Biochimica et Biophysica Acta, 375 (1975) 10–21

BBA 76840

INCREASE IN FLUORESCENCE ENERGY TRANSFER ACROSS LIPID BILAYERS INDUCED BY VALINOMYCIN

FRANCE BESSETTE and WOLF D. SEUFERT

Département de Biophysique, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, Québec (Canada)

(Received May 24th, 1974)

SUMMARY

The translocator antibiotic, valinomycin, increases the energy transfer between fluorophores across a lipid bilayer membrane, contrary to the effect of an inert protein adsorbate. The distance separating the fluorophores is reduced, suggesting that this translocator provokes a perturbation in the palisade arrangement of lipid molecules in the bilayer.

INTRODUCTION

The principle governing ion transfer processes across biological membranes seems closely associated with the nature of lipid–protein interaction. The model approach towards an eventual explanation of the molecular basis of these phenomena has met with considerable success: molecules of defined physical and chemical characteristics can impart on lipid bilayers practically all the functions by which an excitable membrane's behavior is characterized [1, 2]. Interaction of these translocators with bilayers is thus regarded as one model case for the lipid–protein cooperativity in biological membranes.

Several current hypotheses derived from the effects of translocators on bilayers imply that the palisade array of lipid molecules is perturbed. The existence of such perturbations could be demonstrated and their extent assessed by extrapolation from changes in the membranes' electrical characteristics. With a system of energy-coupled fluorophores as recently described by Bessette and Seufert [3], dimensional membrane parameters can be monitored directly. We used this resonance method and the results reported here indicate that the translocator antibiotic valinomycin induces perturbations in the lipid bilayer membrane.

A transfer of energy between two fluorophores across a separating phase of optimal thickness is possible when their respective emission and excitation bands overlap [4, 5]. A donor fluorophore is selected with an emission band in the region of excitation of an acceptor molecule which, in turn, starts fluorescing at its specific emission wavelength. This transfer of energy permits the detection of variations in the distance of closest approach between the fluorophores [6] provided they are

fixed in space and their physical separation is assured. Both requirements are fulfilled with our preparation of vesicles in suspension, bounded by a single lipid bilayer membrane [7]. The vesicles can be prepared to trap solutions of water-soluble molecules [8]. In the experiments given here, their lumina contain acriflavine and they are suspended in an acriflavine-free aqueous phase. 8-Anilino-1-naphthalene sulfonate (ANS) added to the outside aqueous medium starts fluorescing only once attached to the lipid. A shift of the fluorescence emission from the ANS to the acriflavine peak in this combined system indicates a transfer of energy across the bilayer membranes. We should then be able to detect persistent perturbations in the molecular arrangement of the separating bilayer as changes in the resonance mode on which an energy transfer is based, with respect to the unmodified system.

The relative yield of ANS fluorescence in a particular solvent can, by itself, give information as to the immediate dielectric environment of this fluorophore. Systematic studies on such fluorescence enhancement and its maximum emission wavelength have led to the conclusion that ANS binds to lipid molecules in their polar–apolar region [9, 10]. Eventual conformational changes induced by translocators in membranes could, however, go undetected since the immediate environment of the fluorophore conceivably remains unaltered during a perturbation of the membrane's structure. A transfer of energy between two fluorophores provides us with an additional parameter, namely, the relative ANS displacement under the perturbing influences of the translocator. As has been demonstrated previously [3], the efficiency of energy transfer, as given either by a shift of the maximum emission wavelength and/or changes in the emission amplitude, are the criteria by which the question as to eventual conformational changes in the bilayer can be resolved.

MATERIALS AND METHODS

Preparation of lipid bilayer vesicles. Asolectin (Associated Concentrates Inc., Woodside, New York) was homogenized in pH 7.4 buffer of low ionic strength (10 mM tris, 0.5 mM Na–EDTA, 0.25 M sucrose). Suspensions of single lipid bilayer vesicles were then prepared by high-energy sonication and subsequent centrifugation as described by Seufert [7]. For the experiments requiring an acriflavine solution in the vesicles' lumina only, the suspensions were prepared by homogenizing lipid in tris as above, but to which buffer an amount of acriflavine to give the desired 'inside' concentration had been added. In order to then eliminate acriflavine from the 'outside' suspending fluid, the preparations were chromatographed on Sephadex G-25 columns. Tests to assure that the suspending fluid was cleared of acriflavine are described in detail later.

Reagents. The suppliers of the principal chemicals were: K and K Laboratories Inc., Plainview New York, for 8-anilino-1-naphthalene sulfonic acid, sodium salt (ANS) and for acriflavine; Schwarz/Mann, Orangeburg New York, for protamine sulfate, salmine; Calbiochem, La Jolla Calif., for valinomycin, grade A. Solutions of these materials were prepared with the tris buffer also used in the preparation of the vesicles. Valinomycin was dissolved in tris from a 10^{-4} g/ml ethanol stock solution.

Instrumentation. A Zeiss model DMR-21 double-beam recording spectrophotometer was employed, with all accessories for conversion to a spectrofluorimeter including excitation monochromator, polarizing and analyzing filters. The sample

compartment was thermostated at 22 ± 0.1 °C throughout this series of experiments. Polarization was measured with unpolarized and horizontally polarized incident light. The results were corrected for instrumental depolarization and for depolarization due to scattering. All pertinent equations are given by Perrin [11], Kasai et al. [12], or Udenfriend [13]. The spectral scans were reproducible to within 1 %.

RESULTS AND DISCUSSION

As a basis for a valid comparison with eventual translocator effects, a substance had to be found which binds to the membrane but presumably leaves the palisade arrangement of lipid molecules in the bilayer unaltered. Protamine sulfate, a large polypeptide, is a very inert adsorbate judging by the fact that its addition does not provoke any changes in the electrical characteristics of unmodified lipid films. It has no translocator function of its own but can induce anion-permselectivity in artificial membranes modified by 'excitability-inducing material' [14] or alamethicin [15], or it can block gating phenomena produced by monazomycin [16]. These reactions imply that protamine adsorbs to the membrane. The final concentration of protamine sulfate in our experiments ($5 \cdot 10^{-6}$ g/ml) is approx. the same as that used in the studies on planar bilayers. The depsipeptide antibiotic valinomycin, on the other hand, is known to profoundly affect electrical membrane phenomena [16] and several mechanisms by which it could interact with the membrane to fulfill its role as ion translocator have been suggested. Again, the final concentration of valinomycin in the experiments presented here (10^{-6} g/ml) is identical to that producing the 'active' behavior of planar bilayer films, summarized in the N-shaped current-voltage curves of membranes thus treated [1].

All precautions were taken to make the experiments described in the following sections directly comparable: the measurements were performed, when feasible, on the same lipid preparation (usually one day old), they were directly preceded by reference experiments, and they were always made with identical volume ratios of lipid suspension to the solutions of fluorophore, protamine, or translocator. Unless otherwise noted, C/10 lipid preparations were used, i.e. lipid suspensions diluted to 1/10 the original concentration of 0.1 g lipid per ml buffer. The absolute fluorescence intensities were, generally, reproducible to within 1 per cent. Slight variations occurred at times from one series of experiments to the next. However, the proportionality of intensities in each set was always maintained.

In order to ascertain that the membrane reagents added had not aggregated or destroyed the vesicles and thereby rendered spurious the recorded fluorescence levels, only concentrations of protamine or valinomycin were used that did not bring about a change in the scattering level as compared to the vesicle/ANS system. Both the exciting and the emitted light are subject to multiple scattering with different intensity loss functions and different angular patterns depending on the type of scatterer present. We minimize differences in the scattering level of the exciting light, and the intensity of the incident light cascading with multiple scattering to the nth degree will prompt emission of a proportional loss profile. The incorporation of protamine or the translocator antibiotic into lipid bilayers in the concentrations used here produces only negligible changes in the refractive index of the membranes. This is not surprising in view of the virtually identical values ($n = 1.46$) reported for bilayers

formed from different lipids [17, 7, 18]. As important variations in the membrane's refractive index are unlikely in the situation described, we then measure actual differences in the fluorescence intensity, not superimposed scattering changes. The final concentrations of protamine and valinomycin under these precautionary measures conveniently fell within the range for which their electrical effects on bilayers had been described. We can then be reasonably sure that variations in the fluorescence intensity represent changes in the state of the intact membrane.

I. On the distribution of ANS within the bilayer

The possibility that ANS detaches itself from its initial site at the membrane and diffuses through it, was deemed of consequence for later transfer experiments. Should ANS leave its binding site at the outside membrane interface and go deeper into the hydrophobic core, a change in the maximum emission wavelength would be expected [19]. If ANS were to diffuse through this paraffinic core and bind to the lipid polar–apolar region on the inside bilayer interface, a different time course to reach equilibrium ought to be seen between the case where ANS is added all at once and the case where it is added in fractions over a period of time to give the same final concentration. In the latter case, the membrane is initially offered a lower gradient.

Fluorescence spectra of C/10 lipid were taken in intervals of 3 min and over a 21 min period with 50, 100, 150, 200, and 250 μM ANS. A stationary state of fluorescence was reached after 12 min, and after 21 min 50 μM ANS corresponding to the next higher initial concentration in the set were added to each of these samples. The time course was followed again until no further change in intensity was observed. In all cases, polarization was identical and maximum emission was at the same wavelength (482 nm). The dI/dt functions for the same final concentrations went closely parallel and the same plateau was attained after the same time. It therefore appears not likely that ANS diffuses through the membrane within the period during which all later experiments were performed.

II. The effects of protamine and valinomycin on ANS fluorescence

The efficiency of an energy transfer between the fluorophores ANS and acriflavine has to be based on the ANS fluorescence pattern under the various experimental conditions to which we refer here. Not only can the addition of a membrane reagent result in a change of the ANS fluorescence yield with the lipid membrane as its solvent but the emission maximum can also be displaced. This phenomenon is, in fact, used to determine a fluorophore's specific dielectric environment and to localize it in a heterogeneous matrix [20, 21]. Pre-requisite to an interpretation of later transfer results was, therefore, a series of experiments accounting for the effects of protamine and valinomycin on the ANS/lipid fluorescence alone. For further identification of possible structural re-arrangements in the membrane proper, the ANS yield had to be determined in relative terms before and after the addition of protamine or valinomycin to the lipid suspension. The results of these experiments are given in Fig. 1.

The slight variation in the absolute scattering intensities (approx. 10 %) at 390 nm between the two sets of experiments illustrated are partly due to small variations in the lipid content of the preparations and, in part, to long-term fluctuations

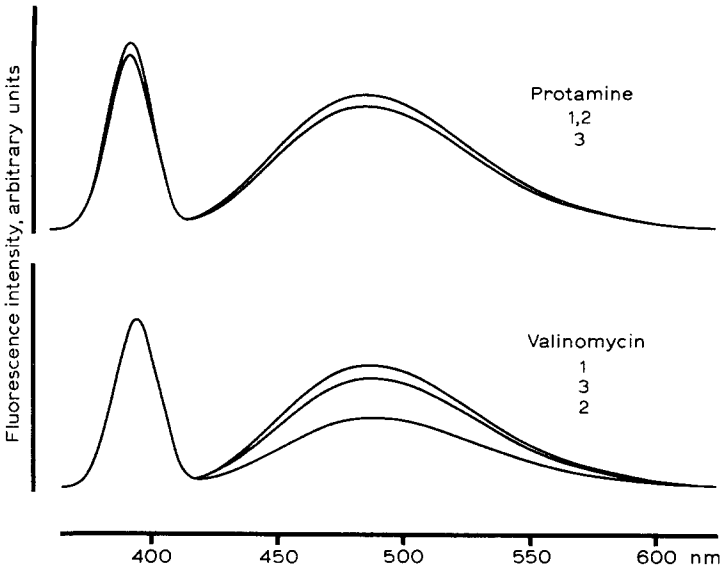

Fig. 1. Effect of protamine or valinomycin on ANS fluorescence in bilayer membranes. Emission spectra of 100 μM ANS (final concentration) in C/10 lipid. Intensity given in arbitrary but equal units; λ_{exc} = 390 nm, peak emission in all cases at 482 nm. Reference spectra designated 1, lipid/ANS +tris; 2 and 3 refer to the order in which ANS and protamine or valinomycin were added to the system; 2, ANS, then membrane reagent after 15 min; 3, membrane reagent first, ANS added after 15 min. Final concentrations of protamine $5 \cdot 10^{-6}$ g/ml, of valinomycin 10^{-6} g/ml.

in the Xenon lamp's output. The response in fluorescence and in scattering to such fluctuations is not parallel. When these experiments were repeated with different lipid preparations, the relation of fluorescence intensities in each family of curves was quantitatively reproduced.

Each set of measurements comprises 3 curves. For the reference spectrum (curve 1), a volume of tris buffer corresponding to that of the reagent solution was added to lipid+ANS. To obtain curve 2, protamine or valinomycin were added after, for curve 3 before, the fluorophore ANS. The maximum in all emission spectra is at 482 nm indicating that the immediate environment of ANS in the lipid remains unchanged by the membrane reagents irrespective of the order of addition and even in the presence of valinomycin.

Protamine binds to the membrane but the fact that curves 1 and 2 are coincident demonstrates that it does not influence the ANS/lipid interaction when added after the fluorophore. The 9 % decrease in fluorescence of curve 3 as compared to its reference cannot be explained with a decreased negativity in the bilayer's surface charge under protamine since results by Rubalcava et al. [9] seem to indicate that ANS fluorescence is generally enhanced when positive charges are introduced. Protamine could, rather, shield the membrane from ANS. No ANS fluorescence is observed in the presence of protamine alone at the concentrations used.

Lipid/ANS fluorescence is decreased by valinomycin. Curve 2 (sequence of addition ANS/valinomycin): the 42 % decrease could be interpreted as either resulting from a strong competition between ANS and the electrically neutral ionophore

for the lipid, or it could mean that the membrane is somehow re-arranged by valinomycin with ANS following and recording any possible perturbations. The much lesser diminution seen in curve 3 (11 %; order of addition valinomycin/ANS) could imply that the antibiotic goes well into the membrane leaving the interface still available to ANS. No ANS fluorescence was observed with valinomycin alone.

In order to eliminate the possibility that the results obtained reflect a direct interaction between ANS and valinomycin either within or outside the membrane, the following experiments were performed: (1) Valinomycin was added to lipid vesicles (C/10) equilibrated with 200 μM ANS; (2) valinomycin was pre-incubated with 100 μM ANS and this solution then added to lipid vesicles equilibrated with 100 μM ANS; (3) ANS (100 μM) was added to lipid vesicles that had been equilibrated with 100 μM ANS and valinomycin. In all three cases, the fluorescence intensity reached the same plateau after the same time. The time courses were so nearly identical as to rule out a direct interaction of ANS with valinomycin under the experimental conditions given.

It is to be noted that the same low salt concentrations were maintained through all experiments described in this and the following sections. Variations in ANS fluorescence amplitude were thus not caused by possible variations in the membrane's surface charge due to electrolytes as reported by Vanderkooi and Martonosi [22] and by Feinstein et al. [23], but reflect changes in the ANS yield brought about by the membrane reagents.

ANS fluorescence results do not permit an unequivocal decision as to the occurrence of translocator-induced perturbations [24]. A change in the overall yield is found here which is not accompanied by a displacement of the ANS emission maximum. Such a shift would, in fluorescence equilibrium experiments, provide the only direct evidence for a conformational change in the bilayer. The fact that no emission shift is observed does not exclude, however, the possibility that a rearrangement in the architecture of the membrane occurs since the lipid palisade array can be changed without affecting the immediate ANS environment.

In a recent paper, Haynes [25] reports changes of the ANS fluorescence yield in the presence of valinomycin and lipid as a function of cation concentration. Emission maxima were found at 480 nm without, and at 475 nm with valinomycin. Responsible for this shift in the peak emission towards the blue end of the spectrum is most probably the fact that phospholipid monolayers were formed around dispersions of an organic solvent into which the lipid hydrocarbon chains will partly dissolve. The preferred binding area of ANS to lipid, i.e., the polar–nonpolar region, is thus in closer contact with the organic solvents trapped in the monolayer vesicles and this could contribute to the ANS fluorescence. The basic premise to an interpretation of these experiments seems to us, therefore, debatable, especially since we show here that the fluorescence effects vary with the order of addition of valinomycin and ANS, and since a blue shift is always accompanied by an increase in the fluorescence yield [26]. Regrettably, the results of the bilayer experiments are neither given nor illustrated.

III. The effects of protamine and valinomycin on energy transfer between ANS and acriflavine

We can now attempt to distinguish between the various alternative inter-

pretations to the translocator effects offered above with transfer experiments from ANS to a second fluorophore, otherwise under the same conditions. Variations in the orientation and distance between ANS and acriflavine trapped inside the vesicles' lumina are monitored as variations of transfer efficiency. The evaluation of the transfer experiments is given relative to the ANS fluorescence yield in the absence and presence of protamine or valinomycin. Affinity constants between the membrane and ANS in the presence of these reagents need, therefore, not be determined.

A suspension of vesicles containing acriflavine in a final concentration of 10^{-4} M both in their lumina and in the suspending fluid was chromatographed on Sephadex. Detailed column specifications are given in a previous paper [3]. Fluorescence spectra of the eluted samples were recorded in order to determine the acriflavine concentrations (excitation maximum at 457 nm), and absorption spectra in order to obtain the lipid concentrations. The results were compared with a set of collected data on acriflavine concentration vs. fluorescence intensity and lipid concentration vs. transmittance. A linear relation between the acriflavine and lipid volume fractions is to be expected if the fluorophore is carried by, or present only inside, the vesicles. The samples falling into the range for which this linear relation holds were lumped, further analyzed, and eventually used for the transfer experiments. At $\lambda_{exc} = 482$ nm (peak ANS emission), fluorescence of 10^{-4} M acriflavine gives maximum emission at 505 nm both in tris and in C/10 lipid. Under these conditions, the lipid does not contribute to the fluorescence. However, at $\lambda_{exc} = 390$ nm, the ANS excitation wavelength used in the transfer experiments, a low lipid fluorescence centered around 470 nm and a low level acriflavine fluorescence were observed. The apparent emission peak of acriflavine in the presence of lipid is shifted towards the lipid band as the acriflavine concentration is systematically lowered from $\geqslant 10^{-5}$ M ($\lambda_{max} = 505$ nm) to 10^{-6} M ($\lambda_{max} = 490$ nm). The samples collected from the column and lumped to yield a C/10 lipid dilution gave an emission maximum at 502 nm. and calibration curves showed that the total acriflavine concentration should amount to approximately $5 \cdot 10^{-6}$ M. The total trapped volume of this lipid dilution comes to about 1 % of the total aqueous volume. Therefore, a suspension of vesicles containing 10^{-4} M acriflavine on the inside should represent a total volume concentration of 10^{-6}. Since we find $5 \cdot 10^{-6}$, and as the inside concentration remains the same, the acriflavine concentration not cleared from the suspending fluid by chromatography is only about 4 per cent of that in the vesicles' lumina.

Transfer experiments in which the fluorophores were separated by the membrane were always performed on the same preparation after elution from the column so that neither the absolute lipid concentration nor the lipid/acriflavine ratio was given to vary from one experiment to the next. The order of addition of ANS and the membrane reagents indicated in Fig. 1 was also followed here. The results of these experiments are given in Fig. 2 (acriflavine inside the vesicles). Correction for background fluorescence was applied. Curve 1 is the reference transfer spectrum of lipid/acriflavine$_{in}$/ANS plus a volume of buffer identical to that of the translocator solution. In all cases, a single maximum at the acriflavine emission wavelength is found (502 ± 2 nm) indicating that the orientation and domain of distances between donor and acceptor fluorophores allow transfer under our experimental conditions. The wavelength of peak emission being invariant, the relative transfer efficiency can then be evaluated from variations of the fluorescence intensity at this wavelength.

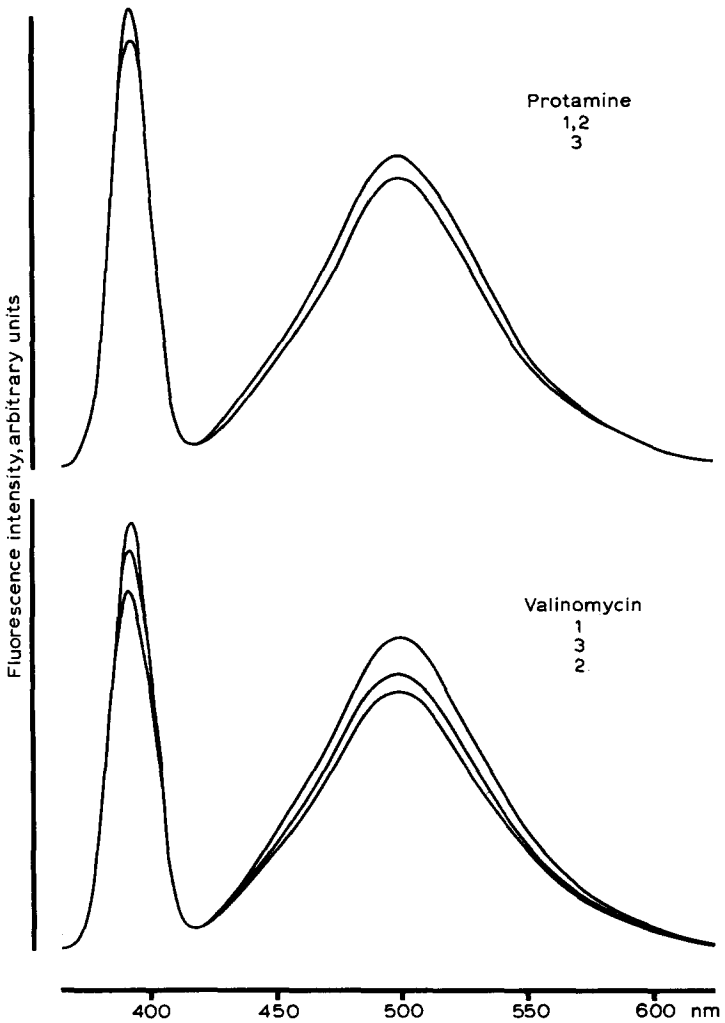

Fig. 2. Effect of protamine or valinomycin on energy transfer between ANS and acriflavine (inside vesicles). Emission spectra, $\lambda_{exc} = 390$ nm, peak emission at 502 ± 2 nm. 1, reference transfer spectrum, i.e. lipid/acriflavine inside/ANS+tris. Other designations and concentrations as in Fig. 1.

We note immediately that protamine added after ANS does not affect the transfer system at all, as is evident from the fact that curve 2 and reference curve 1 are identical. Valinomycin provokes a change in fluorescence for both orders of addition. Before an analysis could be attempted, however, we had to make sure that these results had not been distorted or even invalidated by the presence of the small amount of acriflavine not eliminated from the suspending fluid. A series of experiments was performed on a lipid preparation to which acriflavine had been added only after the formation of vesicles. The 'outside' aqueous volume was brought to a final concentration of $5 \cdot 10^{-6}$ M, roughly equivalent to the total acriflavine content of the preparations in the preceding experiments. All conditions in these trials were the same as

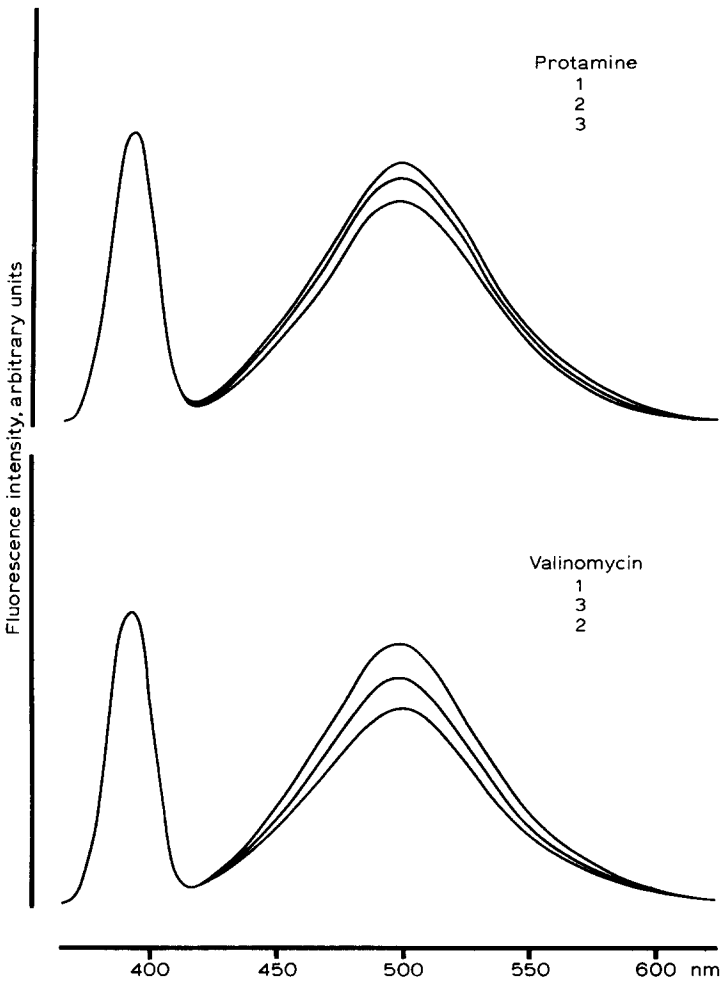

Fig. 3. Effect of protamine or valinomycin on energy transfer between ANS and acriflavine (outside vesicles). Emission spectra; λ_{exc} = 390 nm, peak emission at 502±2 nm. 1, reference transfer spectrum, i.e. lipid/ANS/acriflavine outside+tris. Other designations and concentrations as in Fig. 1.

before, with the lipid concentration adjusted to C/10.

With acriflavine outside the vesicles only, protamine and valinomycin gave the results presented in Fig. 3. The designation of the spectra is the same as in Fig. 1 and 2. In all curves and independent of the order of addition of ANS, protamine or valinomycin, a decrease in transfer intensity over reference curve 1 can be seen.

IV. Analysis: comparison of the effects of protamine and valinomycin on the single and coupled fluorophore systems

Table 1 summarizes the results presented and compares the effects of protamine or the translocator antibiotic on ANS fluorescence alone, on transfer for the two sequences of addition, and on transfer in the preparations with acriflavine either on the inside or the outside of the vesicles only. All changes brought about by prot-

TABLE I

EVALUATION OF FLUORESCENCE INTENSITY IN TRANSFER EXPERIMENTS

	Sequence of addition	Protamine			Valinomycin		
		Normalized fluorescence intensity (%)		Δ %	Normalized fluorescence intensity (%)		Δ %
Lipid plus:	ANS only	(1,1)*	100	—	(1,1)	100	—
	ANS before	(1,2)	100	—	(1,2)	58	—
	ANS after	(1,3)	91	—	(1,3)	89	—
Lipid/acriflavine inside plus:	ANS only	(2,1)	100		(2,1)	100	
	ANS before	(2,2)	100	−(1,2)* 0	(2,2)	67	−(1,2) +9
	ANS after	(2,3)	86	−(1,3) −5	(2,3)	78	−(1,3) −11
Lipid/acriflavine outside plus:	ANS only	(3,1)	100		(3,1)	100	
	ANS before	(3,2)	90	−(1,2) −10	(3,2)	57	−(1,2) −1
	ANS after	(3,3)	77	−(1,3) −14	(3,3)	79	−(1,3) −10

* refers to figure and curve numbers.

amine or valinomycin on ANS fluorescence alone are calculated with the ANS yield in lipid taken as 100 %. Since variations in ANS emission intensity entail corresponding changes in acriflavine excitation, the effect of a membrane reagent added in the same sequence has to be calculated considering transfer between ANS and acriflavine also as 100 %. This double normalization then permits us to detect changes in the actual energy transfer between the two fluorophores involving the separating lipid phase. A decrease in fluorescence transfer does not necessarily express a decrease in transfer efficiency, but an increase in transfer efficiency will become clearly visible as positive deviation from the parallelism in the reagent's behavior on ANS fluorescence and on ANS/acriflavine transfer.

ANS/protamine. ANS fluorescence and transfer to acriflavine$_{in}$ remain the same as without protamine. Transfer to acriflavine$_{out}$ gives a 10 per cent decrease to the reference spectrum: the membrane is shielded by the apposed polypeptide from acriflavine outside.

Protamine/ANS. A more pronounced decrease than in the preceding sequence is observed. Protamine probably adsorbs to the membrane and reduces the number of ANS molecules coming into contact with the bilayer.

The consistency of all results obtained with protamine strongly suggests that this polypeptide only adsorbs to the membrane and leaves the bilayer structure unperturbed.

ANS/valinomycin. The reduction of ANS fluorescence by valinomycin to 58 % is not followed by a decrease of transfer fluorescence in the experiments with acriflavine in the vesicles' lumina only. Quite to the contrary, a 9 % increase is found, consistent with the interpretation that either the relative ANS/acriflavine orientation has become more favorable for energy transfer and/or that the ANS/acriflavine distance across the membrane is diminished. In the experiments on transfer between ANS and acriflavine$_{out}$, the fluorescence intensity is reduced. It is important to insist here again that the ANS and the transfer emission maxima are the same as in the reference experiments without valinomycin.

Valinomycin/ANS. The ANS fluorescence is only reduced to 86 % when valinomycin is added before ANS. The ionophore seems to penetrate into the membrane leaving unoccupied the preferred binding area for ANS to the lipid. A decrease in relative transfer intensity is seen with acriflavine both inside and outside the vesicles.

Responsible for the transfer increment induced by valinomycin in the experiments with acriflavine inside the vesicles could be either a local or an overall thinning out of the lipid matrix separating the fluorophores, and/or a re-orientation of the ANS emission vector relative to the acriflavine excitation vector. Badley et al. [10] have shown that the axial orientation of the absorption vector of ANS in a lipid matrix remains unchanged even under the condensing effect of cholesterol. Any eventual variation of this angle θ in our transfer system is taken into account since we use as reference the relative per cent fluorescence of the lipid/ANS and the lipid/acriflavine/ANS systems, both in the presence of valinomycin. An increase in transfer then has to be due to either a change in the sweep of the emission vector during the excited lifetime or a decrease in the distance between the donor and the acceptor fluorophore.

Polarization values for ANS/lipid in the presence and absence of valinomycin were determined in order to detect changes in the sweep of the emission vector: P remains constant at 0.236 ± 0.003. The contribution of lipid was found to be insignificant. The value of $\alpha = 26°$ for ANS in lipid was determined from a Perrin plot and it agrees well with the literature [20, 10]. Polarization was also observed to remain constant for lipid/acriflavine with or without valinomycin ($P = 0.250$).

The increase in energy transfer recorded can then be explained as a decrease in the distance between the fluorophores. Since a change of θ is unlikely to occur when the angle swept by the emission vector is unaltered, the rotational freedom of ANS in the lipid does not appear to be restricted by valinomycin.

CONCLUSIONS

The translocator antibiotic valinomycin seems to provoke a change in the bilayer's architecture when dissolving into the membrane. The perturbation thus induced is expressed as decrease in the distance between the two energy-coupled fluorophores ANS and acriflavine. We find that a relative re-orientation of the fluorophores cannot account for the increase in transfer observed as both ANS and acriflavine polarization remain unchanged when valinomycin is added to the system. The lack of a shift in the ANS emission peak and the constant polarization values make it unlikely that the lipid molecules are more densely packed by valinomycin. The specific type and the extent of the perturbations in the bilayer can not be assessed at the present time. An overall thinning-out of the membrane under valinomycin seems more plausible than a micellization of the lipid since it is widely accepted that this antibiotic does not form channels through bilayers [27].

ACKNOWLEDGMENTS

This work was supported by grants from the Medical Research Council of Canada and from the Conseil de recherches médicales du Québec.

REFERENCES

1 Mueller, P. and Rudin, D. O. (1967) Nature 213, 603–604
2 Mueller, P. and Rudin, D. O. (1967) Biochem. Biophys. Res. Commun. 26, 398–404
3 Bessette, F. and Seufert, W. D. (1973) Biophysik 9, 325–334
4 Foerster, Th. (1959) Disc. Faraday Soc. 27, 7–17
5 Zwick, M. M. and Kuhn, H. (1962) Z. Naturforsch. 17A, 411–414
6 Kuhn, H. (1967) Naturwiss. 54, 429–435
7 Seufert, W. D. (1970) Biophysik 7, 60–73
8 Seufert, W. D. (1972) Biophysik 8, 292–301
9 Rubalcava, B., deMunoz, D. M. and Gitler, C. (1969) Biochemistry 8, 2742–2747
10 Badley, R. A., Martin, W. G. and Schneider, H. (1973) Biochemistry 12, 268–275
11 Perrin, F. (1929) Ann. de Phys. 12, 169–275
12 Kasai, M., Podleski, T. R. and Changeux, J. P. (1970) FEBS Lett. 7, 13–19
13 Udenfriend, S. (1969) Fluorescence Assay in Biology and Medicine, Vol. II, pp. 29–41, Academic Press, New York
14 Mueller, P., Rudin, D. O., Tien, H. T. and Wescott, W. C. (1962) Nature 194, 979–980
15 Mueller, P. and Rudin, D. O. (1968) Nature 217, 713–719
16 Mueller, P. and Rudin, D. O. (1969) in Current Topics in Bioenergetics (Sanadi, D. R., ed.), Vol. 3, pp. 157–249, Academic Press, New York
17 Cherry, R. J. and Chapman, D. (1969) J. Mol. Biol. 40, 19–32
18 Yi, P. N. and Macdonald, R. C. (1973) Chem. Phys. Lipids 11, 114–134
19 Gulik-Krzywicki, T., Shechter, E., Iwatsubo, M., Ranck, J. L. and Luzzati, V. (1970) Biochim. Biophys. Acta 219, 1–10
20 Freedman, R. B. and Radda, G. K. (1969) FEBS Lett. 3, 150–152
21 Brocklehurst, J. R., Freedman, R. B., Hancock, D. J. and Radda, G. K. (1970) Biochem. J. 116, 721–731
22 Vanderkooi, J. and Martonosi, A. (1969) Arch. Biochem. Biophys. 133, 153–163
23 Feinstein, M. B., Spero, L. and Felsenfield, H. (1970) FEBS Lett. 6, 245–248
24 Radda, G. K. and Vanderkooi, J. (1972) Biochim. Biophys. Acta 265, 509–549
25 Haynes, D. (1972) Biochim. Biophys. Acta 255, 406–410
26 Stryer, L. (1965) J. Mol. Biol. 13, 482–495
27 Eisenman, G., Szabo, G., Ciani, S., McLaughlin, S. and Krasne, S. (1973) in Progress in Surface and Membrane Science (Danielli, J. F., Rosenberg, M. D., Cadenhead, D. A., eds), Vol. 6, pp. 139–241, Academic Press, New York