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EVIDENCE OF A THROMBOXANE A_2-Ca^{2+} COMPLEX : A CONFORMATIONAL APPROACH

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ABSTRACT

Thromboxane A_2 has been proposed as an ionophore capable to transport calcium from the platelet dense tubular system to the cytoplasm to activate the contractile proteins. However the half-life (30 sec.) of this compound limits considerably the experimental approach. The conformational analysis is proposed here as a possible way to identify such transient conformations. We bring evidence of the existence of two Ca^{2+}-thromboxane A_2 isomeric complexes : one structure responsible for the complexation-decomplexation process at the interface and one lipophilic structure able to cross the lipid membrane.

INTRODUCTION

During the last ten years, novel compounds derived from arachidonic acid have been demonstrated to have profound but probably not exclusive effects on platelet aggregation. Thromboxane A_2 (TXA_2) (Hamberg et al., 1975) was believed to be an absolute requirement for platelet aggregation (Gorman, 1979) even if in some cases endoperoxide PGH_2 can initiate this process. Prostacyclin (PGI_2) discovered one year later (Moncada et al., 1976) is the most potent endogenous inhibitor of platelet aggregation. If the mechanism of action of these compounds has been extensively studied, little is known about the process permitting to these hydrophilic molecules to cross the lipid bilayers. It seems indeed probable that to function effectively TXA_2 would need to be able to move from the inside layer of the dense tubular system bilayers across the membrane to the cytosol. An experimental answer to this question is difficult. This is primarily due to the structure of TXA_2 which exhibits a half-life of approximatively 30 sec. at physiological temperature and pH. Consequently, it is not possible to isolate and purify thromboxane A_2 in amounts usually needed for classical experimental procedures (RX diffraction, circular dichroism spectroscopy...). We present another approach based on a

conformational analysis procedure allowing a molecular description of the conformation of amphiphilic molecules (Brasseur et al, 1981; Brasseur et al.,1982; Brasseur and Hurwitz, 1983). We bring here evidence of the conformation of a $Ca^{2+}-(TXA_2)_2$ complex which may adopt two conformations : a lipophilic structure capable of moving across the hydrocarbon region of a cell membrane and an interfacial structure responsible for the ion decomplexation-complexation process at the lipid-water interface.

METHOD

The conformation of the isolated molecule and its orientation at the lipid-water interface has been established as described elsewhere (Brasseur et al., 1981; Brasseur et al., 1982). The total conformational energy is 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 (Brasseur and Hurwitz, 1983; De Coen et al., 1967)

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

where ij=1,2,... are non bonded atoms, r_{ij} their distances from each other and A_{ij},B_{ij} and C_{ij} are coefficients assigned to atom pairs. The values of these coefficients have been reported by Liquori and Giglio (Liquori et al., 1968; Giglio et al., 1968). Like other quantum mechanical results (Scordamaglia et al., 1977), these values emerge in part as the solution of Schrödinger equation and in part as heuristic variables. They have been applied with success to conformational analysis of molecular crystals, proteins, polypeptides and lipids (Brasseur et al., 1981; Giglio, 1969; Liquori, 1969; De Coen and Ralston, 1971). In order to compensate 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 :

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

where e_i and e_j are expressed in electron charge unit and r_{ij} in Å. The value

of the atomic point charges are similar to the values used for polypeptides.

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 are similar to those determined experimentally by numerous authors and summarized elsewhere (Tanford, 1973).

In the calculation procedure, six changes of 60° each were first imposed to 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, i.e. those with a statistical weight of at least 1%. The values used for the valence angles and bond lengths were currently used in conformational analysis (Hopfinger, 1973). After systematic analysis, conformations selected for their lowest internal energy were submitted to a simplex minimization procedure (Nelder and Mead, 1965). To simulate the membrane interface, we have assumed a dielectric constant ε_{ij} equal to 3 above the interface while the atom at the bottom of the Ca^{2+}-$(TXA_2)_2$ configuration was fixed at a plane where the dielectric constant was 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 :

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

in which $\vec{r}_i$ are the coordinates of the i atom. The hydrophobic 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 (I) is defined by the equation :

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

Calculations were performed at 25°C on a CDC-CYBER 170 Computer coupled to a CALCOMP 1051 drawing table.

RESULTS

The $(TXA_2)_2\text{-}Ca^{2+}$ complex presents 28 rotational angles (Fig.1).

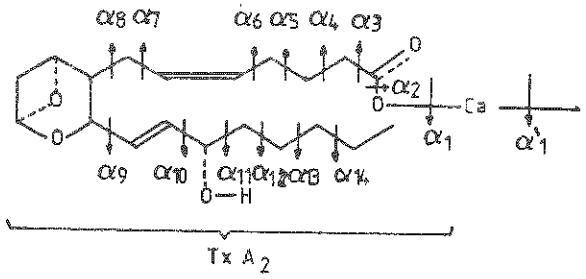
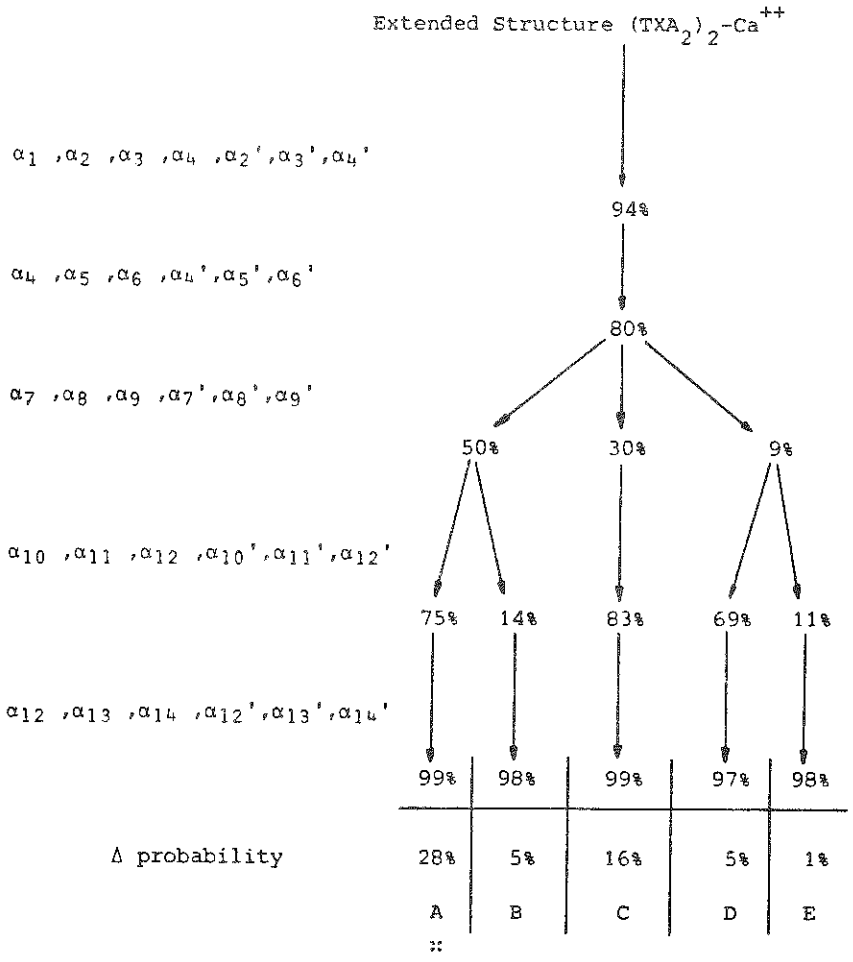


Figure 1. : Initial all trans conformation of thromboxane $A_2\text{-}Ca^{2+}$ complex showing the numbering of the torsional angles.

The Ca-O distance was taken equal to 2.3 Å as determined by X-ray analysis for a similar bond (Brasseur et al., 1982). If all angles were modified by steps of 60°, more than 10^{21} conformers could be obtained. Therefore, the conformational analysis was performed on five different parts of the molecule. First, a systematic study was carried out on the angles $\alpha_1,\alpha_2,\alpha_3,\alpha_4,\alpha'_2,\alpha'_3,\alpha'_4$ allowing us to design one conformer with a probability of 94% (Table I). Second, these conformers were used for a systematic study on the angles $\alpha_4,\alpha_5,\alpha_6,\alpha'_4,\alpha'_5,\alpha'_6$. This study yielded one conformer with a probability equal to 80% (Table I). Third, this conformer was used for a systematic study on the angles $\alpha_7,\alpha_8,\alpha_9,\alpha'_7,\alpha'_8,\alpha'_9$. This study yielded three conformers with a probability equal to 50%, 30% and 9% (Table I). These conformers were used for a systematic study on the angles $\alpha_{10},\alpha_{11},\alpha_{12},\alpha'_{10},\alpha'_{11},\alpha'_{12}$. This analysis yielded two structures for the first conformer (75% and 14%), one structure for the second conformer (83%) and two structures for the last conformer (69% and 11%) (Table I).

Finally, a systematic analysis was performed with the angles $\alpha_{12},\alpha_{13},\alpha_{14},\alpha'_{12},\alpha'_{13},\alpha'_{14}$. This study yielded, in each case, one conformer with a probability in excess of 97%-namely A,B,C,D and E (Table I). The probability of each conformer is equal to the product of the probability along a branch.

Table I : Structure obtained after four successive conformational analysis bearing on indicated rotational angles.



Σ probability = 55%

Conformations with probability of existence below 5% were rejected. Δ is the product of probabilities. Σ is the sum of each probability of each branch. :: is the most probable conformer.

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Table II : Most probable conformers of $(TXA_2)_2$-Ca^{++} complex after systematic analysis.

	A 28%	B 5%	C 16%	D 5%	E 1%
α_1	180	180	180	180	180
α_2	180	180	180	180	180
α_3	300	300	300	300	300
α_4	60	60	60	60	60
α_5	300	300	300	300	300
α_6	300	300	300	300	300
α_7	180	180	180	180	180
α_8	180	180	180	180	180
α_9	180	180	180	180	180
α_{10}	180	180	180	180	180
α_{11}	60	60	60	60	60
α_{12}	180	180	180	180	180
α_{13}	180	180	180	180	180
α_{14}	180	180	180	180	180
α_1'	180	180	180	180	180
α_2'	180	180	180	180	180
α_3'	120	120	120	120	120
α_4'	180	180	180	180	180
α_5'	180	180	180	180	180
α_6'	120	120	120	120	120
α_7'	180	180	180	180	180
α_8'	180	180	180	180	180
α_9'	0	0	0	60	60
α_{10}'	300	300	300	240	300
α_{11}'	120	300	120	120	120
α_{12}'	180	120	240	180	240
α_{13}'	180	180	180	180	180
α_{14}'	180	180	180	180	180

A, B, C, D and E are the conformers of the extremity of the branchs shown in table I.

Table III : Most probable conformers after minimization procedure.

	A'	
	a	b
α_1	167	167
α_2	112	110
α_3	280	285
α_4	176	183
α_5	285	300
α_6	157	161
α_7	167	169
α_8	300	301
α_9	196	181
α_{10}	172	170
α_{11}	69	72
α_{12}	171	180
α_{13}	181	185
α_{14}	169	150
α_1'	162	169
α_2'	176	112
α_3'	50	282
α_4'	204	178
α_5'	169	295
α_6'	204	305
α_7'	151	163
α_8'	91	165
α_9'	52	298
α_{10}'	272	175
α_{11}'	101	171
α_{12}'	182	78
α_{13}'	160	181
α_{14}'	169	181

a : at the lipid-water interface

b : in an hydrophobic medium.

(Denoted by Δ in Table I)

$$P = \prod_{i=1}^{n} P_i$$

The conformer A was the most probable conformer (28%). All conformers obtained after systematic analysis represented a total probability equal to 55%. The torsional angles associated to each conformers are given in Table II. Further calculations by the simplex procedure on conformers A,B,C,D and E in hydrophobic media and at the lipid-water interface yielded five conformers A',B', C',D' and E'. For each medium, all conformers give an unique structure A' after simplex procedure (Table III). Fig.2 shows stereoviews of the conformer A' in the

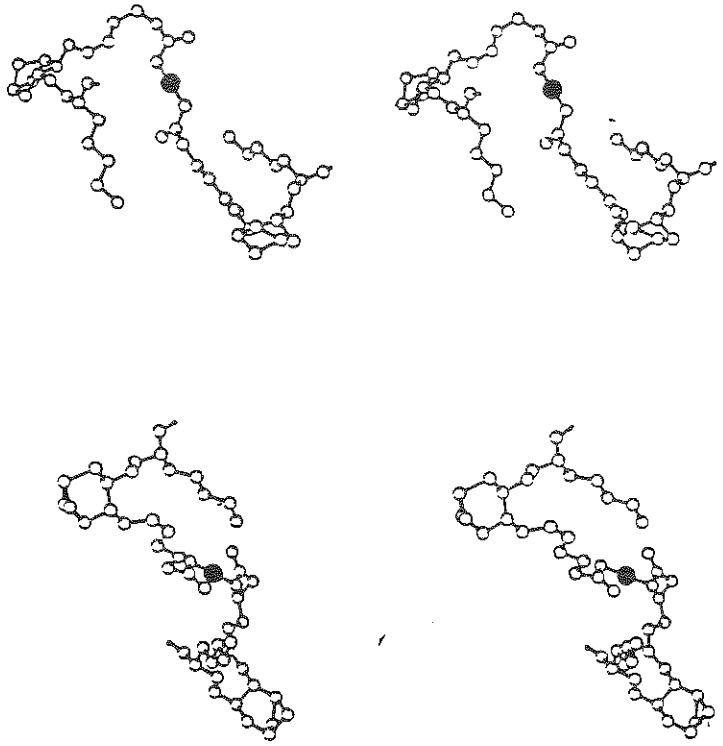

Figure 2.: Stereoscopic views of the most probable conformation of thromboxane A_2-Ca^{2+} complex in an hydrophobic medium after application of the simplex minimization procedure. The views were taken along two directions perpendicular to each other.

hydrophobic medium. The views were taken along two perpendicular axes. The black circles refers to the Ca^{2+} ion. It shows that the $(TXA_2)_2$-Ca^{2+} complex may assume a suitable configuration for the insertion of a Ca^{2+} ion in a cryptic hydrophilic cavity, screening the Ca^{2+} ion from the hydrophobic medium. This conformation appears quite favorable for the transport of Ca^{2+} ion by the $(TXA_2)_2$-Ca^{2+} complex across the hydrophobic media. Fig.3 shows stereoviews of $(TXA_2)_2$-Ca^{2+} complex at

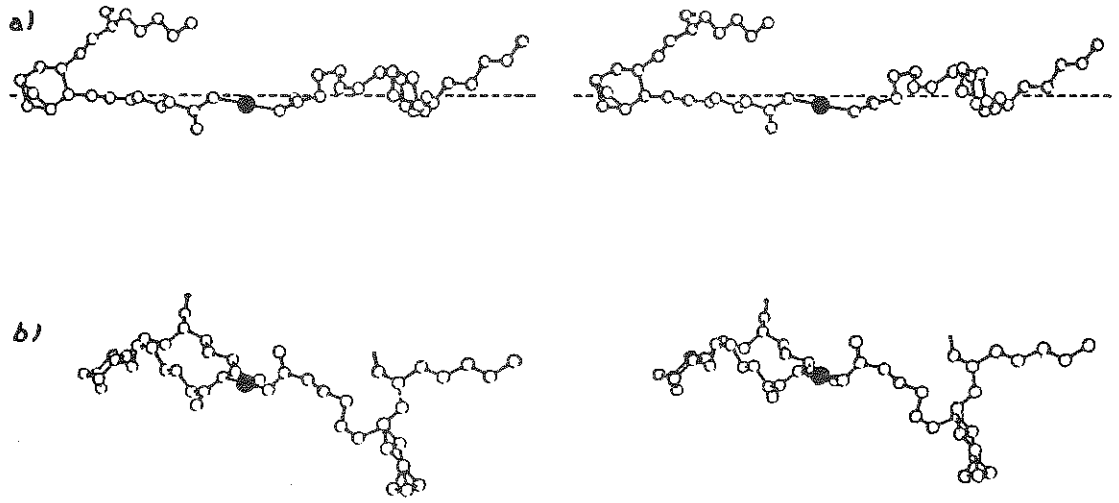

Figure 3.: Stereoscopic views of the most probable conformation of thromboxane A_2-Ca^{2+} complex at the interface after application of the simplex minimization procedure. The views were taken along the interface plane (a) and along the z axis perpendicular to the interface plane (b).

the lipid-water interface along the interface plane (Fig.3a) and along the z axis perpendicular to the interface plane (Fig.3b). The molecule was oriented at the interface as described in "Methods". The Ca^{2+} ion, leaving its cryptic orientation is in position favorable to its complexation-decomplexation at the interface.

DISCUSSION

It was found that thromboxane A_2 can transport calcium from a water phase into diethylether (Gerrard et al., 1978). Ca^{2+} transport by thromboxane A_2 was generated by addition of PGG_2 to platelet microsomes. Microsomes and PGG_2 alone

were much less effective. Addition of a thromboxane synthetase inhibitor and a brief incubation at 37°C, reduces completely the calcium transport (Gerrard et al., 1978). However, no information were available about the molecular structure of the thromboxane A_2-Ca^{2+} complex, neither about it capability to convey Ca^{2+} across the membrane barrier. Our conformational analysis predicts two possible conformers for this complex. The transconformation of one structure into another is probably mediated by the degree of immersion of the complex into the aqueous phase as demonstrated recently for the ionomycin-Ca^{2+} structure (Brasseur et al., 1983). How the lipid packing will modulate thromboxane A_2 structure and how the lipid interface will mediate the transformation of one isomer into the other is presently under investigation. These results suggest that thromboxane A_2, as A23187 ionophore, can transport calcium across the cell membrane. Further experiments using isolated dense tubular system membranes and artificial phospholipid liposomes are necessary to answer definitively to this question. It would however be premature to conclude that the $(TXA_2)_2$-Ca^{2+} complex acts as a physiological ionophore even if it causes platelet aggregation just as A23187 and if the effects of inhibitors on the two agents are similar (White et al., 1974). Finally, the conformational procedure used in this approach allows to predict ionophoretic properties of molecules even before their synthesis and offers an unique possibility to identify transient conformations.

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