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THE INTERACTION OF DETERGENTS WITH BILAYER LIPID MEMBRANES

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Summary

Detergents are widely used for extracting and purifying membrane proteins. Four such detergents have been studied to find the extent to which they alone can alter black lipid film conductances. The slope of the plot of conductivity versus concentration for Triton X-100 is 4.54 in the range 0.025—0.15 mM; dodecyl sulphate 0.82 in the range 0.01—1 mM; sodium deoxycholate 1.03 in the range 0.01—1 mM and sodium cholate 1.37 in the range 0.1—10 mM. These ranges are below the respective critical micelle concentrations; above these concentrations the membranes break. Bilayer lipid membrane conductivity measured at constant detergent concentration increases with the conductivity of the bathing salt solution with a slope greater than 1, indicating an effect on the putative pore structures induced by detergents.

Introduction

Detergents are widely used in the study of components of natural membranes. Extensive reviews in this field include Kagawa [1], Helenius and Simons [2], Gulik-Krzywicki [3] and Razin [4]. Detergents can be used to gently remove unwanted membrane components; for example a ten-fold purification of kidney outer medulla ($Na^+ + K^+$)-ATPase can be achieved using low concentrations of sodium dodecyl sulphate (SDS) [5], though it can be purified by other detergents [6,7]. Higher concentrations of detergents will disrupt natural membranes more completely resulting in a mixture of predominantly lipid-containing micelles and predominantly protein-containing micelles, thereby allowing the proteins to be fractionated; for example, SDS-polyacrylamide gel electrophoresis allows finger printing of peptide chains. In the presence of detergent, membrane proteins can also be re-associated with (chosen) lipids. For example, purified membrane fragments have been mixed with lipids in the presence of approximately 2 mM cholate [8]. Membrane proteins which have

been isolated or reconstituted using detergents include the calcium ATPase of sarcoplasmic reticulum [9,10], bovine rhodopsin [11], acetylcholine receptor [12], mitochondrial membrane [13,14] sodium-dependent glucose transporter [15] and bacteriorhodopsin [16].

Membrane proteins are expected to have anisotropic functions and, therefore, tests on their function when isolated or reconstituted often include direct or indirect measures of membrane permeability and the way it has been modified by the procedures to which it has been subjected.

It appears that detergents can on their own increase the permeability of liposomal membranes [17—19] and bilayer lipid membranes [20—25] and that this effect must be allowed for or excluded when membranes are studied after detergent treatment. In this paper an account is given of the effect of commonly used detergents on bilayer lipid membranes as a function of detergent and salt concentration. It is shown that substantial effects can be measured at detergent concentrations less than one tenth their critical micelle concentration.

Materials and Methods

Phosphatidylcholine and phosphatidylethanolamine were prepared from egg yolks using modifications of the methods of Singleton et al. [26] and Ansell and Hawthorne [27]. *n*-Decane, obtained from Hopkin and Williams, was purified by redistillation and passage through an alumina column. Cholic acid and deoxycholic acid were obtained from Maybridge Chemical Co., U.K.,; the sodium dodecyl sulphate was specially purified by BDH and a gift from Unilever (Port Sunlight). Water was double distilled in glass from potassium permanganate; all other reagents were analytical grade. Bilayer lipid membranes were formed on the ends of a set of five polyethylene tubes modified as shown in Fig. 1. [28]. Changing solutions from one composition to another was achieved by withdrawing the tubes from the trough, refilling it and then, after re-immersing, using a water pump to suck the solution from the inside of the top of each tube. This ensured that no air bubbles were trapped inside the tube or on the tube mouth. In this way a "run" took about 15 min, 10 min for the membranes to thin and 5 min to change solutions and remake the five membranes. 0.1 M chloride salt was used except where otherwise stated, together with 5 mM Tris, pH 7.4.

Membrane resistance and capacitance were measured as shown in Fig. 2. The measurements were made successively on each membrane of the set. The clean dry tubes were initially precoated with a solution of 2.5 mg phosphatidylcholine per ml of heptane and left for 15 min before immersing. Then five membranes were painted and repainted on each tube before the resistance reached a plateau high value. If the tubes were not pre-coated it took longer to reach the plateau steady state. Membranes were made from a solution of 2.5 mg egg phosphatidylcholine plus 0.8 mg phosphatidylethanolamine per ml in redistilled decane, which was painted across the hole with a clean sable brush. At the end of the day the tubes were cleaned by rinsing with distilled water and ethanol and finally dried with nitrogen.

The advantage of this method was that it was fast and reliable, allowing a

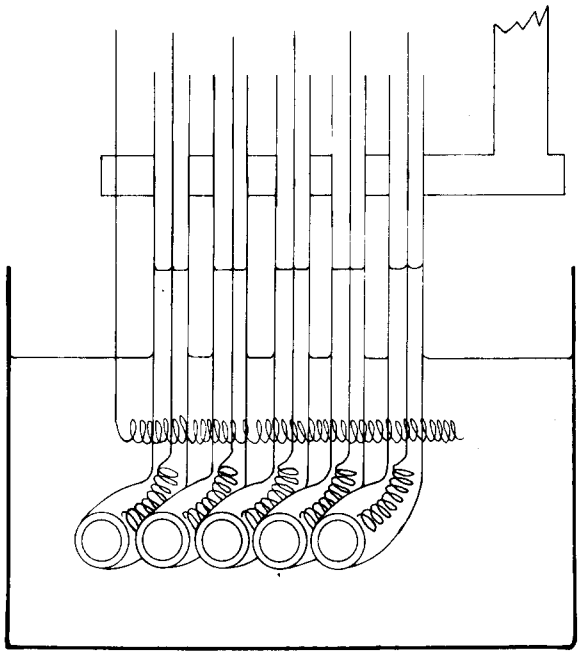
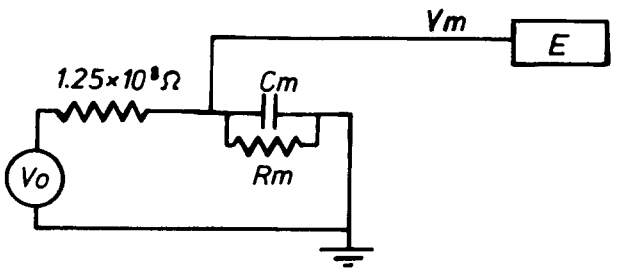


Fig. 1. Polythene tubes 2 mm outer diameter, on the lower ends of which bilayers were formed. Chlorided silver wires were used as electrodes.



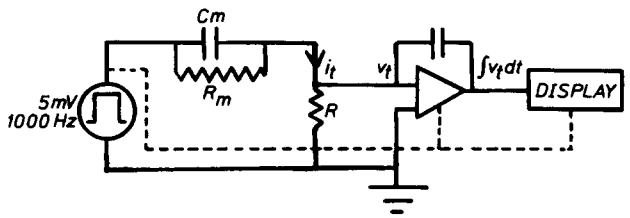


Fig. 2. (a) shows the circuit used for measurement of resistance. (b) The block diagram of the circuitry used to measure membrane capacitance by integration of the charging current as a result of the application of a 5 mV pulse to the membrane. Specific conductance was calculated from the resistance assuming a value of 0.0038 $F \cdot m^{-2}$ for membrane capacitance.

number of statistical comparisons to be readily effected. This rendered bi-ionic experiments for comparing cation selection unnecessary in the present context though the technique can readily be adapted to these rather tedious experiments.

Results

When a membrane is first formed it exhibits negligible capacitance and conductance; thereafter it thins, and in these experiments the evolution of film capacitance (n_F) and specific conductance ($S \cdot m^{-2}$) of the bilayer membrane were followed for 10 min. It can be seen (Table I) that despite an increase in area of black membrane (as evidenced by increase in capacitance) the specific conductance does not change detectably between 5 and 10 min. Henceforth the specific conductance was measured as soon as practicable, usually after 5 min. It was observed that detergents caused membranes to thin more slowly. This is indicated in Table II; moreover, it was found that thinning took longer as the detergent concentration was increased.

Previous workers [20,21] have established that anionic and non-ionic detergents select cations over anions; therefore, we compared membrane conductances in sodium chloride and potassium chloride. Table III shows that these conductances are indistinguishable. Consequently, subsequent experiments were performed with only one salt, usually sodium chloride.

Dependence of conductivity on detergent concentration. Figs. 3 and 4 show the dependence of specific conductivity on concentration for the detergents studied. In Fig. 3a specific conductivities of individual membranes have been plotted. In the remaining graphs the mean of each set of up to five membranes has been plotted. The effect of the non-ionic detergent Triton X-100 on bilayer lipid membranes differs markedly from that of the other detergents studied in that the specific conductance depends on the fourth or more power of the detergent concentration, whereas for the others it depends on a power of 1—2.

Dependence of the membrane conductivity on the conductivity of the salt solution at fixed detergent concentration. Figs. 5 and 6 show that the permeability of the membranes in the presence of detergent depends on the conductivity of the salt solution in the same way for all the detergents studied. The

TABLE I

This table shows that the change in specific conductance G ($S \cdot m^{-2}$) of the bilayer lipid membrane is small with respect to changes in capacitance C between 5 and 10 min after formation. Subscripts denote times.

	$(G_5-G_{10})/G_{10}$		Number of sets of data	$(C_5-C_{10})/C_{10}$	
	Mean	S.E.		Mean	S.E.
Control	0.13	0.20	18	—0.30	0.017
0.1 mM SDS	0.02	0.067	14	—0.40	0.034
1 mM sodium cholate	0.21	0.230	23	—0.40	0.014
0.2 mM sodium deoxycholate	0.061	0.048	12	—0.33	0.028
0.1 mM Triton	—0.02	0.340	8	—0.24	0.065

TABLE II

This table shows the effect of detergents on thinning. The capacitance of each set of 5 bilayer lipid membranes was measured after 5 min. The mean of n (given in square brackets) sets was calculated. The thinning rate decreases with increasing detergent concentration in proportion to its effect on G. The detergent concentrations used were chosen from Figs. 3 and 4.

Detergent	Concn. (mM)	Capacitance at 5 min (n_F)	
		Mean	S.E.
Control		7.59	0.26 [26]
SDS	0.1	1.95	0.37 [8]
Sodium cholate	1	4.25	0.19 [22]
Sodium deoxycholate	0.2	4.88	0.17 [32]
Triton	0.1	4.01	0.31 [12]

TABLE III

This table shows the specific conductance G ($S \cdot m^{-2}$) of the bilayer lipid membrane in sodium and potassium chloride solutions. 0.1 M buffered at pH 7.4 with 0.005 M Tris.

Detergent	Concn. (mM)	Bilayer lipid membrane conductance ($S \cdot m^{-2}$) Mean ± S.E.		t
		In NaCl	In KCl	
SDS	0.1	$(1.25 \pm 0.36) \cdot 10^{-2}$ [6]	$(2.43 \pm 0.73) \cdot 10^{-2}$ [5]	1.46
Triton	0.9	$(4.96 \pm 2.78) \cdot 10^{-2}$ [8]	$(9.37 \pm 4.33) \cdot 10^{-2}$ [11]	0.8
Sodium deoxycholate	0.2	$(4.95 \pm 1.18) \cdot 10^{-3}$ [8]	$(2.57 \pm 0.70) \cdot 10^{-3}$ [6]	1.4
Sodium cholate	1.0	$(6.84 \pm 2.42) \cdot 10^{-2}$ [8]	$(4.52 \pm 2.42) \cdot 10^{-2}$ [8]	0.87

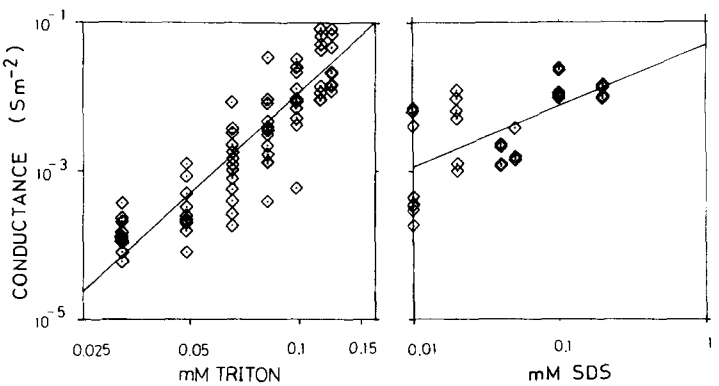


Fig. 3. (a) Log conductance plotted as a function of log concentration for Triton. The regression line drawn has a slope of 4.54 ± 0.27. (b) Log conductance plotted as a function of log concentration for SDS. The regression line drawn has a slope of 0.82 ± 0.17.

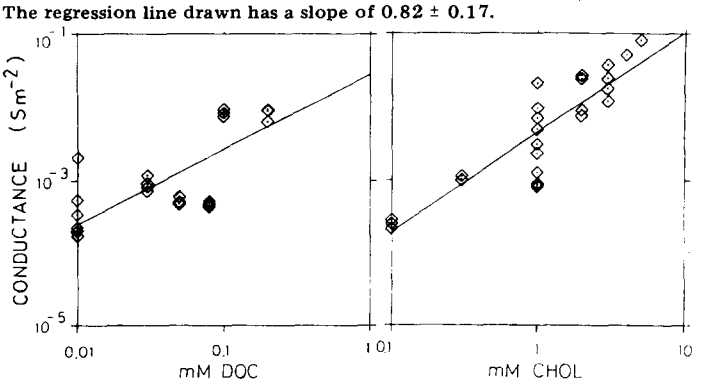


Fig. 4. (a) Log conductance plotted as a function of log concentration for sodium deoxycholate (DOC). The regression line drawn has a slope of 1.03 ± 0.17. (b) Log conductance plotted as a function of log concentration for sodium cholate (CHOL). The regression line drawn has a slope of 1.37 ± 0.12.

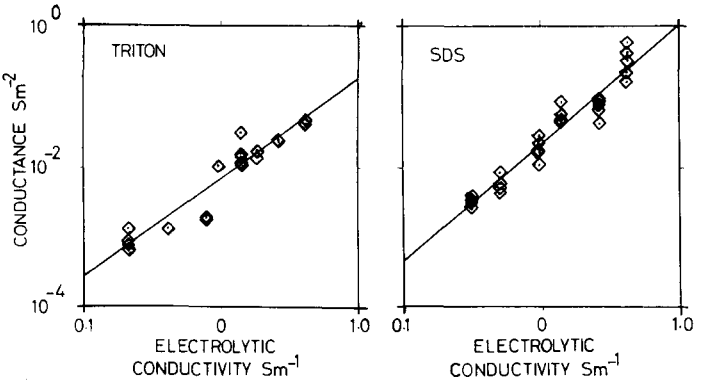


Fig. 5. (a) Log conductance plotted as a function of log electrolytic conductivity in the presence of a fixed concentration of Triton X-100. The regression line drawn has a slope of 1.42 ± 0.10. (b) Log conductance plotted as a function of log electrolytic conductivity in the presence of a fixed concentration of SDS. The regression line drawn has a slope of 1.68 ± 0.09.

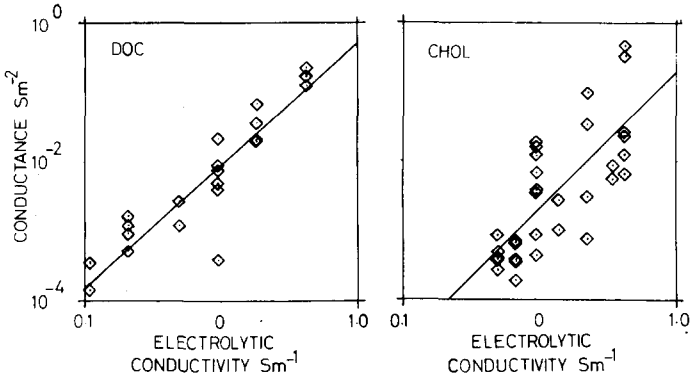


Fig. 6. (a) Log conductance plotted as a function of log electrolytic conductivity in the presence of a fixed concentration of sodium deoxycholate (DOC). The regression line drawn has a slope of 1.77 ± 0.16. (b) Log conductance plotted as a function of log electrolytic conductivity in the presence of a fixed concentration of sodium cholate (CHOL). The regression line drawn has a slope of 1.98 ± 0.31.

specific conductance approached 10^{-1} S · m^{-2} at which point they broke, which suggests that conductance (which varies with salt concentration, etc.) and membrane stability are related.

Discussion

From the recent history of membrane studies two inescapable conclusions have emerged.

1. Non-ionic and mildly ionic detergents appear to be essential tools for the separation of both extrinsic and intrinsic membrane proteins from their associated lipids [1—4].

2. In concentrations below their critical micelle concentrations, detergents interact with bilayer lipid membranes and liposomes in such a way as to increase conductivity to ions and non-electrolytes [17—25].

In this paper we have set out to describe a detailed study of the effects of some biologically useful detergents on bilayer lipid membranes to provide a

foundation for work involving the reassembly of purified membrane proteins with lipids, and the subsequent formation of bilayers containing them.

We have shown that four commonly used detergents, Triton X-100, SDS, deoxycholate and cholate act, on the black bimolecular portion of the membrane increasing its electrical conductivity. That the effects are not due to the torus is demonstrated by the constancy of the conductivity over a period when the ratio of black area to torus is increasing (Table I).

Studies of van Zutphen et al. [22] and Seufert [21] include both non-ionic and anionic detergents. Both studies concentrate on the time course of potential difference and conductance changes following the addition of detergent to one side of a membrane. Interpretation of their results is complicated by problems of asymmetry potentials and equilibrating detergent concentrations. These obscure the effects the detergents have when present on both sides of the membrane in equal concentrations as they are in our experiments. The transient conductance changes, reported previously, when detergent was added to one side of the membrane can probably be accounted for by irregularities in mixing. The transient conductances were particularly noticeable for Triton X-100. According to our results presented in Fig. 3a, a change in detergent concentration from 0.1 mM to 0.05 mM in the vicinity of the membrane would reduce its conductivity by two orders of magnitude. Presumably in the previous studies, the concentration would change as a result of diffusive or mechanical mixing. Our symmetrical system in which the detergent concentration is the same on both sides of the membrane, is uncomplicated by transient surface potentials which are due to the binding of charged molecules to one face of the membrane altering its zeta potential [29]. Qualitatively the present results are consistent with those of van Zutphen et al. [22] and Seufert [21]. Quantitatively, however, a useful comparison cannot be made for reasons outlined above. The results described herein are also consistent with those of Inoue and Kitagawa [18] and Herz and Barenholz [19] who found that the concentration of Triton X-100 required to cause half-maximum release of glucose trapped inside egg phosphatidylcholine liposomes was approximately 0.2 mM. This indicates that the mode of action of Triton X-100 on bilayer lipid membranes is not dependent on the presence of decane.

The most obvious explanation for the observed dependence of conductivity on detergent concentration is that the exponent represents the number of monomers interacting to form a conducting unit as has been suggested for the polyene antibiotics. Such an explanation, however, attractive as it might seem to be, especially for high values of exponent, should be regarded cautiously in the light of the fact that cooperative effects between conducting units or subunits may influence their interpretation [30,31]. It has been suggested that detergents can fold into configurations which clearly resemble the cyclic polyethers [32] and it has been observed that in common with the cyclic polyethers, detergents accompanied by cations may be extracted into organic solvents [33]. Until detailed studies have been made of random fluctuations of current under voltage clamp, however, these observations and suggestions will remain matters for speculation.

Preliminary reports of such fluctuations have been made for SDS, cetyl trimethyl ammonium bromide [23,24] and Triton X-100 [25]. In none of the

TABLE IV

DETERGENTS

The approximate concentration of detergent required to double the intrinsic conductivity of bilayer lipid membranes is shown as well as the approximate concentration neeeded to start releasing glucose from liposomes. Molecular weight, aggregation number, and critical micelle concentration data are from refs. 2 and 19.

Detergent	Mol. wt.	Aggregation number	Critical micelle concentration	Detergent concentration for an effect on permeability (mM)
SDS	288.4	62	8.2	0.01
Triton	640	140	0.240	0.05
				0.05 *
Sodium cholate	431.5	2—4	13—15	0.6
				1.0 **
Sodium deoxycholate	414.57	4—10	4—6	0.07

* From ref. 18.

** From ref. 34.

cases reported has lipid composition been involved in the magnitude or average life-time of fluctuations observed.

The effect of conductivity on salt concentration in the presence of detergents is also reported here.

The conductivity of g_r of a single channel section a_r and length L can be described by the relation $g_r = k\, a_r/L$ where k is the electrolytic conductivity.

The conductivity G of an assembly of p_1 channels of section a_1, p_2 channels of section a_2 etc., may thus be written:

$$G = \frac{k}{L} \sum_r p_r a_r$$

Since, in these experiments, the amount of detergent is constant, whilst the salt concentration and, thus, electrolytic conductivity k is varied, it would seem that $\Sigma_r p_r a_r$ should be constant and that a plot of log G vs. log k should be linear with a gradient of 1.

For none of the detergents has this been found to be the case, which implies that $\Sigma_r p_r a_r$ is a function of salt concentration. This is not too surprising in view of the fact that the critical micelle concentration (which reflects the interaction of detergent molecules with one another) changes with salt concentration. Precisely what the nature of the channel distribution $\Sigma_r p_r a_r$ is will have to await investigation of channel noise studies.

Table IV summaries some data on detergents indicating the concentrations at which they interfere with artificial lipid membranes causing an increase of permeability.

References

1 Kagawa, Y. (1972) Biochim. Biophys. Acta 265, 297—338
2 Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29—79
3 Gulik-Krzywicki, T. (1975) Biochim. Biophys. Acta 415, 1—28
4 Razin, S. (1972) Biochim. Biophys. Acta 265, 241—296

5 Jorgensen, P.L. (1974) Quart. Rev. Biophys. 7, 239—274
6 Kyte, J. (1971) J. Biol. Chem. 246, 4157—4165
7 Dahl, J.L. and Hokin, L.E. (1974) Annu. Rev. Biochem. 43, 327—356
8 Slack, J.R., Anderton, B.B. and Day, W.A. (1973) Biochim. Biophys. Acta 323, 547—559
9 MacLennan, D.H. (1970) J. Biol. Chem. 245, 4508—4518
10 Warren, G.B., Toon, P.A., Birdsall, N.J.M., Lee, A.G. and Metcalfe, J.C. (1974) Proc. Natl. Acad. Sci. U.S. 71, 622—626
11 Osborne, H.B., Sardet, C. and Helenius, A. (1974) Eur. J. Biochem. 44, 383—390
12 Martinez-Carrion, M., Sator, V. and Raftery, M.A. (1975) Biochem. Biophys. Res. Commun. 65, 129—137
13 Racker, E. (1975) Biochem. Soc. Trans. 3, 785—802
14 Riccio, P., Aquila, H. and Klingenberg, M. (1975) FEBS Lett. 56, 129—132
15 Crane, R.K., Malathi, P. and Preiser, H. (1976) FEBS Lett. 67, 214—216
16 Racker, E. and Stoeckenius, W. (1974) J. Biol. Chem. 249, 662—663
17 Weissman, G., Sessa, G. and Weissman, S. (1966) Biochem. Pharmacol. 15, 1537—1551
18 Inoue, K. and Kitagawa, T. (1976) Biochem. Biophys. Acta 426, 1—16
19 Herz, R. and Barenholz, Y. (1977) J. Coll. Interface Sci. 60, 188—200
20 Seufert, W.D. (1965) Nature 207, 174—176
21 Seufert, W.D. (1973) Biophysik 10, 281—292
22 Van Zutphen, H., Merola, A.J., Brierley, G.P. and Cornwall, D.G. (1972) Arch. Biophys. Biochem. 152, 755—766
23 Ksenzhek, O.S., Gevod, V.S., Omel'chenko, A.M. and Koganov, M.M. (1975) Elektrokhimya 11, 1566—1570
24 Ksenzhek, O.S., Bogach, P.G., Omel'chenko, A.M., Jorvorski, J.Z. and Rybal'chenko, V.K. (1977) DOP-UKR. B. (3) 259—262
25 Schlieper, P. and de Robertis, E. (1977) Arch. Biochem. Biophys. 184, 204—208
26 Singleton, W.S., Gray, M.S., Brown, M.L. and White, J.L. (1965) J. Am. Oil Chem. Soc. 42, 53—56
27 Ansell, G.B. and Hawthorne, J.N. (1964) Phospholipids, p. 99, Elsevier, Amsterdam
28 Lea, E.J.A. and Croghan, P.C. (1969) J. Membrane Biol. 1, 225—237
29 Haydon, D.A. and Myers, V.P. (1973) Biochim. Biophys. Acta 307, 429—433
30 Cass, A., Finkelstein, A. and Krespi, V. (1970) J. Gen. Physiol. 56, 100—124
31 Lea, E.J.A. and Collins, J.C. (1976) in Electrical Phenomena at the Biological Membrane Level (Roux, E., Ed.), pp. 195—202, Elsevier, Amsterdam
32 Christiensen, J.J., Hill, J.O. and Izatt, R.M. (1971) Science 174, 459—467
33 Brierley, G.P., Jurkowitz, M., Merola, A.J. and Scott, K.M. (1972) Arch. Biochem. Biophys. 152, 744—754
34 Goldin, S.M. and Tong S.W. (1974) J. Biol. Chem. 249, 5907—5915