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LIPID-WATER INTERFACE MEDIATES REVERSIBLE IONOPHORE CONFORMATIONAL CHANGE

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SUMMARY : A new procedure of conformational analysis was used to demonstrate that the ionophore conformation is mediated by its membrane environment. In the hydrophobic lipid matrix, the ionomycin-Ca^{++} complex adopts a conformation well suited for translocation across the interior of the membrane whereas at the lipid-water interface, the Ca^{++} ion is immersed into the aqueous phase in a position favorable to its complexation or decomplexation. The translocation of Ca^{++} across the lipid bilayer supposes a reversible transformation of the two conformers. The conformational analysis shows how the dielectric constant discontinuity existing at the lipid-water interface mediates the reversible transformation of one structure into the other.

Ionomycin is a recently described polyether antibiotic. It extracts Ca^{++} from an aqueous phase into an organic phase with a 1/1 stoichiometry (1) and is capable of transporting Ca^{++} across biological membranes (2). This permeation process postulates the existence of two conformations for the ionomycin-Ca^{++} complex : a lipophilic conformer capable to convey Ca^{++} across the hydrocarbon region of the cell membrane and an interfacial conformer responsible of the ion-complexation-decomplexation process at the lipid-water interface. To our knowledge, these structures remain hypothetical. We report here about their configurations predicted by conformational analysis (3) and show how the dielectric constant discontinuity existing at the lipid-water interface mediates the transformation of one structure into the other.

METHODS

The conformation of the isolated molecule and its orientation at a simulated lipid-water interface were established by a method described elsewhere (3,4). The total conformational energy was empirically calculated as the sum of the contributions resulting from the Van der Waals interaction, the torsional potential and the electrostatic interaction. To simulate a lipid-water interface, the latter was calculated for a dielectric constant of 3 and 30 respectively in the hydrophobic and hydrophilic media (4). The values for valence angles, boundary lengths, atomic charges and torsional potentials were those currently used in conformational analysis (5). Selected conformers were then submitted to a simplex minimization procedure (6). Their orientations at the interface were defined by calculation of the hydrophilic and hydrophobic gravity centers. These centers were established taking into

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account the transfer energy of each part of the molecule. The hydrophilic gravity center ($\vec{C}_w$) is defined by equation :

$$\vec{C}_w = \sum_{i=1}^{n} [E^+_{transfer_i} \vec{r}_i] / \sum_{i=1}^{n} E^+_{transfer_i}$$

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 values of the transfer energies used were identical to those determined experimentally by numerous authors and summarized elsewhere (7).
The interface position ($\vec{I}$) is defined by the equation :

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

In the second step of the procedure, the assembly of the molecules (3) in the monolayer was computed as follows. The position of a molecule B relative to a reference molecule A was assessed by successive changes of the following parameters: distance between hydrophilic centers of A and B (by steps of 0,05 nm along the X axis), rotation of molecule B around its own Z axis and around molecule A (by steps of 30°), migration of molecule B along the Z axis perpendicular to the lipid-water interfaces (by steps of 0,05 nm) and oscillation of molecule B around its Z axis (by steps of 2°30' each). Interaction between molecules A and B was evaluated in term of Van der Waals and electrostatic energies. The conformation of molecule A and molecule B yielding the lowest energy was then used as a reference to define the position of a third molecule. The same procedure could be repeated up to a total of 10 molecules. The mean molecular area was estimated from the projection of the molecule on the X-Y plane using a grid of squares, each with a 0,1 nm side. Calculations were performed on a CDC-Cyber 170 Computer coupled to a Benson drawing table.

RESULTS AND DISCUSSION

Ionomycin presents 27 rotational angles (Fig.1). The Ca-O distance was taken equal to 2,3 Å as determined by X-Ray analysis (8). If the angles are modified by steps of 60°, more than 10^{21} conformers could be obtained. Therefore, the calculation of conformational analysis was performed on 2 different parts of the molecule. A first systematic study was performed on the angles labelled ($\alpha_1, \alpha_2, \alpha_3, \alpha_4, \alpha_5, \alpha_6, \alpha_7, \alpha_8, \alpha_9$) allowing to design a conformer with a probability of 81 %. A second systematic study was carried out for another angles ($\alpha_{10}, \alpha_{11}, \alpha_{12}, \alpha_{13}, \alpha_{14}, \alpha_{15}, \alpha_{16}, \alpha_{17}, \alpha_{18}, \alpha_{19}, \alpha_{20}, \alpha_{21}, \alpha_{22}$) maintaining unchanged the angles of the first segment.
The conformations derived from this study and yielding a low internal energy, i.e. those with a statistical weight of at least 5 %, were then submitted to a second analysis using a simplex minimization procedure (6). Figure 2 illustrates the most probable conformer displaying a 86 % probability. It takes a cyclic form, screening the ion from the hydrophobic interior of the membrane. RX diffraction analysis (8) and N.M.R. study (9) demonstrated that the enolized ß diketone anion together with a carboxylate group and 3 other oxygen atoms are coordinated to the central Ca^{++} ion.

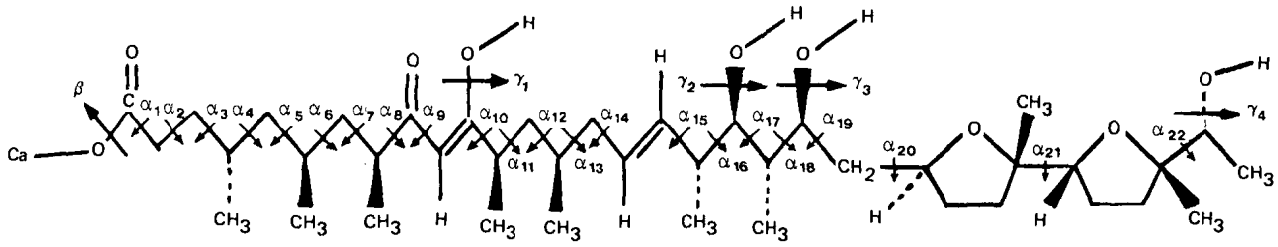

Figure 1. : Ionomycin chemical formula and position of the torsional angles.

Table I : Most probable conformers after minimization of the ionomycin at the lipid-water interface.

Torsional angles	α1	α2	α3	α4	α5	α6	α7	α8	α9	α10	α11	α12	α13	α14	α15	α16	α17	α18	α19	α20	α21	α22	β	γ1	γ2	γ3	γ4
Conformer A	51	64	-76	179	178	-45	133	175	73	-29	192	62	54	179	196	184	-56	-76	172	180	178	179	189	153	183	180	167
Conformer B	55	77	-67	151	192	-55	107	156	81	-13	-66	194	-60	145	177	181	-66	-81	175	182	176	181	183	146	197	221	174

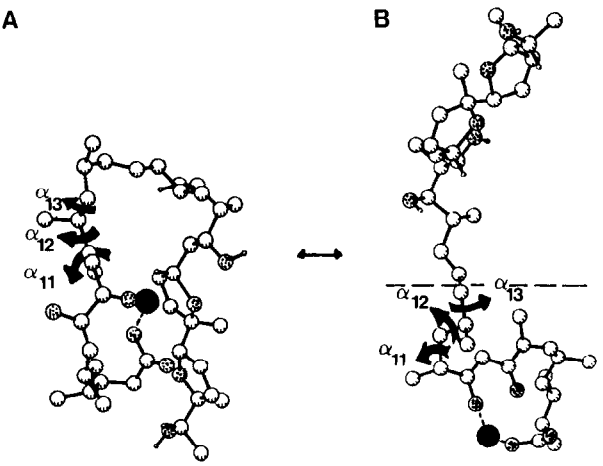

Figure 2. : Conformation of the ionomycin-Ca^{++} complex
A. Hydrophobic complex
B. Interfacial complex
The Ca^{++} ion is shown as a closed circle. Open circles refer to carbon atoms and dotted circles to oxygen atoms. The dotted line delineates the hydrophobic (ε=3) and the hydrophilic medium (ε=30).

Comparison of the rotation angles for our calculated structure and the RX conformation indicates that, even if the 2 forms are large lipophilic, they are however not identical probably as a consequence of the influence of the lipid environment. It is known that lipid rigidity inhibits the ionomycin-mediated Ca^{++} transport (10). How the lipid packing will modulate ionomycin structure is presently under investigation. However it is obvious that if the obtained conformer is well suited for translocation across the hydrophobic interior of membrane, it is difficult to imagine how this species will capture or release Ca^{++} at the membrane-water interface. We demonstrate here that the lipid-water interface can mediate the needed conformational change. To simulate a lipid-water interface, the dielectric constant of the hydrophilic and hydrophobic medium was taken as 30 and 3. The dotted line delineates these two domains (Fig.2). From a biological point of view, this simulation will mimic the immersion of the membrane bound ionophore into the aqueous phase. In these conditions, the hydrophobic structure is transformed into the interfacial structure (Fig.2). Table I indicates which rotations are necessary to induce the conformational change. The transconformation can easily occur by rotation of 3 angles (α_{11},α_{12},α_{13} (Table I)). The Ca^{++} ion leaving its cryptic position within the lipid layer is immersed into the aqueous phase in a position favorable to its complexation or decomplexation at the interface. No experimental information is available on the conformation of this ionophore-ion complex formed at the lipid-water interface. It can however be mentionned (our unpublished results) that the area occupied per ionophore molecule (124 $\AA^2$) in a monolayer spread on an aqueous phase

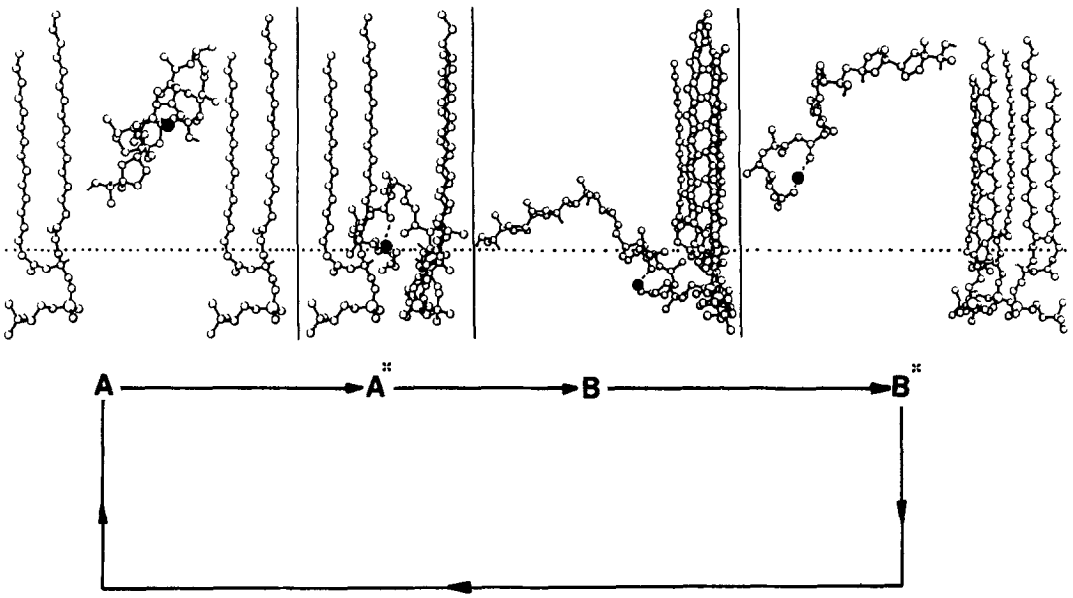

Figure 3. : Transition between the ionomycin-Ca^{++} interfacial complex and the hydrophobic complex. The dotted line has the same meaning as in Fig.2. Ionomycin was inserted into the lipid layer (DL-α-dipalmitoylphosphatidylcholine) using a procedure described elsewhere (3).

$A \rightarrow A^{*}$: vertical displacement of the Ca^{++}-ionomycin complex. The molecule is partially immersed into the aqueous phase (ε=3, ε=30).

$A^{*} \rightarrow B$: conformational change.

$B \rightarrow B^{*}$: vertical displacement of the Ca^{++}-ionomycin complex. The molecule is embedded into the lipid layer (ε=3).

$B^{*} \rightarrow A$: conformational change.

containing Ca^{++} is very similar to the calculated area occupied per ionophore molecule (127 $\mathring{A}^2$). The calculated energy of interaction between ionomycin and lipid is much lower than the lipid-lipid interaction (-13 Kcal/mole). Values of -0,7 Kcal/mole and -3,54 Kcal/mole were respectively found for the interfacial ionomycin-Ca^{++} complex and the hydrophobic ionomycin-Ca^{++} complex. This weak interaction with the lipid phase will render possible the complex migration through the bilayer.

The process is entirely reversible. When the conformational analysis is applied to the interfacial structure B, in a medium of dielectric constant equal to 3, structure A is obtained (Fig.3). The lipid organization is not modified after the ionomycin insertion probably as a consequence of the similitude between the calculated molecular area of the ionophore (60 $\mathring{A}^2$) and of the lipid molecule (60 $\mathring{A}^2$). Finally, our results suggest that a modification in the degree of penetration of a molecule into the lipid layer can induce its conformational change. They could explain conformational changes associated to membrane enzyme-substrate and hormone-receptor inter-

actions. These interactions can mediate even during a very short time, a modification of the hydrophobic-hydrophilic balance of these new entities, a change of their depth of insertion into the lipid layer and consequently a transient conformational state. Experiments of insulin binding to liver plasma membranes support this hypothesis (11). Binding of insulin to its receptor mediated an increase of the degree of exposure of the membrane protein and probably a change of its vertical displacement in the lipid layer which could modulate the response to insulin.

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REFERENCES

1. Lin, C.M. and Hermann, T.E. (1978) J. Biol. Chem. 253, 5892-94.
2. Kauffman, R.F., Taylor, R.W. and Pfeiffer, R. (1980) J. Biol. Chem. 255, 2735-2739.
3. Brasseur, R., Goormaghtigh, E. and Ruysschaert, J.M. (1981) Biochem. Biophys. Res. Commun 103, 301-310.
4. Brasseur, R., Deleers, M., Malaisse, W.J. and Ruysschaert, J.M. (1982) Proc. Natl. Acad. Sci., USA, 79, 2895-2897.
5. Hopfinger, A.J. (1973) Conformational Properties of Macromolecules (Academic, New York, London).
6. Nelder, J.A. and Mead, R. (1965) Computer J. 7, 308-313.
7. Tanford, C. (1973) in The Hydrophobic Effect. Formation of Micelles and Biological Membranes (John Wiley & Sons, Eds, New York).
8. Toeplitz, B.K., Cohen, A.I., Funke, P.T., Parker, W.L. and Gougoutas, J.Z. (1979) J. Am. Chem. Soc. 101, 12, 3344-3353.
9. Anteunis, M.J.O. and Verhegge, G. (1981) Bull. Soc. Chim. Belge 90, 11, 1153-1165.
10. Deleers, M., Couturier, E. and Malaisse, W.J. (1981) Cell Calcium 2, 159-171.
11. Luly, P. and Shinitzky, M. (1979) Biochemistry 18, 445-450.