treatment may actually represent slowed *h*-gating kinetics of drug-altered channels rather than, as suggested above, the rate of drug leaving the channel. In this case different size drugs may promote different repriming kinetics by producing different degrees of stabilization of the closed conformations of *h*-gates.

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ON THE NATURE OF THE MOLECULAR MECHANISM UNDERLYING THE VOLTAGE DEPENDENCE OF THE CHANNEL-FORMING PROTEIN, VOLTAGE-DEPENDENT ANION-SELECTIVE CHANNEL (VDAC)

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We have probed the molecular mechanism underlying the voltage-dependence of the channel-forming protein, voltage dependent anion-selective channel (VDAC) (1). VDAC forms large channels (40 Å in diameter) (2) open at low electric fields and closed at high fields. VDAC, purified from rat liver mitochondria, was introduced into planar phospholipid bilayers by spontaneous insertion from the aqueous phase as described previously (3). After the behavior of the inserted channels was recorded, 20 μl of succinic anhydride (167 mg/ml DMSO) was added to one or both of the aqueous phases (1.0 M LiCl, 50 mM LiMOPS pH 7.2, 5 mM $CaCl_2$). The anhydride modifies accessible amino groups as follows (4):

$$R{-}NH_3^+ + CH_2CH_2C_2O_3 \longrightarrow R{-}NHCOCH_2CH_2COO^- + 2H^+.$$

The medium pH never fell below 6.7.

We have reported (5) that succinic anhydride inhibits VDAC voltage dependence and changes the channel's ion preference from anions to cations. Here we will show how the conformational state of VDAC influences the reaction with the anhydride. The results provide clues to the protein's structure, its symmetry, and the location of the gating charges.

RESULTS

When succinic anhydride was added to membranes containing VDAC in the open state, the channels lost their voltage dependence as measured by the lack of closure in response to an increased field (Fig. 1). A 40-mV voltage difference induced rapid channel closure prior to modification but not after anhydride treatment. (Note that excess anhydride is rapidly destroyed by the water, $t_{1/2}$ = 2 min.) Fig. 2 shows how the voltage dependence is reduced by the modification.

The effects of anhydride modification on VDAC properties when the channel is modified in the closed state depends both on the side of the membrane to which the

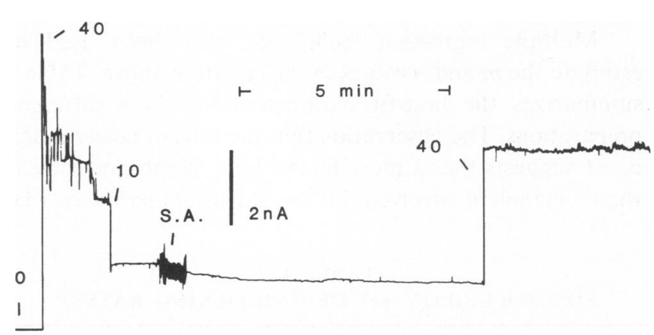

FIGURE 1 Reaction of succinic anhydride with VDAC channels in the open state. VDAC was inserted into planar phospholipid bilayers as described previously (2, 3) and controlled under voltage-clamp conditions. The numbers without units in the figure indicate a change in voltage in millivolts; the sign refers to the *cis* side (the side to which VDAC was added). Succinic anhydride (SA) was added where indicated. The vertical bar indicates 0.2 nA of current.

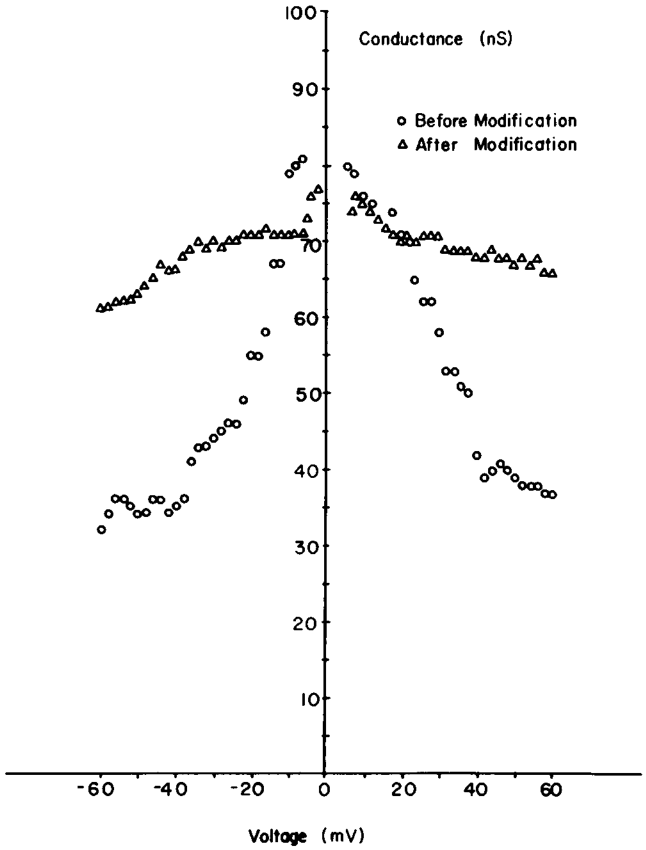

FIGURE 2 GV plot for VDAC before and after modification with succinic anhydride. The voltage was varied by means of a triangular wave at 5 mHz. The data in the figure were obtained when the field was changing from high to low.

anhydride is added and the sign of the applied field used to close VDAC. Fig. 3 shows examples of results obtained using four different protocols. In panels *A* and *B* the potential was positive on the *cis* side (the aqueous phase to which VDAC was added to induce channel insertion). In panels *A* and *C* the anhydride was added to the *cis* side, while in panels *B* and *D* it was added to the *trans* side. However, in panels *C* and *D* the potential was negative on the *cis* side.

Addition of anhydride to the *cis* side produced the mildest modification, whether the field used to close the channels was positive or negative. VDAC remained closed during the modification process and opened when the field was reduced. Subsequent increase in field resulted in some channel closure.

Addition of anhydride to the *trans* side in the presence of a positive field resulted in channel opening during the reaction. These channels did not close when the field was reduced and then again increased. Reaction under negative fields may also have resulted in channel opening during the reaction. Single-channel experiments have not yet been done to check this possibility. The fact that these channels do close to some extent when the field is reduced and then raised indicates that they may have been open at the end of the reaction despite the low conductance.

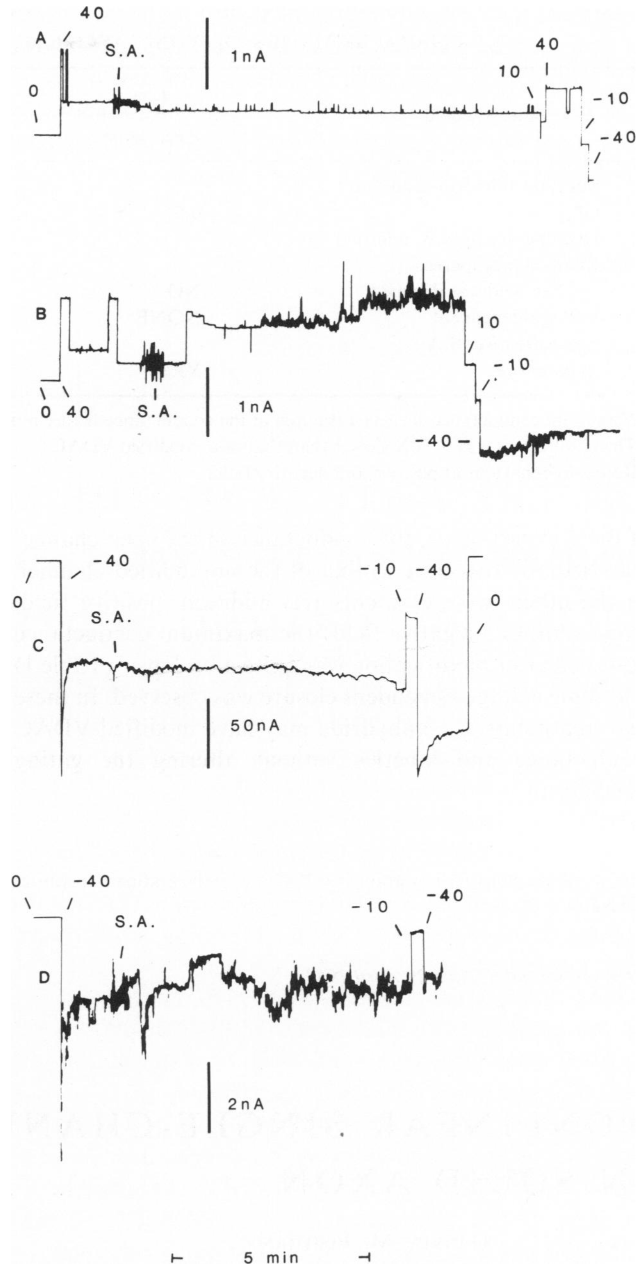

FIGURE 3 Reaction of succinic anhydride with VDAC in the closed state. *A* and *C*, succinic anhydride added to the *cis* side. *B* and *D*, succinic anhydride added to the *trans* side. Except for different current scales, the conditions were the same as in Fig. 1.

VDAC's ability to close with both positive and negative fields indicates the presence of two gating processes. All the treatments described here affected both processes to about the same extent. Modification by adding anhydride to the *trans* side in the presence of a positive field seems to have modified the gating charges associated with both gating processes, while the reciprocal experiment (*cis* addition, negative field) had little effect. The possible slow rise in Fig. 3 *C* may be a hint of a reciprocal effect. In both

TABLE I

CHARACTERISTICS OF VDAC AFTER MODIFICATION UNDER VARIOUS CONDITIONS

Electric field	Low	High positive		High negative	
	cis or *trans*	*cis*	*trans*	*cis*	*trans*
Succinic anhydride addition					
G^{*}_{max}	0.6	0.6	1.0	0.9	0.3
Opening during S.A. addition	—	NO	YES	NO	YES?
Conductance increase when field was dropped	NO	YES	NO	YES	NO
Voltage-dependent closure after S.A.	NONE	SOME SLOW‡	NONE	SOME	SOME
Symmetry§	YES	YES	YES	YES	YES

*Maximum conductance seen as a fraction of the conductance before modification.
‡The closure rate was > 10X slower than that of unmodified VDAC.
§Refers to behaviour at positive and negative fields.

of these experiments, the conductance of the open channel was virtually the same as that of the unmodified channel. In the other two treatments (*cis* addition, positive field; *trans* addition, negative field) the maximum conductance observed after modification was quite a bit lower (Table I) and some voltage-dependent closure was observed. In these two treatments, the anhydride may have modified VDAC conductance and kinetics without altering the gating mechanism.

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NONLINEAR SINGLE-CHANNEL SODIUM-CONDUCTANCE IN SQUID AXON

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Spectral analyses of Na current fluctuations in squid axon (1) and frog node of Ranvier (2, 3), have required the removal of background noise before curves could be fitted. In both studies, the spectral data in tetrodotoxin (TTX) were subtracted from the data before TTX was applied (TTX difference spectrum) at the corresponding membrane potential to obtain a presumedly background-corrected spectrum. We show here that this procedure can produce significant distortion in the corrected spectrum and consequently in model fits and noise-variance estimates with which single channel conductance is calculated. The proper background current-noise spectrum, S_{IB}, can be obtained from the relation

$$S_{IB} = S_V|Y|^2, \quad (1)$$

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