Autodirected Insertion: Preinserted VDAC Channels Greatly Shorten the Delay to the Insertion of New Channels

Xiaofeng Xu and Marco Colombini
Laboratory of Cell Biology, Department of Zoology, University of Maryland, College Park, Maryland 20742 USA

ABSTRACT VDAC, a mitochondrial outer membrane channel, has the ability to catalyze and direct the insertion of other VDAC channels into planar phospholipid membranes. The spontaneous rate of insertion of detergent-solubilized VDAC channels into phospholipid membranes is estimated to be 1.5×10^{-5} channels min^{-1} μm^{-2}. VDAC channels already in the membrane can increase this rate by a factor of 10^9. The presence of 5 M urea on the opposite side of the membrane increases this 10-fold to 4.5×10^5 channels min^{-1} μm^{-2}. Similar but weaker effects are observed with Triton X100 addition (10^{-3} % (v/v)). These agents are not acting on uninserted channels because they do not affect the delay from sample addition to first insertion. Under the chosen conditions, this delay is long (240 s) without preinserted channels. However, the presence of a few VDAC channels in the membrane reduces this delay to 14 s, close to the diffusion limit. Therefore, urea and Triton, added to the side of the membrane opposite that to which the VDAC sample was added, likely increase the flexibility of the VDAC channels in the membrane, allowing them to be more efficient catalysts for VDAC insertion. There are obvious implications for membrane protein insertion and targeting.

INTRODUCTION

The ability of a protein to spontaneously insert into a membrane raises energetic and kinetic questions that are very complex and difficult to address. The enormous energy barrier to such a process must be circumvented by a mechanistic pathway consisting of smaller barriers not too far above *kT*. Numerous toxins have solved this problem (e.g., Gould and Cramer, 1977; Kagan et al., 1981; Merrill et al., 1990) and insert spontaneously without the need for specialized structures. However, biological cells routinely use enzyme catalysts to accelerate reactions impeded by high energy barriers. Thus the proposal that catalysis might be used to accelerate and guide the insertion of proteins into membranes is quite reasonable. Protein complexes, which become more elaborate with each successive experiment, have been identified for the insertion of proteins into membranes in both the rough endoplasmic reticulum and the mitochondrial membranes and less studied systems for other cellular organelles. However, evidence has also been presented for the existence of a protein that can catalyze the insertion of fellow proteins into membranes.

Two reports (Zizi et al., 1995; Xu and Colombini, 1996) provide evidence that the mitochondrial channel, VDAC, can act as an insertion catalyst. More precisely, VDAC is responsible for a process we have termed "Autodirected Insertion." Using mutants with asymmetrical voltage-gating properties, Zizi et al. (1995) showed that, in multichannel membranes, virtually all of the channels inserted in the same direction as monitored by measuring the asymmetry of the voltage dependence. However, the direction of insertion was apparently random from membrane to membrane and was unaffected by the membrane potential. The conclusion was that the first channel inserted in a random direction and virtually all subsequent channels followed suit. Considering the target size of one channel as opposed to that of the entire planar phospholipid membrane, a catalytic acceleration of 10^9 was estimated.

Direct evidence that VDAC channels in the membrane are the sites of insertion was obtained by examining, in detail, the ability of urea and guanidinium chloride (GdmCl) to accelerate the rate of VDAC insertion into planar membranes (Xu and Colombini, 1996). These agents can increase the insertion rate by as much as 60-fold when added to the aqueous phase on the side opposite that to which the detergent-solubilized VDAC channels were added. This effect is rapidly reversible by washing out the urea or GdmCl and inhibited by sarcosine, a substance that makes proteins more rigid. Osmotic and salt effects were eliminated as underlying causal factors (Xu and Colombini, 1996). Therefore urea and GdmCl were proposed to induce discrete structural changes in VDAC that caused VDAC to be a better insertion catalyst.

The novel nature of the proposed autodirected insertion process requires that alternative explanations and other consequences of this process be explored. First of all, there is a possibility that urea or GdmCl might somehow disturb the phospholipid structure of the membrane in such a way that this disturbance is transmitted to the opposite leaflet and somehow facilitates VDAC insertion. Second, if the insertion is catalyzed, how does the first channel insert into an unmodified, virgin membrane? Third, because the detergent, Triton X100, is added along with VDAC, can it be responsible for the catalysis? Finally, if the weakening of H bonds through the use of urea or GdmCl induces VDAC to

Received for publication 30 September 1996 and in final form 31 January 1997.

Address reprint requests to Dr. Marco Colombini, Laboratory of Cell Biology, Department of Zoology, University of Maryland, College Park, MD 20742. Tel.: 301-405-6925; Fax: 301-314-9358; E-mail: colombini@zool.umd.edu.

0006-3495/97/05/2129/08 $2.00

be a better insertion catalyst, would the weakening of the hydrophobic interactions within VDAC, through the addition of a detergent, result in a similar change? As we explore these issues, the results presented strengthen the case for autodirected insertion.

MATERIALS AND METHODS

All experiments were conducted using the lipid bilayer method of Montal and Mueller (1972) as revised by Schein et al. (1976) and Colombini (1987), as described in a previous paper (Xu and Colombini, 1996). Briefly, a Teflon chamber was divided into two compartments by a Saran partition with a 0.15-mm hole. A phospholipid bilayer was formed across this hole using 1 part diphytanoyl phosphatidylcholine (DPyPC) and 0.2 part cholesterol. The membrane was bathed in the standard solution (1 M KCl, 1 mM $CaCl_2$, 1 mM 2-(*N*-morpholino)ethanesulfonic acid, pH 5.8) or other solutions as indicated. Then 0.5–3 μl *Neurospora crassa* VDAC was added to one side of the chamber. The VDAC inserted spontaneously and was studied under the voltage-clamp conditions and recorded on a chart recorder (see Colombini, 1987, for details).

The two compartments of the chamber were named *cis* and *trans*, respectively. The voltage on *trans* side was maintained at virtual ground by the amplifier, and the desired voltage was applied to the *cis* side. Calomel electrodes were used to interface with the solutions. For all experiments reported in this paper, the voltage applied was −10 mV. The currents were converted to conductances for the convenience of the reader.

Perfusion was performed only in the *cis* compartment. The solution was gradually displaced with a denser solution by delivering the new solution by gravity feed via a Teflon tube to the bottom of the chamber. Simultaneously, the level of the aqueous solution was maintained fairly constant by aspiration of the surface through a P-10 pipette tip.

All chemicals were purchased from Sigma Chemical Co. unless otherwise indicated.

RESULTS AND DISCUSSION

The addition, with stirring, of 0.5–3 μl of VDAC (from *N. crassa*) dissolved in 2.5% Triton X100 to 5 ml of 1 M KCl solution (buffered to pH 5.8) bathing a planar phospholipid membrane results, after a delay, in the insertion of VDAC channels into the membrane at some rate. In the experiments performed under the present conditions this averaged 5.2 channels/min, with a standard deviation of 3.9 (see Fig. 2 for the distribution of the insertion rate). Fig. 1 shows this insertion process. Note that discrete single channels inserted into the membrane, and this continued after the scale changed (at the "*20"). At the point indicated by "5 M urea," the solution on the side of the membrane that was opposite that to which VDAC had been added was perfused with the same KCl solution supplemented with 5 M urea (the molarities of the ingredients were kept constant). The urea on the *cis* side greatly increased the rate of VDAC insertion from the *trans* side (typically 10- to 60-fold). Control experiments (Xu and Colombini, 1996) showed that this effect was not due to the osmotic gradient.

Urea accelerates VDAC insertion by acting on inserted VDAC channels

The effect of urea on the insertion rate coincided with the time needed to reach the channels already in the membrane.

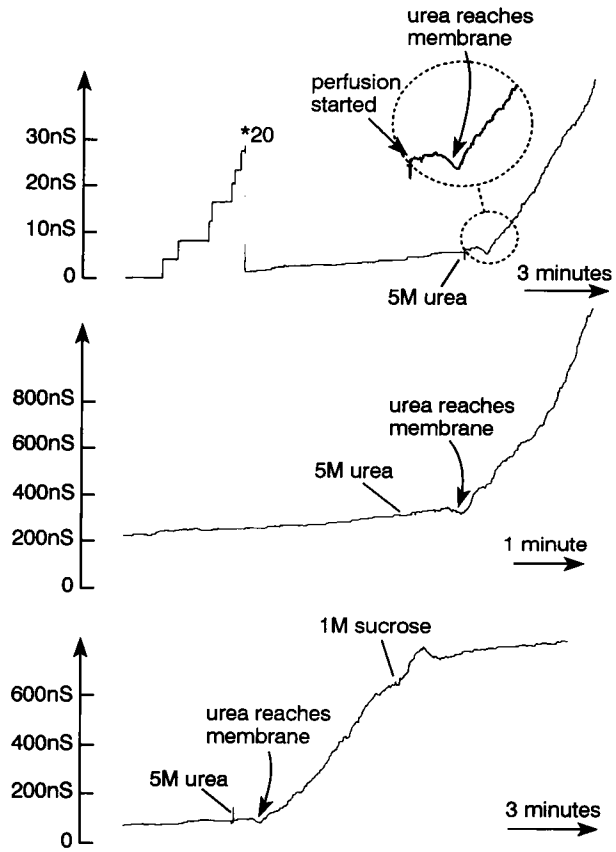

FIGURE 1 Records of experiments in which VDAC was added to the *trans* side of the membrane and urea was perfused into the *cis* side. The membranes were bathed in the standard solution (see Materials and Methods). A small aliquot (1.6 μl) of VDAC solution was added on *trans* side of the membrane. Once channel insertion was proceeding at a steady rate, the *cis* compartment was perfused with about 40 ml of the standard solution supplemented with 5 M urea. The top panel shows the stepwise insertion of VDAC channels; at "*20" the scale was decreased by a factor of 20. In the bottom panel, a second perfusion was carried out, with the standard solution supplemented with 1 M sucrose. The acceleration of insertion caused by urea was 19-, 30-, and 17-fold for the upper, middle, and lower panels, respectively.

The urea decreased the current flow through the channels in the membrane. Thus a drop in membrane conductance signaled the arrival of the urea at the mouth of the channels. This drop was variable (see three experiments in Fig. 1), depending on the experiment, because at times it was partially obscured by channel insertion. Thus there is a temporal correlation between urea reaching the channels in the membrane and the accelerated rate of insertion.

The possibility that sufficient urea crossed the membrane to affect the channels colliding with the membrane from the aqueous phase or adsorbed to the membrane surface is remote because the urea concentration at the membrane surface of the VDAC-containing side is far below the effective level. The permeability of phospholipid membranes for urea was reported to be 4×10^{-6} cm/s (Orbach and Finkelstein, 1980; Vreeman, 1966; Gallucci et al., 1971). For the membranes used in these experiments (150 μm

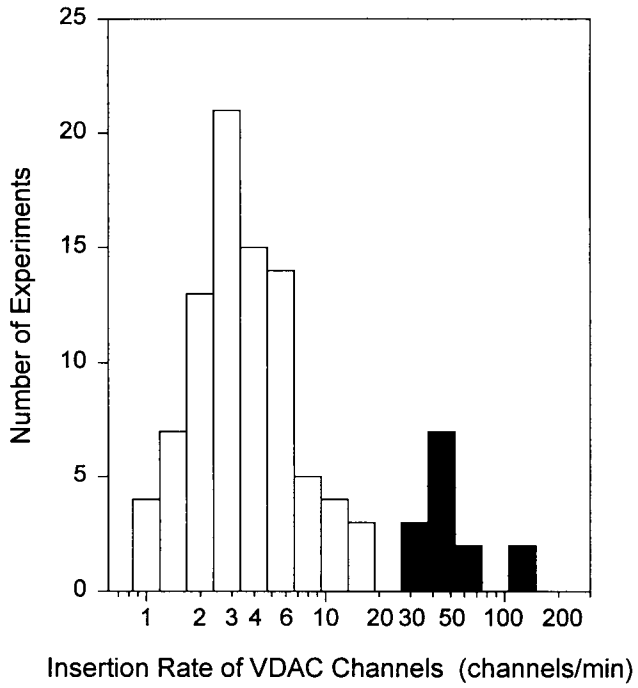

FIGURE 2 The distribution of the insertion rates of VDAC channels in the absence and presence of urea. 0.5–3 μl of the VDAC-containing sample was used in each experiment. The number of experiments without urea (□) and in the presence of 5 M urea (■) are shown as a function of the observed rate of VDAC insertion. In these experiments urea was present on the side of the membrane opposite that to which VDAC was added.

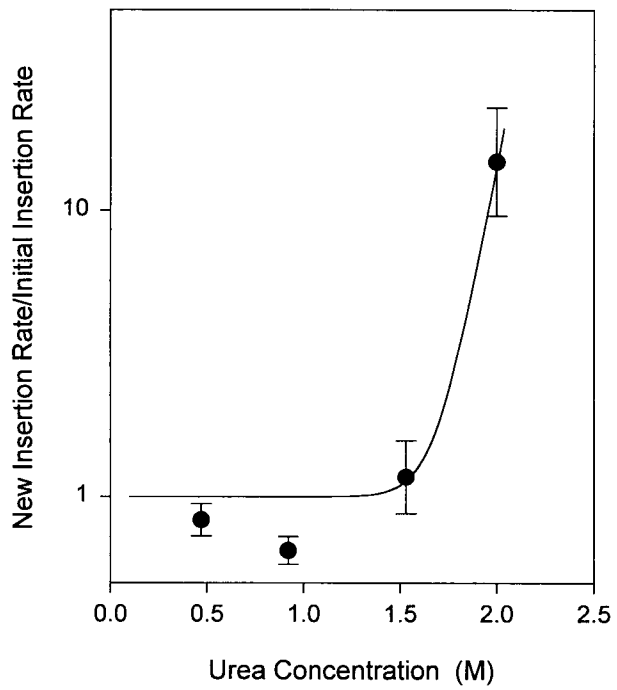

FIGURE 3 Dependence of VDAC insertion rate on the urea concentration. The ratio of the insertion rate after urea addition to that before urea addition is plotted as a function of the final urea concentration. The membranes were bathed in the standard solutions. VDAC was added to the *cis* compartment, and the channels were inserted at a normal rate. Urea was then added directly to the *cis* side, the VDAC-containing side, to the indicated final concentration. The results of 28 experiments are shown as arithmetic means with standard error bars. To work with data the distribution of which approximates a Gaussian, the data were log transformed and the standard error determined on the transformed data. The antilog of the standard error of the transformed data is the number that the mean of the original data should be multiplied or divided by to determine the limits of the standard error. This is what is shown in the figure by the error bars. The solid line is a theoretical line for the equation Ratio = 1 + 0.0001*[urea][17]. (Reprinted with permission from Xu and Colombini, 1996.)

diameter), the flux from the 5 M urea side should be 3.5×10^{-12} mol s^{-1}. The same flux must be sustained in the access resistance region. Because the membrane is but a small disc on a large thin partition, one can use the calculations of Jeans (1925) and Hall (1975) to determine the effective permeability of the access region: $p = 4aD$.

The radius of the membrane $a = 75$ μm, and the diffusion constant of urea $D = 1.38 \times 10^{-5}$ cm^2 s^{-1} (Gosting and Akeley, 1952). Thus $p = 4.1 \times 10^{-7}$ cm^3 s^{-1}, and the urea concentration at the surface must be 8.5 mM, 100 times smaller than the minimum concentration of urea needed to begin to accelerate the VDAC insertion when added to the same side (Fig. 3). Thus urea coming through the membrane is insignificant.

Urea coming through the pore is more significant but highly localized to the mouth of the channel. To calculate the urea concentration as a function of distance from the mouth of the channel, the pore is assumed to be a simple cylinder (as indicated by electron microscopy of negatively stained and frozen-hydrated two-dimensional crystals; Mannella et al., 1984, 1992), and thus its permeability is $P_c = D\pi r^2/L$ (length $L = 5$ nm; radius $r = 1.5$ nm; Mannella et al., 1992). The access resistance was divided into two regions (Hall, 1975): the bulk phase to the surface of a hemisphere at the mouth of the pore ($P_s = 2\pi Dr$) and the hemisphere ($P_h = 4\pi Dr/(\pi - 2)$). The permeability of a hemispherical shell (thickness $= x - r$) extending from the surface of the above hemisphere toward the bulk by distance, $x - r$, is $2\pi D(x - r)r/x$. With these expressions the concentration of urea at the surface of the hemisphere at the mouth of the pore is 0.51 M, and at a distance 5 nm further it drops to 0.12 M. This is an order of magnitude less than that concentration needed to accelerate VDAC insertion when added to the same side (Fig. 3). Therefore, rather than acting on the added channels on the *trans* side, urea probably acts on the channels already in the membrane, making them more effective catalysts.

If urea were to alter the nature of the phospholipid membrane to make channel insertion easier, it should facilitate channel insertion into a membrane lacking any channels (virgin membrane). This was examined by looking at the delay before channel insertion into a virgin membrane. In the absence of urea, there is a lag between sample addition and channel insertion (Fig. 4). That is far longer than expected from diffusion through the unstirred layer. For the

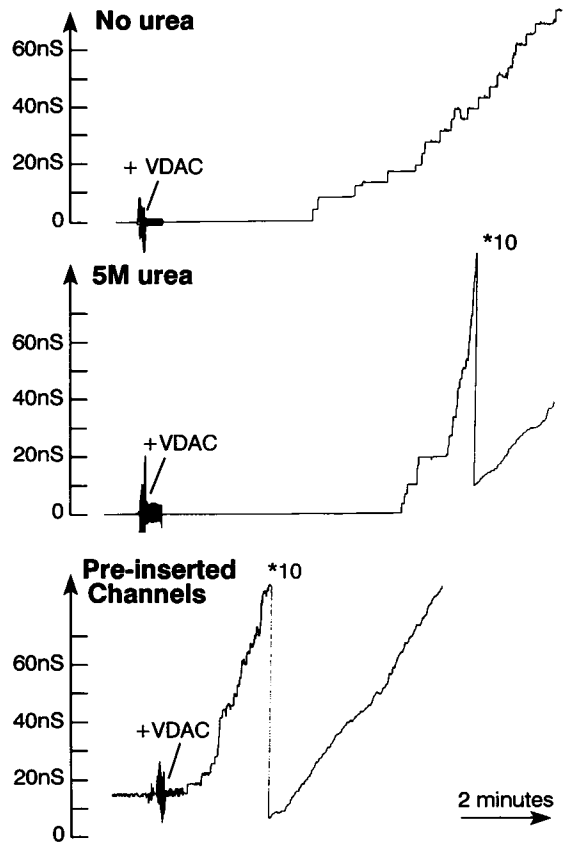

FIGURE 4 Preinserted channels dramatically shorten the delay to the insertion of new channels. In the top panel, the membrane was bathed in standard solutions on both sides. In the lower two panels, the membranes were formed with the standard solution on the *trans* side and the same solution supplemented with 5 M urea on the *cis* side. There were four preinserted channels in the bottom panel. Exactly the same amount of VDAC protein (1.2 μl) was added to the *trans* compartment in each experiment and stirred for 25 s. In the experiment shown in the top panel, VDAC channels were inserted at a rate of 2.9 channels/min after a delay of 200 s. In the middle panel, VDAC was inserted at 42 channels/min with a delay of 321 s. In the bottom panel, VDAC was inserted at 60 channels/min after a delay of 6 s. At "*10" the scale was decreased by a factor of 10.

chamber used in these experiments, the unstirred layer thickness is 50 μm (Negrete et al., 1996). Putting this into Einstein's diffusion equation ($t = \langle x^2 \rangle / 2D$) for the displacement ($x$), and calculating the diffusion constant for VDAC ($D = kT/(6\pi\eta r)$) from the estimated radius of 2.5–5.4 nm (depending on the amount of associated detergent; Linden and Gellerfors, 1983), it should only take about 15–32 s for the VDAC channels to traverse the unstirred layer. However, the measured lag time under our conditions was considerably longer (Fig. 4).

The conditions were strictly controlled so that 1.2 μl of the same VDAC sample was added and the solution stirred for 25 s. On average, the delay before insertion was 240 ± 40 s. After the first insertion the rate of insertion proceeded at a moderate 4.5 channels/min, on average (Table 1). Thus the channels do not insert as soon as they reach the membrane. The presence of urea on the opposite side of the membrane did not alter the delay significantly (Figs. 4 and 5). The average delay was 250 ± 30 s, but as expected, the rate of subsequent insertions was much higher, 35 channels/min on average. Thus urea did not change the nature of the phospholipid membrane in such a way as to facilitate the channel insertion, but once the first channel inserted, urea did increase the insertion rate, presumably by changing the structure of the inserted channel.

VDAC accelerates the rate of insertion

If VDAC channels in the membrane catalyze the insertion of more channels, they should reduce the lag time. A procedure was developed to insert just a few channels without significantly altering the conditions used for the experiments. Membranes were made with the *cis* compartment containing 5 M urea, and then an aliquot of VDAC-containing sample was stirred into this compartment. Insertion of VDAC under these conditions was very rare. When it occurred it did not lead to further insertion. Previous results (Xu and Colombini, 1996) showed that perfusing 5 M urea into the same compartment where VDAC was inserting into the membrane did not result in accelerated insertion. Presumably the urea is too high to achieve the correct protein-protein interaction. In any event, the addition of VDAC-containing sample resulted, on some occasions, in the insertion of one or a few channels into the membrane. There was generally no further insertion. Thus after waiting 10–15 min to ensure no further insertion, the same aliquot of VDAC-containing sample (1.2 μl) was added to the oppo-

TABLE 1 Preinserted channels shorten the delay to insertion of new channels

Initial number of channels in the membrane	Composition of bathing solutions	Amount of VDAC added	Insertion delay* (s)	Insertion rate* (channels/min)
0	*cis*: 1 M KCl *trans*: 1 M KCl	1.2 μl *trans*	240 ± 40 (4)	4.5 ± 0.6 (4)
0	*cis*: 1 M KCl +5 M urea *trans*: 1 M KCl	1.2 μl *trans*	250 ± 30 (3)	35 ± 3 (3)
1 to 4	*cis*: 1 M KCl +5 M urea *trans*: 1 M KCl	1.2 μl *trans*:	14 ± 6 (3)	100 ± 20 (3)

*Mean ± SE (number of observations).

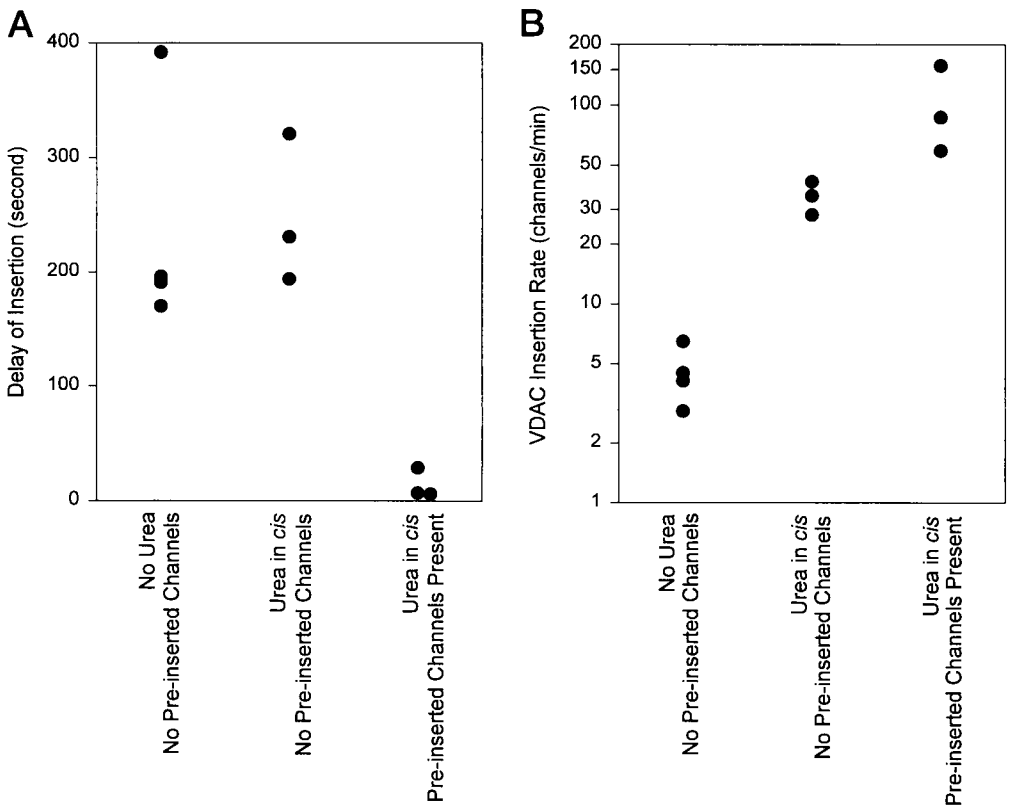

FIGURE 5 The effects of preinserted VDAC channels and the presence of urea on the distribution of the delay from sample addition to insertion of the first VDAC channel and the distribution of the subsequent rate of channel insertion. The results of experiments such as those presented in Fig. 4 are summarized here. When present, urea was located on the side of the membrane opposite that on which channels were inserted.

site (*trans*) side. Again, the stirring was fixed at 25 s. Under these conditions, the lag phase was reduced to an average of 14 ± 6 s (Figs. 4 and 5, Table 1). The rate of subsequent insertions was fast because of the presence of the urea (average of 100 channels/min). This lag time is very close to that expected for diffusion through the unstirred layer, indicating that the rate of catalyzed insertion in the presence of urea may be diffusion limited.

From the increased lag time observed for channel insertion into virgin membranes, one can calculate the rate of spontaneous insertion into such a membrane, i.e., the rate of uncatalyzed insertion. The average increase in lag time was 226 s, indicating an insertion rate of 0.27 channels/min. The target area is the membrane area, 1.8×10^4 μm^2. Thus the uncatalyzed insertion rate is 1.5×10^{-5} channels min^{-1} μm^{-2}.

To calculate the rates of catalyzed insertion, it is necessary to assume a target area. The diameter of VDAC at the membrane surface is 5 nm (Mannella et al., 1992), and thus a target area of 80 nm^2 (a disk 10 nm in diameter) is a high-end estimate. Using this value, the catalyzed insertion rate in the absence of urea is 5.7×10^4 channels min^{-1} μm^{-2}, an acceleration of over 10^9. In the presence of urea on the opposite side, the catalyzed insertion rate is 4.5×10^5 channels min^{-1} μm^{-2}, an acceleration of 10^{10}.

Triton can also facilitate the autodirected insertion of VDAC channels

The addition of 1.2–1.5 μl of VDAC-containing sample to both sides of a membrane resulted in a fast insertion rate comparable to that observed with urea on the opposite side. When the sample was added to one side (*cis*), VDAC inserted at the slow, normal rate (Fig. 6 *A*). When the same dose was added to the opposite side (*trans*), the insertion rate increased dramatically, much more than the expected doubling, even when taking into consideration the variation in the insertion rate from experiment to experiment. The possibility that this was due to some two-channel complex, reminiscent of nystatin, was tested by allowing channels to insert from one side and then washing out the uninserted channels before adding the VDAC-containing sample to the other side (Fig. 6 *A*). The newly added channels inserted at the normal rate, apparently unaware of the presence of the channels that inserted from the other side (Fig. 7).

The acceleration seen from the original two-sided addition may be due to the presence of a very low concentration of Triton (8×10^{-4} % v/v for 1.5 μl VDAC sample used). The VDAC sample contained Triton as a solubilizing agent. This would be washed out by the perfusion process in the second experiment (Fig. 6 *A*). The experiment was repeated

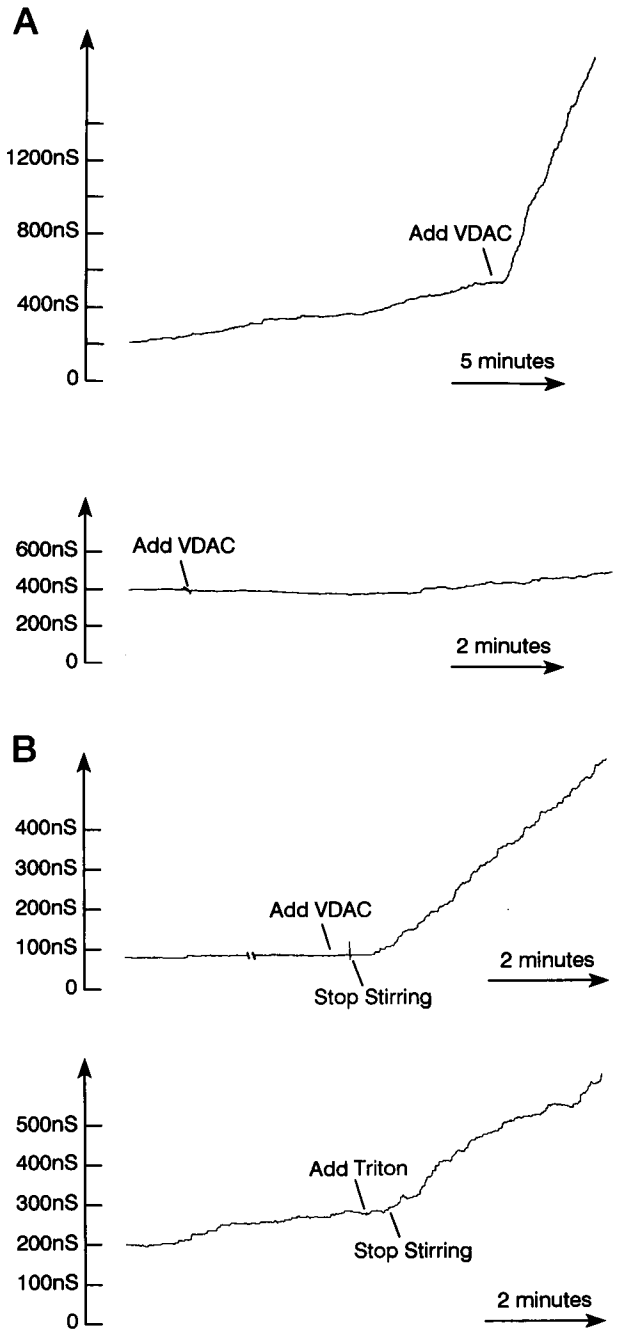

FIGURE 6 Triton's effect on the insertion of VDAC channels. (*A, upper panel*) A 1.2 μl VDAC-containing sample was added to the *cis* side (not indicated), and VDAC was inserted at 5.5 channels/min. A second dose (1.2 μl) was added to the *trans* side where indicated. The insertion rate increased to 93 channels/min. (*A, lower panel*) The experiment was performed as above, except that the *cis* side was perfused with the standard solution supplemented with 0.5 M sucrose before the addition of the second dose of VDAC to the *trans* compartment. The insertion rate was 6.4 channels/min. (*B, upper panel*) Experiments were done with the same procedure as that in *A* (*lower panel*), except that the perfusing solution also contained 1.5×10^{-3} % v/v Triton X100. The insertion rate after the second dose of VDAC was 38 channels/min as compared to 7.9 channels/min after the first addition and before perfusion (not shown). The break was 1 min. (*B, lower panel*) VDAC inserted at 5.0 channels/min after sample addition to the *cis* side. A small amount of 2.5% Triton was added to the *trans* side (final concentration 5×10^{-4} % v/v). This caused an increase in the insertion rate to 25 channels/min.

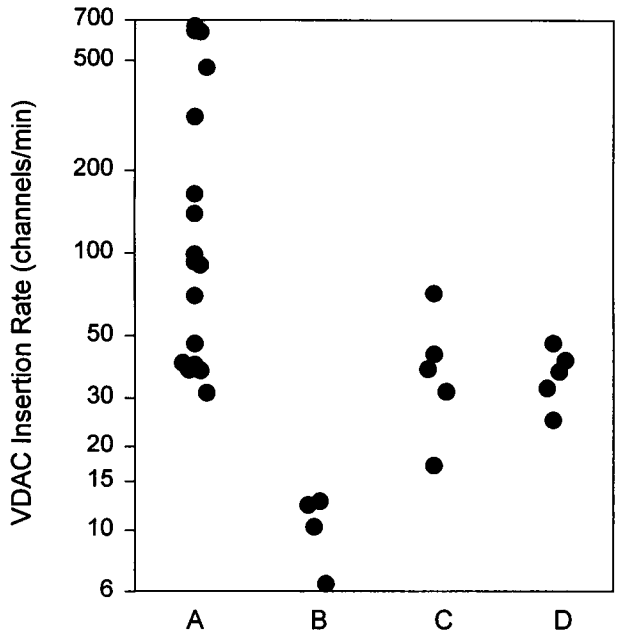

FIGURE 7 The distribution of the insertion rates of VDAC channels under different experimental conditions, in the absence or presence of Triton. The results of experiments such as those illustrated in Fig. 6 are summarized here. All of the values are final insertion rates. (*A*) An aliquot of VDAC-containing solution was first added to the *cis* side, and channels were allowed to insert for a while. Then a second aliquot was added to the *trans* side. (*B*) The VDAC-containing aliquot was first added to the *cis* compartment and channels were allowed to insert as in *A*. Then the *cis* side was perfused with 40 ml or more of the standard solution enriched with 0.5 M sucrose. After the perfusion, a VDAC-containing aliquot was added to the *trans* side. (*C*) Same procedure as in *B*, except that the *cis* side was perfused with the standard solutions supplemented with both 0.5 M sucrose and 1.5×10^{-3} % v/v Triton X100. (*D*) The VDAC-containing aliquot was first added to the *cis* compartment and channels were allowed to insert as in *A*. Then sufficient 2.5% Triton was added to the *trans* side to produce a final concentration of 5×10^{-4} % v/v. The insertion rate indicated is that observed after this addition of Triton.

(Fig. 6 *B*), but the perfusion solution was supplemented with 1.5×10^{-3} % (v/v) Triton. This time, the addition of VDAC-containing sample to the *trans* side resulted in fast channel insertion (Fig. 7).

The direct addition of Triton to the opposite side (*trans*) of the membrane as VDAC was inserting (*cis*) increased the VDAC insertion rate (Figs. 6 *B* and 7). Thus, like urea, Triton seems to affect the VDAC channels in the membrane and augment their ability to catalyze channel insertion. This action is weaker than that observed with urea or GdmCl. On the average, a 5.3-fold increase was observed. Note that before Triton addition to the opposite side, Triton was already present on the side of VDAC addition at a similar level.

Triton is a perturbant of phospholipid membranes. The final concentration in solution used to accelerate the insertion rate is more than 10 times less than the CMC (critical micellization concentration), and at this level, the permeability of the membrane is unaffected. However, to eliminate the possibility that the low concentration of Triton

catalyzed the insertion by acting directly on the membrane structure, Triton's effect on the delay of VDAC insertion was examined. Without Triton on either side, VDAC inserted with a normal delay and slow insertion rate (Fig. 8). If there was Triton (1.5×10^{-3} % v/v) *cis* and VDAC was added *trans*, the delay did not change, but the insertion was faster (Fig. 8). This indicates that Triton does not accelerate VDAC insertion by changing membrane structure; instead, it changes the structure of preinserted channels, making them more effective insertion catalysts.

The effect of Triton could be similar to that of urea. Both may cause limited conformational changes in VDAC structure and therefore facilitate the autodirected insertion process. Thus partial unfolding of VDAC on the opposite side, by weakening H bonds or by weakening hydrophobic interactions, increases the catalytic ability of VDAC channels in the membrane. In the presence of these agents, the channels remain functional. The channels conduct and voltage-gate normally. The fact that both urea and Triton only increased the insertion rate of VDAC and did not affect the delay before insertion indicates that they influence the insertion process by affecting VDAC, not the membrane structure. They increase the autodirected insertion process and provide further evidence that VDAC channels in membranes can direct and accelerate the insertion of other VDAC channels into the same membrane.

These results have important implications for protein targeting in cells. The various membrane structures in cells maintain their unique composition by precise targeting of new constituents, such as proteins, to the appropriate location. Membrane structures grow by expansion of existing membranes. Thus the ability of a membrane protein to catalyze the insertion of its newly formed kin would greatly enhance the fidelity of the targeting process. Indeed, an acceleration of 10^9 would mean that even just one VDAC channel in the outer membrane of a mitochondrion would increase the likelihood of a newly formed VDAC inserting into that membrane as opposed to any other membrane of equivalent area by a factor of 10^5. The presence of many VDAC channels would increase this number further. Thus autodirected insertion would greatly enhance the fidelity of the known targeting machinery.

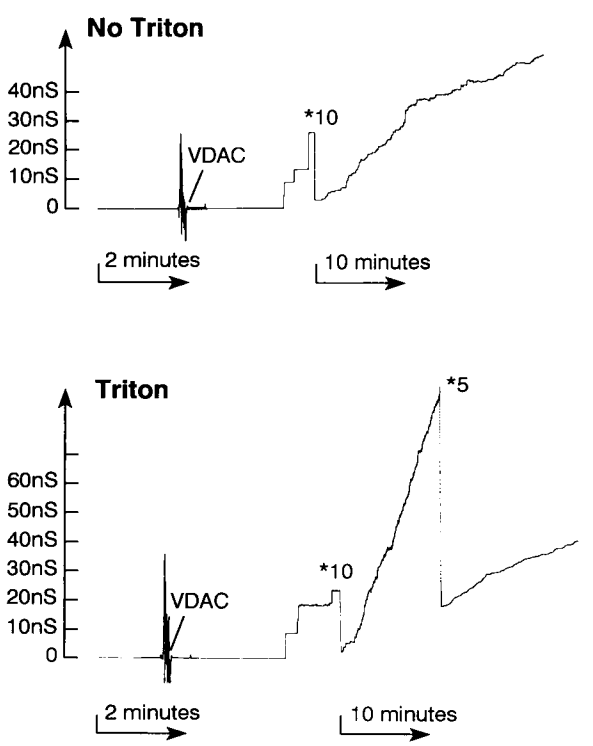

FIGURE 8 Triton does not alter the delay to channel insertion. In both experiments, a 1.5-μl VDAC-containing sample was added to the *trans* compartment and stirred for 25 s. The top panel is the control. In the experiment in the lower panel the standard solution on the *cis* side was enriched with 1.5×10^{-3} % v/v Triton (final) before VDAC addition. At "*10" the conductance scale decreased by a factor of 10, and the time scale changed as indicated.

This work was supported by National Institutes of Health grant GM35759.

REFERENCES

Colombini, M. 1987. Characterization of channels isolated from plant mitochondria. *Methods Enzymol.* 148:465–475.

Gallucci, E., S. Micelli, and C. Lippe. 1971. Non-electrolyte permeability across thin lipid membranes. *Arch. Int. Physiol. Biochim.* 79:881–887.

Gosting, L. J., and D. F. Akeley. 1952. A study of the diffusion of urea in water at 25° with the Gouy interference method. *J. Am. Chem. Soc.* 74:2058–2060.

Gould, J. M., and W. A. Cramer. 1977. Studies on the depolarization of *Escherichia coli* cell membrane by colicin E1. *J. Biol. Chem.* 252: 5491–5497.

Hall, J. E. 1975. Access resistance of a small circular pole. *J. Gen. Physiol.* 66:531–532.

Jeans, J. 1925. The Mathematical Theory of Electricity and Magnetism, 5th Ed. Cambridge University Press, Cambridge.

Kagan, B. L., A. Finkelstein, and M. Colombini. 1981. Diphtheria toxin fragment forms large pores in phospholipid bilayer membranes. *Proc. Natl. Acad. Sci. USA.* 78:4950–4954.

Linden, M., and P. Gellerfors. 1983. Hydrodynamic properties of porin isolated from outer membranes of rat liver mitochondria. *Biochim. Biophys. Acta.* 736:125–129.

Mannella, C. A., M. Forte, and M. Colombini. 1992. Toward the molecular structure of the mitochondrial channel, VDAC. *J. Bioenerg. Biomembr.* 24:7–19.

Mannella, C. A., M. Radermacher, and J. Frank. 1984. Three-dimensional structure of mitochondrial outer-membrane channels from fungus and liver. *In* Proceedings of the 42nd Annual Meeting of the Electron Microscopy Society of America. G. W. Bailey, editor. San Francisco Press, San Francisco. 644–645.

Merrill, A. R., F. S. Cohen, and W. A. Cramer. 1990. On the nature of the structural change of the colicin E1 channel peptide necessary for its translocation-competent state. *Biochemistry.* 29:5828–5836.

Montal, M., and P. Mueller. 1972. Formation of bimolecular membranes from lipid monolayers and a study of their electrical properties. *Proc. Natl. Acad. Sci. USA.* 69:3561–3566.

Negrete, H., R. Rivers, A. H. Gough, M. Colombini, and M. L. Zeidel. 1996. Individual leaflets of a membrane bilayer can independently regulate permeability. *J. Biol. Chem.* 271:11627–11630.

Orbach, E., and A. Finkelstein. 1980. The nonelectrolyte permeability of planar lipid bilayer membranes. *J. Gen. Physiol.* 75:427–436.

Schein, S. J., M. Colombini, and A. V. Finkelstein. 1976. Reconstitution in planar lipid bilayers of a voltage-dependent anion-selective channel obtained from Paramecium mitochondria. *J. Membr. Biol.* 30:99–120.

Vreeman, H. J. 1966. Permeability of thin phospholipid films. III. Experimental method and results. *K. Ned. Akad. Wet. Proc. Ser. B.* 216: 564–577.

Xu, X., and M. Colombini. 1996. Self-catalyzed insertion of proteins into phospholipid membranes. *J. Biol. Chem.* 271:23675–23682.

Zizi, M., L. Thomas, E. Blachly-Dyson, M. Forte, and M. Colombini. 1995. Oriented channel insertion reveals the motion of a transmembrane beta strand during voltage gating of VDAC. *J. Membr. Biol.* 144: 121–129.