Mitochondrial H+-ATPase Activation by an Amine Oxide Detergent*

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Lauryl dimethylamine oxide activates ATP hydrolysis by the mitochondrial H+-ATPase. Activation is observed in systems with a high content of inhibitor protein as described by Pullman and Monroy (Pullman, M. E., and Monroy, G. C. (1963) J. Biol. Chem. 238, 3762-3769), i.e. Mg-ATP submitochondrial particles and a Triton X-100-solubilized H+-ATPase from the same particles. Detergent activation of ATP hydrolysis is also present in inhibitor-reconstituted systems, i.e. submitochondrial particles, Triton extracts, and soluble F1-ATPase.

In submitochondrial particles depleted of inhibitor protein, lauryl dimethylamine oxide induced a biphasic response which is characterized by a drop-in activity induced by relatively low concentrations of LDAO; at higher concentrations the detergent activates to an extent never greater than the initial activity. In inhibitor protein-depleted oligomycin-sensitive Triton extracts, lauryl dimethylamine oxide stimulates ATP hydrolysis to very high values (30 μmol min⁻¹ mg⁻¹). These findings suggest that in addition to the inhibitor protein ATP hydrolysis is controlled by other subunit interactions.

The mitochondrial H+-ATPase catalyzes the synthesis and hydrolysis of ATP (1, 2). It is composed of a membrane sector (F₀) which is responsible for the movement of protons across the membrane (3) and a water-soluble moiety (F₁) which contains the catalytic sites (4). An ATPase inhibitor protein (IF) was originally isolated from mitochondria by Pullman and Monroy (5); for review see Ref. 35. It is a basic protein with a molecular weight of approximately 10,000 (6). It interacts with the β subunit with a stoichiometry of 1 IF₁/molecule of F₁ (7, 8) with high affinity (K₀, 10⁻⁵ M) (9). It is believed that the inhibitor protein regulates both ATP hydrolysis and synthesis (10, 11) and that its interaction with F₁ depends on the existence of electrochemical H⁺ gradients (12, 13). Recently, Löscher et al. (14) reported stimulation of ATPase activity by lauryl dimethylamine oxide (LDAO) in Escherichia coli H⁺-ATPase through the derangement of the interaction between β and ε subunits. By the same token, the Ca⁺²⁺-ATPase activity from chloroplast coupling factor is also stimulated by detergents (15). In this work, the effect of LDAO on the ATPase activity of various mitochondrial systems with and without inhibitor protein was tested. The results show that the detergent releases the inhibitory action of the protein and that it stimulates ATPase activity of systems largely devoid of inhibitor protein.

MATERIALS AND METHODS

Bovine heart mitochondria were prepared as described by Löw and Vallin (16) and stored at −70 °C until used. MgATP submitochondrial particles were prepared as described in Ref. 17, and inhibitor-depleted particles were prepared according to the method of Klein et al. (18), here referred to as Klein particles. They were stored in liquid nitrogen until used. Soluble F₁-ATPase and the inhibitor protein were prepared as described in Refs. 19 and 20, respectively.

Triton X-100 extracts were prepared as follows. 0.8% Triton was added to an equal volume of submitochondrial particles at room temperature; the final protein concentration was 1.5 mg/ml. The mixture was immediately centrifuged for 30 min at 45,000 rpm in a 50 Ti Beckman rotor; the supernatant was collected and used in the experiments described under "Results."

The effect of LDAO on the ATPase activity of the various preparations was tested by adding variable amounts of LDAO from either a 1 or 0.1 M stock solution to 0.3 ml of the enzyme. After 5 min, 0.3 ml of the reaction medium for assay of ATP hydrolysis was added. The reaction medium contained (final concentrations) 50 mM Tris acetate, pH 7.8, 3 mM MgCl₂, 2 mM ATP, 5 mM phosphoenoxypruvate, 150 μg of pyruvate kinase, and 30 μl of [γ-3²P]ATP (2 × 10⁶ cpm). Incubation was at 30 °C. The reactions were stopped by the addition of 0.2 ml of 30% trichloroacetic acid. The mixtures were centrifuged for 10 min, and the supernatant was used for determination of phosphate. It was realized that in the course of ATP hydrolysis the specific activity of [γ-3²P]ATP is diminished due to formation of nonradioactive ATP through pyruvate kinase and phosphoenoxypruvate. However, the experimental conditions were adjusted to have a dilution of not more than 10%. The trichloroacetic acid supernatant (0.7 ml) was added to a tube containing 0.8 ml of water, followed by the addition of 1 ml of 3.3% ammonium molybdate in 3.8 N H₂SO₄. Butyl acetate was added (1 ml), and the tubes were shaken in a Vortex mixer for 2 min and centrifuged for 2 min at 1500 rpm. An aliquot of the organic phase (0.4 ml) was delivered to filter paper (2.5 × 8.0 cm), dried at room temperature, and counted in liquid scintillation vials containing Bray’s scintillation mixture. The detergents did not modify the partition of ³²P, or [γ-3²P]ATP into the organic phase.

The inhibition of ATP hydrolysis by IF₁ was assayed by incubating 30 μg of submitochondrial particles, 7 μg of Triton extract, or 1 μg of F₁-ATPase with excess IF₁ (0.5 μg) at 30 °C for 10 min in 0.3 ml of medium containing 0.25 mM succinate, 2 mM MOPS, pH 6.3, 10 mM Tris-SO₄, pH 6.7, 1 mM MgCl₂, and 0.8 mM Na-ATP, pH 6.0. The final pH was 6.5.

[γ-3²P]ATP was prepared according to Glynn and Chappell (21) using [³²P]Purchased from New England Nuclear. Lauryl dimethylamine oxide (critical micelle concentration, 125 μM) was a gift from Onyx Chemical Co., Jersey City, NJ. All other reagents were of the highest quality available. Protein determination in the submitochondrial particles was carried out as described in Ref. 22 while that of the soluble material was determined according to the method described in Ref. 23. Bovine serum albumin was used as standard.

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‡ The abbreviations used are: LDAO, lauryl dimethylamine oxide; MOPS, 4-morpholinepropanesulfonic acid; IF₁, inhibitor of the F₁-ATPase; F₁, coupling factor 1; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone.

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7807
RESULTS

The action of increasing amounts of LDAO on particles with high and low content of IF (Mg-ATP and Klein particles, respectively) is shown in Fig. 1, A and B. Aside from a small decrease of the hydrolytic activity induced by 5 mM LDAO, detergent concentrations between 30 and 40 mM increase the ATPase activity of Mg-ATP particles by more than 3-fold. This occurred regardless of whether the detergent was preincubated with the enzyme (Fig. 1, A and B) or when added directly to the reaction mixture.

The effect of LDAO on the ATPase activity of Klein particles is triphasic. It decreases as LDAO concentrations are raised up to 10 mM and starts to increase as the concentration is gradually raised to 50 mM. Noteworthy is that the maximum level does not reach the activity observed in the absence of detergent (Fig. 1B). At higher concentrations of LDAO the activity declines.

The distinct behaviors of the ATPase activity of Mg-ATP and Klein particles to increasing concentrations of LDAO suggested that the detergent could activate the enzyme through two different mechanisms, i.e., one that depends on the presence of IF, and another through a process unrelated to IF1. These possibilities were explored.

Relief of Inhibitory Action of the Inhibitor Protein by LDAO—To determine if the effects of LDAO shown in Fig. 1 were exclusive of a particulate system, its effect was studied in Triton X-100 extracts of both Mg-ATP and Klein particles. Table I shows that it is possible to obtain an oligomycin-sensitive ATPase by exposing both particle preparations to Triton X-100. At the concentration of Triton X-100 employed about 50% of the protein is solubilized and approximately 70% of the total ATPase activity is recovered in the supernatant.

Interestingly, the specific activity of the enzymes solubilized from the two types of particles correlated with that of the starting preparations; in the extract obtained from Klein particles it was several times higher than that obtained from Mg-ATP particles, an enzyme that was still under control by IF1.

The effect of LDAO was tested in the Mg-ATP Triton extract (Fig. 2). Similar to its effect on the Mg-ATP particles, LDAO stimulated ATPase activity several times. However, the LDAO concentration required to induce maximal activity is lower. This could be related to the lower amount of protein used in the experiments of Fig. 2. The effect of a given concentration of the detergent depended on the amount of particles in the system (data not shown).

The ability of LDAO to increase the ATPase activity of an inhibitor-reconstituted preparation was determined. The effect of various concentrations of LDAO on the ATPase activity of Klein particles reconstituted with IF1 and a Triton extract from the same particles also reconstituted with the inhibitor protein was tested. The inhibitor-reconstituted Klein particles which possess a very low hydrolytic activity were activated by LDAO by about 4-fold (Fig. 3A, inset); this effect was on an extent similar to that observed in Mg-ATP particles. The initial drop in hydrolytic activity was also observed in this system (Fig. 3A).

ATP hydrolysis by a Triton extract derived from Klein particles which was reconstituted with IF1 was also stimulated by LDAO (Fig. 3B), but it never attained the levels observed in a Mg-ATP Triton extract (Fig. 2). The reason for this difference in activity could be related to a different interaction of added IF1 with the F1-ATPase, in relation to intrinsic IS1. The activation was 10-fold (Fig. 3B, inset), similar to that of the Mg-ATP particles Triton extract.

The data of Fig. 4 show that LDAO also increases the ATPase activity of soluble F1-ATPase which had been previously inhibited by IF1. However, the response to LDAO was biphasic, and the activity increased to maximal values at 1 mM LDAO. At higher concentrations, the activity of the reconstituted enzyme dropped sharply. The activity of F1-ATPase was unaffected by the concentrations of LDAO studied.

Thus, in Mg-ATP particles, in an oligomycin-sensitive Triton extract, and in soluble and particulate systems reconstituted with IF1, activation of ATP hydrolysis by LDAO was observed.

Activation of Hydrolysis by LDAO in Preparations Devoid of IF1—As shown in Fig. 1B, the ATPase activity of inhibitor-depleted submitochondrial particles shows a biphasic response to LDAO at various concentrations of LDAO. Thus, the overall expression of the ATPase activity may be determined by a factor(s) that is independent of the action of the inhibitor protein.

The ATPase activity of a Triton extract derived from Klein particles is increased to 30 amol min⁻¹ mg⁻¹ by LDAO (Fig. 5). Therefore, even though Klein particles are largely devoid of IF1 (18), the ATPase activity observed in the presence of LDAO is one of the highest reported for an oligomycin-sensitive ATPase (24–30). It is to be noted that the detergent did not increase the hydrolytic activity of untreated soluble F1-ATPase (Fig. 4).

DISCUSSION

Certain detergents like LDAO and short chain alkylglucosides activate soluble H⁺-ATPase of E. coli and chloroplasts.
Mitochondrial $H^+$-ATPase Activation

7809

Fig. 2 (left). Effect of LDAO on the hydrolytic activity of a Triton extract of Mg-ATP particles. Mg-ATP Triton extract was obtained as described under "Materials and Methods." Preincubation with LDAO was carried out by adding increasing amounts of the detergent from a 1 M solution to 0.3-ml samples containing 7 μg of protein. The reaction medium was added (0.3 ml), and FCCP was omitted.

Fig. 3 (center). Effect of LDAO on ATP hydrolysis of IF$_1$-reconstituted Klein particles and Triton extract. Increasing amounts of LDAO were added after preincubation with IF$_1$ (as described under "Materials and Methods"). ATP hydrolysis was carried out as described above. FCCP was added in the case of the Klein particles.

Fig. 4 (right). Effect of LDAO on soluble and inhibitor reconstituted F$_1$-ATPase. F$_1$-ATPase was reconstituted with IF$_1$ as described under "Materials and Methods." The detergent was added in the amounts indicated to 0.3 ml of a medium containing 150 mM sucrose and 5 mM Tris-Cl, pH 7.4, and 1 μg of F$_1$-ATPase. O—O, soluble F$_1$-ATPase; •—•, inhibitor-reconstituted F$_1$-ATPase.

Fig. 5. ATP hydrolysis of a Klein particle Triton extract. Effect of LDAO. Klein Triton extract was obtained as described under "Materials and Methods." LDAO was added, as described before, to 0.3-ml samples which contained 7 μg of protein, FCCP was omitted from the reaction medium.

(14, 15). In both cases it has been postulated that the activation is related to the displacement of the ε subunit. In this work we have found that LDAO may stimulate ATP hydrolysis through two apparently different mechanisms. Each mechanism seems to depend on the nature of the enzyme. An activating effect of LDAO is clearly apparent in enzymes inhibited by IF$_1$ and results from an abolition of the inhibitory action of the protein. The latter conclusion derives mainly from the effect of LDAO on the activity of either soluble or particulate systems that had been inhibited by added IF$_1$.

An additional activation by LDAO becomes apparent in systems that are largely devoid of IF$_1$. Rates of ATP hydrolysis by Triton extracts derived from Klein particles are activated by the detergent to extremely high values. Indeed they are apparently the highest values reported for a heart mitochondrial oligomycin-sensitive system (24-30). On the other hand Kagawa and Racker (31) have shown that detergent-solubilized preparations of F$_0$-F$_1$ catalyze low rates of ATP hydrolysis unless phospholipids were also present. The Triton extract is slightly activated by the addition of phospholipids.

LDAO activates ATP hydrolysis of a Triton extract in the presence or absence of phospholipids (data not shown). Therefore, the activation exerted by LDAO in inhibitor-depleted systems is probably not related to a phospholipid-like effect of the detergent on the ATPase complex. It is also important to note that we have previously reported the solubilization and isolation of a F$_0$-F$_1$ complex with LDAO, and the resulting complex was reconstituted into liposomes that carry out membrane-coupled reactions (29, 36). Therefore, we believe that
Mitochondrial H\textsuperscript{+}-ATPase Activation

it is not the physical separation of F\textsubscript{1} that accounts for the activation of the Klein Triton extract. Furthermore, LDAO also activates ATP hydrolysis in a E. coli F\textsubscript{0}-F\textsubscript{1} complex with no apparent release of the F\textsubscript{1} sector (14).

These observations are highly suggestive that the hydrolytic activity of the ATPase is controlled by factors other than IF\textsubscript{1}. Interestingly this activating effect was only observed in particles and in Triton extracts. In soluble F\textsubscript{1}-ATPase no such activating effect was observed. Therefore, it would appear that the suggested mechanism of control only exists in particular oligomycin-sensitive ATPase.

In bacterial systems the \(\epsilon\) subunit regulates ATP hydrolysis (32-34), but the subunit is an integral part of the enzyme and not a detachable protein as is the case of the mitochondrial IF\textsubscript{1}. In CF\textsubscript{1} detergents also induce an activation (15) similar to that observed in E. coli (14). Thus, it is more or less clear that in E. coli and chloroplasts the \(\epsilon\) subunit represents a regulatory subunit of ATP hydrolysis, which in mitochondria may have its counterpart in the inhibitory protein of Pullman and Monroy (5). However, the fact that LDAO activates ATP hydrolysis, independently of modifications of the action of IF\textsubscript{1}, suggests that in mitochondria, certain subunit interactions may in addition control ATPase activity. This suggested control becomes evident by titrations with LDAO.

An interesting point arises from the studies on the effect of LDAO on soluble F\textsubscript{1}-ATPase and on those reconstituted with IF\textsubscript{1} (Fig. 4). The ATPase activity of free enzyme is unaffected by LDAO at the concentrations employed, whereas the activity of the reconstituted enzyme drops sharply at concentrations higher than those that abolished the action of IF\textsubscript{1}. This suggested that in the IF\textsubscript{1}-inhibited enzyme, once the action of the protein is relieved, the enzyme becomes highly sensitive to the detergent. This could be due to a particular transitory conformation of the enzyme that appears when IF\textsubscript{1} ceases to inhibit ATPase activity.

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