

ATP synthesis by the $(\text{Na}^+ + \text{K}^+)$ -ATPase in the absence of an ionic gradient

Effects of organic solvent

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A 200-fold decrease in the P_i concentration required for half-maximal phosphorylation of the $(\text{Na}^+ + \text{K}^+)$ -ATPase is observed when 40% (v/v) dimethyl sulfoxide is added to the assay medium. The phosphoenzyme formed in the presence of dimethyl sulfoxide is able to transfer its phosphate to ADP to form ATP when 400 mM NaCl is added to the medium. The synthesis of ATP depends on the concentrations of organic solvent and of Na^+ , and is inhibited by ouabain.

ATP synthesis; $(\text{Na}^+ + \text{K}^+)$ -ATPase; Organic solvent effect

1. INTRODUCTION

The catalytic cycle of the $(\text{Na}^+ + \text{K}^+)$ -ATPase includes two distinct functional states of the protein, E_1 and E_2 . These two states can be distinguished by their susceptibility to phosphorylation by either ATP or P_i [1–3]. In both cases, an acyl phosphate residue is formed at the catalytic site of the enzyme. In the E_1 conformation, the protein is phosphorylated by ATP and the phosphoenzyme formed ($\text{E}_1\text{-P}$) is referred to as ‘high-energy’ because it can transfer its phosphoryl group to ADP, leading to ATP synthesis. In the E_2 conformation, the enzyme is phosphorylated by P_i ($\text{E}_2\text{-P}$). This phosphoenzyme is referred to as ‘low-energy’ since it is formed with no apparent source of energy and cannot transfer its phosphoryl group to ADP [2–4].

Evidence obtained with organic solvents and different enzymes involved in energy transduction indicates that the high-energy and low-energy forms of phosphoenzyme may be correlated with the structure of water at the catalytic site of these enzymes [5–10]. Here, the effects of dimethyl sulfoxide on the phosphorylation by P_i and on the ATP synthesis catalyzed by $(\text{Na}^+ + \text{K}^+)$ -ATPase were studied.

2. MATERIALS AND METHODS

The $(\text{Na}^+ + \text{K}^+)$ -ATPase was prepared from sheep kidney using the method of Jørgensen [11], as modified by Munson [12]. $^{32}\text{P}_i$ was purified as described [10]. The conditions for enzyme phosphorylation are described in the figure legends and the radioactive phosphoenzyme was measured as described elsewhere [5,9]. Synthesis of ATP was determined by measuring $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ formed from ADP and $^{32}\text{P}_i$, the excess of $^{32}\text{P}_i$ being extracted as phosphomolybdate with 2-butanol/glucose to remove any contaminating ATP. In two

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experiments the [γ - ^{32}P]ATP synthesized by the enzyme was isolated and identified as follows: 2 ml of the assay medium was mixed with 4 ml of a 6% (w/v) suspension of activated charcoal in 0.1 M HCl and 0.01 ml of 0.1 M ATP solution. The mixture was centrifuged at $2000 \times g$ for 15 min. The charcoal was then washed 5 times, centrifuging and resuspending it the first 3 times in 40 ml of 100 mM P_i solution in 0.1 N HCl, and the last 2 times in 40 ml water. The charcoal was packed into a small column and the radioactive ATP was eluted from it with a mixture containing 6 vols of 1 M NH_4OH solution and 4 vols ethanol. The radioactive material eluted from the column was dried under vacuum and re-suspended in 0.05 ml water. The radioactive ATP contained in 0.02 ml of this suspension was hydrolyzed enzymatically by mixing with 0.18 ml of a solution containing 20 mM Tris-HCl buffer (pH 7.5), 120 mM NaCl, 20 mM KCl, 1 mM MgCl_2 , 0.1 mM ATP and 0.1 mg purified $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$. After 30 min at 37°C , the reaction was quenched with 0.05 ml of 3 N HCl. The radioactive material contained in the samples before and after treatment with ATPase was identified by autoradiography of ascending thin-layer chromatograms on PEI-cellulose sheets, using 0.75 M P_i buffer (pH 3.4) as solvent and Kodak XK-1 film [13]. Before treatment with the ATPase, practically all of the radioactive material moved with the same R_f as ATP. After hydrolysis, the radioactive material had the same R_f value as P_i .

3. RESULTS

Measuring the phosphorylation of the $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ by P_i it was found that the apparent affinity of the enzyme for P_i varied with pH (fig.1). At pH 6.0, the P_i concentration needed for half-maximal enzyme phosphorylation was 1.4 ± 0.1 mM ($N = 6$) and increased to 5.7 ± 0.1 mM ($N = 7$) when the pH of the medium was raised to 8.0. This was determined from double-reciprocal plots of data obtained in experiments performed as described in fig.1. In previous reports it has been observed that the addition to the assay medium of an organic solvent such as Me_2SO promotes a large decrease in the apparent K_m for P_i for the formation of an acyl phosphate residue at the catalytic site of the $\text{Ca}^{2+}\text{-ATPase}$ of both the sarcoplasmic

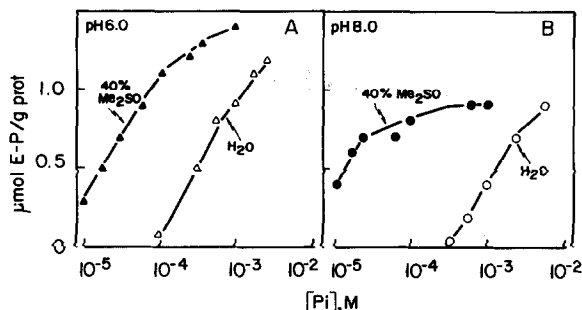


Fig.1. Me_2SO and pH effect on P_i dependence of E-P formation. The assay media contained 10 mM MgCl_2 , 50 mM Tris-maleate buffer, pH 6.0 (A) or pH 8.0 (B), and the concentration of $^{32}\text{P}_i$ shown on the abscissa, either without (Δ , $\circ$) or with 40% (v/v) Me_2SO ($\blacktriangle$, $\bullet$). The reaction was started by the addition of 1.0 mg enzyme protein per ml, and stopped after 2 min at 36°C with 1 vol. of a cold 10% trichloroacetic acid solution.

reticulum [5,6] and red cell [9], and for the formation of tightly bound ATP at the catalytic site of $\text{F}_1\text{-ATPase}$ of mitochondria [6,8]. Fig.1 shows that a decrease in the apparent K_m for P_i was also observed with the use of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ when 40% (v/v) Me_2SO was added to the assay medium. In the presence of the solvent, the concentrations of P_i needed for half-maximal enzyme phosphorylation at pH 6.0 and 8.0 were 0.06 ± 0.01 mM ($N = 6$) and 0.03 ± 0.01 mM ($N = 7$), respectively. The phosphoenzyme formed in the presence of Me_2SO was slowly hydrolyzed when Na^+ was added to the medium (fig.2). The rate of hydrolysis appeared to vary with the concentration of Me_2SO , being slower when a higher concentration of solvent was used (cf. figs 2 and 3B). Taniguchi and co-workers [4] observed that the phosphoenzyme formed in a totally aqueous medium was able to transfer its phosphate to ADP to form ATP when ADP was added to the medium together with Na^+ . We observed that this reaction also occurs in the presence of 40% (v/v) Me_2SO (fig.3A) and was completely inhibited when 1 mM ouabain was added to the medium simultaneously with Na^+ and ADP (not shown). Synthesis of ATP depended on the concentrations of both organic solvent and Na^+ . In the presence of a high Me_2SO concentration (60%), cleavage of the phosphoenzyme was not accompanied by the synthesis of ATP (fig.3B). A similar phenomenon has been

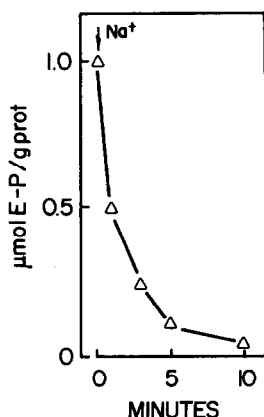


Fig. 2. Effect of Na^+ on the phosphoenzyme formed in the presence of Me_2SO . Phosphorylation was performed in 50 mM Tris-maleate buffer, pH 6.0, 10 mM MgCl_2 , 0.2 mM $^{32}\text{P}_i$ and 40% (v/v) Me_2SO . After 2 min at 36°C , NaCl to 50 mM final concentration was added. At each time interval an aliquot of 1.0 ml was mixed with 1.0 ml cold 10% trichloroacetic acid.

described for the Ca^{2+} -ATPase [5,6]. Fig. 4 shows that in 40% Me_2SO half-maximal synthesis of ATP was attained in the presence of 200 mM NaCl . At NaCl concentrations below 100 mM the

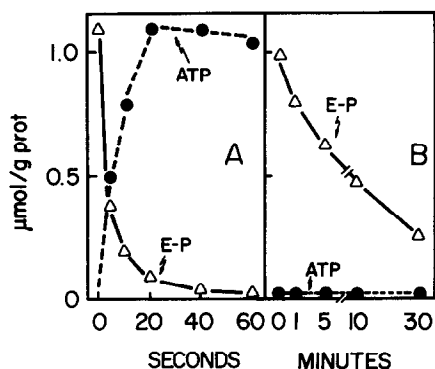


Fig. 3. ATP synthesis in the presence of Me_2SO . The enzyme (1.5 mg/ml) was phosphorylated at 37°C in a medium containing 50 mM Tris-maleate buffer, pH 7.0, 2 mM MgCl_2 , 0.2 mM $^{32}\text{P}_i$ and 40% (v/v) Me_2SO . After 2 min, 0.5 ml of this mixture was diluted in 1.5 ml of a solution containing 50 mM Tris-maleate buffer, pH 7.0, 2 mM MgCl_2 , 0.2 mM $^{32}\text{P}_i$, 400 mM NaCl , 1 mM ADP and 40% (v/v) Me_2SO (A) or 60% (v/v) Me_2SO (B). At each time interval the reaction was stopped with 0.1 ml of 100% cold trichloroacetic acid. Phosphoenzyme (Δ), ATP synthesized ($\bullet$).

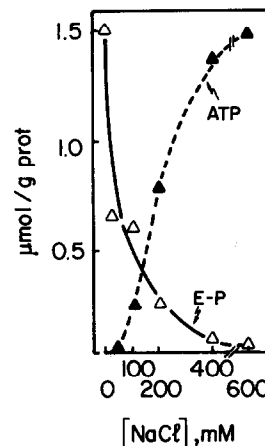


Fig. 4. Na^+ dependence of ATP synthesis in the presence of Me_2SO . The procedure was the same as that described for fig. 3. The dilution was made with 40% (v/v) Me_2SO and the reaction was arrested 5 min after the addition of ADP and different Na^+ concentrations. Phosphoenzyme (Δ), ATP synthesized ($\bullet$).

cleavage of the phosphoenzyme was not accompanied by the synthesis of ATP. As the Na^+ concentration increased in the dilution mixture more phosphoenzyme was able to transfer its phosphate to ADP, synthesizing ATP. A similar Na^+ dependence was previously observed for the $\text{ATP}=\text{P}_i$ exchange reaction catalyzed by the $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ in totally aqueous medium [14]. In two experiments, the $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ synthesized under the conditions of fig. 3 was isolated and identified by autoradiography of thin-layer chromatograms as described in section 2.

4. DISCUSSION

The present data can be interpreted according to the proposal that the enzymes involved in energy transduction undergo a hydrophobic-hydrophilic transition during the catalytic cycle, and that the catalytic site is hydrophobic when the enzyme binds P_i [5–10]. P_i is highly soluble in water and poorly soluble in organic solvents. If the catalytic site of the enzyme is hydrophobic, then the partition of P_i from the assay medium into the catalytic site should be facilitated when the difference in hydrophobicity between these two compartments is reduced [5]. Accordingly, it was found that the addition of Me_2SO to the medium causes a large

decrease in the P_i concentration required for half-maximal phosphorylation of the $(Na^+ + K^+)$ -ATPase (fig.1). A similar phenomenon has been described for different enzymes involved in energy transduction [5–9]. The ATPase phosphorylated in the presence of Me_2SO was able to transfer its phosphate to ADP with the same Na^+ dependence as that described for the phosphoenzyme formed in totally aqueous medium [4,14]. The finding that an excess of Me_2SO blocks the phosphate transfer from the phosphoenzyme to ADP (fig.3B) may indicate that the excess of solvent impairs the entry of water into the catalytic site, a process which is essential for the subsequent solvation of the acyl phosphate residue via a mechanism similar to that proposed for the Ca^{2+} -ATPase [5]. Alternatively, it may be that excess Me_2SO stabilizes the E_2 -P form and impairs its conversion into E_1 -P. The role of ions in the interconversion of phosphorylated forms of $(Na^+ + K^+)$ -ATPase during its catalytic cycle has been discussed in detail in recent reviews [2,3].

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