

RECONSTITUTION OF AN ANION CHANNEL FROM THE CHLOROPLAST ENVELOPE IN PLANAR LIPID BILAYERS

A. VLÉRICK and F. HOMBLÉ

Laboratoire de Physiologie Végétale, U.L.B., CP 206/2, B-1050 Brussels, Belgium

The photosynthetic activity is modulated by anion exchanges between the chloroplast and the cytoplasm. It has been shown previously that anions can be transported across the chloroplast envelope through either carriers or channels. Here we report on the characterization of a chloride channel purified from the chloroplast envelope.

Experiments were performed on planar lipid bilayers formed from DiPhyPC painted over a 300 μm hole drilled in a polystyrene cup. Voltages were applied to the cis side of the membrane while the trans side was held to ground. Chloroplast envelopes were purified from *Spinacia oleracea*. Membrane proteins from the chloroplast envelopes were solubilized in the presence of 1% Genapol X-080 and 26% sucrose. Unsolubilized material was pelleted (150000g, 90 min) and discarded. Dry hydroxylapatite was added to the detergent-solubilized protein fraction. Elution with MgCl_2 5 mM yielded a fraction highly enriched in a 29 kDa protein.

Reconstitution of a channel into a planar lipid bilayer was achieved by addition of an aliquot of the protein sample to the trans side of the planar lipid bilayer under continuous stirring. In symmetrical 150 mM KCl, single channel records indicate that the channel had several conducting states, the fully open state (222 ± 3 pS, $n=15$) and the lowest conducting state (28 ± 2 pS, $n=15$) occurring most frequently. The current-voltage relationship of the different conducting states were linear in this voltage range. In order to measure the ion selectivity of the channel, the current-voltage relationship was obtained in symmetrical 150 mM KCl condition and then in asymmetrical 540/150 mM KCl (cis/trans) concentration. Increasing the salt concentration of the cis compartment shifted the reversal potential of the current-voltage curve by about +20 mV. According to the Goldman-Hodgkin-Katz equation this corresponds to a permeability ratio $P_{\text{Cl}}/P_{\text{K}}$ of about 5. This indicates that the channel is anion selective. In order to check if the channel displays saturation of ion flux, the current-voltage relationship of the fully open state was measured in different symmetrical KCl concentrations (150 mM, 300 mM, 1 M). The conductance of the fully open state increased non-linearly with the salt concentration. However, no saturation was observed in the range of concentration tested. The total amplitude histogram of records lasting 50 seconds was used to investigate the voltage-dependence of the fully open state. The probability of being in the fully open state followed a symmetrical bell-shaped curve. The maximal value of the open probability was centred around zero millivolt and increased linearly with the ion strength. ATP is one of the main products of photosynthesis and is known to modulate the activity of the chloroplast envelope K^+ channel. Addition of 1 mM to either cis or trans compartment or on both sides of the membrane induced a long lasting burst and a fast flickering of the channel. Flickering occurred so rapidly that individual openings were blurred out by the limited bandwidth of the amplifier (1 kHz). The effect of ATP could arise either from an increase of the gating kinetic or from a channel block.