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Effect of Physiological Electron Donors and Acceptors on F_1-ATPase *

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The hydrolytic activity of F_1-ATPase isolated from rat liver was enhanced in the presence of NADH, $FADH_2$, QH_2 or reduced cyt c. The extent of this activation depended largely on substrate concentration. F_1-ATPase sensitivity to bicarbonate or dinitrophenol activators decreased in the presence of any of those electron donors, which originated as well a slight sensitivity to oligomycin and a sensitivity increase to the inhibitory anion OCN^-. In the presence of oxidized carriers the sensitivity to bicarbonate, dinitrophenol, or OCN^- was not modified, and the enzyme remained oligomycin insensitive.

It has been reported that ATPase of mitochondria obtained from animals subjected to a variety of metabolic situations becomes insensitive to the activating anion bicarbonate; this change in properties was related to an increase in serum fatty acids (4). SIESS and WIELAND (12), and more recently REQUERO *et al.* (8) have found that an increase in circulating fatty acids is accompanied by an elevation of the reducing power and by an increase in the ATP/ADP ratio within the mitochondria. On the other hand, it has also been observed that the reducing agent dithionite increased the activity of mitochondrial ATPase with a concomitant modification of its sensitivity to bicarbonate (11), and to other activators and inhibitors (5). This change in properties was interpreted as due to a redox modification of mitochondrial ATPase (11).

These results prompted us to study the effect of physiological electron carriers, oxidized or reduced, on the hydrolytic activity of F_1-ATPase. It has now been found that a stimulation of the activity takes place in the presence of NADH, $FADH_2$, QH_2, or reduced cyt c. The extent of this activation depended largely on the substrate concentration. In the presence of electron donors the enzyme exhibited a decrease in sensitivity to the

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activators bicarbonate or dinitrophenol (DNP), together with the appearance of a slight sensitivity to oligomycin, and an increase in sensitivity to the inhibitory anion OCN^-.

In the presence of oxidized carriers, whether stimulatory or not, the sensitivity to bicarbonate, DNP, or OCN^- was not modified, and the enzyme remained oligomycin insensitive.

The results reported seem to support an early suggestion (11) that ATPase might be modified through a redox reaction.

Materials and Methods

Mitochondria were isolated by the method of HOGEBOOM (2). 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 (3). ATPase activity was determined essentially as described by PULLMAN *et al.* (7) in the absence of an ATP generating system. Aliquots of the F_1-ATPase were preincubated for 5 min at 30° C in 0.8 ml of a medium containing 50 μmoles of Tris-acetate, pH 7.4. The reaction was initiated by the addition of appropriate amounts of ATP-Mg dissolved in 0.2 ml of distilled water to give the required final concentrations in the incubation mixture. 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 (1). Reagent and enzyme blanks were determined in each experiment.

$FADH_2$ was prepared by reduction of FAD with hydrogen gas. Coenzyme Q, dissolved in absolute ethanol, was reduced in a similar way. Cyt c was reduced with sodium borohydride as described by SOTTOCASA *et al.* (13) using 0.05 M Tris-acetate buffer, pH 7.4, instead of phosphate buffer.

Results

The hydrolytic activity of F_1-ATPase has been measured at three fixed ATP-Mg concentrations (3-0.6-0.06 mM) and varying concentrations of the physiological electron carriers NADH, $FADH_2$, coenzyme QH_2 and reduced cyt c. Figure 1 shows that the hydrolytic rate increased with increasing concentrations of any of these reduced electron carriers. It may be observed that the percent stimulation elicited by NADH and coenzyme QH_2 was more pronounced at higher than at lower substrate concentrations. However, in the presence of $FADH_2$ larger stimula-

Tabla. I. *Effect of electron donors on the sensitivity of F_1-ATPase to bicarbonate, dinitrophenol, oligomycin and cyanate at different substrate concentrations.*
When present the concentrations were as follows: 10^{-2} M HCO_3^-, 5×10^{-4} M DNP, 5×10^{-5} M oligomycin, 2×10^{-3} M KOCN, 5×10^{-4} M NADH, 5×10^{-4} M $FADH_2$, 5×10^{-4} red. cyt. c, 5×10^{-5} M QH_2.

Additions	3 mM ATP-Mg^{++}				0.6 mM ATP-Mg^{++}				0.06 mM ATP-Mg^{++}			
	% Stimulation		% Inhibition		% Stimulation		% Inhibition		% Stimulation		% Inhibition	
	HCO_3^-	DNP	Oligomycin	KOCN	HCO_3^-	DNP	Oligomycin	KOCN	HCO_3^-	DNP	Oligomycin	KOCN
None	120	111	0	49	59	46	0	32	17	17	0	19
NADH	80	78	19	61	35	32	11	45	11	10	7	24
$FADH_2$	69	64	27	68	33	28	18	43	0	0	25	29
Red. cyt. c	70	72	22	60	28	25	16	46	3	4	12	22
QH_2	68	65	20	62	31	26	17	45	5	4	12	23

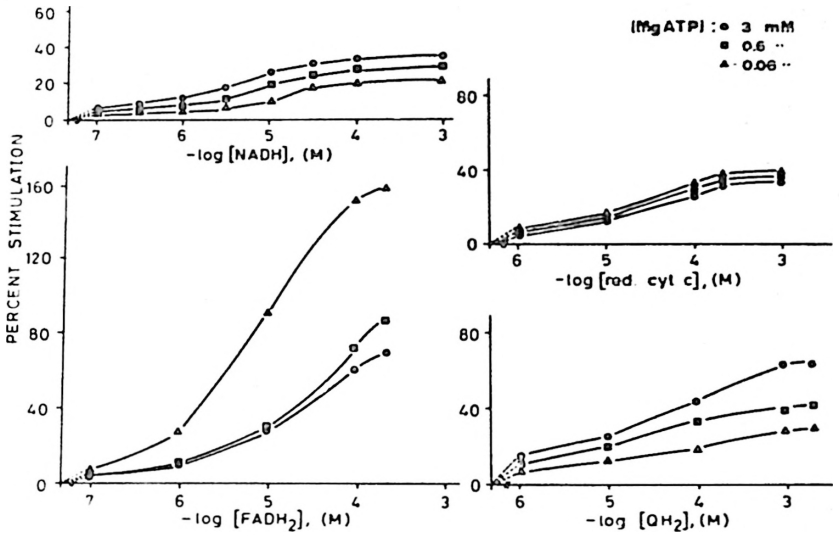

Fig. 1. *Effect of NADH, $FADH_2$, reduced cytochrome c and QH_2 on the hydrolytic activity of F_1-ATPase at different substrate concentrations.*

tions were obtained at lower than at higher substrate concentrations. With reduced cyt c the percent stimulation did not depend on the concentration of ATP-Mg.

The sensitivity of F_1-ATPase to the activators bicarbonate or DNP, and to the inhibitors oligomycin and KOCN has also been studied. Table I shows that the percent stimulation obtained with the activating anions bicarbonate and DNP was much lower in the presence of any of the physiological electron donors regardless the substrate concentration used. However, the percent inhibition produced by the inhibitory anion KOCN was higher in the presence of any of the electron donors at any of the three substrate concentrations used. It should also be noticed in table I that F_1-ATPase acquired some sensitivity to the inhibitor oligomycin in the presence of any of the physiological electron donors; in their absence the enzyme was completely oligomycin-insensitive. The effect of the electron carriers in their oxidized form on the activity of F_1-ATPase and on its sensitivity to activators and inhibitors was also studied. Table II shows that FAD and coenzyme Q stimulated ATPase activity; this stimulation was more pronounced at high substrate concentrations. However, no stimulatory effect of ATPase activity was observed with NAD^+ or cyt c. The activating effect of bicarbonate did not decrease in the presence of electron acceptors, contrary to what happened with the electron donors. The percent stimula-

Table II. *Effect of NAD^+, coenzyme Q, FAD and cytochrome c (10^{-4} n) on F_1-ATPase.* ATPase activity has been referred to 5 μg of F_1-ATPase.

Additions	ATPase activity (nmol min^{-1})			% Stimulation 10 mM HCO_3^-		
	ATP-Mg			ATP-Mg		
	3 mM	0.6 mM	0.06 mM	3 mM	0.6 mM	0.06 mM
None	65	44	20	120	59	17
NAD^+	67	45	21	119	60	18
Q	85	50	25	132	65	22
FAD	94	57	24	130	64	21
Cyc. c	68	46	21	122	58	20

tion caused by DNP was not affected by the oxidized electron carriers; under these conditions no oligomycin sensitivity was observed and the inhibition due to cyanate was not potentiated (data not shown).

Discussion

The effects of reduced physiological electron carriers on the properties of F_1-ATPase seem to suggest a modification of the enzyme by reduction. The change in properties, besides an increase in its hydrolyzing activity, affected the sensitivity of the enzyme to activators, such as bicarbonate or dinitrophenol, and to the inhibitor cyanate. Moreover, in the presence of physiological electron donors a slight sensitivity to oligomycin was elicited. These effects were not observed if the electron carriers NAD^+ or cyt c were present in these oxidized forms; furthermore the increase in activity in the presence of either FAD or coenzyme Q was not accompanied by a change in sensitivity to either activators, such as dinitrophenol or bicarbonate, or to the inhibitor cyanate, and no sensitivity to oligomycin appeared. These observations would support a previous suggestion that mitochondrial ATPase might be modified by a redox reaction (11) after the finding that a change in properties, similar to that now described, takes place when the hydrolytic reaction catalyzed by rat liver F_1-ATPase is carried out in the presence of the reducing agent dithionite. A modification of mitochondrial ATPase by a redox reaction might have some relevance from the point of view of the regulation of the catalytic activities of the enzyme linked to the hydrolysis of ATP. In addition, a redox modification of the enzyme could also be related to the mechanism of oxidative phosphorylation as recently suggested by SANTIAGO and LÓPEZ-MORATALLA (10).

The extent of the activating effect of the reduced electron carriers, NADH, $FADH_2$, QH_2, depended on substrate concentration. These results would be compatible with the existence of several catalytic sites in F_1-ATPase differing in affinity for the substrate (9). It might be suggested that these catalytic sites could have a different affinity for the reduced electron carriers, and also that possible physiological interactions of electron donors and acceptors with each catalytic site of ATPase could be mediated by their corresponding redox potentials and by their precise topographical distribution within the membrane.

Resumen

La actividad hidrolítica de la F_1-ATPasa aislada a partir de hígado de rata aumenta en presencia de NADH, $FADH_2$, QH_2 o citocromo c reducido. El grado de activación depende en gran medida de la concentración de substrato. La presencia de cualquiera de estos donadores de electrones origina una disminución de la sensibilidad de la F_1-ATPasa frente a los activadores bicarbonato o dinitrofenol, así como también la aparición de una ligera sensibilidad a la oligomicina y una disminución de la sensibilidad frente al anión inhibidor OCN^-. Las formas oxidadas de los transportadores de electrones no modifican la sensibilidad del enzima frente al bicarbonato, dinitrofenol o OCN^-, y el enzima permanece insensible a la oligomicina.

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