Bioscience Reports **4**, 651-658 (1984)
Printed in Great Britain

Sequence of ionophore conformational changes induced by a simulated membrane/water interface

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(Received 17 July 1984)

We demonstrate how the progressive passage through an interface simulated by a linear increase in dielectric constant may mediate a reversible transconformation of Na^+/X537A (lasalocid A) from a cyclic to an extended structure. During the passage through the interface, the complex adopts progressively a more extended conformation favorable to the Na^+ complexation or decomplexation at the interface.

The carboxylic ionophores lasalocid A (X537A) and A23187 have been widely studied in experimental biology and model membranes (1-14). Since its X-ray analysis by Johnson et al. (15-16) conformation of lasalocid, which complexes alkali, alakaline earths, and amines, has been extensively investigated. Because of the presence of a relatively long and flexible chain bearing polar groups, the ionophore can adopt a wide variety of conformations in solvents of different polarities (17-22). The ion transfer across biological membranes supposes however the existence of two conformations: a lipophilic conformation capable of conveying Na^+ across the hydrocarbon region of the cell membrane and an interfacial conformer responsible for the ion complexation/decomplexation process at the lipid/water interface. It is however very difficult to obtain experimental information on the transient conformations of the ionophore/ion complex formed at a membrane/water interface. In these conditions, the dielectric constant will vary greatly (ranging from approx. 2.5 to 40) (23) over a distance smaller than the ionophore size so that one part of the molecule may remain in a pure hydrophobic environment while another part may reach a high-polarity environment. We demonstrate here how the progressive passage of the ionophore through the interface simulated by a linear increase in dielectric constant may mediate a reversible transconformation of Na^+/X537A from a cyclic to an acyclic form. The conformations of the Na^+/lasalocid complex and its orientation at a simulated lipid/water interface were established by a method described elsewhere (24-28).

Materials and Methods

The total conformational energy was empirically calculated as the sum of the following terms:

1. The London-Van der Waals energy of interaction between all pairs of non-mutually bonded atoms. Buckingham's pairwise atom-atom interaction functions have been used (29,30)

$$E^{VdW} = \sum_{ij} [A_{ij} \exp(-B_{ij}r_{ij}) - C_{ij}r_{ij} - 6]$$

where i,j = 1,2,... are non-bonded atoms, r_{ij} their distances from each other, and A_{ij} and C_{ij} are coefficients assigned to atom pairs. The values of these coefficients have been reported by Liquori and Giglio (31,32). Like other quantum-mechanical results (33), these values emerge in part as the solution of the Schrödinger equation and in part as heuristic variables. They have been applied with success to conformational analysis of molecular crystals, proteins, polypeptides, and lipids (25,34-36). In order to compensate for the decrease of the function E^{VdW} at small r_{ij}, we have imposed an arbitrary cut-off value of E^{VdW} = 100 Kcal mol^{-1} at $r_{ij} < 1$Å.

2. The generalized Keesom-Van der Waals interaction or electrostatic interaction between atomic point charges.

$$E^{cb} = 332 \left(\sum_{ij} \frac{e_i e_j}{r_{ij} \varepsilon_{ij}} \right)$$

where e_i and e_j are expressed in electron charge units and r_{ij} in Å. The electron density mapping of X537A is given in Fig. 1 where each atom has been identified by means of an alphanumeric code. The values of the atomic point charges are similar to the values used for polypeptide (37).

3. The potential energy of rotation of torsional angles. This rotation around the C-C or C-O bonds was calculated by the equation:

$$E^{Tor} = \frac{U_{ij}}{2} (1 + \cos \phi_{ij})$$

where U_{ij} corresponds to the energy barrier in the eclipsed conformation during the rotation of the angle and ϕ_{ij} the torsional angle.

4. The transfer energy of each part of molecule. The values of the transfer energies used (Table 1) are similar to those determined experimentally by numerous authors and summarized elsewhere (38). In the calculation procedure, six changes of 60° each were first imposed on each of n torsional angles, yielding 6^n conformers. The conformational energy was calculated for each of these conformers. The most probable configurations were taken as those yielding the lowest internal energy. Such a selection is based on the statistical weight (Boltzmann) of all individual configurations.

Table 1. Transfer energy (in cal/mol) fron a hydrophilic phase into a hydrophobic domain derived from reference 38

	: 833	C	: -853
	: 4260	one degree of unsaturation	: -1503
	: 2574		

The values used for the valence angles and bond lengths were currently used in conformational analysis (37). After systematic analysis, conformations selected for their lowest internal engery were submitted to a simplex minimization procedure (39). To simulate the membrane interface and to mimic the passage of X537A-Na through the interface, we have arbitrarily and successively fixed atoms of X537A at a plane above which the dielectric constant (ε_{ij}) is assumed to be 3 while the atom at the bottom of the configuration was fixed at a plane where the dielectric constant is assumed to be 30. Between these 2 planes, the dielectric constant was assumed to increase linearly along the z axis perpendicular to the interface. The molecule is finally oriented with the line joining the hydrophilic and hydrophobic gravity centers perpendicular to the interface. The hydrophilic gravity center ($\vec{C}_W$) is defined by the following equation:

$$\vec{C}_W = \sum_{i=i}^{n} [E^+_{transfer_i} \vec{r}_i] / \sum_{i=i}^{n} E^+_{transfer_i}$$

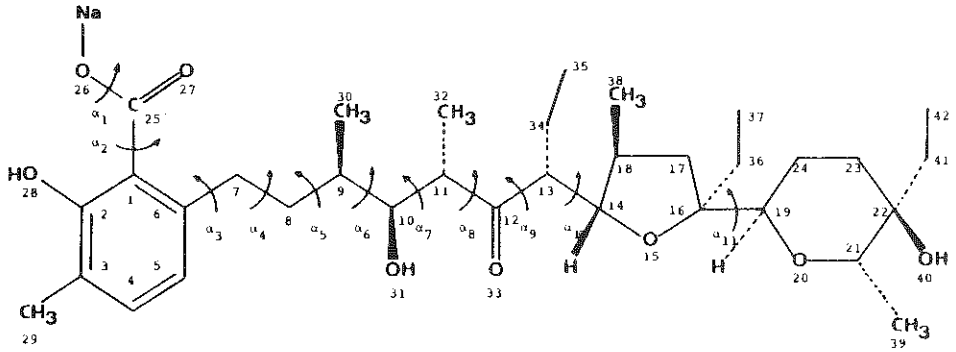
in which $\vec{r}_i$ are the coordinates of the i atom. The hydrophobic gravity center located in the hydrocarbon domain ($\vec{C}_{Hc}$) is defined by the same equation, except that the negative transfer energies are taken into account. The interface position ($\vec{I}$) is defined by the equation:

$$\frac{\sum_{i=1}^{n} E^+_{transfer_i}}{\vec{C}_W - \vec{I}} = \frac{\sum_{j=1}^{m} E^-_{transfer_i}}{\vec{C}_{Hc} - \vec{I}}$$

The molecular structure, the numbering of the torsional angles, the position of successive interfaces, together with the all transconformations of the complex lasalocid A/Na+ taken as our initial model are illustrated in Fig. 1.

Results and Discussion

A first systematic study performed on the angles labelled $\alpha_1 \rightarrow \alpha_6$ made it possible to obtain a conformation with a 99% probability of existence. All other conformations presented a probability of existence below 0.5%. A second study carried out on the angles $\alpha_6 \rightarrow$



Na	= +1	O_{33}	= -.3
O_{26}	= -1.	$C_{14} = C_{16}$	= .15
O_{27}	= -.3	O_{15}	= -.3
O_{28}	= -.27	$C_{19} = C_{21}$	= .15
H_{28}	= .27	O_{20}	= -.3
O_{31}	= -.27	O_{40}	= -.27
H_{31}	= .27	H_{40}	= .27
C_{12}	= .3		

Fig. 1. Structural formula of lasalocid/Na^+ complex with numbering of atoms, torsional angles, and the electron density.

α_{11} maintaining the first segment unchanged (except for angles α_6) gave a structure with a probability of 99%. After application of the simplex minimization procedure to this conformation, the complex adopts a cyclic structure screening the ion from the hydrophobic medium (Fig. 2a). This structure is very similar to the bulk-phase configuration derived from X-ray analysis (21). The sodium ion is complexed by five oxygen atoms in the RX determination (21) (O_{15},O_{20},O_{31},O_{33},O_{40}) and by four oxygen atoms in our structure (O_{15},O_{20},O_{31},O_{33}). Only the position of the O_{40} differs in the two configurations. This difference is probably due to the crystallization conditions. Indeed, one molecule of methanol is associated to one molecule of the lasalocid A/Na^+ complex and the O_{40} acts as hydrogen-bond donor to the methanol molecule. A detailed comparison of our theoretical data with the X-ray analysis values will be presented elsewhere.

To mimic the immersion of the membrane-bound ionophore into the aqueous phase, the interface simulated by the dielectic constant change was imposed at several positions of the ionophore (atom position listed in Table 2 and Fig. 1) and for each case the procedure of minimization and reorientation at the interface was applied. The

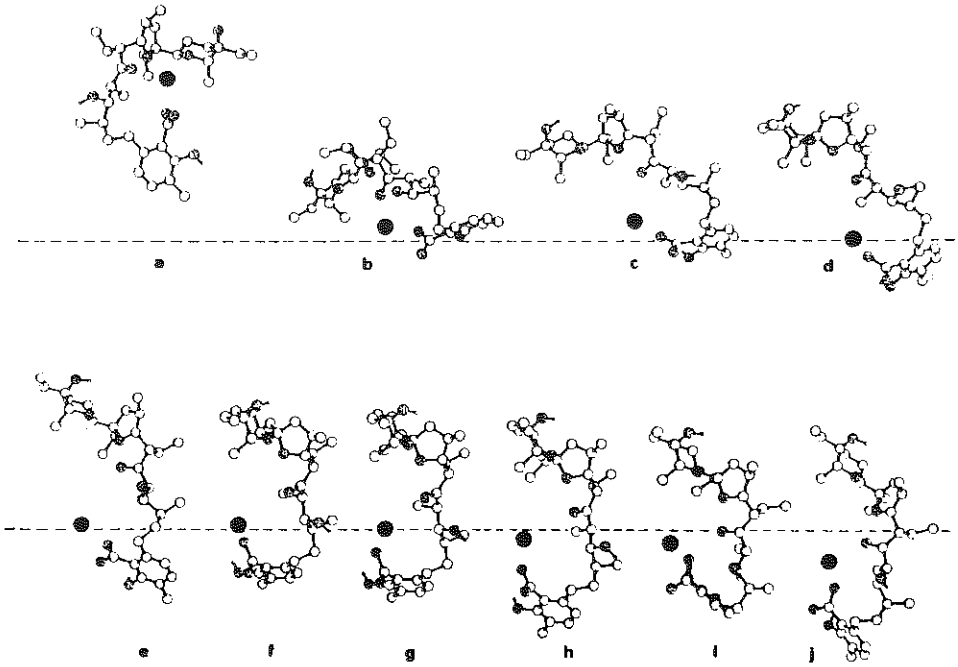


Fig. 2. Sequence of decomplexation process of lasalocid/Na^+ complex at a simulated lipid/water interface. The complex is seen to be transformed from a cyclic form in an apolar environment (a) to a quasi-linear conformation in the high dielectric medium (j) when it is pushed through the interface. Black, dotted, and open circles refer to Na, O, and C atoms respectively. The dotted line represents the membrane interface discontinuity (see text). Letters have the same meaning as in Table 1.

angles of each conformation are listed in Table 2. Each position corresponds to a different degree of immersion of the ionophore into the aqueous phase. It is obvious from Fig. 2 that during the passage through the interface the Na^+ ion leaves its cryptic position. The ionophore adopts progressively a much more extended conformation more favorable to the Na^+ complexation or decomplexation at the interface. This transconformation of a lipophilic complex (Fig. 2a) into an amphiphilic structure (Fig. 2j) is well illustrated by the evolution of the distance between the hydrophobic and hydrophilic gravity centers (Table 2). Painter et al. (14) have proposed that in high-polarity solvents, the uncomplexed anionic ionophore assumes an acyclic conformation and as the solvent polarity decreases, the conformation shifts toward a cyclic structure. Cyclization proceeds by rotation about three carbon-carbon hinge bonds (α_5, α_8, α_9). Our scenario suggests that the transconformation of the Na^+/X537A complex from a cyclic form to an acyclic form is obtained essentially by the rotation about five carbon-carbon hinge bonds (α_4,α_5, α_7, α_8, α_9.

Table 2. Torsional angles of the lasalocid/Na^+ complex

Letters indicate the conformers obtained for a simulated interface imposed at a specific atom position of the ionophore. Atom numbering is given in Fig. 1.

Torsional angles	Atom numbering									
	a	b 25	c 6	d 7	e 8	f 9	g 10	h 11	i 12	j 13
α_1	345	322	323	308	336	325	296	311	344	317
α_2	260	263	231	265	253	260	260	245	261	260
α_3	132	138	137	141	146	149	147	154	148	168
α_4	249	290	299	287	289	270	279	272	292	284
α_5	317	296	304	285	292	295	286	274	323	293
α_6	159	175	165	181	175	180	182	175	164	171
α_7	193	160	171	149	154	163	166	184	153	158
α_8	199	227	226	242	226	227	223	214	213	224
α_9	238	198	200	186	187	194	190	184	187	194
α_{10}	183	173	175	175	167	174	170	169	160	169
α_{11}	317	308	305	309	309	284	304	306	303	291
Distance between hydrophobic and hydrophilic gravity center (Å)										
	0.9	1.52	1.92	2.24	2.39	2.22	1.98	1.90	1.85	1.76

The approach used by Painter et al. considers neither the orientation of the molecule at the membrane/water interface nor the Na^+/lasalocid complex. Conformational change is the result, in this case, of a change of the solvent dielectric constant but the authors do not consider the possibility of a structure modification mediated by the membrane-water interface. We demonstrate here the importance of such a contribution. The method here described makes it possible to predict the inophoretic properties of drugs before their synthesis and offers a unique way to identify transient conformations of ionophores crossing lipid membranes.

Acknowledgements

We thank the Computing Centre of the Université Libre de Bruxelles, where the calculations were performed on a C.D.C. Cyber 170 Computer coupled to a Calcomp drawing table with the Pluto drawing program/B.C. Motherwell and W. Clegg, Pluto Cambridge, Engl. (1978).

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