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The gate of mitochondrial porin channel is controlled by a number of negative and positive charges

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Negatively charged carboxyl groups of mitochondrial porin have been converted into positively charged ones by means of reaction with water-soluble carbodiimide in the presence of ethylenediamine. Properties of channels formed in a planar lipid bilayer by native and modified porins are compared. Amidation has only little influence on the porin channel-forming activity as well as on the open-state conductance of the channel. However, the modification results in a significant enhancement of the voltage dependence of the channel gating and in an increase of the anionic selectivity. It is suggested that the voltage sensor of the porin channel gate is composed of a number of negative (> 14) and positive (> 22) charges.

Porin channel; Gating charge; Amidation; (Mitochondria)

1. INTRODUCTION

When incorporated into a planar lipid bilayer, mitochondrial porin forms voltage-gated channels which exist in the most conducting state at zero and low (< 15 mV) membrane potentials and switch to substates of a lower conductance on the application of a higher positive or negative voltage [1,2]. In a multichannel membrane, this results in a gradual decrease of the conductance with an increase of the membrane potential [1]. The steepness of the conductance-voltage dependence is lowered by raising the pH [3] or by the conversion of positively charged amino groups of porin molecules to negative ones due to succinylation [4]. These results led Bowen and co-workers [3] to suggest that the voltage dependence of the channel closure is caused by the movement of 3–5 positive charges, presumably those of lysine residues.

Rat liver mitochondrial porin contains, besides positive residues of arginine and lysine (about 7 and 27 per molecules, respectively), also negative radicals of aspartic and glutamic acids [5]. Therefore, voltage-dependent transitions may be accompanied by the movement of both positive and negative residues in the membrane potential field. To clarify the role of negatively charged amino acid radicals in the voltage dependence of a channel we converted the negative charges of the carboxyl groups of the protein into positive charges by means of the reaction with positively charged carbodiimide in the presence of ethylenediamine. In the conditions used, this reaction results in the charge conversion due to attachment of either a carbodiimide or an ethylenediamine residue [6,7].

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2. MATERIALS AND METHODS

Porin was obtained from rat liver mitochondria as described in [8]. For amidation of COO^- groups, the porin solution was diluted with a solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide-HCl (Serva) and ethylenediamine-2HCl (Fluka) to a final content of 10 mg protein/ml, 10 mM Na_2SO_4, 10 mM Mops, 1% Triton X-100, 100 mM carbodiimide, 1 M ethylenediamine at pH 4.75 and thermostated at 25°C. Membranes from an *n*-heptane solution of soybean phospholipids (Type II-S, Sigma) purified as in [9] were formed on a 0.2 mm hole in a teflon partition which separated aqueous solutions of 1 M KCl, 20 mM Tris-HCl at pH 7.5 and 22°C. After a mem-

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brane was formed, the aqueous solution on one side was substituted with a porin-containing solution (3–10 ng/ml) of identical ionic content. The solution was prepared immediately before use to avoid protein inactivation. After the required conductance was achieved, the solution was again replaced by a porin-free one. The chamber to which porin was added was maintained at virtual ground.

3. RESULTS

Fig.1 (lower record) shows a typical current response of a porin-containing bilayer to voltage steps. On the application of a low voltage (5 mV) the current instantaneously approaches a steady-state value corresponding to conductance of all the channels in the most conducting state. At higher voltages (15–20 mV) the current decreases with time due to transition of a part of the channels to the states of a lower conductance. With amidated porin (upper record), the decrease occurs at a much lower voltage (1–3 mV).

The characteristic time of the modification of porin carboxyl groups was determined in preliminary experiments like those shown in fig.1 with samples of the incubation medium taken every 30 min for 10 h. The bilayer conductance properties changed noticeably only during the first 4 h of incubation. We have concluded that all COO^- groups accessible for amidation had been modified by this time. The channel-forming activity of porin as determined by the steady-state conductance for a given protein concentration did not change in the course of chemical modification.

In fig.2 the normalized steady-state conductance is shown versus the membrane potential for native and modified porins. The amidation led to a significant enhancement of the voltage dependence of the conductance. The effective gating charge was calculated from this dependence as in [2] and was found to be about 4 with native and about 18 with amidated porin. Note that the protein modification did not influence the symmetry of the $G - V$ plot.

Single-channel records revealed that the charge conversion influenced both the gate and the ionic pathway. Both systems remained symmetric in relation to the voltage polarity (see fig.3). The con-

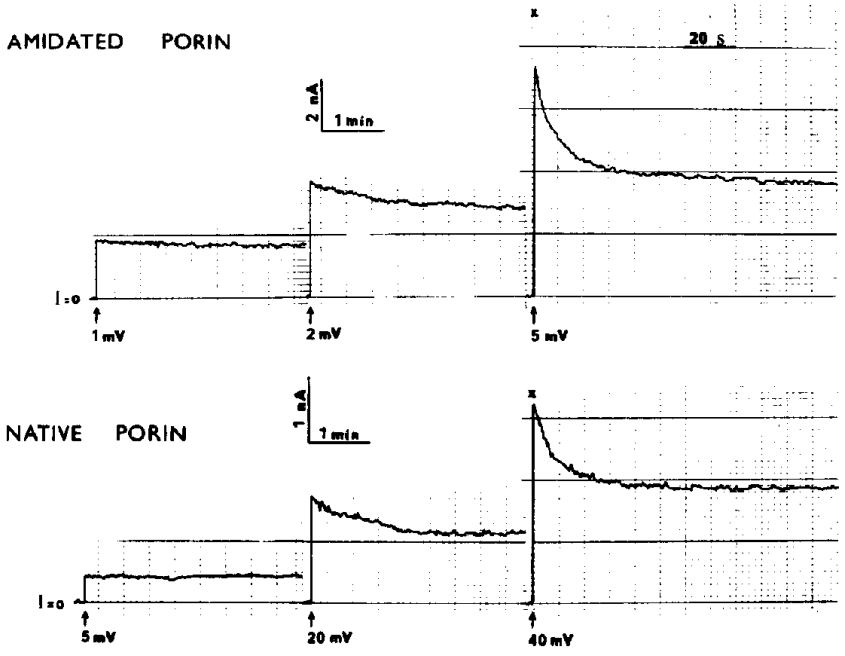

Fig.1. Current response of multichannel membrane with native porin (lower trace) and porin amidated for 6 h to steps of voltage from zero to the values shown below the vertical arrows. Values of the initial current not resolvable on a chart recorder were measured with an oscilloscope (crosses). 20-s time bar relates only to the record obtained for amidated porin at 5 mV. Voltage values were chosen so that similar responses could be obtained from the two membranes. Note that with amidated porin about the same decrease in the conductance occurs at much lower voltages.

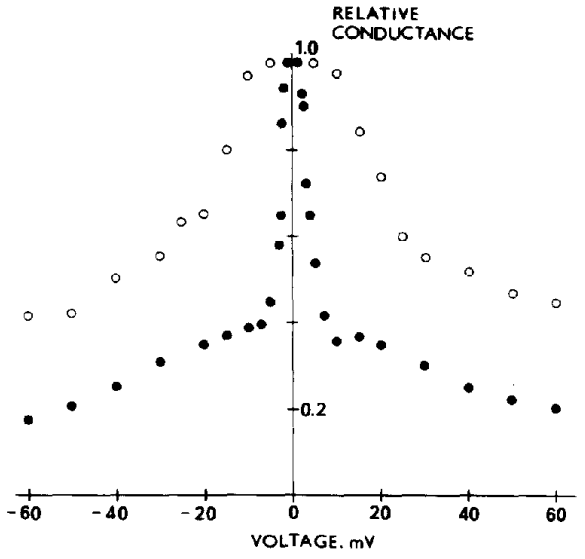

Fig.2. Normalized steady-state conductance vs membrane voltage for multichannel membranes with native (○) and amidated (●) porin. Values of G were obtained from records like those in fig.1 as a ratio of the steady-state current (5 min after the voltage jump) to the initial current. Each point is the average of three experiments. The time of amidation is 4 h.

ductance of each state decreased (see table 1) to a different extent. For example, the open-state conductance decreased by only 15% whereas that of the first substate by 50%. These changes in the conductance are presumably caused by a decrease in the size of the pore cavity due to the covalent modification. The increase of the anionic selectivity of the open state (see table 1) may be explained by the appearance of additional positive charges on the walls of the pore.

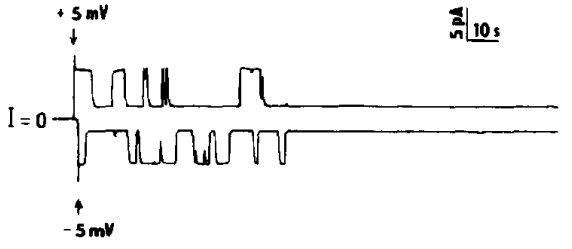

Fig.3. Fluctuation of the current through a single channel formed by amidated porin at voltages of different polarity. At the voltage value indicated the channel fluctuates between the open and the first subconductance states. The records illustrate a symmetry of the channel gating in relation to the voltage polarity.

Table 1

Parameters of native and amidated porin channels in a lipid bilayer

	Conductance level	Conductance (nS) mean ± SE	N	P_{Cl}/P_K	n
Native porin	0	4.0 ± 0.3	103	1.8 ± 0.3	+4 ± 1
	1	1.8 ± 0.2	21		
	2	0.8 ± 0.1	17		
	3	0.3 ± 0.1	6		
	4	0.1 ± 0	4		
Amidated porin	0	3.4 ± 0.2	169	>3.5	+ 18 ± 3
	1	0.9 ± 0.2	18		
	2	0.4 ± 0.1	12		
	3	0.2 ± 0.1	11		
	4	0.1 ± 0	8		

N is the number of single channels observed with a given conductance level. N is used for calculating the mean and the standard error in the second column. P_{Cl}/P_K is the ratio of the permeability coefficients of a channel in the open state. The ratio was calculated from the values of the zero current potential at a low KCl concentration difference according to the Goldman-Hodgkin-Katz equation. n is the value of the effective charge which determines the potential dependence of the transitions between the open and the first subconductance states. n was obtained as in [4] from the slope of the linear dependence

$$\ln(G_{max} - G)/(G - G_1) = (V - V_0)nF/RT$$

where G_{max} is the membrane conductance at zero voltage with all the channels open. G_1 is the conductance with all the channels in state 1. The normalized values used were $G_{max} = 1$, $G_1 = 0.4$ for native and 0.26 for amidated porin in accordance with the data of table 1. V_0 is a potential value at which $G = 0.5\ (G_{max} + G_1)$. Values of G were taken from fig.2. For estimating n the voltage values were used in the range where single channels fluctuated between the two most conducting states ($V \leqslant 6$ mV for amidated and $\leqslant 25$ mV for native porin). F, R and T have their usual meanings

4. DISCUSSION

It has previously been shown that the potential dependence of porin channels may be explained by the movement of about 4 positive charges in the external field [2]. The positive sign of the charge has been inferred from the observation that the potential dependence can be eliminated by neutralizing some amino groups via raising the pH or by converting some of them to negative groups via succinylation. The results presented in this paper show that the modification of side-chain carboxyl groups in porin molecules strongly influences the

potential-dependent equilibrium between the open and the first subconductance state. Thus the carboxyl groups move in the field of the membrane potential during the transition and compose part of the gating charge. All this means that the gating charge of a channel formed by native porin consists of many negative and positive charges with an excess of about 4 or more positive charges at a neutral pH. The number of charges may be evaluated in the following way. Since the conversion of charges resulted in an increase of the effective charge from 4 to 18 the number of negative gating charges should be equal to (or greater than) $(18 - 4)/2 = 7$ and the number of positive charges should not be less than 11 in unmodified porin. This calculation is done on the assumption that each charged group moves through the entire membrane voltage. However, from the symmetry of the gating system of a channel it follows that each charge can pass through not more than half of the field (or, alternatively, not more than half of the total number of gating charges pass through the entire field). Therefore, the total gating charge is composed of not less than 14 negative and 22 positive charges. Another reason for underestimating the charge numbers is that not all of the carboxyl groups which contribute to the gating charge might be accessible for amidation.

Our belief that amidation does not result in a gross structural change of the channel is supported by the following observations. First, both the channel-forming activity and the open-channel conductance are influenced only insignificantly. Second, preliminary experiments on the influence of the pH on the properties of the channels formed by native porin have also revealed a significant enhancement of its voltage dependence at pH 3–4, i.e. in the range of titration of protein carboxyl groups.

Thus, even if the channel is formed by two porin molecules, as sedimentation experiments suggest [10], as many as one third of the positively charged groups (22 of 68) should move through half of the externally applied voltage (or a greater part of the charges move through a smaller fraction of the field). This apparently means that no localized voltage sensor exists in the porin channel structure. The sensor seems to be distributed over the whole channel structure or a significant part of it and this structure undergoes a substantial rearrangement in the course of the potential-dependent transition.

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