Biochimica et Biophysica Acta 890 (1987) 392–394
Elsevier

BBA 40090

BBA Report

The rate of ATP synthesis catalyzed by reconstituted CF_0F_1-liposomes: dependence on ΔpH and $\Delta\psi$

Günter Schmidt and Peter Gräber

Max-Volmer-Institut für Biophysikalische und Physikalische Chemie, Technische Universität Berlin, Berlin (Germany)

(Received 10 October 1986)

Key words: ATP synthesis; Coupling factor; Membrane reconstitution; Proton gradient

The kinetics of proton-transport coupled ATP synthesis in CF_0F_1 reconstituted into asolectin liposomes was investigated upon energization of the membrane by an artificially generated ΔpH and $\Delta\psi$. With a rapid mixing system the rate of ATP synthesis was measured at short reaction times (under 200 ms) where all parameters (ΔpH, $\Delta\psi$, substrate and product concentrations) remain practically constant at their initial values. The rate of ATP synthesis depends, in a sigmoidal way, on ΔpH, the maximal rate being 200 ATP per CF_0F_1 per s. At constant ΔpH, an additional diffusion potential increases the rate until the maximal rate is reached.

The chloroplast ATP synthase (ATPase), CF_0F_1, has been isolated, purified and reconstituted into liposomes [1]. The reconstitution has been optimized, and the rate of ATP synthesis has been measured after generation of ΔpH and $\Delta\psi$ (acid–base transition and K^+/valinomycin diffusion potential) with a rapid mixing apparatus. A rate of 200 ATP per CF_0F_1 per s was obtained, which is about half the rate obtained with chloroplasts under the same experimental conditions [2].

CF_0F_1 was isolated and reconstituted into asolectin liposomes according to Refs. 1 and 2. The enzyme concentration in the reconstitution medium was 0.05–0.1 mg/ml, the lipid concentration 45 mg/ml, and bovine serum albumine was 1 mg/ml. The ATP yield was measured as a function of reaction time with a rapid mixing quenched-flow apparatus and the rate was determined as described earlier [3]. The proteoliposomes were incubated with the acid solution I for 20–40 s (protein concentration 10–20 μg/ml). Solution I contained 30 mM sodium succinate, 0.50 mM KOH, 2 mM MgCl, 5 mM NaH_2PO_4, and 1 μM valinomycin. The pH was adjusted with NaOH. The different K^+ concentrations were obtained by addition of KCl. The solution was then mixed in the mixing chamber with the basic solution II, which contained 200 mM Tricine, 120 mM KOH, 5 mM NaH_2PO_4, 2 mM MgCl and 0.2 mM ADP. The pH of the basic solution II was adjusted with NaOH so that the final pH was 8.35 ± 0.05. The reaction was allowed to proceed for a definite time (50–300 ms) and was then stopped by mixing with 4% trichloroacetic acid in the second mixing chamber. The amount of ATP synthesized was measured with luciferin/luciferase.

Fig. 1 shows the ATP yield as a function of the reaction time measured with the quenched flow technique. The pH_{out} was 8.35, the K^+ concentration inside was 6 mM and outside 63 mM. The linear increase of the yield demonstrates that under these conditions the transmembrane ΔpH and

Correspondence: G. Schmidt, Max-Volmer-Institut für Biophysikalische und Physikalische Chemie, Technische Universität Berlin, Strasse des 17. Juni 135, 1000 Berlin 12, Germany.

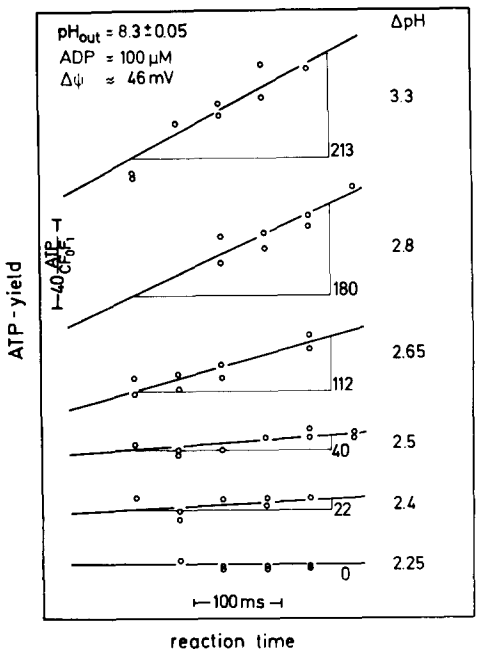

Fig. 1. ATP yield as a function of the reaction time. The slope of these curves gives the rate of ATP synthesis, the numbers give the rate in ATP per CF_0F_1 per s. For further details see Materials and Methods and text.

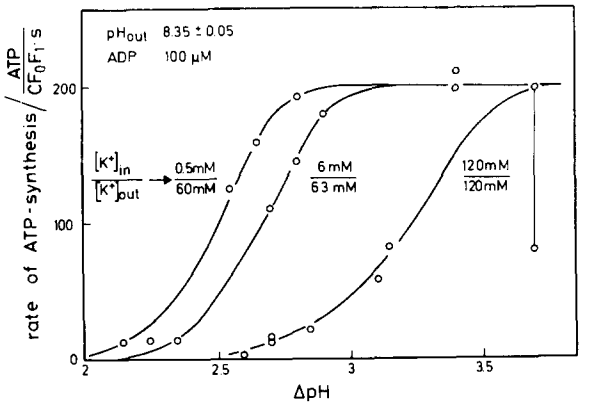

Fig. 2. The rate of ATP synthesis as a function of ΔpH and Δψ. Data are from Fig. 1 and similar sets of experiments. The parameter given at the curve is the ratio of inner and outer K^+ concentration. Open circles are directly measured rates; the square is a corrected rate as described in the text; the dashed line connects the directly measured rate with the corrected one. For further details see text.

Δψ do not change significantly. Thus, the slope of the curves directly gives the rate of ATP synthesis at constant ΔpH and Δψ. Increase of the transmembrane ΔpH increased the rate.

Fig. 2 shows the rate of ATP synthesis as a function of ΔpH when different K^+/valinomycin diffusion potentials are generated simultaneously with ΔpH. The data are from Fig. 1 and similar sets of measurements at other diffusion potentials. The dependence of the rate on ΔpH is sigmoidal, with a maximum of about 200 s^{-1}.

When no diffusion potential is generated, such a high rate cannot be observed directly: if the reconstituted liposomes are incubated at a pH $\lesssim$ 5, an inactivation (possibly denaturation of the enzyme) occurs. For correction of this inactivation the following procedure was applied as described in detail in Ref. 3:

(1) Measurement of the ATP synthesis rate, v_1, at the required ΔpH; e.g. at ΔpH = 3.7 (pH$_{in}$ = 4.7).
(2) Measurement of ATP synthesis rate, v_2, at ΔpH = 3.2. (This rate is called the standard rate; at this ΔpH (pH$_{in}$ = 5.1) no inactivation occurs.
(3) Incubation of the reconstituted liposomes for 30 s at the pH used for measurement 1 (e.g., at pH$_{in}$ = 4.7), then incubation for 30 s at pH$_{in}$ = 5.1 and measurement of the rate, v_3.

The corrected rate, v(corr), that would have been measured if no inactivation had occurred is given by $v(\text{corr}) = v_1 \cdot (v_2/v_3)$. The only experiment at higher ΔpH which requires such a correction is indicated in Fig. 2. The measured rate (circle) is converted into the corrected rate (square) as indicated by the dashed line.

At constant ΔpH an additional diffusion potential (the parameter given in Fig. 2 is the ratio between inner and outer K^+ concentration) increases the rate unless the maximum has been reached. If we assume the same permeability coefficients as in chloroplasts, diffusion potentials of 68 mV, 47 mV and 0 mV are calculated for the ratios of $[K^+]_{in}/[K^+]_{out}$: 0.5 mM/60 mM, 6 mM/63 mM and 120 mM/120 mM at ΔpH = 2.7 from the Goldman-Hodgkin-Katz equation. Using these potentials, the sum of ΔpH and Δψ does not determine the rate of ATP synthesis as found for chloroplasts [3]. This is probably due to different permeability coefficients for asolectin and the thylakoid membranes. Table I lists the ion concentration in solution 1 (corresponding to the

TABLE I

ION CONCENTRATION INSIDE AND OUTSIDE OF THE LIPOSOMES FOR THE THREE DIFFERENT REACTION CONDITIONS

Values quoted in mM

ion	inside	outside	inside	outside	inside	outside
K^+	0.5	60	6	63	120	120
Na^+	37–60 [a]	37	37–60 [a]	37	37–60 [a]	37
Cl^-	4	4	10	7	124	64
Mg^{2+}	2	2	2	2	2	2
Succinate	30	15	30	15	30	15
Phosphate	5	5	5	5	5	5
Tricine	0	100	0	100	0	100

[a] Depending on the pH of solution I.

internal concentrations) and in solution 1 + 2 (corresponding to the outer concentrations). When permeability coefficients become available, the diffusion potentials can be calculated from these data.

The half-maximal rate at $\Delta\psi = 0$ mV is obtained at $\Delta pH = 3.25$. This is nearly identical with the result from chloroplasts at $\Delta pH = 3.35$ [3]. The maximal rate observed here was 200 s^{-1}. This is almost half the rate observed with chloroplasts under the same conditions [3]. Since the calculation of the rate is based on the amount of protein added to the lipid/detergent mixture before dialysis, it is possible that not all CF_0F_1 is actively reconstituted into the liposomes. Therefore, the rate given here is the minimum and any correction for inactivation would increase the rate.

In comparison with earlier work on the reconstitution of CF_0F_1, very high rates have been obtained here [4–7]. We assume that, aside from the optimization of the reconstitution and assay procedures, this is due to the combination of high energization and short reaction time. The reaction time at which a constant rate of ATP synthesis is found (approx. 500 ms) is very short in view of other reports giving reaction times up to 60 min, when ATPases from the thermophilic cyanobacterium *Synechococcus* 6716 are reconstituted into liposomes made from native lipids [7]. However, the decay of the transmembrane electric potential in asolectin liposomes is much faster than in liposomes made from the lipids of thermophilic organisms [8,9]. In addition to this property of the asolectin lipids, the distribution of the reconstituted CF_0F_1 between the vesicles is inhomogeneous. Although we have chosen the lipid/protein ratio in such a way that approximately one CF_0F_1 per vesicle should be found; electromicrographs show that this distribution is inhomogeneous, i.e., a few vesicles contain more CF_0F_1 and others none et all. This effect leads to a strongly increased proton efflux from the protein-containing vesicles.

Since our result reported here depend only on the initial conditions which are identical for all vesicles, this effect does not influence these results. However,it might lead to the considerable shortening of the duration of constant energization.

This work has been supported by a grant from the Deutsche Forschungsgemeinschaft.

References

1 Pick, U. and Racker, E. (1979) J. Biol. Chem. 254, 2793–2799
2 Schmidt, G. and Gräber, P. (1985) Biochim. Biophys. Acta 808, 46–51
3 Gräber, P., Junesch, U. and Schatz, G.H. (1984) Ber. Bunsenges. Physik. Chem. 88, 599–608
4 Hauska, G., Samoray, D., Orlich, G. and Nelson, N. (1980) Eur. J. Biochem. 111, 535–543
5 Dewey, T.G. and Hammes, G.G. (1981) J. Biol. Chem. 256, 8941–8946
6 Dinant, M. and Kaminsky, K. (1984) Z. Naturforsch. 39c, 320–321
7 van Walraven, H.S., Hagendorn, M.J.M., Krab, K., Haak, N.P. and Kraayenhof, R. (1985) Biochim. Biophys. Acta 809, 236–244
8 Krab, K., van Walraven, H.S., Scholts, M.J.C. and Kraayenhof, R. (1985) Biochim. Biophys. Acta 809, 228–235
9 Sone, N., Yoshida, M., Hirata, H. and Kagawa, Y. (1977) J. Biol. Chem. 252, 2956–2960