Influence of Acylation on the Channel Characteristics of Gramicidin A†

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ABSTRACT: The influence of acylation on the conductance, average duration, and channel-forming potency of channels formed by gramicidin A analogues was investigated using single-channel and multichannel techniques. Lauroyl-, myristoyl-, palmitoyl-, stearoyl-, and oleoylgramicidin A were prepared by covalent coupling of that fatty acid to the C-terminal ethanolamine group. Acylation of gramicidin A does not affect the single-channel conductance or the minichannel frequency in diphytanoylphosphatidylcholine/*n*-decane black lipid membranes. However, the average duration of all acylgramicidin channels was increased ~5-fold as compared to unmodified gramicidin A, which has a duration of 0.9 s at 200-mV applied potential. Somewhat surprisingly the rate of channel formation of the acylgramicidins is decreased relative to gramicidin A: lauroyl- and stearoylgramicidin are ~200 times less effective in channel formation as compared to gramicidin A. We conclude that channels formed by the acylgramicidins and by gramicidin A are structurally and conformationally equivalent.

Many proteins are co- or posttranslationally modified by fatty acylation [for a review, see Schmidt (1989)]. Little is known about the structural or functional significance of this covalent modification. Fatty acylation is not confined to large proteins; polypeptides can also be acylated. The linear pentadecapeptide antibiotic gramicidin, for example, occurs in an acylated form (Koeppe et al., 1985). Gramicidin K, an acylated gramicidin purified from the commercially available mixture of gramicidins, has a fatty acid covalently coupled through an ester bond to the C-terminal ethanolamine group (Koeppe et al., 1985; Williams et al., 1992). A number of fatty acids including palmitic and stearic acid are covalently coupled to gramicidin K (Koeppe et al., 1988).

The linear gramicidins are well-known for their channel-forming ability (Andersen, 1984) and for their ability to influence the lipid organization in phospholipid bilayers (De Kruijff et al., 1988). Gramidicin is extensively used as a prototypical membrane-spanning structure. The aspects of function, conductance, duration, and channel-forming potency of the gramicidin channel, have been elucidated using, among others, techniques like single-channel and multichannel measurements performed on planar lipid bilayers (Andersen, 1984; Andersen et al., 1988; Hladky & Haydon, 1984).

Although disputed over the years, there is now convincing evidence that gramicidin channels are head-to-head (formyl-NH to formyl-NH) single-stranded $\beta^{6.3}$ helical dimers, as originally proposed by Urry (Urry, 1971; Urry et al., 1971); for a recent review, see Andersen et al. (1992). In this structure, the channel conformation, the C-terminal ethanolamine group is near the membrane interface. The covalently coupled fatty acid of acylgramicidins is expected to be inserted into the bilayer and aligned along the channel.

Previously, we prepared a series of acylated gramicidins, with fatty acids that differed in length and unsaturation, and examined their molecular characteristics (Vogt et al., 1991). It was shown that the interfacial properties of acylgramicidins at the air/water interface are equivalent to those of gramicidin A. It was further demonstrated by circular dichroism (CD) spectroscopy that, as for gramicidin A, the single-stranded $\beta^{6.3}$ helix was the preferred conformation of the acylgramicidins in a lipid bilayer.

In this study we examined the influence of acylation on the conductance, average duration and channel-forming potency using single-channel and multichannel measurements in black lipid membranes. The single-channel measurements show that the conductance of gramicidin A channels is unaffected by acylation but that the average duration of the acylgramicidin channel is increased ~5-fold. These results are comparable to those found for gramicidin K (Peart-Williams et al., 1988; Williams et al., 1992). However, in contrast to the naturally occurring gramicidin K, there was not an increased conductance heterogeneity for channels formed by these synthetic acylgramicidins. Surprisingly, the multichannel measurements show that the rate of channel formation for the acylgramicidins is decreased relative to gramicidin A.

EXPERIMENTAL SECTION

Materials. Gramicidin A was purified from the naturally occurring mixture of gramicidins A, B, and C (from Sigma Chemical Co, St. Louis, MO) as described by Vogt et al. (1991). The fatty acids lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), and oleic acid (18:1c) were from Merck (Darmstadt, Germany). They were covalently coupled to gramicidin A as described previously (Vogt et al., 1991). Analytical HPLC confirmed that the acylgramicidins were at least 99.9% pure. NaCl (Suprapure grade) was from Merck (through MCB, Cincinnati, OH); water was deionized Millipore Corp. Milli-Q water (Bedford, MA). 1,2-Diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC) was from Avanti Polar Lipids (Birmingham, AL), and *n*-decane was from Wiley Organics (Columbus, OH). Gramicidin K was a generous gift from Prof. Dr. R. E. Koeppe, II, and was used without further purification.

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Single-Channel Conductivity Measurements. The experimental setup for planar lipid bilayer single-channel experiments using the bilayer punch technique is described in detail by Andersen (1983). For this study we used a Dagan Model 3900 (+ 3910 expander + 3902 headstage) integrating patch clamp to apply the membrane potential across and measure the current through the membrane. Planar bilayers were formed from DPhPC dissolved in *n*-decane (~25 mg/mL). The electrolyte was unbuffered 1.0 M NaCl solution. The temperature of the solution was maintained at 25 ± 1 °C in all experiments. (Acyl)gramicidin was added in equal amounts to both sides of the black lipid membrane from an ethanolic solution. The final (acyl)gramicidin concentration was ~10^{-10} M. Current transition amplitude histograms and lifetime survivor plots were calculated by following the procedure described previously (Andersen, 1983; Sawyer et al., 1989; Durkin et al., 1990). Only channels with conductances that fall inside the main peak of the amplitude histogram were used for the determination of the average duration. For each gramicidin analogue, the reported results are based on experiments done over several days.

Multichannel Conductance Measurements. For these experiments, the membrane current was measured with a home-built four-electrode circuit. The membrane potential was determined as the potential difference between two Ag/AgCl electrodes at opposite sides of the planar lipid bilayer. The potential was maintained at 50 mV by passing a current through the membrane using a manually controlled source and two platinum electrodes placed at opposite sides of the planar lipid bilayer. The electrolyte was again unbuffered 1.0 M NaCl at 25 ± 1 °C. The (acyl)gramicidin was codissolved with the lipid at molar ratios varying between 1.10^{-8} and 1.10^{-5} gramicidin/lipid. The (acyl)gramicidin concentration was determined by fluorescence spectroscopy, following procedures described (Vogt et al., 1991). The concentration of DPhPC was determined by weight.

Initial experiments, using only *n*-decane as the (membrane-forming) lipid solvent, did not provide reproducible results and there was no clear relation between the gramicidin/lipid molar ratio and the membrane conductance. We therefore shifted to bilayers formed from (acyl)gramicidin and DPhPC codissolved in a mixture of *n*-decane/ethanol (1/1, v/v). This addition of ethanol to the membrane-forming solution allowed for a reproducible conductance of the large membrane. The membrane conductance was followed in time using a chart recorder. For both gramicidin A and the acylgramicidins a similar time dependence of the membrane conductance was observed. The maximum conductance of the black lipid membrane was reached within 1 min (when the membrane was completely "black") for *n*-decane/ethanol membranes as compared to 5–10 min for *n*-decane membranes, which thinned much slower. Single-channel experiments indicated that, for gramicidin A as well as the acylgramicidins, the channel conductance, average duration, and minichannel frequency were not significantly affected by the addition of ethanol (results not shown).

RESULTS

Figure 1 compares the properties of the channels formed by the acylgramicidins, as reflected in their single-channel current steps and durations, to those formed by gramicidin A. Gramicidin A channels in the presence of equimolar amounts of free palmitic acid (16:0) were used as a control.

Current traces for gramicidin A channels at 200-mV applied potential (Figure 1A, top) show current transitions of ~3.0 pA. These fall in a single predominant peak in the transition current amplitude histogram (Figure 1B, top), which contains 87% of all transitions. The channel durations are exponentially distributed (Figure 1C, top); an average duration of 0.9 s (200 mV) was calculated from the normalized survivor histogram. These observations are in good agreement with published data (Andersen & Procopio, 1980; Durkin et al., 1990).

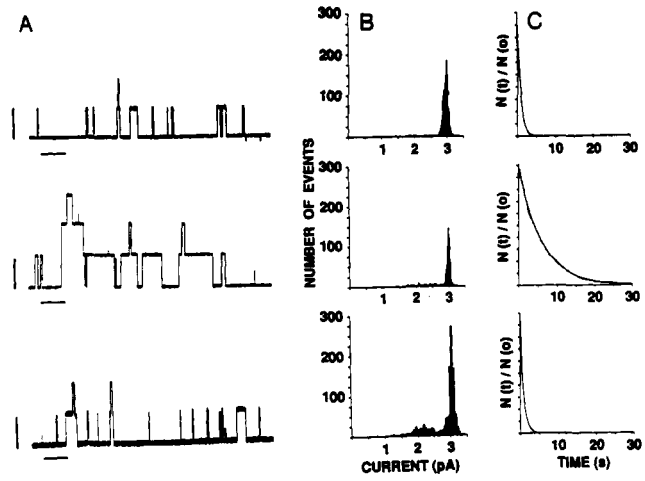

FIGURE 1: Current traces (A), amplitude histograms (B) and lifetime survivor plots (C) of gramicidin A (top), palmitoylgramicidin A (middle), and gramicidin A plus equimolar amounts of noncovalently coupled palmitic acid (bottom). The vertical and horizontal bars represent 3 pA and 10 s, respectively. The traces were recorded at 25 °C, 200-mV applied potential, and filtered at 50 Hz. The amplitude histograms contain more than 1500 transitions. The lifetime survivor plots contain more than 500 channels selected from the main peak of the amplitude histogram.

The middle row in Figure 1 shows examples of single-channel current traces obtained with palmitoylgramicidin (Figure 1A, middle). One is struck immediately by the longer average duration of channels formed by the acylgramicidins. The average duration was 5.5 s (200 mV). The amplitude histogram (Figure 1B, middle), in contrast, shows no change from gramicidin A channels. The main single-channel current of channels formed by the palmitoylgramicidin is comparable to that of gramicidin A. Likewise the conductance dispersity was indistinguishable (main peak containing 87% of all transitions). Figure 2 shows that the current traces obtained with all the synthetic acylgramicidins studied here are very similar. Occasionally a transition from the main conductance level to a lower conductance level was observed, as illustrated in Figure 2C for palmitoylgramicidin. Also the main single-channel current of the acylgramicidins was identical at all applied potentials (Figure 3). On the basis of the amplitude histograms of the acylgramicidins at 200 mV, the main peak contained 85%, 87%, 87%, 84%, and 90% of the transitions for lauroyl-, myristoyl-, palmitoyl-, stearoyl-, and oleoylgramicidin, respectively. The average durations of the various (acyl)gramicidin channels including gramicidin K are shown in Table I. At all applied potentials it was found that the acylgramicidins form channels that exhibit approximately a 5-fold increase in channel duration as compared to gramicidin A channels, with the exception of stearoylgramicidin for which a 4-fold increase in channel duration was observed. The results obtained with gramicidin K are in good agreement with those reported by Williams et al. (1992) and correspond to the results obtained for the synthetic acylgramicidins. The only difference in channel behavior was the larger conductance dispersity (more minichannels) for gramicidin K channels [this work and Williams et al. (1992)].

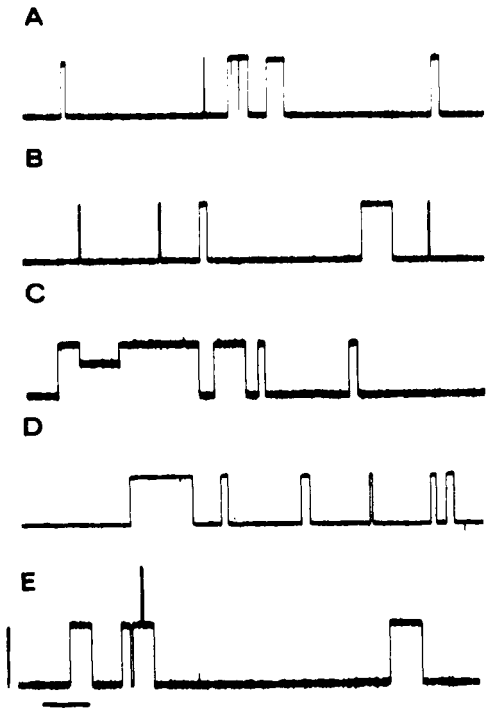

FIGURE 2: Current traces of lauroyl- (A), myristoyl- (B), palmitoyl- (C), stearoyl- (D), and oleoylgramicidin A (E) recorded at 200 mV and filtered at 50 Hz. The vertical and horizontal bars represent 3 pA and 10 s, respectively.

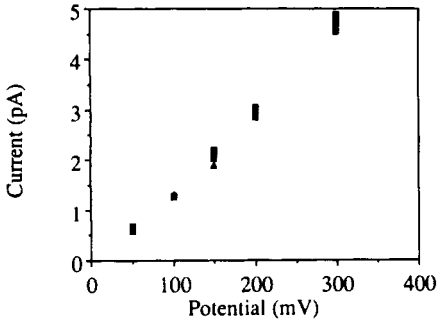

FIGURE 3: Main current transitions of channels formed by gramicidin A (■) and lauroyl- (□), myristoyl- (△), palmitoyl- (▲), stearoyl- (○), and oleoylgramicidin A (●), gramicidin plus equimolar amounts of free palmitic acid (+), and gramicidin K (×) as a function of the applied potential. Each point represents a superposition of these symbols.

Table I: Average Durations of Gramicidin A and Acylgramicidin A Analogues Determined at Various Applied Potentials[a]

peptides	average duration (s)		
	50 mV	100 mV	200 mV
gramicidin A	0.6	0.8	0.9
gramicidin A = 16:0	0.8	1.0	1.0
lauroylgramicidin	4.1	3.9	5.1
myristoylgramicidin	3.7	4.0	4.8
palmitoylgramicidin	3.8	3.7	5.5
stearoylgramicidin	2.2	3.0	3.8
oleoylgramicidin	3.7	3.6	5.0
gramicidin K	2.5	3.9	4.9

[a] Over 500 channels are selected from over 2000 current transitions that fall in the main peak of the amplitude histogram. The results obtained from experiments performed on different days are combined. The estimated error in the average duration measurements is 10%.

A current trace obtained after addition of an ethanolic gramicidin A solution containing equimolar amounts of free fatty acid (16:0) is shown in Figure 1A (bottom). The amplitude histogram (Figure 1B, bottom) shows an increase in channels

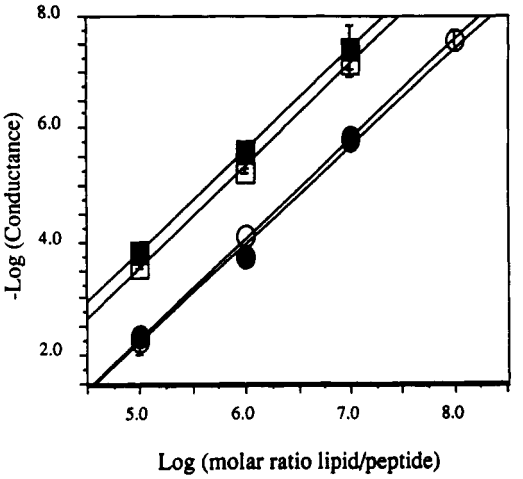

FIGURE 4: Total membrane conductance (S) as a function of the molar ratio DPhPC/(acyl)gramicidin. Plotted are the maximum conductances of black lipid membranes from solutions of gramicidin A (○), equimolar mixtures of gramicidin A and free fatty acid (●), lauroylgramicidin (□), stearoylgramicidin (■), and DPhPC codissolved in decane/ethanol (1/1, v/v).

with current transitions that fall outside the main peak (minichannels). Only 70% of the transitions fall inside the main peak. The main value of the current transitions for the events in the main peak, however, is identical to the value found for gramicidin A. The average duration of gramicidin channels is increased slightly (1.0 s, 200 mV) upon addition of free fatty acid to the channel-forming gramicidin solution (Table I).

Compared to gramicidin A, 10–100 times more acylgramicidin had to be added to the electrolyte solution in order to obtain a comparable single-channel appearance rate [see also Williams et al. (1992)]. To investigate whether this was due to a decreased insertion of the acylgramicidins into the membrane, multichannel experiments were performed in which the total membrane conductance of black lipid membranes, formed by solutions of (acyl)gramicidin and DPhPC codissolved in a mixture of decane/ethanol (1/1, v/v), was measured.

The total membrane conductance (G) is a function of the main channel conductance (g) and the average number of channels ($[D]$):

$$G = g[D] \tag{1}$$

The average number of channels is a function of the rate of channel formation (k_{for}), the rate of channel dissociation (k_{dis}) or channel duration (τ) ($k_{dis} \approx 1/\tau$), and the free monomer concentration ([M]):

$$K = k_{for}/k_{dis} = k_{for}\tau = [D]/[M]^2 \tag{2}$$

At the high lipid/gramicidin ratios used in the planar bilayer experiments, [M] is inversely proportional to the lipid/gramicidin ratio. Combination of eqs 1 and 2 yields

$$G = gk_{for}\tau[M]^2 \tag{3}$$

When the total membrane conductance is plotted vs the lipid/gramicidin ratio, one expects (Appel et al., 1977) a linear relation between the log (conductance) and log (ratio). We found such a linear relationship for gramicidin A (Figure 4). The slope, 1.8 ± 0.1, is close to 2, which is the value expected for a dimeric channel. Addition of free fatty acid to the mixture of gramicidin A and DPhPC, in an equimolar ratio of gramicidin A to fatty acid, gave results similar to those found for gramicidin A (Figure 4).

The conductance of planar bilayers formed from a lauroylgramicidin/DPhPC mixture showed a linear relationship similar to the results for gramicidin A. However, despite the increased channel duration for lauroylgramicidin (Table I), the total membrane conductance of the lauroylgramicidin/DPhPC membranes was decreased (Figure 4) as compared to gramicidin A/DPhPC membranes, which according to eq 3 implies that the rate of channel formation is decreased for lauroylgramicidin. A similar result was obtained with stearoylgramicidin/DPhPC membranes (Figure 4). Lauroyl- and stearoylgramicidin are, respectively, 190 ± 30 and 210 ± 30 times less effective in channel formation relative to gramicidin A.

DISCUSSION

The fundamental behavior of channels formed by acylgramicidins is similar to that of gramicidin channels. Current traces of (acyl)gramicidins show rectangular-shaped current steps (Figures 1 and 2). These current steps correspond to the appearance and disappearance of the conducting channels. The current transitions usually have a uniform amplitude.

The current–voltage characteristics of the channels formed by the acylgramicidins studied here are identical to those of gramicidin A channels. This is a surprising observation because minor changes to the gramicidin side chains can result in significant differences in channel conductance (Russell et al., 1986; Prasad et al., 1986), and because the ethanolamine has been implicated as being important for ion permeation (Etchebest & Pullman, 1984; Trudelle et al., 1987). In any case, these results suggest that the acylation does not induce major conformational changes.

In fact, earlier experiments using circular dichroism techniques indicated that acylgramicidins, when incorporated into lipid vesicles, are conformationally equivalent to gramicidin A (Vogt et al., 1991). Although identical CD spectra imply conformational equivalence, it does not imply structural equivalence of the channel-forming unit. However, a strong indication that the channel-forming units of gramicidin A and the acylgramicidins are structurally equivalent was obtained from the single-channel black lipid membrane conductivity measurements. This general question is further discussed by Williams et al. (1992) for gramicidin K (a family of naturally occurring acylated gramicidins), which has channel characteristics very similar to the synthetic acylgramicidins.

Additional evidence that acylation does not produce gross alterations in the gramicidin backbone structure was obtained by the observation that hybrid channels could be formed between [F_3 Val^1]gramicidin A and palmitoylgramicidin (results not shown), using the method of Durkin et al. (1990). Moreover, the properties of these hybrid channels are very similar to those reported for hybrid channels formed by gramicidin K and gramicidin A with [F_3 Val^1]gramicidin A, since the ratio $f_h/(2(f_a f_b)^{0.5})$ was 2.1 ± 0.3 (mean ± SD) as compared to 1.4 ± 0.2 for gramicidin K (Williams et al., 1990) and 1.2 ± 0.4 for gramicidin A (Durkin et al., 1990), where f_h, f_a, and f_b denote the appearance rate of the hybrid and two symmetrical channel types, respectively.

The channels formed by acylgramicidins have an approximately 5-times longer average duration than channels formed by gramicidin A. If covalent and noncovalent coupled fatty acids do not alter the structural or conformational properties of the gramicidin channel, how do they influence the channel duration?

The increase in average duration of channels formed by acylgramicidins can be understood in the light of the theory of Huang (1986) and Helfrich and Jakobsson (1990). These authors proposed that the average duration of a gramicidin channel will be (inversely) correlated to the magnitude of the energy for membrane deformation (dimpling) needed for docking of two gramicidin monomers. The covalently coupled fatty acid is expected to preferentially contribute to the cross-sectional area of the gramicidin monomer within the hydrocarbon area. Following the shape–structure concept of lipid polymorphism (Israelachvili et al., 1976; Cullis & De Kruijff, 1979), this can be expected to stabilize the deformation in the bilayer originating from the dimpling effect, thereby decreasing the magnitude of the deformation energy (Andersen et al., 1992) and thus increasing the channel's average duration.

One would then predict a fatty acid chain length dependent increase in average channel duration. Indeed, Benayad et al. (1991) recently showed a significant decrease in channel duration with much shorter fatty acid chain length. However, our results show no obvious length dependence of the covalently coupled fatty acid in the investigated region of 12–18 C-atoms, except for channels formed by stearoylgramicidin. Channels formed by this analogue had durations shorter than all other acylgramicidin channels. We believe the difference is significant because it was found at all applied potentials (and over the course of several days). This could arise because stearic acid is the only covalently coupled fatty acid longer than the gramicidin monomer (Watnick & Chan, 1990). Such length mismatch between the gramicidin monomer and the covalently coupled stearic acid could slightly destabilize the channel and thereby result in a relative decrease in channel duration.

Because the covalently coupled fatty acid is always next to the gramicidin, it is more effective in increasing the channel duration as compared to noncovalently coupled fatty acids, which are expected to be homogeneously distributed in the bilayer.

The multichannel experiments show that gramicidin A and the acylgramicidins have similar channel-forming characteristics (Figure 4). The rate constant for channel formation of the acylgramicidins, however, is reduced (~200 fold) as compared to gramicidin A. This decrease in the rate of channel formation is observed not only in planar bilayers. Analysis of (acyl)gramicidin-induced K^+ leakage from dioleoylphosphatidylcholine large unilamellar vesicles (data not shown) also indicates that the rate constant for channel formation by the acylgramicidins is decreased relative to that of gramicidin A.

In a kinetic description of gramicidin channel formation [cf. Hladky and Haydon (1984)], the rate constants for channel association and dissociation are interconnected. The observed decrease in the rate constant for channel formation therefore appears to be in conflict with an increase in the average channel duration. The finding that the conducting units of the acylgramicidins and gramicidin A have identical current–voltage characteristics indicates that the observed changes in rate constants cannot be an effect of the conducting unit. The decrease in the rate of channel formation can be explained by an increase in nonconducting units [cf. Cifu et al. (1992)]. Possible explanations are differences in conformational equilibrium, differences in (acyl)gramicidin/lipid interactions, or a more efficient blocking of the gramicidin channel by the modified ethanolamine group.

We expect to gain more insight in the acylgramicidin/lipid interaction by studying the influence of these gramicidin analogues on lipid polymorphism in model membrane systems.

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REFERENCES

Andersen, O. S. (1983) *Biophys. J. 41*, 119–133.
Andersen, O. S. (1984) *Annu. Rev. Physiol. 46*, 531–548.
Andersen, O. S., & Procopio, J. (1980) *Acta Physiol. Scand. Suppl. 481*, 27–35.
Andersen, O. S., Durkin, J. T., & Koeppe, R. E., II (1988) in *Transport Through Membranes: Carriers, Channels and Pumps* (Pullman, A., et al., Eds.) pp 115–132, Kluwer Academic Publishers, Dordrecht, The Netherlands.
Andersen, O. S., Sawyer, D. B., & Koeppe, R. E., II (1992) in *Biomembrane Structure and Function* (Gaber, B. P., & Easwaran, K. R. K., Eds.) pp 227–244, Adenine Press, Schenecttedy, NY.
Appel, H.-J., Bamberg, E., Alpes, H., & Läuger, P. (1977) *J. Membr. Biol. 31*, 171–188.
Barret Russell, E. W., Weis, L. B., Navetta, F. I., Koeppe, R. E., II, & Andersen, O. S. (1986) *Biophys. J. 49*, 673–686.
Benayad, A., Benamar, D., Van Mau, N., Page, G., & Heitz, F. (1991) *Eur. Biophys. J. 20*, 209–213.
Cifu, A. S., Koeppe, R. E., & Andersen, O. S. (1992) *Biophys. J. 61*, 1–15.
Cullis, A. S., & De Kruijff, B. (1979) *Biochim. Biophys. Acta 559*, 399–420.
De Kruijff, B., Killian, J. A., & Tournois, H. (1988) in *Transport Through Membranes: Carriers, Channels and Pumps* (Pulmann, A., et al., Eds.) pp 267–287, Kluwer Academic Publishers, Dordrecht, The Netherlands.
Durkin, J. T., Andersen, O. S., Blout, E. R., Heitz, F., Koeppe, R. E., II, & Trudelle, Y. (1986) *Biophys. J. 49*, 118–121.
Durkin, J. T., Koeppe, R. E., II, & Andersen, O. S. (1990) *J. Mol. Biol. 211*, 221–234.
Etchebest, C., & Pullman, A. (1984) *FEBS Lett. 170*, 191–195.
Helfrich, P., & Jakobson, E. (1990) *Biophys. J. 57*, 1075–1084.
Hladky, S. B., & Haydon, D. A. (1984) *Curr. Top. Membr. Transp. 21*, 327–372.
Huang, H. W. (1986) *Biophys. J. 50*, 1061–1070.
Israelachvili, J. N., Mitchel, D. J., & Ninham, B. W. (1976) *J. Chem. Soc., Faraday Trans. 72*, 1525–1568.
Koeppe, R. E., II, Paczkowski, J. A., & Whaley, W. L. (1985) *Biochemistry 24*, 2822–2826.
Koeppe, R. E., II, Corder, R. A., Narcessian, E. J., Peart-Williams, L. M., & Waller, G. R. (1988) *Biophys. J. 53*, 328a.
Peart-Williams, L. M., Narcessian, E. J., Andersen, O. S., & Koeppe, R. E., II (1988) *Biophys. J. 53*, 329a.
Prasad, K. V., Alonso-Romanowski, S., Venkatachalam, C. M., Trapane, T. L., & Urry, D. W. (1986) *Biochemistry 25*, 456–463.
Russell, E. W. B., Weiss, L. B., Navetta, F. I., Koeppe, R. E., & Andersen, O. S. (1986) *Biophys. J. 49*, 673–686.
Sawyer, D. B., Koeppe, R. E., & Andersen, O. S. (1989) *Biochemistry 28*, 6571–6583.
Schmidt, M. F. G. (1989) *Biochim. Biophys. Acta 988*, 411–426.
Trudelle, Y., Daumas, P., Heitz, F., Etchebest, C., & Pullman, A. (1987) *FEBS Lett. 216*, 11–16.
Urry, D. W. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 672–676.
Urry, D. W., Goodall, M. C., Glickson, J. D., & Mayers, D. F. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 1907–1911.
Vogt, T. C. B., Killian, J. A., Demel, R. A., & De Kruijff, B. (1991) *Biochim. Biophys. Acta 1069*, 157–164.
Watnick, R. I., & Chan, S. I. (1990) *Biochemistry 29*, 6215–6221.
Williams, L. P., Narcessian, E. J., Andersen, O. S., Waller, G. R., Taylor, M. J., Lazenby, J. P., Hinton, J. F., & Koeppe, R. E., II (1992) *Biochemistry* (preceding paper in this issue).