CATION CHANNELS BY RECONSTITUTION OF CF₀CF₁
AND BY SUBUNIT III OF CF₀

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INTRODUCTION

CF₀, the channel portion of the ATP-synthase in the thylakoid membrane has a very high proton selectivity and a proton conductivity in the pS-range (1,2,3). Single-channel H⁺ currents through the membrane part of CF₀CF₁ have been reported for the ATP-synthase incorporated into lipid bilayers (3). The thylakoid membrane has proved difficult to be inquired by patch pipettes, except, so far, in giant chloroplasts of P. metallica, where a voltage-dependent chloride channel has been detected (4). For a further electrophysiological inquiry of the ATP-synthase we used the strategy of Tank and Miller (5): CF₀, CF₁ and subunit III of CF₀ were isolated and reconstituted into lipid vesicles which were then fused to form large liposomes suitable for single-channel recordings (Fig. 1). At membrane voltages exceeding 40 mV cation channels were observed which were still sensitive to Venturicidin (as is CF₀) but had lost proton selectivity.

MATERIAL AND METHODS

CF₀ CF₁ was purified and reconstituted into azolectin vesicles using the dialysis technique. The integrity of the preparation was tested by measuring ATP synthesis driven by an artificial pH-gradient (6). From dialysis vesicles large liposomes were formed by a dehydration/rehydration procedure (7). Subunit III of CF₀ was isolated by electroelution from SDS-gels (8) and added to lipid vesicles prior to dehydration. Single bilayer inside-out patches were isolated and single-channel recordings performed as in (9). All potentials given refer to the pipette.

Fig. 1: Schematic diagram: From membrane protein purification over reconstitution into dialysis vesicles to patch clamp technique (adapted from ref. 5).
RESULTS
After forming an inside-out patch a potential difference was applied and with an induction period of a few ten seconds we observed the voltage-dependent onset of single-channel activity. Under asymmetrical KCl concentrations, the reversal potential of the single-channel currents shifted in direction of the Nernst potential for potassium, as expected for a cation channel. Liposomes reconstituted with CF$_0$CF$_1$ showed most frequently channel openings with a conductance of about 13 pS (in 100 mM KCl). A detailed analysis showed 3 subconductance levels (10 pS, 13 pS and 18,5 pS) (Fig. 2). 1 µM Venturicidin (a blocker of CF$_0$) decreased the open probability (P$_{open}$) of these cation channels by more than a factor of 2 (Fig. 2). Additionally single-channel currents with an open channel conductance of 60 pS were observed with reconstituted CF$_0$CF$_1$ (Fig. 3).

Subunit III of CF$_0$ was electroeluted from SDS-gels, either from the 8 kD band (monomer) or the 48 kD band (hexamer (10)) and incorporated into large liposomes. They showed no differences in electrophysiological behaviour. Aside from a conductance level of about 13 pS (in 100 mM KCl) at least two further conductance levels (of about 21 pS and 33 pS) were detected. They showed ohmic behaviour between +100 mV and -100 mV (Fig. 5). EF$_0$, the homologous channel portion of the ATP-synthase of E. coli (11) yielded similar single-channel activities (Fig.4).

Fig.2: Single-channel recordings of liposomes reconstituted with CF$_0$CF$_1$ at -80 mV in 100 mM KCl, 5 mM MgCl$_2$, 10 mM Tricine/KOH pH 7.9. Single-channel traces (left) and corresponding total amplitude histograms (right). The histogram shows the amplitude relative to the baseline of each digitized point of a 85 s (top) / 45 s (below) record. After perfusion of 1 µM Venturicidin to the same patch channel gating was dramatically changed. Even digitized at a rate of 100 µs instead of 1 ms channel openings were hardly resolved.
Fig. 3 (left): Single-channel recordings and corresponding total amplitude histogram of reconstituted CF$_{0}$CF$_{1}$ in 100 mM KCl, 2 mM CaCl$_{2}$, 10 mM Tris/KOH pH 7.5.

Fig. 4 (right): Single-channel recordings of liposomes reconstituted with EF$_{0}$ in 100 mM KCl, 5 mM MgCl$_{2}$, 20 mM Tris/Tricine pH 7.8 and total amplitude histogram.

Fig. 5: Single-channel recordings of liposomes reconstituted with subunit III of CF$_{0}$ in 100 mM KCl, 5 mM MgCl$_{2}$, 20 mM Tris/Tricine pH 7.8. Top: Single-channel records at +100 mV and the corresponding total amplitude histogram (Record length: 8 min / Digitization rate: 2 ms). Right: Equivalent histograms for +50 mV (8 min / 2 ms) and -50 mV (6 min / 2 ms). Lower left: Current-voltage relationship of open channels. Data points were derived from total amplitude histograms.
CONCLUSION
In liposomes, reconstituted with CF_0 CF_1, we observed potassium channels. This was surprising, as it is known, that the intact enzyme conducts protons only (12,13), and even in CF_1-depleted thylakoid membranes, proton conductance is dominant during the first 100 ms after flash induced generation of a potential difference (1,2). Dialysis vesicles containing CF_0 CF were competent to synthesize ATP in an acid-base jump and showed single-channel currents carried by H^+ when incorporated into a lipid bilayer (3). To explain the lack of H^+ specificity in our patch clamp experiments it was conceivable, that some CF_0 CF-complexes had disintegrated during the dehydration/rehydration procedure and their components gave rise to cation channels. The dominance of proton conductance in CF_1-depleted thylakoid membranes (during the first 100 ms after a saturating flash of light) may be due either to the still intact structure of CF_0-complexes or to a time lag in channel formation (from CF_0-subunits) after the onset of a membrane potential.

Venturicidin, a highly specific CF_0 blocker, decreased the channel open probability significantly (Fig.2). Therefore it was likely, that the described cation channels did not arise from contaminations in purified CF_0 CF_1. Compared to the intact ATP-synthase, where 0.5 μM Venturicidin completely abolish ATP synthesis, the K_0 seemed to be higher. Venturicidin is assumed to bind to subunit III of the channel portion of the ATP-synthase (14). In experiments with purified subunit III of CF_0 (Fig.5) cation channels with conductance states and gating behaviour similar to CF_0 CF_1 were observed. These results implied that subunit III (liberated from CF_0 CF_1-complexes) was responsible for the formation of cation channels.

A closer look at the single-channel recordings obtained with CF_0 CF_1 (Fig. 2 and Fig. 3) and with isolated subunit III of CF_0 (Fig. 5), revealed some differences. With subunit III we hardly observed only one type of channel for a longer time period, and we never detected pronounced subconductance levels as shown in Fig. 2 for CF_0 CF_1. Obviously, subunit III alone had channel forming ability. Yet it might be that in the presence of the other subunits of CF_0 (as in liposomes with CF_0 CF_1 incorporated), channel formation by subunit III was modified.

ACKNOWLEDGEMENT
Purified EF_0 was a friendly gift of Dr. G. Dekker-Hebestreit. We thank Dr. R. Hedrich and Dr. W. Hanke for introducing us into patch clamp electrophysiology, and H. Kenneweg for photographs. Financial support by the Deutsche Forschungsgemeinschaft (SFB 171 / B2 and B3).

REFERENCES