ATP Synthesis Driven by a Valinomycine Induced K^+ Diffusion Potential in Liposomes Bearing Chloroplast ATP Synthase

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Partially purified chloroplast ATP synthase was reconstituted into asolectin liposomes. A valinomycine induced potassium diffusion potential from outside to inside the vesicles promoted a measurable ATP synthesis. If valinomycine was replaced by nigericine, practically no ATP was formed.

Introduction

Unilamellar liposomes containing ATP synthase in their walls are usefull models for studying oxidative- and photo-phosphorylations [1–5]. The essential condition for ATP formation is the energization of the membrane with a transmembrane pH gradient, ΔpH or a transmembrane potential difference, $\Delta\Psi$ [6]. In this paper, we show that a K^+ diffusion potential induced by the ionophore, valinomycine is sufficient to get measurable ATP synthesis.

Experimental

Liposomes were prepared by sonication to clarity of soybean phospholipids (40 mg/ml) in 50 mM Na-Tricine (pH 8.0) and 0.5 mM EDTA. ATP synthase was isolated from spinach chloroplasts according to [1]. The ammonium sulfate (37.5–45%) precipitated fraction was reconstituted into liposomes $\left(\frac{\text{phospholipids}}{\text{proteins}}\ \text{w/w} = 20\right)$ using the freeze-thaw technique [7] or by a 10 min incubation at 20 °C.

The reconstituted vesicles (0.2 ml) were then passed through a 1 ml Sephadex G50 column [8] equilibrated with 50 mM Na-Tricine (pH 8.0) and 0.5 mM EDTA. The phosphorylation reaction was started by addition of 0.8 ml reaction mixture containing 50 mM Na-Tricine (pH 8.0), 5 mM $MgCl_2$, 5 mM Na-ADP, 2 mM phosphate (5 µCi $^{32}P_i$) 0.25% bovine serum albumine (defatted), 100 mM KCl, 20 mM glucose and 10 units hexokinase. After 5 min incubation at room temperature, the reaction was stopped by addition of 50 µl of 50% trichloracetic acid. [^{32}P] ATP formed was determined after removal of the $^{32}P_i$ by the isobutanol-benzene extraction of the phosphomolybdate complex [9]. Radioactivity was counted with Lumagel scintillator in a Packard scintillation counter.

In each series a control was run (trichloroacetic acid was added before reaction mixture) and its radioactivity after extraction (10–15 counts/min) was negligeable. All the reagents used were of analytical grade.

Results and Discussion

The results are summarized in Table I. The addition of 1 µM valinomycine to the phosphorylation medium promotes a measurable ATP synthesis: the values obtained are twice as high when the reconstitution is made by freeze-thaw compared to incubation at 20 °C. If valinomycine is replaced by nigericine, practically no ATP is formed. The small quantity of ATP observed (in case of freeze-thaw reconstitution) cannot be attributed to phosphorylation. Indeed, in this case, the transmembrane K^+ diffusion is accompanied by an antiport proton movement, without energization of the membrane.

Table I. ATP synthesis driven by a valinomycine induced K^+ diffusion potential.

Conditions	ATP, nmol × mg protein^{-1}	
	Reconstitution by freeze-thaw	10 min 20 °C
Reconstituted liposomes	0	0
Reconstituted liposomes + 1 µM valinomycine	30	15
Liposomes without ATP synthase + 1 µM valinomycine	0	0
Reconstituted liposomes + 1 µM nigericine	3.5	0

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It was shown formerly [1] that the ATP synthesis coupled to a transmembrane pH gradient driven by an acid-to-base transition is enhanced by a K^+ diffusion potential induced by valinomycine. It is found here that the energy of the membrane potential alone is sufficient to get ATP synthesis.

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