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Reversible Modification of Rat Liver F_1-ATPase by a Redox Reaction

N. López-Moratalla, E. Santiago, A. J. Iriarte and M. J. López-Zabalza

Departamento de Bioquímica
Universidad de Navarra
Pamplona (Spain)

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F_1-ATPase from rat liver mitochondria exhibited a change in properties when reduced by dithionite: an increase in activity together with a disappearance of its sensitivity to bicarbonate stimulation was observed. A complete reversion to the original properties was achieved with the oxidizing agent 2,6-dichlorophenolindophenol.

It is now widely accepted that F_1-ATPase, besides catalyzing the hydrolysis of ATP, also catalyzes its synthesis when properly coupled to the electron transport chain (9, 10). A knowledge of the properties of the hydrolytic activity of F_1-ATPase may be useful not only for the understanding of the mechanism of this reaction but also for that of oxidative phosphorylation.

Upon studying the properties of purified rat liver F_1-ATPase a rather surprising observation has been made. The properties of this enzyme were affected by a reducing agent, and its effects reverted by an oxidizing agent. Among the properties affected by redox reactions was that of its sensitivity to bicarbonate, an anion known to be an activator of the enzyme (1, 2, 5, 8).

The results here presented show that the reduction of purified mitochondrial ATPase (F_1-ATPase) with dithionite led to a form of the enzyme with a much higher ATPase hydrolyzing activity than that of the control. Furthermore, the reduced form of the enzyme was practically insensitive to bicarbonate. The reversibility of the modification was verified after reoxidizing the enzyme with 2,6-dichlorophenolindophenol (DCPIP); the oxidized form of the enzyme exhibited a much lower specific activity than the reduced form, and at the same time its sensitivity to bicarbonate stimulation was completely recovered.

Materials and Methods

Mitochondria were isolated by the method of HOGEBOOM (4). Protein determination was carried out following the reaction of LOWRY *et al.* (6). F_1-ATPase was prepared from rat liver mitochondria by the procedure of LAMBETH and LARDY (5). ATPase activity was determined essentially as described by PULLMAN *et al.* (10) in the absence of an ATP generating system. Aliquots of the F_1-ATPase were preincubated for 5 minutes at 30° C in 0.8 ml of a medium containing 50 μmoles of Tris-acetate, pH 7.4, and 3 μmoles of $MgCl_2$; when present, the amount of sodium bicarbonate was 10 μmoles. The reaction was initiated by the addition of 3 μmoles of sodium ATP, pH 7.4, dissolved in 0.2 ml of distilled water. The incubation was continued for 2 min and stopped by the addition of 0.1 ml of 50 % trichloroacetic acid. Inorganic phosphorus was determined according to FISKE and SUBBAROW (3). Reagent and enzyme blanks were determined in each experiment.

Results

The effect of dithionite concentration, both on ATPase activity and on its stimulation by bicarbonate, has been studied using a fixed substrate concentration, 3 mM ATP-Mg^{++} (fig. 1). ATPase activity increased with increasing dithionite concentrations reaching a maximum value, which was almost three times higher than that of the control. These results agree with those previously found by MYERS and SLATER (7) in rat liver mitochondria. In the absence of dithionite the ATP hydrolyzing activity of ATPase was stimulated over 100 % by 10 mM bicarbonate. This stimulation notably decreased with increasing dithionite concentration; at 40 μM dithionite the bicarbonate stimulation completely disappeared; this dithionite concentration coincided with that giving the maximum value of ATPase activity in the absence of bicarbonate.

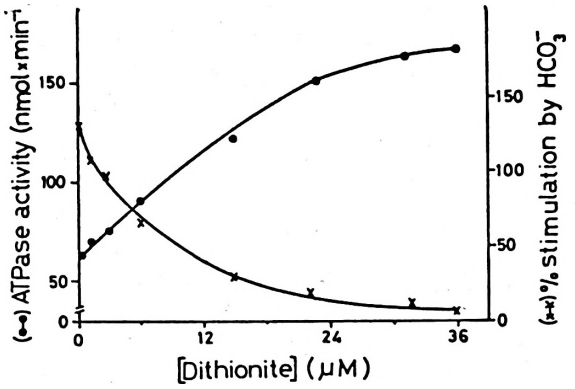

Fig. 1. *ATP hydrolyzing activity of mitochondrial ATPase and its stimulation by bicarbonate in the presence of dithionite.* Final concentration of bicarbonate, when present, 10 mM. The addition of bicarbonate followed always that of the enzyme and dithionite. The activity has been referred to 4.8 μg of purified enzyme protein in 1 ml of incubation mixture. Number of experiments, 10.

Table I. *Effect of DCPIP on the hydrolyzing activity of ATPase and on its sensitivity to bicarbonate.*
Final concentration of bicarbonate, when present, 10 mM. The activity has been referred to 4.8 μg of purified enzyme protein in 1 ml of incubation mixture. Number of experiments, 5.

Addition	ATPase activity (μmol × min^{-1})	Stimulation by HCO_3^- (%)
None	65±2	130±5
50 μM DCPIP	69±2	132±6

To verify whether the effects of dithionite on the enzyme were reversible, the reduced form of ATPase was treated with the oxidizing agent, 2,6-dichlorophenolindophenol (DCPIP). As shown in table I DCPIP had no manifest effect on the original form of F_1-ATPase. Figure 2 shows the rate of the ATP hydrolyzing activity of ATPase, and its stimulation by

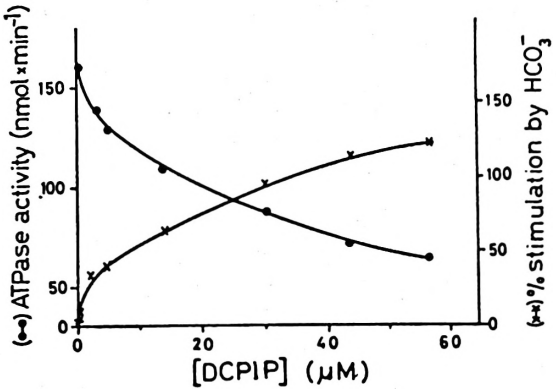

Fig. 2. *ATP hydrolyzing activity of mitochondrial ATPase and its stimulation by bicarbonate after reoxidation with DCPIP of previously reduced ATPase.*
The enzyme was kept at room temperature for 2 minutes in the presence of dithionite before adding the DCPIP. Final concentration of dithionite was 35 μM and that of bicarbonate, 10 mM. The activity has been referred to 4.8 μg of purified enzyme protein in 1 ml of incubation mixture. Number of experiments, 6.

bicarbonate, after the addition of different concentrations of DCPIP to an enzyme preparation previously reduced by dithionite. It may be observed that the rate of ATPase hydrolysis progressively decreased with increasing concentrations of DCPIP and finally became equal to the values given by the original preparation of the enzyme. At the same time the sensitivity to bicarbonate reappeared and the percent stimulation reached values which were precisely equal to those given by the original purified ATPase.

Discussion

The data of figures 1 and 2 could be interpreted in the sense that mitochondrial ATPase might be an interconvertible enzyme with two possible forms differing in activity and also in their sensitivity to the activation by bicarbonate. The reduced form of the enzyme would be more active and at the same time less sensitive to bicarbonate stimulation.

The observation that changes in activity and properties of F_1-ATPase took place in the presence of an electron donor, and that these changes were readily reverted by an electron acceptor, might be of importance in the understanding of the mechanisms of energy transduction in mitochondria.

Resumen

Cuando la F_1-ATPasa, aislada de mitocondrias de hígado de rata, se reduce con ditionito tiene lugar un cambio en sus propiedades: se ha observado un aumento de la actividad junto con una pérdida de su sensibilidad a la estimulación por bicarbonato. Mediante tratamiento con el agente oxidante 2,6-clorofenolindofenol se consigue la completa recuperación de sus propiedades originales.

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