained from Eastman Kodak Co. (Rochester, NY, U.S.A.); Coomassie R-250 was from Schwarz-Mann (Orangeburg, NY, U.S.A.) and sodium dodecyl sulfate (sodium lauryl sulfate) from British Drug Chemicals Ltd. (Poole, U.K.). All other chemicals of the highest quality commercially available were from Merck (Darmstadt, F.R.G.).

Purification of F_1-ATPase

Micrococcus lysodeikticus (*M. luteus*) strain A was grown and harvested as described [14,19]. Membranes were obtained from protoplasts prepared by using lysozyme and by subsequent osmotic shock [14] carried out in the presence of 10 mM $MgCl_2$ [8]. The crude F_1-ATPase fraction was obtained by the low-ionic-strength wash procedure [14] and subsequently purified by preparative polyacrylamide gel electrophoresis [20]. F_1-ATPase preparations appeared to be more than 95% homogeneous as judged by analytical gel electrophoresis.

Analytical procedures

Analytical gel electrophoresis in non-dissociating and dissociating conditions was carried out as described [21] and on slab gels according to the method of Laemmli [22].

Protein concentration was determined by the Lowry method [23] with bovine serum albumin as standard.

Detergent binding

Binding of labelled detergents was measured by using the gel chromatography technique of Hummel and Dreyer [24] as described [25]. Samples containing 2.3–3.0 mg of protein were incubated at 25°C for 40 min with a chosen total detergent concentration, as specified in the legends of the figures, in a final volume of 1 ml 30 mM Tris-HCl (pH 7.5). Final protein and detergent concentrations were prepared by careful dilution of concentrated solutions. Then, the mixtures were applied to 0.9 × 25 cm Sephadex G-25 columns, previously equilibrated with the same buffer containing identical detergent concentration as that of the

sample. Column temperature was controlled to 25 ± 0.5°C by means of water jackets and a circulatory bath. Flow was 20 ml/h and fractions of 1.30 ± 0.05 ml were collected. The radioactive detergent concentration was measured throughout the column effluent by taking 0.5 ml aliquots of each fraction, added to 5 ml Bray's scintillant [26], and counted twice in a liquid scintillation spectrometer. Duplicate aliquots were taken in all the fractions. The amount of bound detergent was calculated from the measured increment in eluate radioactivity associated to protein elution. The base line radioactivity was determined from the regions outside the peak and trough.

Molar concentrations of purified F_1-ATPase from *M. lysodeikticus* were based on a molecular weight of 420000, determined by low-speed sedimentation equilibrium [9] and cross-linking studies [10].

Charge-shift electrophoresis

The procedure was that described by Helenius and Simmons [27] with minor modifications. Electrophoreses were performed in 1% agarose on glass plates (8×9 cm), using a water-cooled chamber (Multiphor 2117 LKB) and 100 mM imidazole-HCl (pH 7.2) as a buffer system. The following detergents were used: 0.5% Triton X-100, 0.25% sodium deoxycholate, and 0.05% cetyltrimethylammonium bromide. Runs were performed at 5.5 V/cm for 45 min.

Critical micellar concentration determination

The critical micellar concentration of deoxycholate was measured at 25°C by anilinonaphthalene sulfonate (ANS) fluorescence enhancement, with a Fica MK 55 II spectrofluorimeter (Le Mesnil, St. Denis, France) as described [28]. The excitation wavelength was 370 nm and the emission wavelength was 500 nm.

Enzymatic activity

Adenosine triphosphatase activity was measured by the liberation of P_i from a standard assay which contained in a final volume of 0.5 ml: 4 μmol ATP, 4 μmol $CaCl_2$ (Ca^{2+}-dependent ATPase activity) or 2 μmol $MgCl_2$ (Mg^{2+}-dependent ATPase activity), 12–20 μg of enzyme protein and Tris-HCl buffer (pH 7.5) to give 30 mM as final concentration. The mixture was incubated for 5 min at 37°C, and then the reaction was stopped by the addition of 3.2 ml ice-cold water. The P_i liberated was estimated as reported [14,21]. One unit of enzyme activity is defined as that amount which liberates 1 μmol P_i/min at 37°C under the experimental conditions described. The normal values for pure F_1-ATPase preparations were of 3–5 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ and 1.4–2.2 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ for Ca^{2+}- and Mg^{2+}-dependent ATPase activities respectively.

To increase the sensitivity, the ATPase activity was measured as follows. The standard assay mixture contained, in a final volume of 0.2 ml, 2 μmol [γ-^{32}P]ATP ($(1–2) \cdot 10^5$ cpm), 2 μmol $CaCl_2$ or 1 μmol $MgCl_2$, 12–20 μg enzyme and Tris-HCl buffer (pH 7.5) to a final concentration of 30 mM. Mixtures were incubated for 10 min at 37°C. Then, the reaction was stopped by placing the tubes on ice and the phosphomolybdate extraction procedure of Nishizuka et al. [29] was used to determine $^{32}P_i$ release. Briefly, the successive additions of 1 ml of 1 mM KH_2PO_4, 0.5 ml of 2.5% (w/v) ammonium molybdate and 1.2 ml *n*-butanol/benzene (1 : 1) to the reaction mixture were followed by vigorous mixing in a vortex. Organic phase aliquots of 0.4 ml were removed and counted in a liquid scintillation counter, by adding 2 ml of Triton/toluene scintillant [30]. The Triton/toluene scintillant was composed of a mixture of 2 volumes of toluene, 1 volume of Triton X-100 and 0.5% butyl-PBD (2-(4-butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiozole). The two, colorimetric and radioactive, methods gave similar values of enzyme activity. In some experiments P_i release was determined at 25°C to correlate the binding of detergents to the enzyme with their effect on the ATPase activity. The normal values of Ca^{2+}-dependent ATPase activity at 25°C ranged between 0.4 and 0.5 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ for pure enzyme preparations.

The effect of several detergents on the ATPase activity was tested by preincubating the purified enzyme (12–20 μg) with increasing amounts of the detergents for 10 min at 37°C or at 25°C as specified in the respective legends. The activities were referred to 100% controls performed as described above, applied to F_1-ATPase samples in-

cubated under the same conditions, but in the absence of detergents.

Results

Charge-shift electrophoresis

We applied this technique for a rapid diagnosis and a first indication of the presence of hydrophobic domains in the soluble F_1-ATPase. As shown in Fig. 1, the enzyme is able to bind mild detergents. The purified enzyme underwent an anodic shift in relation to its mobility in neutral detergent when run in Triton X-100 plus deoxycholate, but a cathodic shift when run in Triton X-100 plus cetyltrimethylammonium bromide, indicating the binding of the ionic detergent in each case.

Equilibrium gel chromatography

The binding of deoxycholate and octyl glucoside to F_1-ATPase from *M. lysodeikticus* was measured by gel chromatography in columns previously equilibrated with labelled ligand, and the results are shown in Fig. 2. The chromatography of F_1-ATPase was associated with a well-defined peak and trough of octyl glucoside concentration in the elution pattern (Fig. 2B). The concentration

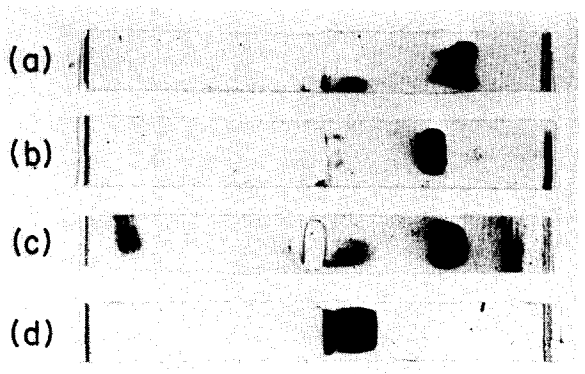

Fig. 1. Charge shift electrophoresis of F_1-ATPase from *M. lysodeikticus*, showing a typical experiment at pH 7.2 of samples containing 10 μg of protein. (a) No detergent; (b) with Triton X-100; (c) with Triton X-100 plus sodium deoxycholate; (d) with Triton X-100 plus cetyltrimethylammonium bromide. Runs were for 45 min. Anode (+) was on the right side. The black lines in both sides are the edges of the plates. For other details, see Materials and Methods.

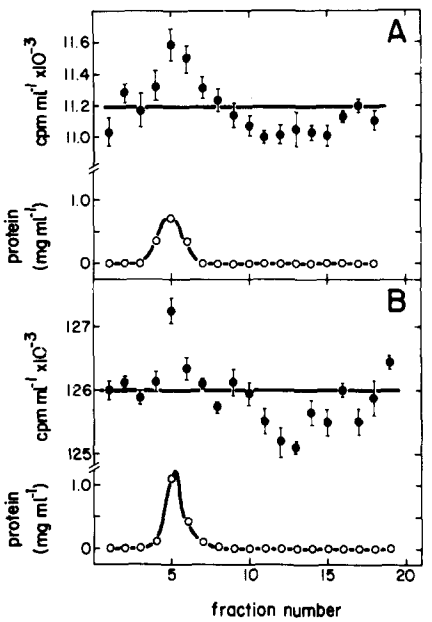

Fig. 2. (A) Binding of [^{3}H]deoxycholate to *M. lysodeikticus* F_1-ATPase. 1 ml of a solution containing 2.65 mg enzyme and $6 \cdot 10^{-3}$ M [^{3}H]deoxycholate in 30 mM Tris-HCl (pH 7.5) was chromatographed on a 0.9×25 cm Sephadex G-25 column previously equilibrated in the same buffer with a free concentration of $6 \cdot 10^{-3}$ M [^{3}H]deoxycholate and thermostated at 25±0.5°C. Flow rate was 20 ml/h and 1.3 ml fractions were collected. ●, Radioactivity elution profile: O——O protein elution profile. (B) Binding of [^{14}C]octyl glucoside to *M. lysodeikticus* F_1-ATPase. Experimental conditions were as in (A), except that $2 \cdot 10^{-2}$ M [^{14}C]octyl glucoside was used as detergent. ●, Radioactivity elution profile; O——O, protein elution profile. Bars indicate standard error of counting. For other details, see Materials and Methods.

of free octyl glucoside in this experiment was 20 mM, which was just below the critical micellar concentration of 25 mM [31]. In the case of deoxycholate (Fig. 2A) the peak and trough were closer. Such an effect with this detergent can be avoided employing Sephadex G-50 instead of Sephadex G-25 (Andreu, J.M., unpublished data). Under the experimental conditions used, the critical micellar concentration of deoxycholate was 3.6 ± 0.5 mM, while the concentration of deoxycholate in the experiment was 6 mM. The quantitation of the increments observed in Fig. 2 resulted in 0.12 ± 0.01 g [^{3}H]deoxycholate per g protein (119 ± 14 mol detergent bound per mol of BF_1 factor) and 0.06 ± 0.01 g [^{14}C]octyl glucoside per g protein

(81 ± 6 mol of detergent bound per mol of BF_1 factor).

In order to rule out the possible binding of detergents to lipid contaminants of the purified BF_1 factor from *M. lysodeikticus*, the enzyme preparations was methanolysed and subjected to fatty-acid analysis by gas–liquid chromatography [32]. It was found that the enzyme preparation contained less than 0.2% (w/w) of fatty acids. This result agrees with previous estimations indicating the absence of extractable lipids [14] and the lack of biosynthetically labelled phospholipids [33] in the purified F_1-ATPase from *M. lysodeikticus*.

Effect of detergents on the enzymatic activity

The effects of several detergents (octyl glucoside, deoxycholate and Triton X-100) on the Ca^{2+}-dependent ATPase activity of the BF_1 factor of *M. lysodeikticus* are shown in Fig. 3. Octyl glucoside and deoxycholate inhibited completely the enzymatic activity at 37°C, whereas Triton X-100 showed a very slight effect, even at concentrations higher than the critical micellar concentration, 0.34 mM [31]. In order to show that the inhibitory effect was not due to temperature and to compare it with the binding experiments, the effect of octyl glucoside on the ATPase activity of the enzyme was measured also at 25°C. These results are shown by the open circles in Fig. 3A. Hill plots of these results (inset in Fig. 3A) gave Hill coefficients of 2.9 (at 37°C) and 2.4 (at 25°C) at the midpoint of inhibition, indicating moderate cooperativity in the detergent effect.

On the other hand, incubation of the enzyme with octyl glucoside in the presence of ATP did not protect the BF_1 factor against inhibition by the detergents (compare Table I with Fig. 3). Furthermore, the Mg^{2+}-dependent ATPase activity of the enzyme resulted also inhibited by octyl glucoside as illustrated in Table I. Although there is a small difference in the absolute percentage of inhibition on the Ca^{2+}- and Mg^{2+}-dependent ATPase activity by octyl glucoside, the patterns of inhibition for both divalent cations-dependent ATPase activities are qualitatively similar.

Effect of detergents on the quaternary structure of the enzyme

To check the effect of detergent binding on the

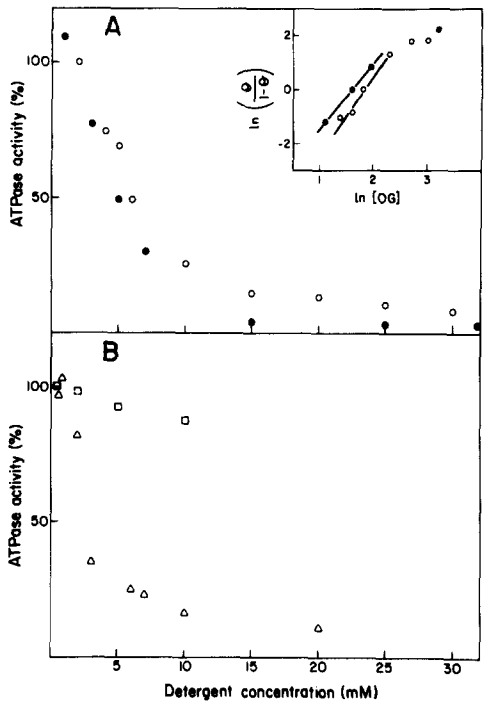

Fig. 3. Effect of detergents on the Ca^{2+}-dependent ATPase activity of *M. lysodeikticus* F_1-ATPase. (A) The enzyme was incubated with different concentrations of octyl glucoside for 10 min at 37°C (○) and 25°C (●) and subsequently analysed for Ca^{2+}-dependent ATPase activity at 37°C and 25°C, respectively. The inset illustrates the Hill plot of the inhibitory effect of octyl glucoside at 37°C (○) and 25°C (●), where θ represents the fractional inhibition. (B) The enzyme was incubated with different concentrations of deoxycholate (△) and Triton X-100 (□) for 10 min at 37°C and subsequently analysed for Ca^{2+}-dependent ATPase activity at 37°C. For other details, see Materials and Methods.

TABLE I

EFFECT OF OCTYL GLUCOSIDE ON THE Ca^{2+}- AND Mg^{2+}-DEPENDENT ATPase ACTIVITY OF THE BF_1 FACTOR OF *MICROCOCCUS LYSODEIKTICUS*

Samples were incubated with octyl glucoside for 10 min at 37°C in a final volume of 0.45 ml, containing 30 mM Tris-HCl (pH 7.5) and 4.5 mM ATP, and then the ATPase activity was assayed as described in Materials and Methods. Mean values ± the standard errors of three experiments are reported.

Octyl glucoside (mM)	%ATPase activity	
	Ca^{2+}-dependent	Mg^{2+}-dependent
0	100	100
10	41.0 ± 4.4	53.4 ± 14.3
22	14.2 ± 0.1	25.7 ± 5.8
40	10.0 ± 1.0	18.4 ± 3.8

substructure of the *M. lysodeikticus* F_1-ATPase, this protein was subjected to gel chromatography in Sephacryl S-300 in the presence of detergents under the same conditions as those employed in the binding experiments. The fractions were analysed by SDS-polyacrylamide gel electrophoresis and compared to controls of enzyme without any treatment. The polypeptide pattern of these fractions in the presence of deoxycholate or octyl glucoside was similar to controls, that showed the native structure of F_1-ATPase from *M. lysodeikticus* made up of four subunits, namely α, β, γ and δ [8]. The only effect was an increase in the spontaneous release of the δ subunit from the core of the enzyme after deoxycholate treatment (not shown). This partial dissociation of the δ subunit from the enzyme confirms previous reports [2,8] indicating the relatively weak association of that polypeptide with the core of this BF_1 factor. In this regard, a recent report described the isolation of a F_1-ATPase form from *M. lysodeikticus* defective in γ and δ subunits by hydrophobic interaction chromatography [34]. At any case, removal of the δ subunit from the enzyme by controlled proteolytic digestion [8] or by EDTA treatment (Mollinedo, F., unpublished data) did not inhibit the enzymatic activity of this BF_1 factor. On the contrary, a slight increase in the specific ATPase activity was observed in these three subunit forms of the enzyme (Ref. 8; see also Mollinedo, F., unpublished data).

Discussion

The results herein reported demonstrate the presence of extensive areas capable of hydrophobic interaction on the purified F_1-ATPase from *M. lysodeikticus (M. luteus)*. First, the charge shift electrophoresis experiments indicate that purified F_1-ATPase shows amphiphilic character, due to its ability to interact with mild detergents. Second, the chromatographic results illustrated in Fig. 2 show conclusively the binding to purified F_1-ATPase of large amounts of a mild anionic detergent (deoxycholate, 119 ± 14 mol/mol protein) and of a non-ionic detergent (octyl glucoside, 81 ± 6 mol/mol protein). While the binding of deoxycholate was measured well above the critical micellar concentration, the binding of octyl glucoside was measured below the critical micellar concentration and does not necessarily correspond to saturation. Employing the latter detergent above the critical micellar concentration may lead to protein aggregation and disturb binding patterns [25]. Furthermore, the practical absence of lipids in the purified *M. lysodeikticus* F_1-ATPase, as demonstrated by gas–liquid chromatography, rules out the participation of lipids in the detergent binding to the enzyme. To our knowledge, this is the first report demonstrating conclusively the presence of hydrophobic domains on a purified F_1 factor. This feature may be highly relevant to the structural and catalytic properties of the enzyme as well as to its interaction with the membrane.

Interaction of anilinonaphthalene sulfonate with *M. lysodeikticus* F_1-ATPase has been previously observed [35]. Nevertheless, the hydrophobic probe anilinonaphthalene sulfonate is able to interact with small hydrophobic regions present in the protein, differing from the massive binding of detergent molecules to the enzyme herein reported.

Prediction of hydrophobic domains can be now attempted from the knowledge of the primary structures of a number of different F_1-ATPases [36,37]. However, we have not found any significant large hydrophobic region in the primary structure of the different subunits of the homologous enzyme from *E. coli* [36], after applying the method of Kyte and Doolittle [38] to their respective amino acid sequences. The hydrophobicity profiles were similar to those of globular proteins [38] and clearly different from those of intrinsic membrane proteins, such as the F_0 components [36], bacteriorhodopsin and cytochrome b_5 [38]. This suggests that the hydrophobic domains detected by mild detergent binding to *M. lysodeikticus* F_1-ATPase are not due to the primary structure of the protein, but to higher-order structures.

Employing gel chromatography and electrophoresis, no major changes were detected in the quaternary structure of the enzyme by interaction of 6 mM deoxycholate and of 20 mM octyl glucoside with the enzyme. Only deoxycholate treatment enhanced the spontaneous release of the δ subunit from the core of the enzyme. These results suggest that binding regions of detergents would not be localized at the binding interfaces between subunits, but at some other areas. Such regions are

not spontaneously exposed by the protein, but induced by detergent binding, since the former possibility would cause aggregation in the absence of detergent, a process which has not been observed [21]. It is conceivable that these hydrophobic regions might play a role in the interaction of BF_1 factor with the membrane, as previously suggested [9], being hidden in soluble BF_1, but interacting with hydrophobic membrane components in the native BF_1-F_0 complex.

Incubation of deoxycholate and octyl glucoside with F_1-ATPase from *M. lysodeikticus* inhibits its ATP hydrolytic activity. However, Triton X-100 has a negligible effect on the ATPase activity of the enzyme. These results indicate a certain specificity in the enzyme inactivation by detergents, which may be due to specific moieties of the detergent molecule and/or to the size of the detergent micelles. The partial dissociation of the δ subunit from the BF_1 factor of *M. lysodeikticus* cannot account for this inhibition, since forms of the enzyme lacking the δ subunit preserve their normal ATPase activity (Ref. 8, see also Mollinedo, F., unpublished data). Thus, this inhibitory effect of mild detergents (deoxycholate and octyl glucoside) on the ATP hydrolytic activity of the BF_1 factor, without any effect detected on its quaternary structure, suggests that it is the binding to hydrophobic areas of the enzyme what inhibits the enzymatic activity. These hydrophobic regions might be responsible for an apolar environment of the F_1-ATPase catalytic site facilitating the ATP synthesis. However, the presence of ATP did not protect the BF_1 factor against inhibition by detergents. Alternatively, inhibition of the F_1-ATPase by the detergents herein studied may take place by inducing conformational changes or/and by prevention of critical structural transitions in the enzyme required for its proper functioning. It is noteworthy to mention that the hydrophobic reagent DCCD (*N*, *N'*-dicyclohexylcarbodiimide) inhibits the ATPase activity of several F_1-ATPases by modification of a glutamic acid residue of the β subunit of the enzyme [39,40].

It is worth mentioning the results reported by Pick and Bassilian [41,42] on the activating effect of octyl glucoside on the Mg^{2+}-dependent ATPase activity, but not on the Ca^{2+}-dependent ATPase activity of the chloroplast F_1-ATPase or CF_1 factor in a defined medium containing ATP. These investigators suggested the presence of a regulatory site for free divalent cations, which show a distinct accessibility depending on the physical state of the enzyme. The presence of specific sites for divalent cations is supported by a recent and elegant work by Noumi et al. [43]. These investigators showed that substitution of the serine residue 174 of the β subunit of the *E. coli* F_1-ATPase by phenylalanine resulted in strong inhibition of the Mg^{2+}-dependent ATPase activity, but not of the Ca^{2+}-dependent ATPase activity, as well as in changes in the hydrophobicity profile and the secondary structure prediction of the region in the vicinity of the mutated residue.

Unlike CF_1, we found that octyl glucoside inhibited both Mg^{2+}- and Ca^{2+}-dependent ATPase activity of the *M. lysodeikticus* F_1-ATPase, either in the presence or in the absence of ATP in the assay medium, even though the Ca^{2+}-dependent activity was slightly more sensitive to the detergent. In this context, it has been recently reported [44] that the presence of the detergent lauryldimethylamine oxide increased the ATPase activity of both F_1 and F_0F_1-ATPase from *E. coli*, without any effect on the cation specificity of the enzyme. On these grounds, it is reasonable to suggest that all the F_1 factors, irrespective of their origin, may possess areas capable of hydrophobic interaction which are involved in the enzymatic activity. However, as mentioned above, there are differences in the effect of that interaction depending on the system considered. These distinct behaviours could reflect structural differences among the different F_1 factors. In this context, it should be worth to remind the notion of a hydrophobicity gradient among the different F_1-ATPase molecules as discussed in an earlier report [2].

Taking together the results herein reported on the binding and on the inhibitory effect of mild detergents on the BF_1 factor from *M. lysodeikticus*, it is feasible to propose the following hypothesis for the interaction of non-denaturing detergents with this *M. lysodeikticus* BF_1 factor, which could be extensive to other F_1 factors. Detergents bind cooperatively to the purified enzyme, inducing structural changes on the protein. These changes in the protein conformation involve the appearance on the enzyme surface of previously

buried hydrophobic regions and lead to inhibition of its ATP hydrolytic activity. In this position, the F_1-ATPase shows a weak capacity for ATP hydrolysis, likely due to the localization of ATP in a hydrophobic environment where H_2O is extruded. On the contrary, it may be suggested that an enzyme containing bound ADP and P_i shows an enhanced capacity to synthesize ATP by releasing H_2O from the catalytic site, localized in a hydrophobic environment. Thus, the enzyme would be localized in an environment, hydrophobic or hydrophilic, depending on its function to synthesize or hydrolyze ATP. These ideas are in agreement with the notion of an abrupt change in the F_1 factor position in the membrane during its functioning, previously proposed by Kozlov and Skulachev [7]. In this context, there is evidence for a different enzyme conformation between the latent and activated chloroplast F_1-ATPase [1,45]. On the other hand, the low ATPase activity of the membrane-bound F_1 factor is well known [46,47]. These observations seem to indicate that mild detergents might mimick the membrane environment in their interaction with the F_1-ATPase. Thus, studies on the interaction of F_1-ATPase with detergents might be considered as a simplified model for the knowledge of membrane-F_1 factor interactions.

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