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

M. Dinant and K. Kaminski

Institut de Botanique, Université de Liège, Sart Tilman, Bât. B22, B-4000 Liège, Belgium

Z. Naturforsch. **39c**, 320–321 (1984);
received November 25, 1983/January 20, 1984

ATP Synthesis, Valinomycine, $K^+$ Diffusion Potential, Liposomes, Chloroplast ATP Synthase

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, $\Delta$pH 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 |

Reprint requests to Dr. M. Dinant.

0341-0382/84/0300-0320 $ 01.30/0

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.

*Acknowledgements*

The authors gratefully acknowledge the kind help and advices of Dr. J. P. Dufour. This research was supported by a grant from the Belgian Government (Actions Concertées n° 80/85-18).

[1] U. Pick and E. Racker, J. Biol. Chem. **254,** 2793 (1979).
[2] G. D. Winget, N. Kanner, and E. Racker, Biochim. Biophys. Acta **460,** 490 (1977).
[3] G. Hauska, G. Orlich, D. Samoray, E. Hurt, and P. V. Sane, Proc. 5th Internat. Congr. Photosynth. Halkidiki 1980, **vol. 2,** pp. 903–914 (1981).
[4] M. Rögner, K. Ohno, T. Hamamoto, N. Sone, and Y. Kagawa, Biochem. Biophys. Res. Commun. **91,** 362 (1979).
[5] P. Gräber, M. Rögner, H. E. Buchwald, D. Samoray, and G. Hauska, FEBS Lett. **145,** 35 (1982).
[6] P. Mitchell, Science **206,** 1148 (1979).
[7] M. Kasahara and P. C. Hinkle, J. Biol. Chem. **252,** 7384 (1977).
[8] H. S. Penefsky, J. Biol. Chem. **252,** 2891 (1977).
[9] G. Hauska, in Methods in Enzymol. (S. P. Colowick and N. O. Kaplan, eds.), **vol. 69,** pp. 648–658, Academic Press, New York, London 1980.