Biochimica et Biophysica Acta, 1104 (1992) 147–150

BBAMEM 75519

Aggregation of lasalocid A in membranes: a fluorescence study

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(Received 21 May 1991)
(Revised manuscript received 22 October 1991)

Key words: Lasalocid A; Aggregation; Ionophore; Model membrane; Fluorescence self quenching

The aggregation behavior of the carboxylic ionophore, lasalocid A, has been studied in egg phosphatidylcholine vesicles by monitoring the intrinsic fluorescence of lasalocid A. Self quenching of lasalocid A fluorescence in vesicles of egg phosphatidylcholine suggests aggregation of lasalocid A. When aggregated lasalocid A is treated with increasing concentrations of lipid, there is an increase in fluorescence due to gradual reduction of self quenching on lateral dilution. This confirms the presence of loosely held non-covalent aggregates of lasalocid A in the membrane. This result is relevant in elucidating the molecular mechanism of cation transport by lasalocid A across membranes.

Introduction

Lasalocid A (also known as X537A) is a carboxylic ionophore isolated from *Streptomyces lasaliensis* [1]. It is a linear polyether antibiotic molecule and facilitates the transport of metal ions (especially Ca^{2+} ions) as well as some biogenic amines across biological and model membranes [2–5]. It is generally believed that lasalocid forms specific complexes with cations and encircles the cation to which it is coordinated, rendering it lipophilic, and thus providing a means for its transport across apolar barriers. Lasalocid A is an intrinsically fluorescent molecule. The fluorescence of lasalocid arises from its salicylic acid residue [6]. This group is probably directly involved in complexation with ions and thus lasalocid fluorescence is sensitive to the degree of complexation and protonation of the ionophore [6–11]. The fluorescence quantum yield of lasalocid A is very sensitive to the polarity of the medium [6].

In spite of a number of studies on lasalocid A mediated membrane transport of ions, the detailed kinetics and mechanism of ion transport by lasalocid is not fully understood [8,12–21]. A number of studies on the kinetics and mechanism of ion transport by lasalocid A suggest, based on the concentration dependence of the rates of transport, that lasalocid A could be aggregated in membranes [12,16–18]. In addition, fluorescence lifetime experiments have shown that lasalocid exists in two different environments when bound to membrane vesicles, possibly reflecting two different modes of lasalocid–phospholipid interactions [8]. Although interaction of lasalocid A with phospholipid vesicles has been studied [22–24], aggregation of lasalocid in membranes has not been examined before. In this report, we have investigated the aggregation behavior of lasalocid A in egg PC vesicles as monitored by intrinsic fluorescence. Our studies indicate that at low lipid/lasalocid molar ratio, lasalocid has a tendency to become aggregated in membranes.

Materials and Methods

Sodium salt of lasalocid A and egg PC (type XI-E) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Egg PC was checked for purity by TLC on silica gel plates in chloroform/methanol/water (65:35:5, v/v). Solvents used were of spectroscopic grade. Fluorescence measurements were performed with a Hitachi F-4000 steady state spectrofluorometer with a built-in computer and using 1 cm path-length quartz cuvettes. Excitation and emission slits with a nominal bandpass of 5 nm were used in all experiments. The excitation wavelength used was 310 nm in all cases. Background intensities of samples in

Abbreviations: PC, phosphatidylcholine; TLC, thin-layer chromatography; ULV, unilamellar vesicle.

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which lasalocid was omitted were subtracted from all reported values. For the self quenching experiment (Fig. 2), fluorescence was corrected for inner filter effect, as described previously [25,26]. Absorbance in 1 cm cuvettes was measured using a Hitachi 200–20 spectrophotometer. Unilamellar vesicles (ULV) of egg PC were prepared by the ethanol injection method [27,28] by injecting ethanolic solutions of egg PC into a pH 7.2 buffer containing 10 mM Tris chloride and 150 mM sodium chloride. To incorporate lasalocid A into membranes, small aliquots of lasalocid were added from a stock solution of lasalocid in ethanol to the preformed vesicles and mixed well. Samples were then kept in dark for 16 hours before measuring fluorescence.

Results and Discussion

Fig. 1 shows the chemical structure and fluorescence emission spectrum of lasalocid A in ULVs of egg PC. The maximum of emission occurs around 416 nm. Fig. 2 shows lasalocid fluorescence intensity in ULVs of egg PC as a function of lasalocid concentration. If upon increasing lasalocid concentration, there is no effect on the state of the ionophore molecule, there should be a linear increase in fluorescence with concentration; this

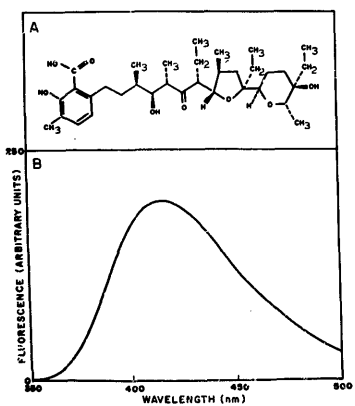

Fig. 1. (A) Structure of lasalocid A. (B) Fluorescence emission spectrum of lasalocid A in unilamellar vesicles of egg PC. The concentration of lasalocid was 5 μM and lasalocid/lipid ratio was 1:20 (mol/mol). The excitation wavelength was 310 nm.

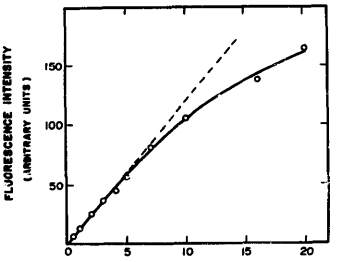

Fig. 2. Change in fluorescence intensity as a function of lasalocid concentration in egg PC vesicles. Fluorescence was monitored at 416 nm. The dotted line denotes theoretically expected fluorescence. Fluorescence values were corrected for inner filter effect (see Materials and Methods). Concentration of egg PC was 0.15 mM in all samples and lipid/lasalocid ratio varied between 7.5 and 300 (mol/mol). Each sample was made separately.

is observed at lower concentrations of lasalocid. However, the plot deviates from linearity for lasalocid concentrations greater than 5 μM which corresponds to a lipid/lasalocid ratio of 30 (mol/mol). This deviation from linearity beyond a certain concentration suggests association of lasalocid molecules in the membrane leading to self quenching of fluorescence. Such self quenching of lasalocid fluorescence on aggregation in organic solvents has been previously observed [17]. There was, however, no significant shift in fluorescence emission maximum of lasalocid on aggregation in the membrane.

To further examine the aggregation behavior of lasalocid A in membranes and to check whether the aggregation process was reversible, fluorescence intensity titration of a fixed concentration of lasalocid by egg PC vesicles was performed with separate samples. Fig. 3 shows a plot of fluorescence intensity of lasalocid A with increasing lipid concentration. The lipid/lasalocid molar ratio in this experiment was carefully chosen so that lasalocid would initially remain aggregated and thus its fluorescence would be self quenched. With the addition of increasing amounts of lipid, lasalocid would get laterally diluted resulting in release of its fluorescence. Fig. 3 shows that with increasing lipid/lasalocid ratio, there is an increase in fluorescence followed by a leveling off at higher lipid/lasalocid ratios indicating that the disaggregation process is complete and monomeric lasalocid molecules are bound to the membrane. This type of titration, based on release of self quenching on lateral dilution, has been previously used

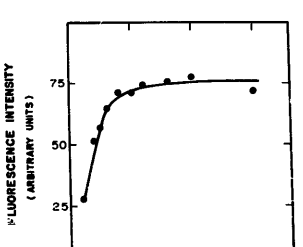

Fig. 3. Fluorescence of lasalocid A (measured at 416 nm) in egg PC vesicles as a function of lipid/lasalocid ratio (mol/mol). Samples corresponding to each data point were separately made. The concentration of lasalocid in each sample was 5 μM. Concentration of egg PC was between 0.05 and 0.75 mM. See Materials and Methods for other details.

to probe the aggregation behavior of membrane active antibiotics [29].

The point upto which there is a linear increase in fluorescence in Fig. 3 corresponds to a lipid/lasalocid ratio of 30 (mol/mol). This matches very well with Fig. 2 in which the deviation from linearity is observed at a lipid/lasalocid ratio of 30. This internal self-consistency ensures that in both the cases we are studying the same phenomenon, that is, self-association of lasalocid A in the membrane. The aggregation-disaggregation process is also independent of the total lasalocid concentration. Thus, Figs. 2 and 3 are not altered if the total lasalocid concentration is doubled with a simultaneous doubling of lipid concentration so as to keep the lipid/lasalocid ratio constant.

Our self quenching and lateral dilution experiments clearly show that lasalocid A is aggregated in membranes. The nature of these aggregates deserves some comment. As is apparent from Fig. 3, the aggregated form having low fluorescence can be diluted away to the highly fluorescent monomeric form, that is, the process of aggregation can be reversed by lateral dilution. The lasalocid molecules are, thus, probably loosely held together in the aggregate by non-covalent forces.

Lasalocid A is a flexible molecule and is capable of adopting a number of different conformations in response to its environment [7,9–11]. It is thus quite possible that lasalocid aggregates in membranes so as to minimize the exposure of its hydrophilic portion to the hydrophobic interior of the lipid bilayer. Self association of lasalocid in organic solvents has been previously reported [17]. Aggregation of membrane fluidity probes such as pyrene [30–33] and membrane potential probes like carbocyanine and merocyanine dyes [34–38] in membranes is well known. Aggregation of other antibiotic ionophores has also been postulated in a number of studies. Alamethicin [39,40], nystatin and amphotericin B [41,42] are all believed to form aggregates in membranes, although the latter also require sterol molecules. A functionally related ionophore, A23187, has been shown to aggregate in organic solvents [43] as well as in membranes [44].

The results described in this report demonstrate that the carboxylic ionophore lasalocid A tends to aggregate in membranes at low lipid/lasalocid ratio. Knowledge of such aggregation behavior is particularly relevant in elucidating the molecular mechanism by which lasalocid transports ions across membranes.

Acknowledgements

We would like to thank Drs. D. Balasubramanian, R. Nagaraj, and D. Chatterji for helpful discussions.

References

1 Berger, J., Rachlin, A.I., Scott, W.E., Sternbach, L.H. and Goldberg, M.W. (1951) J. Am. Chem. Soc. 73, 5295–5298.
2 Pressman, B.C. and Guzman, N.T. (1975) Ann. N.Y. Acad. Sci. 264, 373–385.
3 McLaughlin, S. and Eisenberg, M. (1975) Annu. Rev. Biophys. Bioeng. 4, 335–366.
4 Pressman, B.C. (1976) Annu. Rev. Biochem. 45, 501–530.
5 Painter, G.R. and Pressman, B.C. (1982) Topics Curr. Chem. 101, 83–110.
6 Haynes, D.H. and Pressman, B.C. (1974) J. Membr. Biol. 16, 195–205.
7 Shastri, B.P., Sankaram, M.B. and Easwaran, K.R.K. (1987) Biochemistry 26, 4930–4936.
8 Haynes, D.H., Chiu, V.C.K. and Watson, B. (1980) Arch. Biochem. Biophys. 203, 73–79.
9 Degani, H. and Friedman, H.L. (1974) Biochemistry 13, 5022–5032.
10 Chen, S.-T. and Springer, C.S. (1978) Bioinorg. Chem. 9, 101–122.
11 Richardson, F.S. and Das Gupta, A. (1981) J. Am. Chem. Soc. 103, 5716–5725.
12 Degani, H., Simon, S. and McLaughlin, A.C. (1981) Biochim. Biophys. Acta 646, 320–328.
13 Degani, H. (1978) Biochim. Biophys. Acta 508, 364–369.
14 Hunt, G.R.A. (1975) FEBS Lett. 58, 194–196.
15 Shastri, B.P., Sankaram, M.B. and Easwaran, K.R.K. (1987) Biochemistry 26, 4925–4930.
16 Chen, S.-T. and Springer, C.S. (1981) Biophys. Chem. 14, 375–388.
17 Kinsel, J.F., Melnik, E.I., Lindenbaum, S., Sternson, L.A. and Ovchinnikov, Y.A. (1982) Biochim. Biophys. Acta 684, 233–240.
18 Celis, H., Estrada-O., S. and Montal, M. (1974) J Membr. Biol. 18, 187–199.
19 Cader, B.M. and Horrocks, W. deW. (1989) Biophys. Chem. 33, 265–275.
20 Blau, L., Stern, R.B. and Bittman, R. (1984) Biochim. Biophys. Acta 778, 219–223.
21 Fernandez, M.S., Celis, H. and Montal, M. (1973) Biochim. Biophys. Acta 323, 600–605.

22 Aranda, F.J. and G ez-Fernandez, J.C. (1986) Biochim. Biophys. Acta 860, 125–130.
23 Sankaram, M.B., Shastri, B.P. and Easwaran, K.R.K. (1987) Biochemistry 26, 4936–4941.
24 Grunwald, R. and Painter, G.R. (1990) Biochim. Biophys. Acta 1027, 245–252.
25 Lakowicz, J.R. (1983) in Principles of Fluorescence Spectroscopy, pp. 44–45, Plenum Press, New York.
26 London, E. (1986) Anal. Biochem. 154, 57–63.
27 Batzri, S. and Korn, E.D. (1973) Biochim. Biophys. Acta 298, 1015–1019.
28 Kremer, J.M.H., Esker, M.W.J., Pathmamanoharan, C. and Wiersema, P.H. (1977) Biochemistry 16, 3932–3935.
29 Burke, T.G. and Tritton, T.R. (1984) Anal. Biochem. 143, 135–140.
30 Vanderkooi, J.M. and Callis, J.B. (1974) Biochemistry 13, 4000–4006.
31 Galla, H.-J. and Hartmann, W. (1980) Chem. Phys. Lipids 27, 199–219.
32 L'Heureux, G.P. and Fragata, M. (1988) Biophys. Chem. 30, 293–301.
33 Pownall, H.J. and Smith, L.C. (1989) Chem. Phys. Lipids 50, 191–211.
34 Sims, P.J., Waggoner, A., Wang, C.-H. and Hoffman, J.F. (1974) Biochemistry 13, 3315–3330.
35 Waggoner, A. (1976) J. Membr. Biol. 27, 317–334.
36 Verkman, A.S. and Frosch, M.P. (1985) Biochemistry 24, 7117–7122.
37 Cabrini, G. and Verkman, A.S. (1986) J. Membr. Biol. 90, 163–175.
38 Cabrini, G. and Verkman, A.S. (1986) J. Membr. Biol. 92, 171–182.
39 Eisenberg, M., Kleinberg, M.E. and Shaper, J.H. (1977) Ann. N.Y. Acad. Sci. 303, 281–191.
40 Mathew, M.K., Nagaraj, R. and Balaram, P. (1982) J. Biol. Chem. 257, 2170–2176.
41 De Kruijff, B. and Demel, R.A. (1974) Biochim. Biophys. Acta 339, 57–70.
42 Van Hoogevest, P. and De Kruijff, B. (1978) Biochim. Biophys. Acta 511, 397–407.
43 Case, G.D., Vanderkooi, J.M. and Scarpa, A. (1974) Arch. Biochem. Biophys. 162, 174–185.
44 Balasubramanian, S.V. and Easwaran, K.R.K. (1989) Biochem. Biophys. Res. Commun. 158, 891–897.