Potassium-Selective Amphotericin B Channels Are Predominant in Vesicles Regardless of Sidedness†

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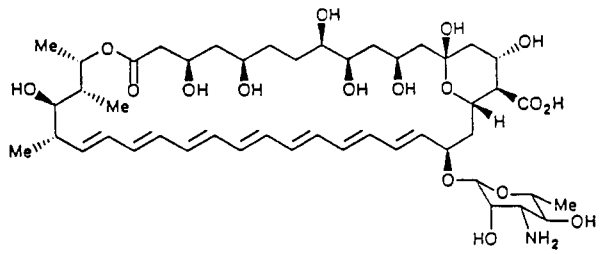
ABSTRACT: Amphotericin B (AmB) is a membrane-active antibiotic which has been shown to increase ion and small molecule permeability in a variety of model and biological membrane systems. A major mechanistic model, based on BLM systems, proposes that amphotericin forms barrellike pores with cholesterol which are cation selective when added to one side of the membrane and anion selective when added to both sides. We have tested this hypothesis on small and reverse-phase large unilamellar vesicles (SUV and REV) with and without cholesterol. The method used to measure K^+, Cl^-, and net ion currents is based on ion/H^+ exchange detected by the entrapped pH probe pyranine. We find that AmB forms channels which have net selectivity for K^+ over Cl^- regardless of sidedness or sterol content in SUV. REV with 10% cholesterol also show net K^+ selectivity with double-sided addition. Differences are noted between cholesterol- and non-sterol-containing vesicles consistent with at least two separate modes of action: (1) cholesterol-containing SUV form some larger diameter pores which allow the passage of larger ions especially when added to both sides; (2) SUV without sterol form pores which are still K^+ over Cl^- selective, but larger ions do not pass. The latter mode of action precludes a sterol/pore type of model but not necessarily a barrellike model consisting only of amphotericin molecules. This sterol-free mode of action and the $K^+ > Cl^-$ selectivity may also be consistent with a membrane defect mechanism. Possible reasons for the failure to observe a preponderance of anion-selective pores upon double-sided addition are that the high radius of curvature of SUV might prevent formation and association of half-pores and K^+-selective pores predominate or simply that high-conductance anion-selective pores are rare events in vesicles and those vesicles are not readily detectable by this technique.

The antifungal polyene antibiotic amphotericin B (AmB)[1] interacts with biomembranes, causing the loss of monovalent and divalent cations and small molecules, and is selectively



amphotericin B

toxic toward fungi [see Bolard (1986) for a comprehensive review]. In many experimental systems, sterols are necessary for maximal AmB activity. Differences in the sensitivity of ergosterol- (the fungal sterol) versus cholesterol-containing membranes are presumably the reason for greater susceptibility of fungal cells for the toxic effects of AmB. One widely accepted model proposes that these antibiotics form 1:1 barrellike pore complexes with sterols (Andreoli, 1973). However, there is evidence that amphotericin B (AmB) may also interact with sterol-free phosphatidylcholine small and large unilamellar vesicles to induce cation permeability (Hartsel et al., 1988; Whyte et al., 1989; Archer, 1976; Hsu Chen & Feingold, 1973; Milhaud et al., 1989). The model of Finkelstein's group (Kleinberg & Finkelstein, 1984; Marty & Finkelstein, 1975) eliminates the need for sterol by suggesting that the related antibiotic nystatin (as well as AmB) self-associates into pores and sterols have a binding or ordering role in promoting sensitivity to this antibiotic. However, significant K^+ permeability has been noted even at very low AmB concentrations (<2 AmB/vesicle), suggesting an additional mechanism for AmB action (Hartsel et al., 1988). Also, differences between AmB-induced H^+/OH^-, Ca^{2+} and large molecule permeability have been observed among vesicles containing ergosterol, cholesterol, or no sterol, indicating a multiplicity of mechanisms (Bolard, 1986; Hartsel et al., 1988; Ramos et al., 1989; Milhaud et al., 1989).

One of the unusual features of the sterol pore model, based on solvent-free planar bilayer lipid membrane (BLM) conductance experiments, is that AmB and other related polyenes appear to produce monovalent cation selective pores when added to one side of the membrane, but monovalent anion selective pores when added to both sides (Kleinberg & Finkelstein, 1984). According to the model, "half-pores" would be cation selective and "whole-pores" which join together from both sides of the bilayer would become anion selective (Andreoli, 1973; Marty & Finkelstein, 1975). High selectivity of this type has not been seen so far in model membrane vesicle systems or in vivo experiments. Using a novel stopped-flow fluorescence method for sensitive detection of transmembrane ion currents, we have addressed this question in SUV and

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[1] Abbreviations: AmB, amphotericin B; REV, reversed-phase unilamellar vesicle(s); SUV, small unilamellar vesicle(s); EPC, egg phosphatidylcholine; MOPS, 3-(*N*-morpholino)propanesulfonic acid; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone; BLM, planar bilayer lipid membrane(s).

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REV. The results of this study do suggest at least two mechanisms of action of AmB, but we find net selectivity for K^+ over Cl^- regardless of conditions.

Materials and Methods

Materials. Egg phosphatidylcholine was isolated from fresh eggs by using the Singleton procedure (Singleton et al., 1965). Purified amphotericin B was a generous gift from Squibb Pharmaceuticals (Princeton, NJ). FCCP [carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone] and valinomycin were obtained from Sigma Chemical Co. (St. Louis, MO). Laser-grade pyranine (1,3,6-pyrenetrisulfonic acid) was purchased from Eastman-Kodak (Rochester, NY).

Preparation and Assay of Lipid Vesicle Samples. Lipids in chloroform were dried under a stream of argon and dried in a vacuum for >24 h to remove the chloroform. The mixtures were dispersed in buffer with 2.2 mM pyranine added and sonicated under argon with a titanium probe-type sonicator until clear; 1.5-mL samples of REV (at 15 mg/mL) were prepared in the presence of 2.2 mM pyranine in the appropriate buffer according to the ether evaporation method of Szoka and Papahadjopoulos (1978). REV samples were diluted to 7 mL, and external pyranine was removed by cellulose tubing dialysis against several 1-L buffer changes for about 2 h at 22 °C in the dark. Pyranine was removed from SUV by gel filtration through a 1.5 × 10 cm column of Sephadex G-25. For double-sided addition experiments, it was determined that 85–90% of the AmB remained with cholesterol-containing vesicles after dialysis. Stock AmB in DMSO was added to vesicles and preequilibrated for 20–30 min in the dark at room temperature for single-sided addition. For double-sided addition experiments, dry AmB was added to the initial lipid mixture in chloroform and dried with the lipids. It was determined by absorption spectroscopy that >90% of amphotericin survived the sonication step if care was taken to maintain darkness and an argon atmosphere. Starting lipid concentrations varied from 15 to 20 mM for SUV and 1–3 mM for REV and AmB concentrations from 0.001 to 0.2 mM. The relative concentrations of AmB and lipid were determined for each sample as described in Hartsel et al. (1988). FCCP was added to all samples at 10 μM shortly before measurements were to be made.

Measurements of K^+, Cl^-, and Net Permeability in SUV and REV. Gradients of K_2SO_4 (for K^+ permeability), arginine chloride (for Cl^-), or KCl (net) were created by variable-ratio syringes in a On Line Instrument Systems (OLIS, Jefferson, GA) converted Durrum D-110 stopped-flow spectrophotometer in the fluorescence mode. The dilution factor from these syringes was determined to be 1:5.7. Dead times were on the order of 5 ms for this instrumental setup. Only traces which could be reproduced by subsequent stopped-flow shots within ±3% were used. The starting SUV suspension contained 100 mM K_2SO_4, KCl, or arginine chloride in 25 mM MOPS at pH 7.20 at 22 ± 1 °C. The solution was diluted with osmolar sucrose/25 mM MOPS at pH 7.20, creating a chemical potential gradient. The fluorescence was filtered with a Kodak 8 Wratten filter in order to minimize scattering problems from unabsorbed light. The sample was excited with 450-nm light from a xenon lamp source. Under these conditions, pyranine fluorescence intensity was linear with respect to pH from 6.25 to 7.75. The initial H^+ ion current density, $I_0(H^+)$, can be measured from the initial rate of change of pH assuming a linear response of pyranine fluorescence in this pH region (Kano & Fendler, 1978; Dencher et al., 1986; Whyte et al., 1989). The initial pH rate of change is extrapolated to $t = 0$ and hence the membrane potential, $\psi = 0$. This allows us to use the approximation $I_0(H^+) \simeq I_0(K^+)$ to determine K^+ currents (Whyte et al., 1989). Permeability coefficients (P_0) for K^+ may also be determined from initial currents (Whyte et al., 1989).

The assumption made in these expressions is that K^+ or Cl^- permeation is much slower than FCCP-mediated H^+ transport, an assumption borne our by experimental determinations of H^+ currents. Briefly, a high concentration of valinomycin (10 μM) was used to create a K^+ ion current. FCCP was added at different concentrations until no further changes in the initial K^+ current were seen (at about 3 μM FCCP). The concentration of FCCP was increased to 10 μM to ensure that H^+ equilibration was not rate limiting. In any case, the H^+/OH^- flux would not be limiting in cholesterol-containing vesicles since AmB induces a high H^+/OH^- permeability in the presence of cholesterol (Hartsel et al., 1988). The counterion of the species to be measured must also be assumed to be impermeant in the presence of AmB. The average sizes of SUV were previously determined (Cafiso & Hubbell, 1978; Hartsel et al., 1988).

A potential problem with the quantitation of ion currents is the fact that they reflect net currents averaged over the entire vesicle population. In fact, it has been shown by NMR that under some conditions amphotericin-induced cation release from vesicles is an "all or none" phenomenon; i.e., some vesicles are completely permeabilized while others are not affected at all (Gary-Bobo, 1989; Cybulska et al., 1986). Unfortunately, it is not possible to make rapid kinetic measurements via NMR, and our technique cannot distinguish between these possibilities. Because of this difficulty, data are presented in the more intuitive form of pH vs time measurements. In experiments utilizing K_2SO_4 gradients across SUV with or without cholesterol, it takes 0.5–4 h (depending on single-sided drug concentrations) to eventually reach ideal K^+-selective equilibrium pH, indicating that all vesicles are ultimately permeabilized under our experimental conditions. All experimental systems were also treated with 1–5 μM valinomycin as an ideally K^+-selective control. At these relatively high valinomycin concentrations, K^+ conductance is dominant. However, in the presence of Cl^-, depending on the drug concentration, the pH gradient evenutally breaks down due to Cl^- leakage depleting the salt gradient. In all cases except where noted, the ΔpH was close to the 0.75 unit drop (±10%) expected theoretically for a weakly buffered system with a 5.7:1 K^+ gradient.

Results

When salt gradients are imposed across SUV, the fluorescence intensity of internally trapped pyranine changes with time if any single ion species is more permeant than the others. This is reflected as an increase or decrease in fluorescence intensity depending on which ion is permeable. For example, if K^+ is more permeant than Cl^- when a KCl gradient is imposed, a decrease in fluorescence is observed. The quenching is due to a lowering of the vesicle internal pH (and subsequent protonation of pyranine) caused by electroneutral exchange of H^+ for K^+. The K^+ gradient imposed on these vesicles and the transmembrane voltage produced by K^+ efflux are the driving force for this exchange. Since the protonophore FCCP is present in all of the vesicle preparations, the rate-limiting step should be K^+ or Cl^- efflux. If the counterion is impermeant, the initial ion current due to K^+ or Cl^- efflux should equal the initial H^+ current carried by FCCP as measured by pyranine fluorescence. Thus, net K^+ currents (or any other electrogenically permeant ion) can be accurately and sensitively determined from the time-dependent change in pyranine

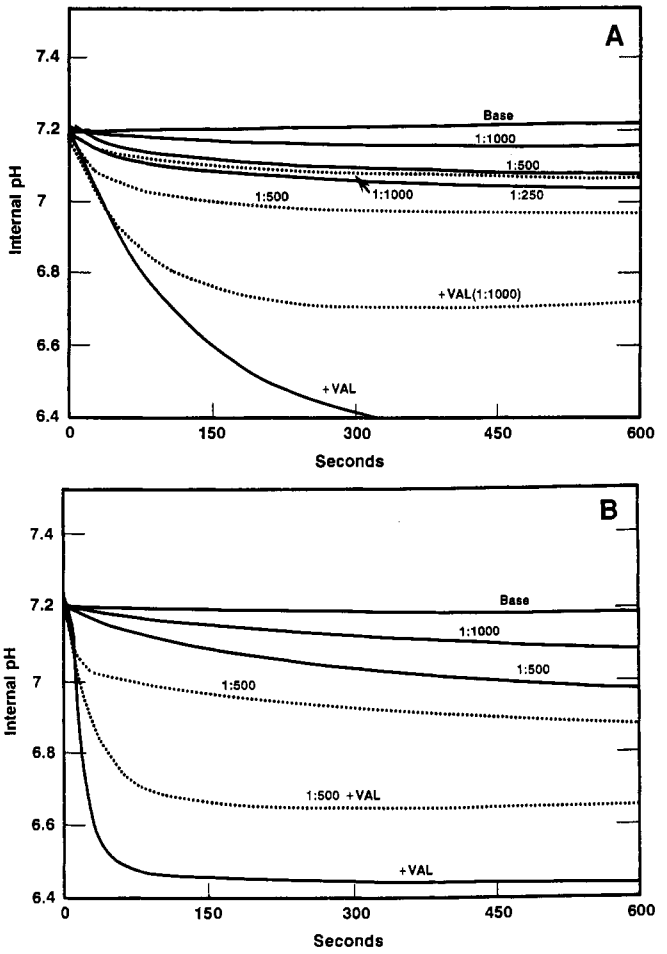

FIGURE 1: Time course of the KCl and K_2SO_4 gradient-driven pH gradient formation caused by AmB addition to SUV with 10 mol % cholesterol. (A) KCl gradients with single (—) and double-sided (···) AmB addition. (B) K_2SO_4 gradients with single- (—) and double-sided (···) AmB addition. AmB concentrations are given in AmB:lipid ratios. Valinomycin was added as a K^+ selectivity control at 1 μM in (A) for double-sided addition and of 5 μM for (B) with single-sided addition. Adding more AmB to the outside or the double-sided addition experiments simply increased net K^+ permeability indicated by an increased fluorescence quenching. Notice that the base line of (A) and all other KCl or arginine chloride SUV experiments showed a slightly greater intrinsic permeability toward Cl^- (indicated by the increasing time-dependent pH). All vesicle suspensions contain 10 μM FCCP.

fluorescence keeping in mind the precautions outlined under Materials and Methods. If both species are permeant, *net* ion selectivity may be determined by the direction of the fluorescence (i.e., pH) change. Since the starting pH inside is known and the experiments are within the linear range of pyranine, calibration curves need not be constructed for each experiment. An added advantage of pyranine over weak acid pH probes such as 9-aminoacridine is that pyranine is membrane-impermeant and does not rely on probe redistribution. Also, since pyranine under these conditions has a −3 or −4 charge, unwanted membrane binding is minimal (Dencher et al., 1986).

Antibiotic-Induced K^+ Permeability. Figure 1A,B illustrates the K^+-selective action of AmB against cholesterol-containing SUV when added to one or both sides. The total change in K^+ permeability when comparable amounts of AmB are added to one side of the SUV (as reflected by the difference in pH after 10 min) is only slightly greater in the K_2SO_4 medium (B) as compared to the KCl medium. This shows that AmB induces little additional Cl^- conductance under single-sided addition to cholesterol-containing membranes. Upon double-sided addition, again there is a slightly greater overall enhancement of K^+-selective permeability in the presence of the divalent sulfate counterion (+18%). The initial phase of K^+ leakage, however, was much faster with the K_2SO_4 buffer. A feature of double-sided addition to cholesterol-containing SUV is the failure to reach the ideal K^+-selective equilibrium pH. Vesicles with AmB added to only one side did reach that equilibrium, in the presence or absence of valinomycin and similar amounts of AmB. This suggests that double-sided addition of AmB to cholesterol-containing SUV may be causing the release of larger molecules or anions (e.g., MOPS buffer, the pyranine dye, SO_4^{2-}) very rapidly (<10 ms) or vesicle lysis and destruction may be occurring. Nonetheless, of those vesicles we can observe, AmB always produces net K^+ permeability.

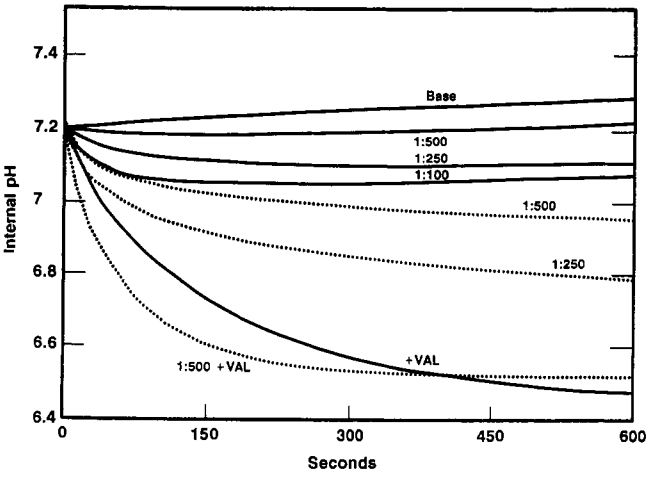

FIGURE 2: Net permeability of EPC sterol-free SUV with a KCl gradient. AmB was added to a single side (—) or double side (···). Valinomycin was added to 1 μM in the controls. The 1:250 AmB/lipid double-sided run consisted of 1:500 vesicles to which additional AmB had been added to the outside for a total AmB:lipid ratio of 1:250. This extra addition promoted K^+ rather than Cl^- permeability.

Figure 2 shows the results of double- and single-sided addition ot sterol-free egg phosphatidylcholine (EPC) SUV in KCl buffer. The results are similar to the cholesterol case in that comparable concentrations of AmB on both sides of the membrane cause greater K^+ permeability than single-sided addition. The results in the presence of K_2SO_4 are similar (Whyte et al., 1989; Hartsel et al., 1988). One noteworthy difference is that double-sided addition does not lead to the rapid leakage of anions or large molecules from an EPC SUV population; in fact, a nearly ideal K^+ selectivity is observed when valinomycin is added. Again, the *net* ion permeability is always K^+ selective.

Antibiotic-Induced Cl^- Permeability. To measure Cl^- currents independently, we chose a counterion which was very large and polar, arginine, in the hope that it would be impermeant. The results of these experiments are shown in Figure 3A,B. SUV with cholesterol show a sharp initial Cl^- current when AmB is added to both sides, followed by a slower relaxation toward the initial pH (Figure 3A). The rapid phase suggests high initial Cl^- permeability followed by permeation of ions other than Cl^-. The results may indicate a leakage of arginine, MOPS, or even pyranine. All of these possibilities would require a large channel and are consistent with the lower equilibrium pH observed with double-sided addition to cholesterol SUV in KCl or K_2SO_4 buffers in Figure 1A,B. Single-sided addition leads to an initial small jump in Cl^- permeability followed by a slight decline in fluorescence relative to the base line. When additional AmB is added to the outside, a reproducible though slight decline in fluorescence

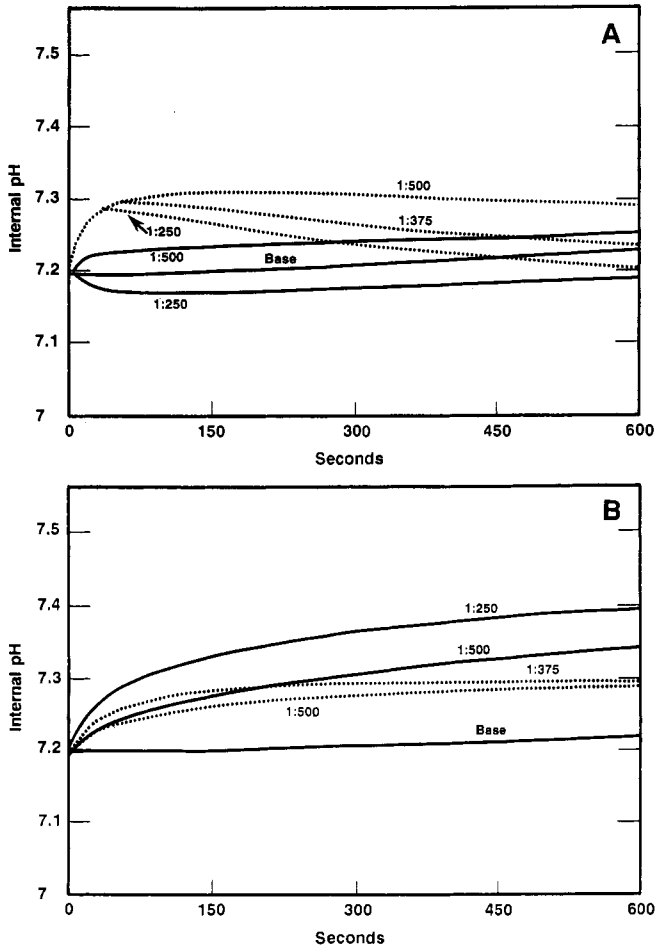

FIGURE 3: AmB-induced ion permeability with arginine chloride gradients. (A) SUV contain 10 mol % cholesterol and AmB was added to one side (—) or incorporated on both sides (···). For the double-sided addition experiments, additional AmB was added to 1:500 SUV from the outside to a total 1:375 or 1:250 AmB/lipid. (B) SUV consisting of only EPC had AmB added to one (—) or both sides (···). Other conditions were the same as in (A).

is seen which suggests a cation-selective component. The results are consistent with the results of addition of extra AmB to the outside of vesicles with AmB already present on both sides in Figure 3A.

For EPC in the absence of sterol, AmB induces a steady electrogenic flow of Cl^- ions when added on one or both sides (Figure 3B). Note that the base-line Cl^- conductance is intrinsically greater than K^+ (Figure 2) or arginine (Figure 3B). This agrees with previous studies which show that Cl^- has a higher permeability coefficient than monovalent cations in untreated membrane bilayers (Hauser et al., 1972). This flow, however, must be less than the AmB-induced conductance of K^+ ions as shown by the KCl conductance of Figure 2.

REV Net Permeability. REV (large predominantly unilamellar vesicles) prepared by the method of Szoka and Papahadjopoulos (1978) are more realistic models of biological membranes than are SUV. For this reason, we determined the net ion selectivity in cholesterol-containing REV using the KCl buffer system (Figure 4). The results of both single- and double-sided addition showed a rapid efflux with very high $K^+ > Cl^-$ selectivity. In fact, there was essentially no difference between the two modes of addition for identical AmB concentrations, unlike the SUV case. One problem with these measurements was that the equilibrium pH change determined by the K^+ ionophore valinomycin was significantly, but reproducibly lower than expected. A likely reason for this is that this technique of producing REV leads to a significant fraction

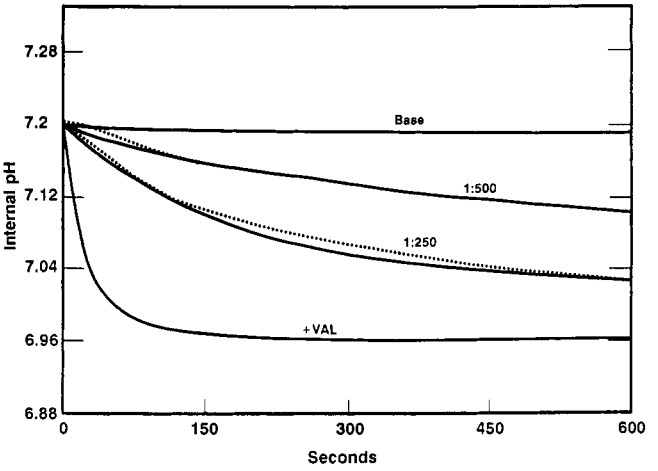

FIGURE 4: Effect of single (—) and double-sided (···) addition of AmB to REV with 10 mol % cholesterol. The double-sided traces had AmB incorporated at 1:500 and 1:250 during vesicle formation. Additional AmB added to the outside of the double-sided REV lead to enhanced K^+ permeability (not shown). The valinomycin control had 1.5 μM valinomycin added. The final pH value for the double-sided valinomycin control (1:500) was 6.92.

of multilamellar vesicles where the internal vesicles would not have a gradient set up across them by the stopped-flow dilution (Hope et al., 1986). The fluorescence would be nearly unchanged for this entrapped population. Another possibility is that external pyranine was not completely removed by dialysis. However, the important result is that in REV with cholesterol, AmB produces very rapid and high selectivity for K^+ over Cl^- regardless of sidedness. We find that REV made of pure EPC, as previously shown by Milhaud et al. (1989), are insensitive to AmB even at high concentrations (unpublished results). Contrast this with the high sensitivity of EPC SUV (Whyte et al., 1989; Hartsel et al., 1988; Figure 2), and it is apparent that the different vesicle geometry of SUV is an important factor for AmB activity.

Discussion

It was the intent of this study to attempt to reconcile observations on the mechanism of action of AmB obtained in different experimental systems such as BLM, SUV, and cultured whole cells. One thing that is clear from the overview of the extensive work which has been done on AmB is that there are several distinct and separable mechanisms of action (Bolard, 1986; Brajtburg et al., 1990). The one distinct characteristic of AmB and the related polyene nystatin in BLM experimental systems that we were unable to directly reproduce in vesicles is the shift to nearly exclusive chloride selectivity for two-sided drug addition in the presence of sterols (Marty & Finkelstein, 1975). We did, however, observe clues suggesting a sterol-dependent difference for two-sided drug addition. For the arginine chloride experiments, a very high initial Cl^- conductance was observed followed by an apparent leakage of larger molecules such as arginine. A channel which allows molecules of this size is not unreasonable. Milhaud et al. (1989) have shown that in the presence of sterol, AmB may allow leakage of molecules as large as carboxyfluorescein and divalent cations such as Ca^{2+}. Considering the fact that double-sided drug addition to cholesterol SUV always produced a less than predicted K^+-selective pH, it is quite possible that a population of vesicles were emptied of their contents by large or highly anion-selective pores before they could be detected by this technique (<10–20 ms). Since SUV have such high surface to volume ratios, a pore of the dimensions predicted by Finkelstein's group (8-Å diameter; Marty & Fink-

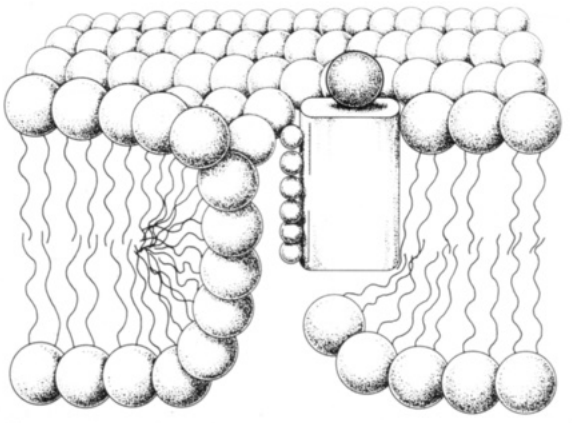

FIGURE 5: Highly schematic model for the formation of AmB-induced membrane defects. The AmB molecule (rectangle) is vertically inserted in the phospholipid bilayer with the hydrophobic polyene region favorably interacting with the acyl chains. The unfavorable juxtaposition of the polyol region of AmB (small spheres) with the acyl chains might induce membrane defects. These defects might also be induced by an AmB aggregate or an AmB/sterol complex. Ion selectivity in a structure like this might occur via molecular sieving, by differences in ion hydration energy (assuming a pore smaller than an ions's hydrated radius), or by electrostatic interaction with the AmB molecule.

elstein, 1975) would very likely do this, and it is a limitation to our technique. Since EPC under all conditions and 10% cholesterol SUV with single-sided addition did reach the ideal K^+-selective pH with valinomycin, this is a double-sided sterol-dependent property. However, in all cases, AmB induced net cation selective permeability in the majority of vesicles.

Experiments with REV are much harder to reconcile with the selectivity properties observed in BLM. In these experiments, very high $K^+ > Cl^-$ selectivity was observed; ideal K^+ selectivity was approached within 20 min for a ratio of 1:250 AmB/lipid. This is a much faster approach compared to cholesterol-containing SUV. In REV, the vesicle packing constraints involved with the joining of "half-pores" would not be as acute as in SUV. Yet we see no increase in net anion permeability (Figure 4) for double-sided addition. However, since it appears that there may be a significant fraction of MLV associated with the REV preparations, experiments with more uniform extruded large unilamellar vesicles (LUV) will be necessary to generalize to all LUV systems (Hope et al., 1986). These experiments also point to a very important dependence of AmB properties to vesicle geometry (i.e., SUV vs REV). Recently Bolard's group (Milhaud et al., 1989) has confirmed that spectroscopically distinct forms of AmB are associated with different vesicle sizes and these forms have different channel characteristics.

Since sterols are not required for AmB action and in all of the experiments K^+ is selected for over Cl^-, there must be a mechanism distinct from the large sterol-dependent pores observed in BLM studies. What additional mechanism might account for the observed results? A possible mode of activity in the absence of sterols could be the formation of ion-conducting membrane defects at the antiobitic–lipid interface (Figure 5). Such defects have been evoked to explain the membrane activities of melittin, abscissic acid, and the complement C9 complex (Terwilliger et al., 1982; Stillwell et al., 1989; Tschopp, 1984). Also, conductive membrane defects formed during phase transitions (Bramhall et al., 1987) or strong electrical pulses (electroporation; El-Mashak & Tsong, 1985) have been observed. These defects might be exacerbated by the high radius of curvature in SUV (r = 150 Å) since AmB shows little effect against sterol-free EPC REV (Ramos et al., 1989; unpublished studies). The increased sensitivity of cholesterol REV may involve an increasingly vertical insertion of AmB into the bilayer as opposed to surface adsorption. Linear dichroism studies have shown that cholesterol promotes orientation of the long axis of AmB toward the membrane normal in monolayers (Ockman, 1984). SUV, because of packing constraints, are expected to have a lower packing density in the outer membrane leaflet and higher inside (Ellena et al., 1985). The increased space between lipids, then, in sterol-free SUV might allow AmB to insert more readily toward the membrane normal. Differences in AmB binding affinity to SUV and REV have been observed in agreement with the above model (Milhaud et al., 1979). A defect of this sort might be produced either by an aggregate or by a monomer, dependent upon lipid composition and AmB concentration in the membrane. It is significant that CD studies of EPC SUV have shown that under conditions where high K^+ permeability is observed in this particular system, the predominant AmB species is monomeric (Hartsel et al., 1988). What would such a defect look like? Figure 5 is a highly schematic diagram of one possibility. The insertion of AmB brings the polar polyol region in direct contact with the lipid hydrocarbon region. This unfavorable juxtaposition may induce some of the local lipid head groups to "fold around" so as to interact with the polyol. This (probably) transient defect could lead to leakage of ions or other solutes. The size of the defect might depend on the aggregation state of AmB or its interaction with sterols. The size and hydration differences of a defect in various environments may be the basis for the differential H^+/OH^- conductances seen between ergosterol- and cholesterol-containing SUV. Direct physical evidence for such a structure is currently slim. However, recent ^{31}P NMR results show that AmB can induce lipid structures with a high radius of curvature (Ellena et al., 1987) or interdigitated lipid phases (Lewis & Levin, 1989).

If AmB can promote membrane defects, how can the $K^+ > Cl^-$ selectivity be accounted for? El-Mashak and Tsong (1985) have observed significant Rb^+ over K^+ selectivity at membrane phase defects in dipalmitoylphosphatidylcholine large unilamellar vesicles caused by the phase transition *or* by electroporation. These authors proposed a difference in hydrated radius between these ions (hydration energy) as a decisive factor. In the case of AmB selectivity, the crystal radii of Cl^- ions are significantly larger than K^+, but they have similar hydration energies, making it possible to select them by molecular sieving (Jain, 1972). If hydration energies (the energy it takes to "strip off" waters) also play a significant role in selectivity, then AmB channels or defects might be expected to be selective for K^+ over Na^+ because of the significantly higher hydration energy of Na^+. In fact, Cybulska et al. (1984) have shown K^+ over Na^+ selectivity for AmB added to erythrocytes. However, charge and dipole enviromments could well produce a different selectivity sequence under different circumstances (Jain, 1972). Molecules as large as carboxyfluorescein may also pass through phase transition induced membrane defects (Bramhall et al., 1987). This model of AmB as a membrane defect inducer does not rule out organized pore models of AmB but does provide a testable hypothesis for an additional mode of action which may be relevant or even dominant under physiological conditions. Recent studies on simple membrane-spanning macrocyclic AmB analogues also show $K^+ > Na^+$ selective membrane activity even though there is no a priori reason to believe that they form organized barrellike pores (Fyles et al., 1990). Further studies on these agents may prove fruitful in clarifying the mechanisms

of action of polyene macrolide antibiotics.

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Registry No. AMB, 1397-89-3; K^+, 7440-09-7; Cl^-, 16887-00-6; cholesterol, 57-88-5.

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Genomic Sequence and Organization of Two Members of a Human Lectin Gene Family[†,‡]

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ABSTRACT: We have isolated and sequenced the genomic DNA encoding a human dimeric soluble lactose-binding lectin. The gene has four exons, and its upstream region contains sequences that suggest control by glucocorticoids, heat (environmental) shock, metals, and other factors. We have also isolated and sequenced three exons of the gene encoding another human putative lectin, the existence of which was first indicated by isolation of its cDNA. Comparisons suggest a general pattern of genomic organization of members of this lectin gene family.

Many animal tissues contain soluble lactose-binding lectins (S-Lac lectins)[1] (Barondes, 1984). In the rat, at least nine have been identified and partially characterized (Cerra et al., 1985; Leffler et al., 1989; Hinek et al., 1988). Although all bind lactose, there is evidence for considerable specificity among them (Leffler & Barondes, 1986; Sparrow et al., 1987). For example, three S-Lac lectins from lung (Leffler & Barondes, 1986; Sparrow et al., 1987) differ greatly in their relative binding of a series of naturally occurring mammalian glycoconjugates, suggesting biologically significant lectin-glycoconjugate interactions.

The best studied of the S-Lac lectins is a dimer with subunit molecular weight of about 14 000, here referred to as L-14. It is abundant in many cell types where it often comprises more

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[1] Abbreviations: S-Lac lectin, soluble lactose-binding lectin; SSC, standard saline citrate buffer; L-14, S-Lac lectin of subunit M_r ~14 000; L-30, S-Lac lectin of M_r ~30 000; bp, base pair(s); kbp, kilobase pair(s); PCR, polymerase chain reaction; IgE, immunoglobulin E; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate.

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