events may be altered in cultured cells. Clonal populations of bone cells therefore represent a convenient system for the study of bone matrix macromolecules and are amenable for investigation of the effects mediated by bone-specific hormones.

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Diffusion Potential Cascade. Convenient Detection of Transferable Membrane Pores†

Leslie M. Loew,* Ian Rosenberg, Mary Bridge, and Carlos Gitler

ABSTRACT: A valinomycin-mediated K^+ diffusion potential across the membrane of multilamellar liposomes is stable for longer than 30 min and can be collapsed by a nonselective channel such as gramicidin. The kinetics of the potential collapse are complex but can be qualitatively broken down into a series of processes involving (1) binding of the gramicidin to the outer membrane, (2) dimerization to form a functional channel, (3) the flow of ions through the channel, (4) the establishment of a new diffusion potential on the next bilayer within the multilamellar liposome, and (5) the dissociation of gramicidin from the outer bilayer into the adjacent internal aqueous space. These processes are then repeated, in turn, for all the internal bilayers until the K^+ concentration gradient (and membrane potential) is completely dissipated. Process 5 appears to be rate limiting at high gramicidin concentrations, but ion flux, process 3, becomes slower at low gramicidin concentrations where the collapse of the K^+ gradient displays voltage dependence. Of course the rates of these processes can also be manipulated by changing the composition or size of the liposome and by varying the ion concentrations. Since the diffusion potential can be conveniently monitored with a voltage-sensitive fluorescent dye, 3,3′-diethylthiodicarbocyanine iodide [diS-C_2-(5)], a simple method for the detection and partial characterization of membrane pores emerges from this investigation.

The maintenance or dissipation of electrolyte concentration gradients across cell memranes is mediated by protein pores, a term by which we intend here to encompass both channels and carriers. A wide variety of toxins and antibiotics have been identified which behave as transferable pores; a subclass of these, the ionophores, are low molecular weight molecules which are freely soluble in detergent-free aqueous solutions. The detection and characterization of pores have relied heavily upon ion current measurements through voltage-clamped planar lipid bilayer membranes (Meuller & Rudin, 1969; Montal & Meuller, 1972) into which the pores have been

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reconstituted. Details of the voltage dependence, ion selectivity, and molecular mechanism of the transport process can be obtained. On the other hand, the apparatus and expertise required for these experiments are quite specialized and therefore not widely available nor are these experiments adaptable for routine screening of biochemical preparations.

A number of pores have been functionally reconstituted into liposome membranes and their activity monitored via radioactive tracers (which must often be congeneric surrogates for the physiologically active ions) [e.g., see Racker et al., (1975), Gasko et al., (1976), and Kasahara & Hinkle (1977)]. These experiments require complex protocols for the separation of released from residually entrapped tracer; also, multiple sampling of large volumes may be required if a full kinetic profile of the pore activity is desired. More recently several new methods have appeared for monitoring pore-mediated ion transport across liposome membranes. In one approach, several elegant NMR-based methods for monitoring the channel-mediated flux of ^{23}Na across liposome membranes were presented (Degani & Elgavish, 1978; Ting et al., 1981; Pike et al., 1982) but again cannot be widely applicable because of the specialized nature of the apparatus. A study of valinomycin-mediated (Clement & Gould, 1981a) and gramicidin-mediated (Clement & Gould, 1981b) transport employed the fluorescence of an entrapped pH indicator to monitor the proton flux across the liposome membrane following addition of external acid; an ion pore forming material dissipates the diffusion potential generated by the proton flux, allowing complete pH equilibration across the liposome and thus providing an indirect detection method for the incorportion of the pore.

In an effort to characterize and purify a recently discovered pore-forming substance associated with *Entamoeba hystolytica* (Lynch et al., 1982; Loew et al., 1982), we have developed an interesting and convenient alternative to the above procedures. It involves the pore-mediated collapse of a diffusion potential which may be monitored fluorometrically. A multilamellar liposome preparation is employed which displays a stable potential in the absence of pore. The decay of the potential in the presence of pore can be described as a combination of the rate of ion flux and the rate of percolation of the pores through the bilayers of the vesicle. We present here a thorough study of this potential cascade for a valinomycin-mediated K^+ diffusion potential in the presence of gramicidin.

Experimental Procedures

Materials. Crude soybean lecithin (type II), partially purified soybean lipid (type IV), egg lecithin, and dimyristoylphosphatidylcholine were obtained from Sigma Chemical Co., St. Louis, MO. 3,3′-Diethylthiodicarbocyanine iodide [diS-C_2-(5)][1] was obtained from Molecular Probes, Inc., Junction City, OR. Gramicidin D was obtained from Sigma Chemical Co. and valinomycin from Calbiochem, San Diego, CA. Buffers were prepared from double-glass-distilled water. "K^+ buffer" consisted of 50 mM K_2SO_4, 0.5 mM Na_2EDTA, and 50 mM Tris–maleate pH 6.8; "Na^+ buffer" is identical except that Na_2SO_4 replaces K_2SO_4.

Liposomes. A 20-mg sample of soybean lipid is suspenced in 0.5 mL of K^+ buffer in a 15 × 125 mm test tube. The suspension is then vortexed for 10 min and sonicated in a bath-type sonicator (Laboratory Supplies, Hicksville, NY) for 5 min under a nitrogen atmosphere. The clear light yellow preparation is then quick-frozen in liquid nitrogen, thawed, and resonicated for 30 s as described by Pick (1981) [cf. Kasahara & Hinkle (1977)], affording a cloudy liposome suspension. Unless otherwise noted, liposomes prepared in this way from soybean type II lecithin were used. Negatively stained samples are examined with a Philips EM201 transmission electron microscope. Grids are prepared by placing a drop of a 1:1 mixture of the liposomes (diluted to 5 mg/mL lipid or less) and 4% ammonium molybdate, pH 7, on a parlodion/carbon grid and blotting off the excess after 1 min of settling.

Diffusion Potential. The procedure of Sims et al. (1974) is used to monitor diffusion potentials across the liposome membranes. A 3-μL aliquot of the liposome suspension is added to 3 mL of Na^+ buffer containing 1 μM of the voltage-sensitive dye diS-C_2-(5). The 1000:1 K^+ concentration gradient results in a diffusion potential upon addition of 10^{-8} M valinomycin. A 90% quench of the dye fluorescence accompanies development of the potential, and fluorescence is recorded as the potential dissipates. Fluorescence is excited at 620 nm and monitored at 670 nm in a magnetically stirred cuvette with either a Perkin-Elmer model 1000M filter fluorometer or a Perkin-Elmer Model MPF44B fluorescence spectrometer. The cuvette temperature is thermostatically maintained at 25 °C unless noted otherwise.

Results

Fluorometric Detection of Pore-Mediated Collapse of a Liposome Diffusion Potential. Under the conditions described under Experimental Procedures and adapted from Sims et al., (1974), the permeant cyanine dye diS-C_2-(5) binds to liposomes, and, if the lipid concentration is sufficiently low, dye aggregation occurs accompanied by fluorescence quenching. Creation of a membrane potential negative on the inside drives more dye into the membrane, leading to additional fluorescence quenching. We have found that a very stable K^+ diffusion potential can be obtained with soybean lipid liposomes if relatively low concentrations of valinomycin are employed to specifically carry K^+ down its concentration gradient. Figure 1 illustrates that with 10^{-5} M valinomycin a potential develops instantaneously and is stable for over 30 min. Concentrations of valinomycin in the micromolar range led to unstable potentials possibly via the onset of facilitated Na^+ transport or the formation of second-order complexes capable of anion transport. Also noteworthy in Figure 1 is the slow downward drift of the fluorescence even before addition of valinomycin; this may be due to a small intrinsic K^+ permeability.

In Figure 2, the presence of 10^{-8} M gramicidin leads to a rapid recovery of the pre-valinomycin fluorescence level. Clearly, an equilibration of ion concentrations occurs upon addition of gramicidin and continues after valinomycin is added until the ion gradients, and therefore the transmembrane potentials, are abolished. The depth of the minimum in the curve and the rate of recovery are only slightly dependent on the time interval between the additions of gramicidin and valinomycin. Also apparent from Figure 2 is that addition of a nonspecific ionophore like gramicidin halts the inital downward drift of the fluorescence, providing a convenient reference level for subsequent fluorescence changes. In general, this would be an important reason for choosing to add the unknown matieral before the valinomycin in an assay protocol.

A quantitative description of the shapes of the curves in Figure 2 is not possible because of the complexity of the experimental system. There are at least two permeant ions, Na^+ and K^+, with transport facilitated by a dimeric channel and carrier, respectively. The diffusion potential at any given point

[1] Abbreviations: diS-C_2-(5), 3,3′-diethylthiodicarbocyanine iodide; EDTA, ethylenediaminetetraacetic acid; tris, tris(hydroxymethyl)-aminomethane.

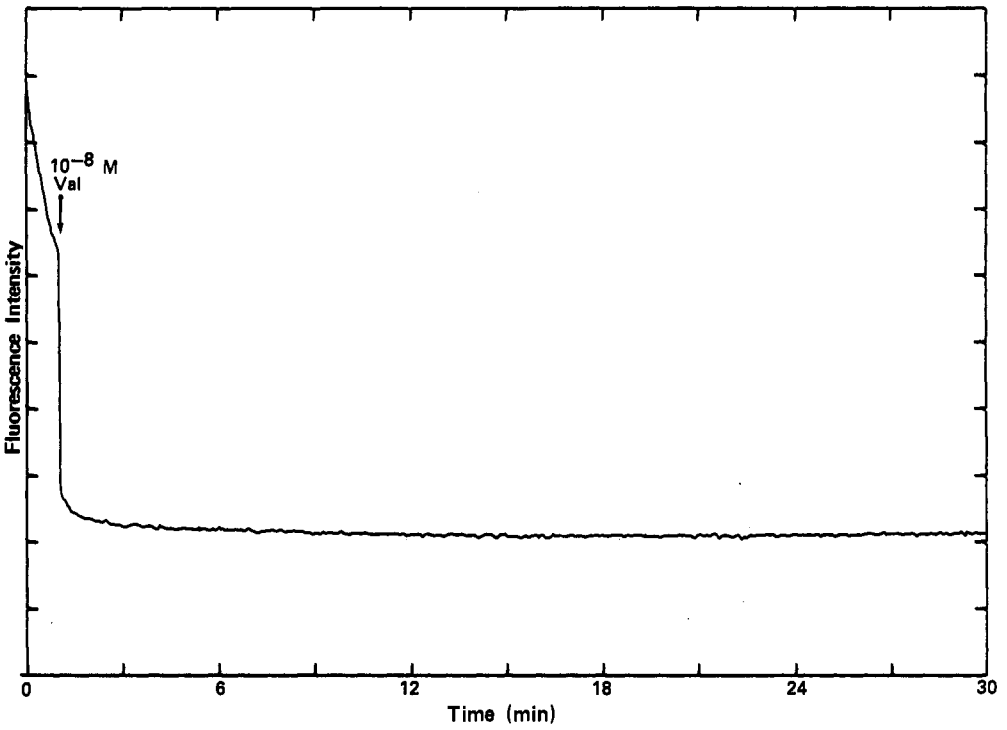

FIGURE 1: Fluorescence of di-S-C_2-(5) is quenched upon addition of valinomycin to a liposome suspension with a 1000:1 inside:outside K^+ concentration gradient. The fluorescence remains quenched for >30 min after which it slowly decays back to the initial level. Conditions are described under Experimental Procedures.

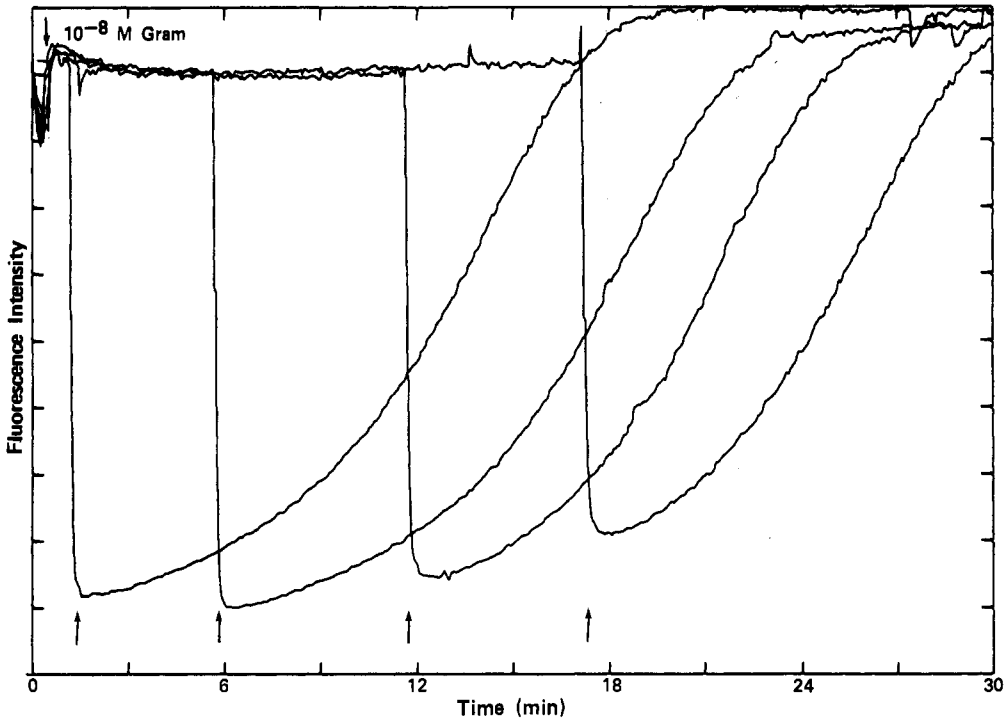

FIGURE 2: Series of four experiments in which valinomycin is added at varying times, indicated by the arrows, following the addition of 10^{-8} M gramicidin to the vesicle–dye preparation.

is logarithmically related to the instantaneous concentration gradients and permeabilities of these ions across the vesicle membranes. As will be detailed later, these concentration gradients and permeabilities are markedly heterogeneous with respect to the bilayers in the vesicle suspension at any given time prior to complete equilibration. The fluorescence quenching is at least approximately linear with potential for a homogeneous system and can therefore give a qualitative indication of ion gradients, however. Further, the simple fact that valinomycin induces such a dramatic quench even in the presence of gramicidin indicates that K^+ is providing the dominant contribution to the diffusion potential and that the fluorescence recovery curves may be related directly to the dissipation of the K^+ concentration gradients; K^+ efflux is, nevertheless, coupled to and limited by the rate of gramicidin-mediated Na^+ influx.

That gramicidin alone effects only a very slow dissipation of concentration gradients can be established by examining

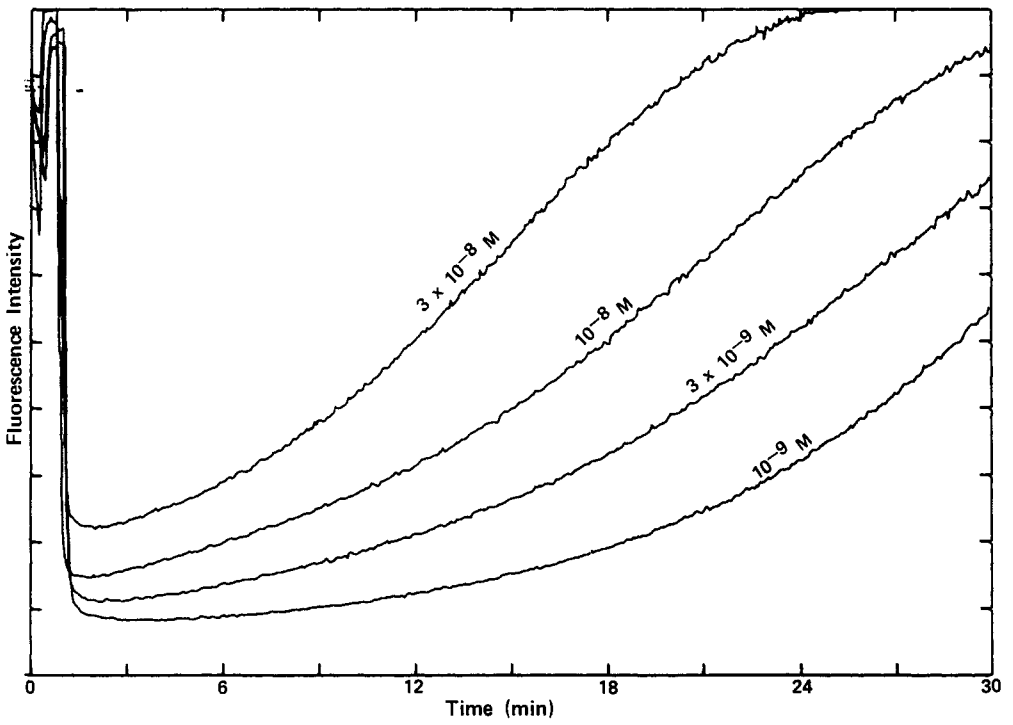

FIGURE 3: Series of experiments with 10^{-8} M valinomycin added immediately following the addition of varying concentrations of gramicidin. The gramicidin concentrations are indicated on the traces.

the levels of fluorescence quenching immediately following the additions of valinomycin in Figure 2. Since the actual K^+ efflux required to establish the diffusion potential is insignificant, these initial post-valinomycin fluorescence levels can be used as an indication of the K^+ gradients following varying incubation times with gramicidin. Clearly, gramicidin alone has a relatively small effect on the K^+ gradient over a 15-min period. On the other hand, the fluorescence recovery time for any one curve is much faster once both ionophores are present, indicating that the K^+ gradient has collapsed almost completely 15 min after valinomycin is introduced. The potential accelerates the influx of Na^+ through the gramicidin channels, allowing the K^+ gradient, in turn, to dissipate more rapidly via valinomycin. Thus, at least qualitatively, the system follows the prediction of the Nernst–Planck flux equation.

Variation of Gramicidin Concentration. Figure 3 shows that the dissipation of the diffusion potential is accelerated with increasing gramicidin concentation. Apparently, however, this concentration dependence is nonlinear and appears to be approaching saturation. This is illustrated more clearly in Figure 4, where the inverse of the time required for 50% recovery of fluorescence is plotted against gramicidin concentration; we have found this to be a convenient parameter which summarizes the rate of fluorescence recovery but attach no special theoretical significance to it.

One might attempt to rationalize this saturation behavior by postulating a Na^+ permeability so high that it no longer limits K^+ efflux. However, if gramicidin-mediated Na^+ permeability were so high as to swamp out the K^+ flux, one would expect a diffusion potential of reversed polarity to reflect the reversal of the concentration gradient for the dominant charge carrier (Goldman, 1943; Hodgkin & Katz, 1949); i.e., valinomycin could not induce a diffusion potential. Furthermore, gramicidin is relatively nonselective with a permeability for K^+ that is actually slightly higher than that for Na^+ (Hladky & Haydon, 1972).

Experiments equivalent to those described in Figure 2, but with a saturating gramicidin concentration, give strikingly

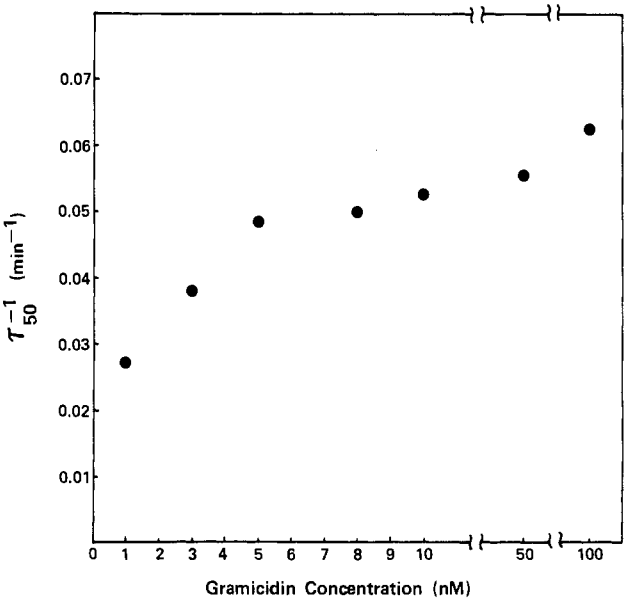

FIGURE 4: Relation between gramicidin concentration and the reciprocal of the time required for 50% recovery of the fluorescence following valinomycin-mediated quench.

different results (Figure 5). In Figure 5, the concentration gradients are being dissipated at the same rate by gramicidin alone as by the combination of both ionophores. Clearly, the decay of the K^+ concentration gradient is no longer significantly accelerated by the electrical potential or by the presence of valinomycin.

Rate-Limiting Percolation of Gramicidin Channels through the Lamella. Upon exposure to 1 μM gramicidin, an instantaneous dissipation of concentration gradients across bilayers composed of only 50 μMn lipid wouild be anticipated [compare Pike et al., (1982), where a 1000-fold greater lipid:gramicidin ratio was employed]. Thus, the behavior in Figure 5 suggests that the membranes in the vesicle suspension

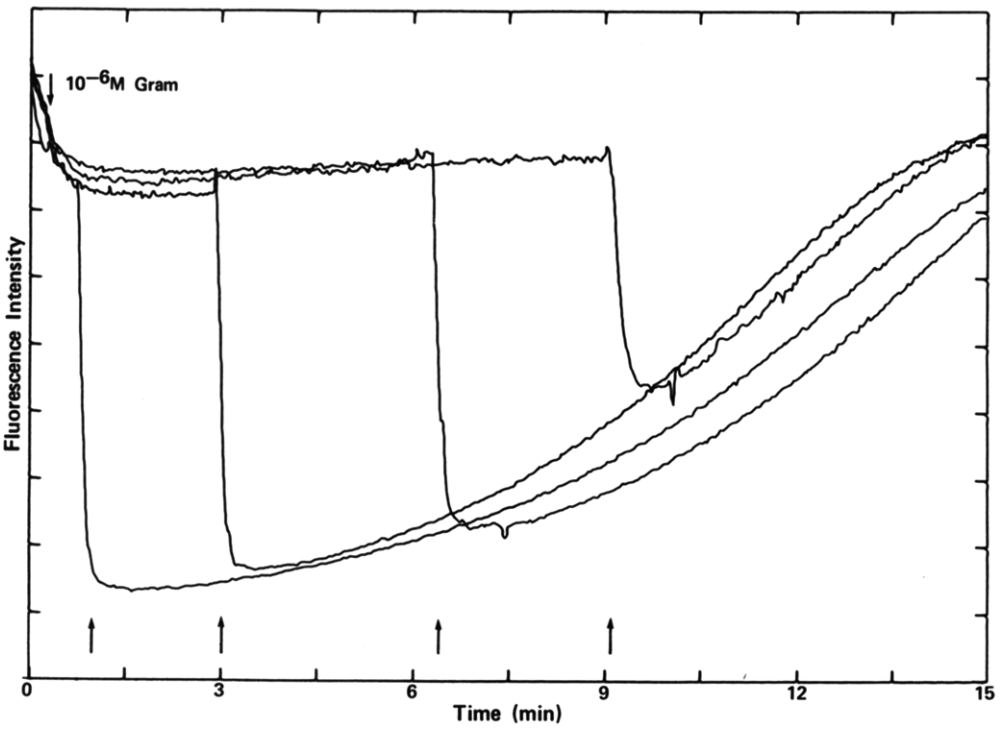

FIGURE 5: Series of four experiments in which valinomycin is added at varying times, indicated by the arrows, following the addition of 10^{-6} M gramicidin.

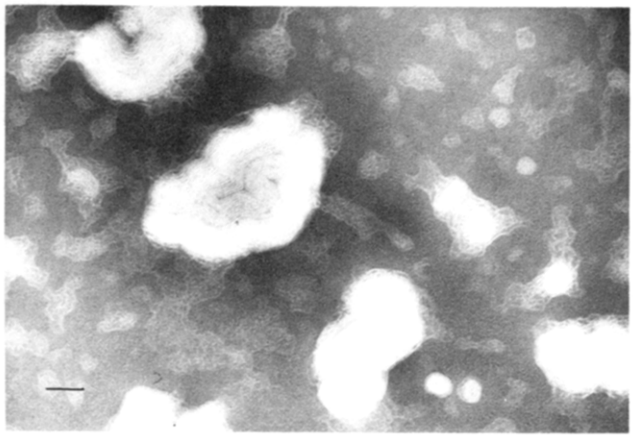

FIGURE 6: Electron micrograph of type II freeze–thawed vesicles. The bar corresponds to 1000 Å.

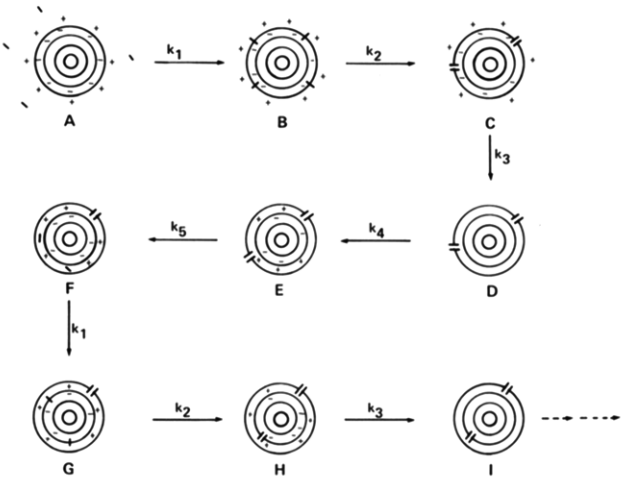

FIGURE 7: Simplified model for the series of processes involved in the potential cascade through multilamellar vesicles. Rate 1 corresponds to binding of gramicidin molecules, represented by the bars, to a bilayer (each circle represents one bilayer); process 2 corresponds to the rate of dimerization of gramicidin molecules within the bilayer to form channels; process 3 is the ion flux through the channels which eliminates the preexisting diffusion potential across the bilayer; process 4 corresponds to the establishment of a new valinomycin-mediated diffusion potential across the next bilayer; the dissociation of gramicidin molecules from the bilayer is represented by k_5. For a population of vesicles, these processes will proceed nonconcertedly through the lamella until all the internal aqueous compartments are equilibrated with the external medium.

are not all equally accessible and that the limiting step for the saturating gramicidin concentrations may be transfer of channels. Electron micrographs of the vesicle preparations reveal well-defined multilamellar structures (Figure 6). The series of processes depicted in Figure 7, therefore, must all be considered in any attempt to rationalize the experimental results. The results of Clement & Gould (1981b) are very helpful in the analysis of the rate process. They employed small unilamellar soybean vesicles at a similar lipid concentration in an investigation of the development of gramicidin channels. It was found that (a) channels developed rapidly (within 30 s for $\sim 10^{-8}$ M gramicidin), (b) ion equilibration was also rapid (unresolved on their time scale), and (c) transfer of gramicidin between vesicles was very slow (undetected after 1 h). Thus, processes 1, 2, and 3 in Figure 7 are unlikely to be rate limiting at 10^{-6} M gramicidin; similarly, the ready migration of valinomycin between lipid and aqueous phases [e.g., see Clement & Gould (1981a)] along with the rapid development of potential, displayed in Figures 1, 2, 3, and 5, attests to the speed of process 4. Process 5, therefore, is most likely to be limiting and, assuming that the gramicidin binding sites are saturated, accounts for the saturation of the gramicidin concentration dependence. It also accounts for the insensitivity to potential of the rate at which the ion concentration gradients are dissipated (Figure 5) at these saturating gramicidin concentrations; a potenital should have the greatest effect on the rate of process 3. Conversely, the fact that potential does accelerate the observed rate at the low gramicidin concentrations (Figure 2) indicates that the rate of

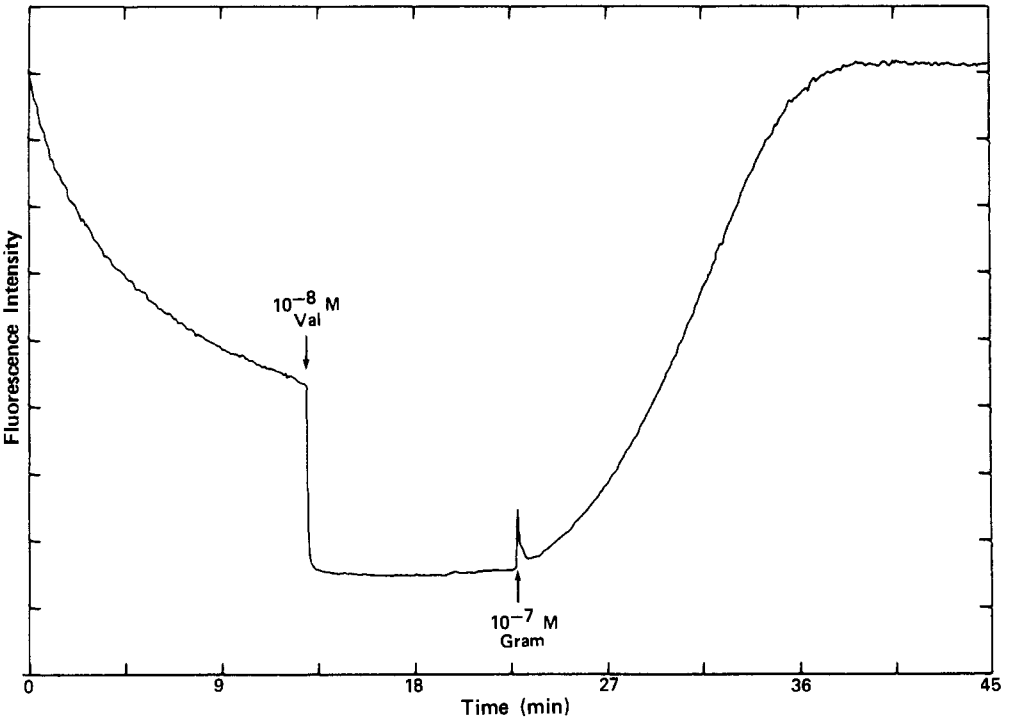

FIGURE 8: Experiment in which valinomycin is added prior to 10^{-7} M gramicidin. The spike in the tracing after gramicidin addition is *not* an artifact and is rationalized in the text.

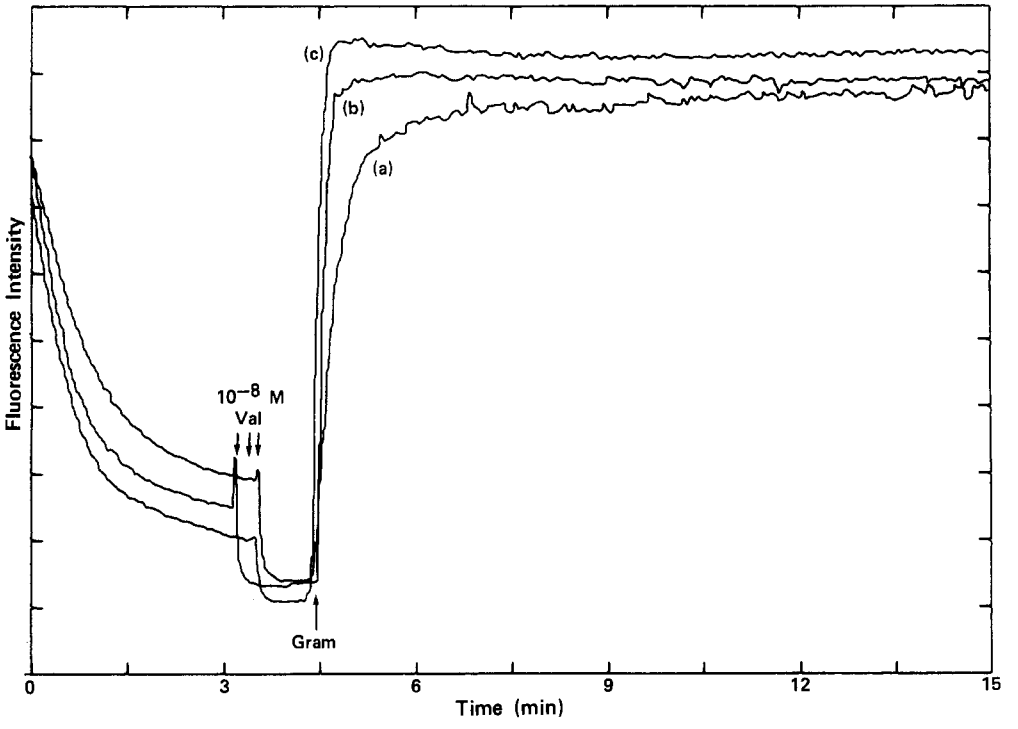

FIGURE 9: Series of experiments with small unilamellar vesicles in which 10^{-8} M valinomycin is added first and is followed by (a) 10^{-8}, (b) 10^{-7}, or (c) 10^{-6} M gramicidin.

process 3 must then be at least comparable with that of process 5.

A corroboration of this reasoning is offered by the rather dramatic results of simply reversing the order of addition of the two ionophores (Figure 8). As expected, addition of 10^{-7} M gramicidin, after the valinomycin-mediated K^+ diffusion potential has already been established, leads to a small but rapid recovery of fluorescence corresponding to fast rates for the first three processes in Figure 7. However, this rapid fluorescence recovery is checked prior to completion by the onset of process 4—a potassium diffusion potential is possible on the second bilayer only after K^+ has been exchanged for Na^+ in the aqueous layer surrounding it. Finally, the fluorescence resumes its recovery, albeit more slowly, with the rate limited, once again by the nonconcerted percolation of gramicidin through the lamella (process 5).

Other Variables Affecting the Rate of Fluorescence Recovery. For comparison, the results of inverted addition experiments with vesicles not subjected to the freeze–thaw step are shown in Figure 9. These vesicles are, of course, much

smaller and are unilamellar to a much greater extent than vesicles after freeze–thawing. As expected, 10^{-6} M gramicidin gives an instantaneous fluorescence recovery with essentially none of the subsequent slow phase seen for the multilamellar vesicles. Only at 10^{-8} M is there an indication of a slower recovery consistent with rate-limiting channel development (Clement & Gould, 1981b). Also noteworthy in these tracings is the steep pre-valinomycin fluorescence drop, indicating a significant unfacilitated K^+ permeability. In general, unstable valinomycin-mediated K^+ diffusion potentials were obtained with these small soybean type II vesicles even in the absence of gramicidin; typical 50% fluorescence recovery times were on the order of only 5 min.

Several other vesicle preparations with differing lipid composition were also tried. Soybean "type IV" lipid gave somewhat more stable vesicles which were more sluggish in their response to gramicidin. The "lightly sonicated" egg lecithin–cholesterol vesicles, described by Sims et al. (1974) in their original examination of voltage-sensitive dyes, proved even more sluggish. Synthetic dimyristoylphosphatidylcholine was used to prepare small unilamellar vesicles by sonication with a probe-tip sonicator above the phase transition temperature. These vesicles did not appear capable of supporting stable diffusion potentials.

Returning to the standard type II freeze–thaw preparation, we examined the temperature dependence of the fluorescence recovery. The experiments described up to now were all carried out in a thermostated cuvette holder set at 25 °C. Lowering the temperature to 15 °C led to no perceptible fluorescence recovery over a 30-min period in the presence of 10^{-8} M gramicidin. On the other hand, increasing the temperature to 37 °C gave a 50% fluorescence recovery time, 6 times faster than that at 25 °C. It should be noted here that higher temperature did somewhat diminish the stability of the diffusion potential in the absence of gramicidin as well.

Cutting the vesicle concentration decreases the fluorescence recovery time proportionately. Likewise, decreasing the initial internal K^+ concentration, while maintaining a constant K^+ gradient, effects a proportional increase in the rate of recovery. In both cases, the experiments were carried out with 10^{-8} M gramicidin and 10^{-8} valinomycin. These results simply reflect the relation between the time required to collapse the potential and the number of ions which must be transported to do so. Of course, the base-line stabilities (i.e., valinomycin only) of the potentials are also quite sensitive to these variations.

As has already been mentioned, high valinomycin concentrations (micromolar) lead to unstable base-line potentials. Systematic variations of the valinomycin concentrations were tested between 5 and 40 nM, in which range no appreciable destabilization of the base line was detected. For 10^{-6} M gramicidin (i.e., saturating concentration), the recovery rate was only slightly enhanced by raising valinomycin levels in this range. For 10^{-9} M gramicidin, however, the recovery rate was nearly proportional to valinomycin concentration with a 50% fluorescence recovery reached after only 25 min for 40 mM valinomycin (compare Figure 3). The origin of this synergistic behavior is not apparent.

Discussion

The large multilamellar vesicles employed as the primary experimental system are easily prepared and provide very stable potentials. The procedure was adapted from that of Pick (1981) which produces mainly large unilamellar vesicles; it is probable that the high ionic strength of the buffers employed in this work causes the differing vesicle structure. The other vesicle types which were surveyed did not provide the combination of stability or reproducibility which may make the method generally useful.

The procedure described in this work represents a simple approach toward detection of transferable pores. It does not require specialized equipment, and the reagents are readily available. The lipid vesicle preparation, in particular, does not require dialysis, gel filtration, or centrifugation steps; 0.5 mL of this lipsome suspension is sufficient for over 150 experiments and can be stored at 4 °C for several days. In some cases, with completely uncharacterized pore-forming activity, it may be necessary to check for lysis of the vesicles; the most convenient way to do this is by monitoring the release of large molecules entrapped in the aqueous interior [although this has been shown to accompany ion transport in at least one study (R. Korenstein, unpublished results)]. Also, the behavior in Figure 2 would not be possible for a lytic process so that such an experiment could be diagnostic for pores.

The distinction of ion flux from pore transfer has been achieved for gramicidin, and the experiments which provide this distinction should be applicable to other pore formers as well. Both processes are important characteristics of a transferable pore. Although the analysis cannot be carried sufficiently far so as to obtain actual rate constants, gramicidin can serve as a standard for comparison with unknown pore-forming activity with respect to the relative efficiencies of these processes.

Variations in buffer composition should allow the further characteristics of ion selectivity to be probed. Gated pores should give particularly interesting voltage-dependent behavior. It will also be of interest to contrast the behavior of a carrier (e.g., monensin) with that of gramicidin. It might be anticipated that carriers would show unsaturable kinetics with much slower ion flux rates. In general, the sensitivity of an assay system may be adjusted to meet these diverse situations by varying cation or vesicle concentrations and temperature.

Acknowledgments

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Registry No. diS-C_2-(5), 514-73-8; K, 7440-09-7; gramicidin D, 1393-88-0; dimyristoylphosphatidylcholine, 13699-48-4; valinomycin, 2001-95-8.

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Properties and Characterization of Binding Protein Dependent Active Transport of Glutamine in Isolated Membrane Vesicles of *Escherichia coli*†

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ABSTRACT: The reconstituted binding protein dependent active transport of glutamine in isolated membrane vesicles of *Escherichia coli* [Hunt, A. G., & Hong, J. (1981) *J. Biol. Chem. 256*, 11988-11991] is characterized in some detail. Transport activity exhibits a rather narrow pH optimum at about 5.8 with apparent pK_as of 5.3 and 6.6, is inhibited by increasing ionic strength, and requires potassium and phosphate ions. However, the binding of glutamine to the glutamine binding protein is unaffected by pH over a range of 5-8, is relatively insensitive to variation in ionic strength up to 1.0 M KCl, and does not require potassium and phosphate ions. Since the internal pH of vesicles does not change over the range of 5-8, the pH-dependent transport profile most probably reflects the interaction of liganded glutamine binding protein with the membrane-bound components of the glutamine transport system. Two classes of compounds can serve as exogenous sources of energy for glutamine transport. One class consists of those compounds that can be metabolized to pyruvate. This class of compounds is effective only if NAD is incorporated into vesicles, and only if vesicles are prepared from strains containing active phosphotransacetylase and acetate kinase. The second class of compounds, of which succinate is the sole member, is effective in vesicles containing only those small molecules present in the lysis buffer, and in vesicles prepared from phosphotransacetylase and acetate kinase mutant strains as well as from the parent PSM116. It appears that succinate or a yet to be determined metabolite derived from succinate or a common product of pyruvate and succinate metabolism is the energy donor for glutamine transport. ATP and/or acetyl phosphate are found to be inactive as a source of energy in vesicles. Substances that abolish the electrochemical proton gradient ($\Delta\bar{\mu}_H$), either by conducting protons across the vesicular membrane or by halting respiration, inhibit glutamine transport in vesicles. However, it appears that $\Delta\bar{\mu}_{H^+}$ is required for glutamine transport for a role other than serving as an energy donor as in the $\Delta\bar{\mu}_{H^+}$-driven shock-resistant transport. The membrane vesicle preparations described here possess considerable metabolic capabilities. Vesicles are capable of incorporating ^{32}P from P_i into ATP, ADP, AMP, GTP, acetyl phosphate, and several unidentified phosphate-containing compounds, indicating the presence of pyruvate dehydrogenase, phosphotransacetylase, acetate kinase, Mg^{2+}-ATPase, adenylate kinase, nucleoside (AMP) phosphatase, nucleotide (adenosine) kinase, and nucleoside diphosphate kinase in vesicles.

The glutamine transport system of *Escherichia coli* is one of those whose activity is abolished by a cold osmotic shock treatment of whole cells [for a review, see Wilson (1978)]. Shock-sensitive transport systems possess this unique property because of the involvement of soluble, periplasmic substrate binding proteins in transport. Osmotic shock releases these proteins from cells, rendering these transport systems inactive. Shock-resistant transport systems, on the other hand, require only cytoplasmic membrane-bound components for their activity and are therefore not affected by the loss of periplasmic proteins.

The involvement of the glutamine binding protein in glutamine transport has been firmly established. This protein is seen to bind glutamine with an affinity for glutamine very similar to that of transport in whole cells (Weiner & Heppel, 1971). Removal of this binding protein, by either osmotic shock (Weiner & Heppel, 1971), mutation (Masters & Hong, 1981a), or lypozyme-ethylenediaminetetraacetic acid (EDTA)[1] treatment (Masters & Hong, 1981b), greatly diminishes glutamine transport. Moreover, addition of purified glutamine binding protein to spheroplasts restores the glutamine transport ability of spheroplasts (Masters & Hong, 1981b).

Very little is known about the membrane-bound components of the glutamine transport system of *E. coli*. The existence of these components has been proven by Masters & Hong (1981b), but their number and properties are not established.

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[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; $\Delta\bar{\mu}_{H^+}$, electrochemical proton gradient; $\Delta\psi$, electrical potential; ΔpH, chemical gradient of hydrogen ions; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; *ack*, acetate kinase; *pta*, phosphotransacetylase; DON, 6-diazo-5-oxo-L-norleucine; PEP, phosphoenolpyruvate; CoA, coenzyme A; Mops, 4-morpholinepropanesulfonic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; PEI, poly(ethylenimine).

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