Conformational analysis of the calcium–A23187 complex at a lipid–water interface

(ionophore/dielectric constant/transfer energy)

ROBERT BRASSEUR*, MICHEL DELEERS†, WILLY J. MALAISSE†, AND JEAN-MARIE RUYSSCHAERT*

*Laboratory of Macromolecules at Interface; and †Laboratory of Experimental Medicine, Brussels Free University, Brussels, Belgium B-1000

ABSTRACT   A possible conformation of the complex formed by one calcium ion and two molecules of the ionophore A23187 at a simulated lipid–water interface was predicted by a variant method for conformational analysis. This method takes into account, in addition to the Van der Waals energy, electrostatic interaction, and torsional potential, the alteration of electrostatic forces attributable to changes in dielectric constant at the interface and the transfer energy for each part of the complex as it moves through the lipid–water interface. The most probable conformer was characterized by a two-fold axial symmetry that was maintained during transition to the hydrophobic bulk conformation. Minor changes in the interfacial structure were sufficient to achieve the configuration characteristic of the hydrophobic bulk phase.

Carboxylic ionophores such as A23187 and bromolasalocid are widely used to facilitate Ca^{2+} transport across natural (1–4) or artificial (5–9) membranes. These ionophores form complexes in which each Ca^{2+} is bound to two molecules of the same (10–12) or different (13–14) ionophores. The conformations of such complexes in nonpolar solvents and in the crystalline form have been extensively investigated (15–20). However, to our knowledge, no information is available on the transient conformation of the complex when it is formed at a lipid–water interface. The stoichiometry of the complex and the pK_a value have been established with liposomes and solvents of different polarity (6, 8, 15, 21). By using a monolayer approach, Ferreira et al. (22) have examined the conformational stability (k_d) and molecular area of A23187 at an air–water interface.

In the present study, we have attempted to define the conformation of the complex between Ca^{2+} and two molecules of A23187 at a simulated lipid–water interface. For this purpose, we took into account for calculation of conformational energy not only the Van der Waals but also the electrostatic and torsional contributions. In addition, the electrostatic contributions were calculated as a function of changes in the dielectric constant, and allowance was made for the transfer energy of each part of the molecule as it moves through the lipid–water interface.

METHODS

The method followed for the conformational analysis of the Ca–A23187 complex is based on a strategy described elsewhere (23) and currently used to study the conformations of polypeptides (23–26) and other molecules (27, 28). This method was modified to incorporate variations in the dielectric constant and the energy of transfer from one environment to another at the simulated lipid–water interface.

Briefly, the total conformational energy of the interfacial complex was empirically calculated as the sum of all contributions resulting from local interactions—i.e., the Van der Waals energy, the torsional potential, the electrostatic interaction, and the transfer energy. The electrostatic energy was calculated as a function of the dielectric constant (ε). To simulate a lipid–water interface, the dielectric constants of the hydrophobic and hydrophilic media were taken as 3 and 30, respectively. Between these two media, the dielectric constant was assumed to increase linearly over a distance of 4.5 Å along the z axis, which is perpendicular to the interface. The length 4.5 Å corresponds to the distance between the Ca^{2+} and the line passing between the N atoms of the two benzoxazole rings of A23187 in the all-trans configuration. The energy of transfer for distinct parts of the molecule has been determined experimentally by numerous authors, as summarized elsewhere (29). The values used for the valence angles, bonding lengths, and atomic charges were those determined by x-ray analysis (17) or currently used in conformational analysis (30, 31). A given torsional angle (j) was defined as the angulation between the ($j-1$) and ($j+1$) bonds. It equals zero if the two bonds are superimposed (eclipsed) when examined along the j bond and increases if the ($j+1$) distal bond is rotated clockwise (32).

In the method of calculation, six changes of 60° each were first imposed on each of n torsional angles, yielding 6^n conformers. The internal energy was calculated for each of these conformers. The most probable configurations were taken as those yielding the lowest internal energy, such a selection based on the statistical weight (Boltzmann) of all individual configurations (31). A simplex minimization procedure (33) was eventually used to further reduce the total internal energy of selected conformations.

RESULTS

The Ca–A23187 complex was assumed to be formed of two molecules of ionophore and one Ca^{2+}. The molecular structure of the ionophore and the nomenclature of the torsional angles, together with the all-trans conformation taken as our initial model, are illustrated in Fig. 1. In this model, the length of the Ca—O bond is 4.2 Å. This value corresponds to half the maximal distance between the carboxyl groups of two molecules of A23187 in the acid form, as calculated for a monolayer of A23187 at an air–water interface (data not shown).

The Ca–A23187 complex has 18 rotational angles. If these angles are affected by 60° changes, more than 10^{14} conformers could be designed. A more economic procedure was used therefore, the calculations of conformational analysis being carried out in a stepwise manner on three different parts of the molecule.

First, a systematic study was carried out on the angles α_1, α_1', β_1, β_1', β_2, and β_2', allowing us to design two conformers with
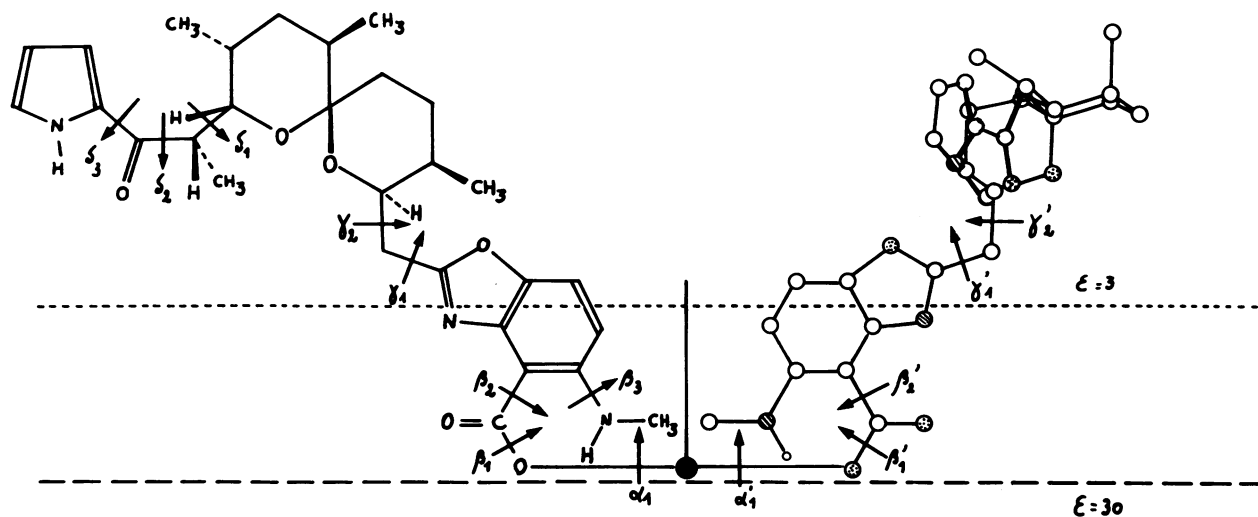
FIG. 1.   Configuration of the Ca–A23187 complex at a simulated lipid–water interface. (*Left*) Structural formula with torsional angles (arrows) indicated. (*Right*) *All-trans* configuration of the initial conformer (frontal view along the x axis). ●, Ca^{2+}; the vertical line passing through the Ca^{2+} is parallel to the z axis and serves as a reference for the torsional angles α_1 and α'_1. The torsional angles are defined by reference to the adjacent bonds located along the shortest chain of atoms connecting the Ca^{2+} to the N atom of the pyrrole group in each moiety of the complex. Hatched and dotted circles refer to N and O atoms, respectively. Open circles refer to carbon atoms (>C<, —CH=, —CH₂—, —CH₃). --- and ——, Zone in which the dielectric constant is assumed to increase linearly from the low value characteristic of the lipid phase ($\varepsilon = 3$) to the high value characteristic of the water phase ($\varepsilon = 30$).

probabilities of 64% (conformer A_1) and 24% (conformer B_1), respectively. Then, these conformers were used for a systematic study on the angles γ_1, γ'_1, γ_2, γ'_2, δ_1, and δ'_1. This study yielded, in each case, one conformer with a probability in excess of 95%—namely, A_{12} and B_{12}. Finally, these conformers were used for a systematic study on the angles δ_2, δ'_2, δ_3, and δ'_3 and yielded in each case a conformer with a probability close to 99% (A_{123} and B_{123}). The conformer A_{123} is shown in Fig. 2, as seen along along either the x or z axis.

Further calculations by the simplex procedure on conformers A_{123} and B_{123} yielded the two conformers A'_{123} and B'_{123} defined in Table 1. Conformer A'_{123} is characterized by a two-fold axial symmetry parallel to the z axis. In conformer B'_{123}, this symmetry is broken, mainly as a result of the major change in the α_1 and α'_1 angles (Table 1). The total energy of conformer A'_{123} is 1 kcal/mol lower (1 cal = 4.18 J) than that of B'_{123}. The configuration of the A'_{123} interfacial conformer and the rotations necessary to convert this conformer into the configuration of the Ca–A23187 complex as derived from x-ray analysis (17) are shown in Fig. 3.

DISCUSSION

Conformational analysis of the Ca–A23187 complex at a simulated lipid–water interface suggests, as the most probable conformer, a configuration characterized by a two-fold axial symmetry. The symmetry axis is perpendicular to the interface and passes through the Ca^{2+}. From this interfacial structure, the transition to the configuration characteristic of the hydrophobic bulk phase can easily occur by rotation of the benzoxazole rings around γ_2 and γ'_2, with concomitant shortening of the Ca—O bond from 4.2 to 2.3 Å (Fig. 3). As a result of these changes, the Ca^{2+} reaches its cryptic position within the complex. The

two-fold axial symmetry is maintained during this conformational transition. Likewise, the spirolactone groups keep the same position during such a transition. The pyrrole groups, however, undergo a slight displacement, which could be merely associated with migration of the Ca^{2+} along the z axis from the lipid–water interface to the hydrophobic bulk phase.

The changes postulated in the configuration of the Ca–A23187 complex as it moves from the lipid–water interface to the hydrophobic bulk phase were essentially identical whether the bulk-phase configuration was derived from x-ray or conformational analysis. In the latter case, calculations on the angles α_1, α'_1, β_1, β'_1, β_2, β'_2, γ_1, γ'_1, γ_2, and γ'_2, with the length of the Ca—O bond reduced to 2.3 Å, led to a conformer with rotated benzoxazole rings (data not shown), as seen in the model defined by x-ray analysis (Fig. 3).

The approach used here for conformational analysis of the Ca–A23187 complex at a simulated lipid–water interface adds two features to the current technique of conformational analysis—i.e., changes in electrostatic forces as a function of the environmental dielectric constant and the energy of transfer for each part of the molecule as it moves from the interface to the hydrophobic bulk phase. This method offers a way to predict the behavior of extracellular molecules as they come in contact with the phospholipid domain of the plasma membrane and, eventually, are incorporated into such a domain.

1.   Scarpa, A., Baldassare, J. & Inesi, G. (1972) *J. Gen. Physiol.* **60**, 735–749.
2.   Caswell, A. H. & Pressman, B. C. (1972) *Biochem. Biophys. Res. Commun.* **49**, 292–298.

Table 1.   Conformers of Ca–A23187 complexes

Conformer	Torsional angle, °																
	$\alpha_1 + \alpha'_1$	β_1	β'_1	β_2	β'_2	β_3	β'_3	γ_1	γ'_1	γ_2	γ'_2	δ_1	δ'_1	δ_2	δ'_2	δ_3	δ'_3
A'_{123}	8	180	175	320	320	235	229	196	194	164	165	245	245	153	154	176	176
B'_{123}	160	181	203	300	303	253	228	187	202	179	168	253	250	149	150	175	177

FIG. 3.   Comparison between A′₁₂₃ interfacial complex (*Lower*) and configuration of the Ca–A23187 complex derived from x-ray analysis (*Upper*) (adapted from ref. 17). Symbols for the various atoms are as in Fig. 1. In *Lower*, heavy lines emphasize proximal part of the complex and interrupted and curved arrows illustrate postulated movements of the ketopyrrole and benzoxazole rings as the complex moves from the lipid–water interface to the bulk organic phase.

FIG. 2.   (*Upper*) Frontal view along the x axis of a most probable conformer (A₁₂₃). Heavy lines emphasize the proximal part of the complex. --- and —— have the same meaning as in Fig. 1. (*Lower*) The A₁₂₃ conformer as observed from the aqueous phase along the z axis. Heavy lines refer to the benzoxazole rings.

3. Case, G. C., Vanderkooi, J. M. & Scarpa, A. (1974) *Arch. Biochem. Biophys.* 162, 174–185.
4. Pressman, B. C. (1976) *Annu. Rev. Biochem.* 45, 501–530.
5. Hyono, A., Hendriks, Th., Daemen, F. J. & Bonting, S. L. (1975) *Biochim. Biophys. Acta* 389, 34–46.
6. Puskin, J. S. & Gunter, T. E. (1975) *Biochemistry* 14, 187–191.
7. Kafka, M. S. & Holz, R. W. (1976) *Biochim. Biophys. Acta* 426, 31–37.
8. Wulf, J. & Pohl, W. G. (1977) *Biochim. Biophys. Acta* 465, 471–485.
9. Deleers, M. & Malaisse, W. J. (1980) *Biochem. Biophys. Res. Commun.* 95, 650–657.
10. Reed, P. W. & Lardy, H. A. (1972) *J. Biol. Chem.* 247, 6970–6977.
11. Chaney, M. O., De Marco, P. V., Jones, N. D. & Occolowitz, J. L. (1974) *J. Am. Chem. Soc.* 96, 1932–1933.
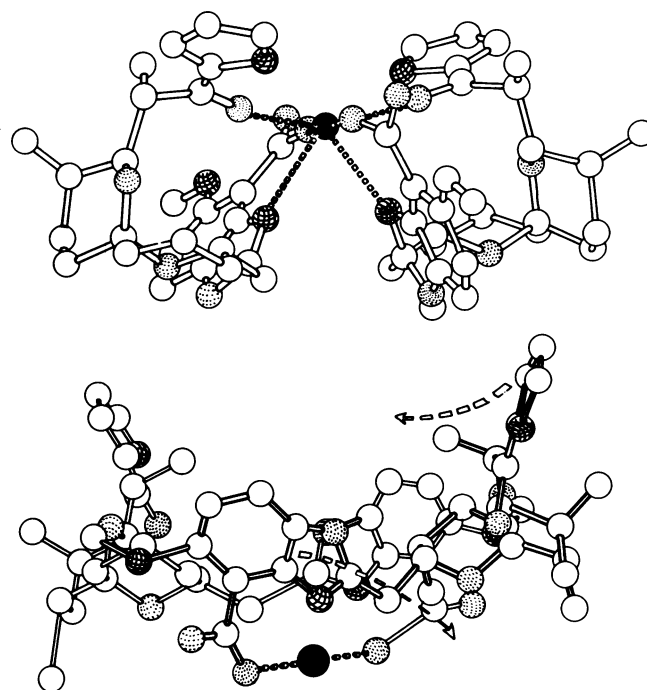12. Pfeiffer, D. R. & Lardy, H. A. (1976) *Biochemistry* 15, 935–943.

13. Couturier, E., Deleers, M. & Malaisse, W. J. (1980) *Pharmacol. Res. Commun.* 12, 651–655.
14. Deleers, M., Gelbcke, M. & Malaisse, W. J. (1981) *Proc. Natl. Acad. Sci. USA* 78, 279–282.
15. Pfeiffer, D. R., Reed, P. W. & Lardy, H. A. (1974) *Biochemistry* 13, 4007–4014.
16. Deber, C. M. & Pfeiffer, D. R. (1976) *Biochemistry* 15, 132–141.
17. Smith, G. D. & Duax, W. L. (1976) *J. Am. Chem. Soc.* 98, 1578–1580.
18. Patel, D. S. & Shen, C. (1976) *Proc. Natl. Acad. Sci. USA* 73, 1786–1790.
19. Shen, C. & Patel, D. S. (1976) *Proc. Natl. Acad. Sci. USA* 73, 4277–4281.
20. Chiang, C. C. & Paul, I. C. (1977) *Science* 196, 1441–1443.
21. Pfeiffer, D. R., Taylor, R. W. & Lardy, H. A. (1978) *Ann. N.Y. Acad. Sci.* 307, 402–423.
22. Ferreira, J., Caspers, J., Brasseur, R. & Ruysschaert, J. M. (1981) *J. Colloid Interface Sci.* 81, 158–168.
23. Ralston, E. & De Coen, J. L. (1974) *J. Mol. Biol.* 83, 393–420.
24. Ralston, E., De Coen, J. L. & Walter, R. (1974) *Proc. Natl. Acad. Sci. USA* 71, 1142–1144.
25. De Coen, J. L. & Ralston, E. (1977) *Biopolymers* 16, 1929–1937.
26. De Coen, J. L., Humblet, C. & Koch, M. H. J. (1977) *FEBS Lett.* 73, 38–42.
27. Deleers, M., Brasseur, R., Gelbcke, M. & Malaisse, W. J. (1982) *J. Inorg. Biochem.*, in press.
28. Brasseur, R., Goormaghtigh, E., Ruysschaert, J. M. (1981) *Biochem. Biophys. Res. Commun.* 103, 301–310.
29. Tanford, C. (1973) *The Hydrophobic Effects. Formation of Micelles and Biological Membranes* (Wiley, New York).
30. Hopfinger, A. J. (1973) *Conformational Properties of Macromolecules* (Academic, New York, London).
31. Brasseur, R. (1981) *Dissertation* (Free University of Brussels).
32. Sundaralingam, M. (1972) *Ann. N.Y. Acad. Sci.* 195, 324–355.
33. Nelder, J. A. & Mead, R. (1965) *Comput. J.* 7, 308–313.