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Effects of organic solvents and orthophosphate on the ATPase activity of F1 ATPase

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The ATPase activity of soluble F1 ATPase of mitochondria is activated by P_i. The concentration of P_i required for half-maximal activation decreases from a value higher than 50 mM to about 1 mM P_i when one of the organic solvents dimethyl sulfoxide (15 to 30%), methanol (7.5 to 15%) or ethylene glycol (10 to 30%) is added to the assay medium. This effect is observed in the presence of $MgCl_2$ but not in the presence of $CaCl_2$.

F_1 ATPase; Organic solvent; Enzyme activation; Orthophosphate

1. INTRODUCTION

In the presence of $MgCl_2$ the ATPase activity of soluble mitochondrial F1 ATPase is activated by P_i concentrations higher than 10 mM [1,2]. Activation is also observed when part of the water of the incubation medium is replaced by an organic solvent such as dimethyl sulfoxide or methanol [3–7]. At present, the mechanism by which P_i and organic solvents activate the hydrolytic activity of F1 ATPase is not clear. P_i is a product of ATP hydrolysis but when the enzyme catalyzes the synthesis of ATP, P_i serves as one of the substrates of F1. Recently it has been shown that organic solvents promote an increase in the affinity for P_i of F1 and other enzymes that catalyze the synthesis of phosphate compounds in processes of energy transduction [8–12].

In this report we studied the combined effects of P_i and organic solvents on the ATPase activity of F1 in the presence of both magnesium and calcium ions. The data obtained indicate that synthesis of tightly bound ATP at one of the catalytic sites of F1 activates the turnover of the enzyme during ATP hydrolysis. This effect seems to depend on the divalent cation used.

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2. MATERIALS AND METHODS

F1 was prepared from bovine heart mitochondria as described [13]. ATPase activity was assayed by measuring the release of $^{32}P_i$ from [γ-^{32}P]ATP [8].

3. RESULTS

The ATPase activity of soluble F1 is much higher in the presence of $MgCl_2$ than in the presence of $CaCl_2$ (fig.1). In the presence of $MgCl_2$, the activity of F1 may be enhanced or inhibited depending on the organic solvent used. At the concentrations shown in fig.1A, methanol and dimethyl sulfoxide activate, while ethylene glycol inhibits the ATPase activity of F1 [3–7]. We now show that in the presence of calcium, the three organic solvents used strongly inhibit the ATPase activity of F1 (fig.1B).

In totally aqueous medium and in the presence of $MgCl_2$ (figs 2A and 3A) a small activation of the ATPase activity is observed as the P_i concentration

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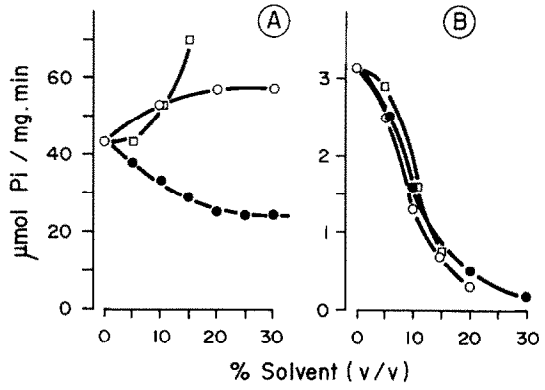

Fig.1. Effects of organic solvents in the presence of $MgCl_2$ or $CaCl_2$. The assay medium composition was 50 mM 3-(*n*-morpholino)propanesulfonic acid-Tris buffer (pH 7.0), 3 mM ATP and either 3 mM $MgCl_2$ (A) or 1 mM $CaCl_2$ (B). The organic solvents used were (□) methanol, (○) dimethyl sulfoxide and (●) ethylene glycol. The assay was performed at 35°C and the F1 concentration was 5 μg protein per ml.

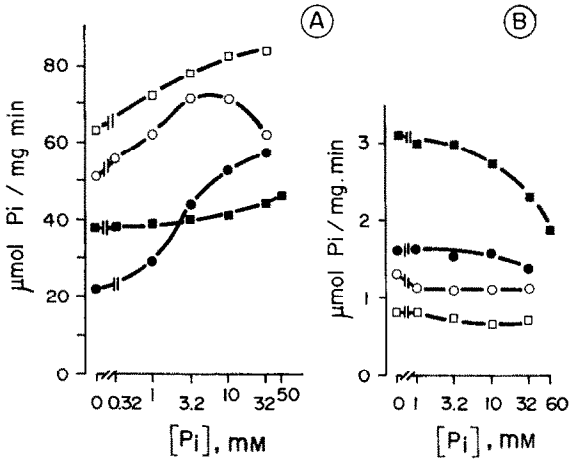

Fig.2. Effects of P_i and of organic solvents in the presence of $MgCl_2$ or $CaCl_2$. The assay medium composition was 50 mM 3-(*n*-morpholino)propanesulfonic acid-Tris buffer (pH 7.0), 3 mM ATP, the concentrations of Tris-P_i indicated on the abscissae, and either 3 mM $MgCl_2$ (A) or 1 mM $CaCl_2$ (B). In (A), the organic solvent concentrations (v/v) used were (■) totally aqueous medium, (□) 15% methanol, (○) 30% dimethyl sulfoxide and (●) 30% ethylene glycol. In (B) the organic solvent concentrations used were (■) totally aqueous medium, (□) 15% methanol, (○) 10% dimethyl sulfoxide and (●) 10% ethylene glycol. Other conditions were as in fig.1.

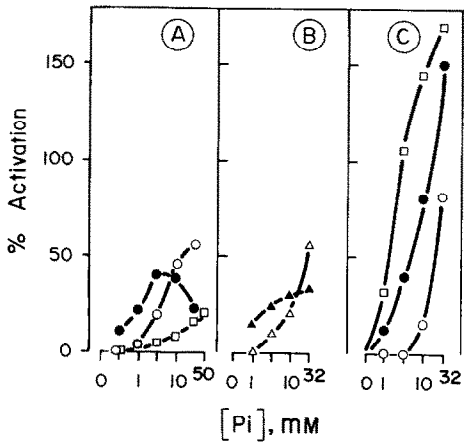

Fig.3. P_i dependence. The assay medium composition was 50 mM 3-(*n*-morpholino)propanesulfonic acid-Tris buffer (pH 7.0), 3 mM ATP, 3 mM $MgCl_2$ and the concentrations of Tris-P_i indicated on the abscissae. Other conditions were as in fig.1. (A) Totally aqueous medium (□), 15% (○) and 30% (●) dimethyl sulfoxide. (B) 7.5% (△) and 15% (▲) methanol. (C) 10% (○), 20% (●) and 30% (□) ethylene glycol.

in the medium is raised from 10 to 50 mM. The activation by P_i becomes much more pronounced when organic solvents are included in the assay medium (figs 2A and 3). Notice in fig.2A that ethylene glycol inhibits the ATPase activity when P_i is not added to the medium. However, similarly to dimethyl sulfoxide and to methanol, ethylene glycol strongly potentiates the effect of P_i on the ATPase activity of F1. In fig.3 it is shown that the concentration of P_i required for half-maximal activation of the ATPase activity decreases as the concentration of organic solvent in the medium is increased. In totally aqueous medium saturation is not attained even after the addition of 50 mM P_i to the medium, but in the presence of 30% (v/v) dimethyl sulfoxide, half-maximal activation requires only 1 to 2 mM P_i (fig.3A). In contrast to its effect on the apparent affinity for P_i, increasing the concentration of organic solvent seems to decrease the maximal rate of ATP hydrolysis attained in the presence of saturating concentrations of P_i. This could be observed most clearly when the concentration of dimethyl sulfoxide was raised from 15 to 30% (fig.3A) and when the concentration of methanol was raised from 7.5 to 15% (fig.3B).

A puzzling finding is that in the presence of $CaCl_2$, P_i does not activate the ATPase activity of F1 in either the absence or presence of organic solvents (fig.2B). On the contrary, in totally aqueous medium P_i inhibits the ATPase activity of F1 (fig.2B).

4. DISCUSSION

The F1 ATPase of mitochondria catalyzes an exchange between water oxygen and the oxygen of phosphate [14–17]. These studies led to the conclusion that ATP can be synthesized at the catalytic site of the soluble F1 without requiring energy. This ATP remains tightly bound to the enzyme, and in intact mitochondria the energy of the proton gradient is not necessary for the synthesis of ATP at the catalytic site of the enzyme but instead serves to increase the affinity of the enzyme for P_i and to permit the dissociation from the enzyme of the tightly bound ATP [14–17]. Several authors have failed to detect the spontaneous synthesis of a significant amount of ATP when the mitochondrial F1 was incubated in media containing ADP and a high P_i concentration (10 to 500 mM). This has been attributed to the very low affinity of soluble F1 for P_i [6,10,11,18,19]. Among the enzymes whose affinity for P_i increases with organic solvents are the Ca^{2+}-ATPase of sarcoplasmic reticulum [8,9], yeast inorganic pyrophosphatase [12], the H^+-ATPase of yeast plasma membrane and soluble F1 ATPase [6,10,11,18,19]. Taking advantage of this effect of organic solvents, it has been possible to measure the synthesis of tightly bound ATP from P_i of the medium in both mitochondria [6,10,11] and bacterial F1 ATPase [19]. In the presence of 40% (v/v) dimethyl sulfoxide and 1 to 10 mM P_i, 0.2–0.6 mol of tightly bound ATP is synthesized per mol of soluble F1 ATPase [6,10,11,18,19]. In these experiments there was no need to add ADP to the medium. Synthesis of ATP occurred at the expense of medium P_i and the ADP that is normally found bound to the enzyme (tightly bound ADP).

The mechanism of catalysis of F1 ATPase is still far from understood. Recently it has been shown that the F1 complex has more than one catalytic site and that the hydrolysis of ATP by F1 involved cooperative interaction between catalytic sites [17,20–24]: the hydrolysis of ATP at one site is accelerated when ATP binds to a second site. The findings of figs 2A and 3 can be interpreted on the basis of the cooperative interaction between different catalytic sites of F1. Organic solvents and P_i would promote the synthesis of tightly bound ATP at one site and this would promote the hydrolysis of ATP bound at a second site. In this view, the ATP to be hydrolyzed would be derived from the assay medium and the ATP molecule which would activate the hydrolysis of the medium ATP would be synthesized at the regulatory site from medium P_i and bound ADP.

In a previous report, evidence has been presented that the cooperative interaction between different catalytic sites of F1 requires the binding of magnesium to the enzyme [11]. It may be that this interaction does not occur in the presence of calcium. This would account for the finding that in the presence of calcium, the ATPase activity of F1 was not activated either by organic solvents (fig.1B) or by P_i (fig.2B).

The mechanism by which organic solvents increase the enzyme affinity for P_i has been discussed in previous reports [8,9].

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