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Conformational analysis of Amphotericin B

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Abstract

Within a theoretical approach to the problem of antifungal action of Amphotericin B (AmB), a conformational analysis of the neutral and zwitterionic form of this antibiotic in vacuo was performed by the MM2P and AM1 methods. The analysis was carried out with regard to the mutual orientation of the macrolidic and glycosidic fragments of the molecule, which is defined by the ϕ and ψ steric angles. This orientation defines the overall shape of the molecule and is postulated to be important for the antifungal action of the drug. As a result of the MM2P calculations, ϕ, ψ steric energy and population maps were prepared. Several conformers were found on these maps but only two of them (one each for the zwitterionic and the neutral forms of the antibiotic) were previously observed experimentally for isolated molecules. Our other calculated conformers were not observed experimentally but we propose that they may also appear in the AmB channel structure. The results of our conformational analysis were compared with experimental NMR data (nuclear Overhauser effects between selected hydrogen atoms) obtained previously [1]. New structural information obtained for AmB in the present work will be useful for building a molecular model of AmB–target interactions as well as for designing new derivatives of AmB. © 1997 Elsevier Science B.V.

Keywords: Amphotericin B; Conformational analysis; Molecular mechanics; MM2; AM1; NMR nuclear Overhauser effects

1. Introduction

Amphotericin B (AmB) is a well-known polyene macrolide antibiotic (Fig. 1) widely used in the treatment of systemic fungal infections [2–4]. After more than 30 years of clinical application, AmB still has potent fungistatic and fungicidal properties against many different species, only a few of which have developed resistance. Despite its wide clinical use, the antibiotic has numerous harmful side effects, the main one being its nephrotoxicity [5]. In order to improve the therapeutic index of AmB, many of its chemical derivatives have been tested and many studies have been devoted to the elucidation of the mechanism of AmB antifungal action [6–9]. Until now, the results of these efforts have been rather modest [10], and new derivatives have not gained wide application. The only rational way of overcom-

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ing the difficulties is to obtain further knowledge of molecular details of the mechanism of AmB antifungal action.

It is commonly accepted that the mechanism of the action of AmB is associated with cell membranes [6,9,11]. The drug binds to membrane sterols, i.e. ergosterol in fungal cells and cholesterol in mammalian cells, causing impairment of their barrier function and loss of the cell contents through membrane ionic channels. It has been postulated that the relative specificity of AmB for fungi is based on differences between the affinity of the drug for ergosterol and for cholesterol [12,13].

During the last few years, many different theoretical approaches, in addition to the experimental efforts, have been directed to the elucidation of the molecular factor involved in the antifungal action of AmB and its specificity towards ergosterol [14–25]. Correct molecular models and precise structural data for the AmB molecule are essential, and are required in this theoretical approach. Some structural studies of AmB and its derivatives have so far been performed using X-ray diffraction (for *N*-iodoacetyl AmB) [26], NMR (for methoxycarbonylmethylamide AmB) [1], and computational chemistry (for AmB) [14,17,19,20,27] methods. It was found that amphotericin B is a heptaene macrolide antibiotic with a mycosamine linked by a β glycosidic bond to the hydroxyl group at C19 on the macrolidic ring (Fig. 1). AmB can be regarded as a semirigid molecule, of which the overall conformation is defined by two rigid fragments having the ability to rotate around the C19–O41 and O41–C42 bonds (Fig. 1). One of these fragments is the macrolidic ring with a chain of seven conjugated trans double bonds. The second one is the mycosamine sugar ring which takes the chair conformation. The mutual positions of the macrolidic and the glycosidic fragments of the antibiotic play a crucial role with respect to the overall shape of AmB. One may also expect that this shape and the spatial location of certain functional groups of AmB are essential for AmB–AmB and AmB–sterol interactions. Since the AmB molecules form ionic transmembrane channels, the relative position of the macrolidic and the glycosidic fragments of the antibiotic may be particularly important for the formation of inter- and intramolecular hydrogen bonds. Considering the structural studies performed so far [1,14,17,19,26,27], it can be stated that the mutual orientation of these fragments is rather similar to that in the crystalline state. This means that the steric angles ϕ and ψ (Fig. 1) take ranges of values from $-92.4°$ to $-122.4°$ and from $83.5°$ to $142.1°$, respectively [14,17,26]. The ϕ, ψ values in the crystalline state (for *N*-iodoacetyl AmB) are equal to $-87.6°$ and $142.0°$, respectively. Very similar results were also suggested for amphotericin B methyl ester [17]. However, after analyzing more precisely the NMR nuclear Overhauser effect (NOE) data [1] for the hydrogen atoms in the macrolidic ring (H17, H18e, H19) and in the aminosugar moiety (H42, H43) of AmB, one can argue that the mutual position of these two fragments is not so fixed.

Since the mutual orientation of the glycosidic and the macrolidic fragments of the antibiotic is so important, this conformational problem is the subject of the present study. A conformational analysis of AmB was carried out using molecular mechanics (MM) and semiempirical quantum chemistry methods. To further investigate the conformation of AmB, the NOEs from the NMR data [1] were also taken into account. The final results of the conformational anal-

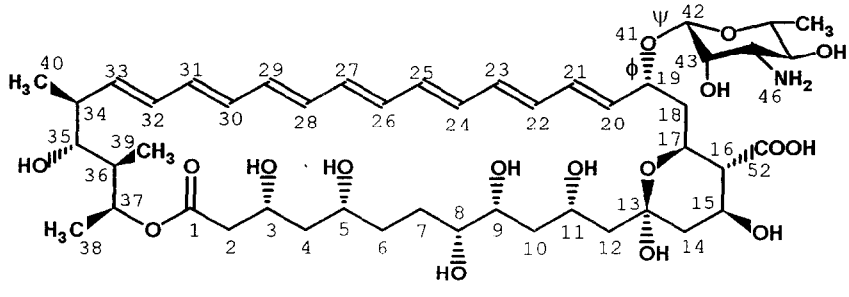

Fig. 1. Structure of the Amphotericin B molecule with partial numbering of atoms.

ysis were compared with previous experimental and computational data.

2. Methods

The MM2P program [28–30] was used for the reported molecular mechanics calculations, while the AM1 [31,32] and MNDO [33] programs were used for the semiempirical calculations. All calculations were performed on the AmB molecule in vacuo. It is worth mentioning here that previous experimental studies were performed on isolated AmB derivatives [1] in a physiologically irrelevant environment (e.g. in the solid state [26] or in pyridine–alcohol solution [1]). Previous theoretical calculations which took into account some water molecules also did not simulate a proper environment. One should remember that AmB acts on non-polar membranes where water molecules play a minimal role, mainly at the surface. Moreover, the AmB molecules form transmembrane channels so it is very likely that the molecules interact with each other in this channel. Of course, the current conformational analysis in vacuo is also an approximation. However, our studies on the charged and the neutral AmB molecules can detect all the possible mutual orientations of the two rigid fragments of AmB that could potentially occur. At the same time, our analysis is complementary to previous experimental and theoretical work.

The initial geometry (only "heavy atoms" without hydrogen atoms) of AmB was taken from crystallographic structural data for the *N*-iodoacetylo derivative of AmB [26]. The hydrogen atoms were allocated using standard values for bonds, valence and steric angles, which are included in the MM2P set of parameters. This initial geometry was optimized using the "dipole" option, and pairs of free electrons were allocated to the oxygen atoms in the hydroxyl groups. Since the AmB molecule has many polar groups, the "charge" option [34–36], instead of the "dipole" one, was used within the MM2P program for further calculations. In this case, a set of potential derived net atomic charges was applied [37–40]. The charges were calculated by the MNDO method with the ESP procedure within the MOPAC'93 program [41]. Calculations of charges were performed for an initially optimized structure of AmB. The molecular electrostatic potential was calculated on four Connolly layers according to the literature [42,43]. A scaling factor of 1.4 was introduced for the van der Waals radii to create the first Connolly layer. The distance between the next layers was 0.2 Å.

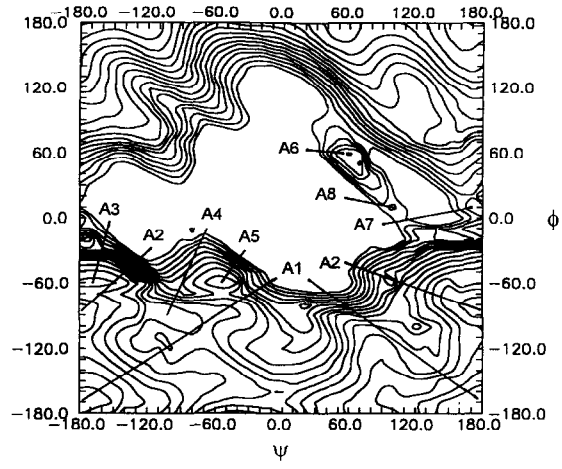

Fig. 2. Steric energy ϕ, ψ map (2Dns) for AmB obtained using MM calculations with a non-scaled set of atomic charges. Isoenergetic lines are drawn every 1 kcal mol^{-1}. The symbols of the minima correspond to the notation presented in Table 1. The solid straight lines indicate the precise positions of the minima.

Taking the initially optimized structure and the two sets of atomic charges, obtained within the MOPAC'93 calculations (i.e. scaled and non-scaled), two-dimensional (2D) conformation maps were determined. In this case, the steric energy, as a function of the ϕ(C42–O41–C19–C18) and ψ(C43–C42–O41–C19) angles, was monitored. During the MM calculations (geometry optimization), all internal coordinates were free except the ϕ, ψ angles. A step of 10° was used for the generation of the ϕ, ψ map. In addition to the steric energy, the distances between three pairs of hydrogen atoms (H17–H43, H18e–H42, H19–H42) were recorded for each grid of the ϕ, ψ map. These distances were used to

[1] The isolated AmB molecule (or its derivative) means a molecule in a medium (e.g. gas, solution or solid state) that does not interact with other AmB molecules via amino or carboxyl functional groups.

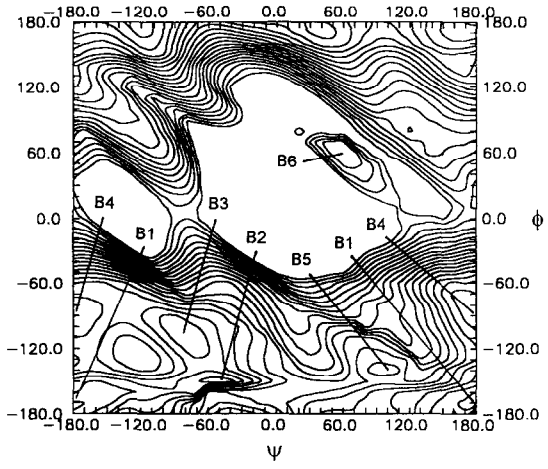

Fig. 3. Steric energy ϕ, ψ map (2Ds) for AmB obtained using MM calculations with a scaled set of atomic charges. Isoenergetic lines are drawn every 1 kcal mol^{-1}. The symbols of the minima correspond to the notation presented in Table 1. The solid straight lines indicate the precise positions of the minima.

prepare the ϕ, ψ maps shown in Figs. 6 and 7. Eventually, the 2D steric energy maps (Figs. 2 and 3) were transformed into 2D population maps (Figs. 4 and 5) according to the method described by Baginski and Piela [44].

Additional calculations were carried on structures corresponding to the minima on the 2D maps using the AM1 Hamiltonian within the MOPAC'93 pro-

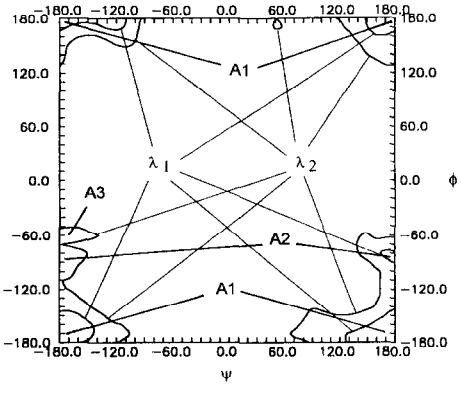

Fig. 4. Population ϕ, ψ map (2DPns) for AmB based on the 2Dns map (Fig. 2). The contours λ_1 and λ_2 define the regions of conformational space containing 85% and 99% of the molecules, respectively. Symbols of populated minima, A1, A2, A3.

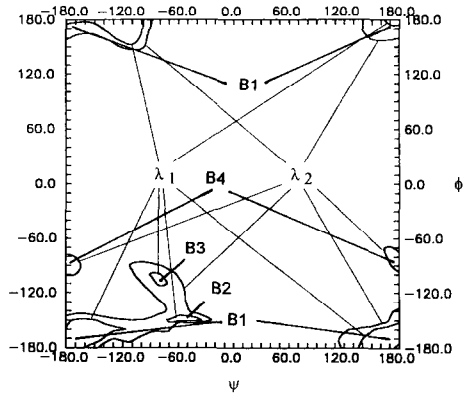

Fig. 5. Population ϕ, ψ map (2DPs) for AmB based on the 2Ds map (Fig. 3). The contours λ_1 and λ_2 define the regions of conformational space containing 85% and 99% of the molecules, respectively. Symbols of populated minima, B1, B2, B3, B4.

gram [40] for the neutral and the zwitterionic forms of the molecule. To build the zwitterion, a hydrogen atom from the carboxyl group was transferred to the amino group. As for the AmB molecule, which contains more than 130 atoms, it was not possible to optimize the whole structure. Only 36 internal coordinates, important for defining the mutual positions of the macrolidic and the glycosidic ring as well as the positions of the carboxyl and the amino groups, were optimized. The starting structures for the zwitterionic form of AmB were based on structures X1–X6 obtained for the neutral form of the antibiotic.

3. Results

The conformational analysis, which describes the mutual position of the macrolidic and glycosidic rings of neutral AmB, was performed using MM methods. Two 2D ϕ, ψ maps of the steric energy (denoted here 2Dns and 2Ds, indicating non-scaled and scaled sets, respectively of the atomic charges used in the MM methods) were obtained in our analysis (Figs. 2 and 3). Several minimum energy conformations were found on each map. The lists of minima for the 2Dns and 2Ds maps are presented in Tables 1 and 2, respectively. Subsequently, the steric energy maps (Figs. 2 and 3), according to the procedure outlined in Section 2, were transformed into

Table 1
List of stable conformers [a] (corresponding to the local minima of steric energy on the 2Dns map) with regard to the ϕ, ψ steric angles

Symbol of minimum	Angle ϕ, C42–O41–C19–C18 (deg)	Angle ψ, C43–C42–O41–C19 (deg)	Relative steric energy (kcal mol^{-1})
A1	−170	±180	0.0
A2	−85	±180	+1.5
A3	−60	−170	+2.3
A4	−90	−100	+6.1
A5	−60	−60	+8.3
A6	+50	+60	+11.7
A7	+10	+170	+14.1
A8	+10	+100	+15.5

[a] Found using the MM calculations with a non-scaled set of atomic charges.

Table 2
List of stable conformers [a] (corresponding to local minima of the steric energy on the 2Ds map) with regard to ϕ, ψ steric angles

Symbol of minimum	Angle ψ, C42–O41–C19–C18 (deg)	Angle ϕ, C43–C42–O41–C19 (deg)	Relative steric energy (kcal mol^{-1})
B1	−170	±180	0.0
B2	−150	−50	+0.6
B3	−110	−80	+1.3
B4	−90	±180	+2.3
B5	−140	+100	+4.9
B6	+60	+60	+16.5

[a] Found using the MM calculations with a scaled set of atomic charges.

population ϕ, ψ maps, denoted 2DPns and 2DPs, respectively (Figs. 4 and 5). In the case of map 2DPns, only the two lowest minima are populated, i.e. A1 and A2 (Fig. 4). The same minima (i.e. B1 and B4) are populated on the map 2DPs (Fig. 5); additionally, two other minima (B2 and B3) are also present on this map (Fig. 5). Taking into account the values of the steric energy (Tables 1 and 2) for all the above-mentioned minima, one can see that the difference between them is not more than 2.3 kcal mol^{-1}. In both cases (i.e. maps 2Dns and 2Ds), the lowest minima are A1 and B1 (Figs. 2 and 3, respectively). It is worth stressing that neither of these two minima nor others shown on both the maps correspond to the AmB conformation (precisely the mutual orientation of studied fragments of the AmB molecule) which was found in the solid state [26].

The next step in the conformational analysis of AmB was the application of a semiempirical quan-

Table 3
List of conformers [a] found within the AM1 calculations for the neutral form of AmB

Symbol of minimum	Angle ϕ, C42–O41–C19–C18 (deg)	Angle ψ, C43–C42–O41–C19 (deg)	Relative steric energy (kcal mol^{-1})
X1	−179	163	+3.5
X2	−85	−177	0.0
X3	−68	−169	+3.4
X4	−102	−75	+0.7
X5	−84	−72	+4.1
X6	+56	+57	+13.4

[a] The conformers correspond to those found within the MM calculations listed in Table 1.

Table 4
List of conformers [a] found within the AM1 calculations for the zwitterionic form of AmB

Symbol of minimum	Angle ϕ, C42–O41–C19–C18 (deg)	Angle ψ, C43–C42–O41–C19 (deg)	Relative steric energy (kcal mol − 1)
Y1	−58	126	0.0
Y2	−81	−69	+45.4

[a] The conformers correspond to those found within the MM calculations listed in Table 1.

tum chemistry method (AM1) to confirm the minimum energy structures (corresponding to the stable conformers) identified using the MM calculations. The AM1 calculations were carried out for two forms of AmB: neutral and zwitterionic.

The calculations for neutral AmB were performed for the six lowest minima found on the 2Dns map. The results are summarized in Table 3. All six conformers obtained by the MM methods were also found within the AM1 calculations. Additionally, two further minima (B2 and B3), mentioned in Table 2 and Fig. 3, were taken into account in our AM1 calculations. This was done so as to cover all regions with minimal energy on the 2D steric energy maps (Figs. 2 and 3). However, in this case the input structural data for AM1 was taken not from the 2Ds (Fig. 3) map but from the 2Dns map (Fig. 2). This step was carried out in order to obtain structural data for AmB (for the part that was not optimized in the AM1 calculations) from the same 2D steric energy map [2]. The calculations for the B2 and B3 conformers gave the conformer X4 (Table 3).

Six conformers of the zwitterion of AmB, for which the input structures were obtained from the X1–X6 conformers by transferring a hydrogen atom (from the carboxyl group to the amino group), gave only two stable conformers Y1 and Y2 (Table 4), according to AM1. The conformer Y1 does not correspond to any conformer found so far in our calculations and is rather similar to that observed in the crystalline state of AmB (precisely the *N*-iodoacetylo derivative of AmB). This conformer can form a weak intramolecular hydrogen bond between the amino group and the hydroxyl group of AmB. The conformer Y2 for the zwitterionic form of AmB corresponds to the conformer X5 for neutral AmB. Comparing the total AM1 energy of the neutral conformers, X1–X6 (Table 3), with the corresponding zwitterionic form, Y1–Y2 (Table 4), it was found that the Y1 conformer has a higher energy than X2 by approximately 32 kcal mol^{-1}. The Y1 conformer forms an intramolecular hydrogen bond but in spite of this, it has high steric energy. This suggests that the zwitterion in vacuo is not as stable as the neutral form of AmB. This may be explained by an analysis of the structure of the Y1 conformer. The formation of the intramolecular hydrogen bond causes steric hindrances (van der Waals repulsion) within the "polar head" of the antibiotic and this may be a reason for the high steric energy of that conformer.

Analyzing the results of the MM calculations, one can also conclude that there is a difference in the location of the minima on the 2D ϕ, ψ maps with regard to the set of atomic charges applied in the program. This difference is difficult to interpret, but it looks as if the calculations with the non-scaled set of charges are more reasonable for the gas phase. This statement can be supported by our AM1 results. As stated in Footnote 2, all the minima found during testing of the AM1 calculations based on the 2Ds map had higher energy compared to the conformers found in the calculations based on the 2Dns map.

The dependence of the MM results on the charges used in the calculations may be explained by comparing the absolute values of the applied charges. It was found that the absolute values of the scaled charges are higher than those of the non-scaled charges for the same atoms. This fact caused many chemically unrealistic distortions (forced by electro-

[2] Some test calculations indicated that when these data came from the 2Ds map (and not the 2Dns map) the conformers obtained in the AM1 calculations had a much higher energy (about 80–100 kcal mol^{-1}). One may expect that this shift was caused by small structural differences in the part of the AmB molecule that later was frozen in the AM1 calculations.

static strong interactions) of the AmB structure during the MM calculations when scaled atomic charges are applied. These electrostatic interactions were particularly strong since the calculations were carried out for a molecule in the gas phase. The distortions were found mainly for bond distances and bond angles in the macrolidic fragment of AmB. This fragment of the AmB molecule was not AM1- optimized, and, therefore, all conformers based on the 2Ds map had a higher energy than conformers based on the 2Dns map.

These observations can also be valuable for other workers who are going to use the MM2P or MM2 program with atomic charges. It seems that the MM2, similar to other force fields, is sensitive to the set of atomic charges used in the calculations. This is especially true for molecules having many polar groups. However, one may expect that the application of a non-scaled set of potential fitting charges (from MNDO) to the MM2 calculations gives reasonable structural data and energy values.

To further investigate the conformation of the AmB molecule, information concerning the NMR NOEs, observed previously [1] for three pairs of hydrogen atoms (i.e. H17–H43, H18e–H42, and H19–H42), was taken into account. It was assumed that a NOE can be observed in a molecule if the distance between hydrogen atoms is equal to or less

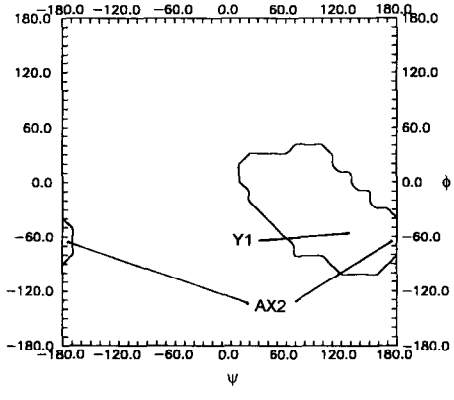

Fig. 6. The ϕ, ψ map with the region "allowed" by the NOE for three pairs of hydrogen atoms (H17–H43, H18e–H42, H19–H42). The map was based on the H–H distance found from the MM calculations with a non-scaled set of charges. Symbols of "allowed" minima, AX2, Y1. The solid straight lines indicate the precise positions of the minima.

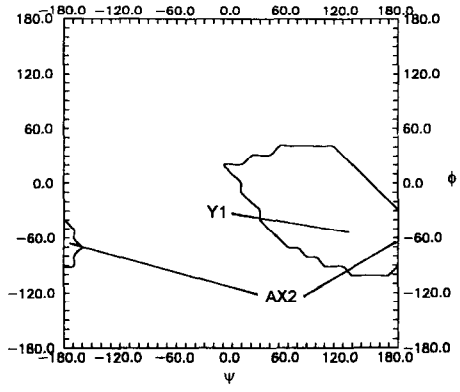

Fig. 7. The ϕ, ψ map with the region "allowed" by the NOE for three pairs of hydrogen atoms (H17–H43, H18e–H42, H19–H42). The map was based on the H–H distance found from the MM calculations with a scaled set of charges. Symbols of "allowed" minima, AX2, Y1. The solid straight lines indicate the precise positions of the minima.

than 3.5 Å. Therefore, during the MM calculations, the distances between studied hydrogen atoms were recorded for each grid of ϕ, ψ angles. Considering this monitored threshold value of the H–H NOE distance (i.e. 3.5 Å) two 2D ϕ, ψ maps were drawn with allowed regions of the ϕ, ψ angles (Figs. 6 and 7). On inspecting these maps it was found that only the conformers A2, A3, A8, B4, X2, X3 (for the neutral form of AmB), and Y1 (for the zwitterionic form of AmB) are allowed. Assuming that two conformers are similar or the same if a given steric angle differs by not more than $\pm 30°$, it is possible to regard the conformers A2, A3, B4, X2, and X3 as being the same conformer (denoted by us as AX2). The conformer A8, possessing high steric energy, can be rejected for further discussion. Thus only the conformer Y1 (zwitterionic form of AmB) differs from the set listed above.

4. Discussion

In our studies, conformational analyses were performed for the neutral and zwitterionic forms of Amphotericin B by MM and AM1 methods. The calculations were carried out with regard to the two steric angles ϕ, ψ which define a mutual placement of the macrolidic and glycosidic fragments of the antibiotic. The orientation of these fragments is pos-

tulated to be important for the biological action of AmB since it defines the overall shape of the molecule. This shape is expected to be essential for creating a stable structure of the AmB membrane channel.

Within the scope of these studies, several conformers were found for both forms of the antibiotic molecule. However, only a few of them have low steric energy and can exist according to the population 2DP maps. Two such conformers were found for the neutral form of AmB, i.e. A1 and A2 (A3 is similar to A2), in the MM calculations and by analogy, X1 (Fig. 8) and X2 (X3 is similar to X2) in the AM1 calculations. Only one conformer with low energy was found for the zwitterionic form of AmB (i.e. Y1). The amino and carboxyl groups in the conformer Y1 form a weak intramolecular hydrogen bond.

A comparison of our results with NMR data revealed that only a few selected low-energy conformers obtained in the theoretical conformational analysis can also be observed experimentally (by NMR) in the solution. It was found that according to the NOE distance barrier (Fig. 6), only two conformers (i.e. AX2 and Y1) are allowed. The Y1 conformer was calculated for the zwitterionic form of AmB. The AX2 conformer was detected for the neutral form of AmB. It is worth mentioning that the NMR measurements we recall here were performed for AmB methoxycarbonylmethylamide in a mixture of pyridine and methanol [1]. In this solvent, one can expect that the amino group is protonated and can form intramolecular hydrogen bonds. Nevertheless, our conformational analysis shows that the same NOEs might correspond both to the AX2 and the Y1 conformer.

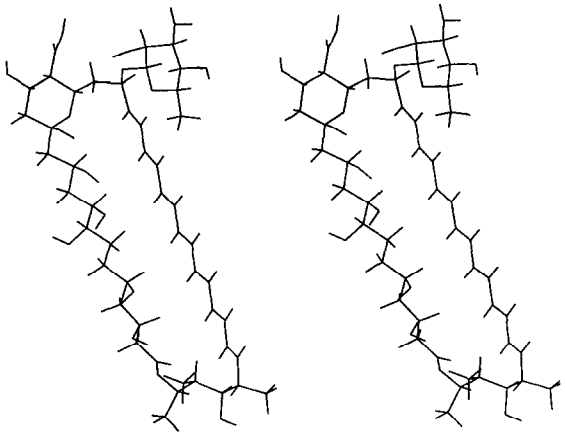

Fig. 8. Stereoview of the most "open" conformer X1.

Further comparison of the theoretical conformational analysis results and the NMR data with crystallographic data revealed that only the calculated conformer Y1 can occur in solution, in the gas phase and in the solid state simultaneously. Nevertheless, one should keep in mind that the AmB molecules form channel structures in a cell membrane. In this case it is very likely, and was also postulated by other workers [16], that the AmB molecule can form intermolecular hydrogen bonds (e.g. AmB–AmB or AmB–sterol) which can stabilize the channel. Taking into account such a possibility, one may expect that some of AmB conformers which were not detected by the NMR or crystallographic studies for the isolated molecules can also be observed in a channel structure. Using names for the AmB conformers similar to those used by Berges et al. [14] (i.e. open and folded structure with regard to the mutual position of the glycosidic and macrolidic fragments of AmB), one can say that the most open conformer found in our conformational analysis is A1 (in MM) or X1 (in AM1) for the neutral form (Fig. 8). It is very likely that this open conformer may also exist for the zwitterionic form of the AmB molecule present in the channel. Our assumption can be supported by the finding that the formation of an intramolecular hydrogen bond in the conformer Y1 causes some steric hindrance. For this reason, the formation of intermolecular hydrogen bonds, instead of intramolecular, may be an advantage. Further support for our finding comes from Anachi et al. [20] who are able to observe in a molecular dynamics simulation a similar value of the ψ angle to that in the A1 or X1 conformer [3]. Theoretical studies of the AmB dimer

[3] Our ψ angle defined by the atoms C43–C42–O41–C19 (Fig. 1) corresponds to the angle ϕ (defined by the atoms H1′–C1′–O–C19) in the work of Anachi et al. [20]. On analyzing our structural data for the X1 conformer, it was found that when the angle ψ takes the value 163°, the angle H42–C42–O41–C19, corresponding to the angle H1′–C1′–O–C19 defined by Anachi et al., takes the value 43°. A value of about 40° was found in the Anachi et al. MD simulations.

in water [24] are also consistent with our finding that the ϕ, ψ angles can take different values compared to those observed in the solid state. One should also remember that our structural studies, similarly to a previous experimental study, were performed for the isolated AmB molecule and therefore it was not possible to observe explicit intermolecular hydrogen bonds. However, theoretical simulations to some extent overpower the experimental approach with regard to this point. The conformational analysis of the neutral and zwitterionic forms of AmB allowed us to sample the whole accessible conformational space for two rigid fragments of the antibiotic. The isolated AmB molecule in our study, in contrast to the isolated AmB molecule studied experimentally (i.e. the zwitterionic form), is not forced to take only conformations that form an intramolecular hydrogen bond. Thus, some conformations of AmB found in our analysis (e.g. A1 or AX2) may correspond to conformations of the antibiotic present in the channel. It is very likely that these conformers form intermolecular hydrogen bonds.

To summarise we present the following points.

- The theoretical conformational analysis revealed that two conformers of isolated AmB (i.e. AX2 for the neutral form and Y1 for the zwitterionic form of AmB) can exist within the region of the ϕ, ψ angles "allowed" by NMR NOEs. Both conformers can appear in solution (NMR data) and in the gas phase (our calculations). However, in the case of a solution they cannot be distinguished by analyzing NMR data.
- The conformer Y1 for the zwitterionic form of AmB found for the gas phase by theoretical calculations is similar to that found in the solid state (X-ray) and in solution (NMR).
- The "open" conformers, found by theoretical conformational analysis and "not allowed" by NMR NOEs (i.e. A1 and X1), may also be important for the biological activity of the antibiotic since they are structurally the most appropriate for forming the intermolecular hydrogen bonds essential for channel stability.

The above-mentioned conclusions may be important and useful for molecular structure studies of AmB and its action mechanism. The results of our studies will be very helpful for creating a model of AmB–sterol and/or AmB–AmB interactions in the channel. We also expect that our results may be useful for designing new derivatives of the antibiotic that can form better intermolecular hydrogen bonds.

The first of the above conclusions points out that both techniques (NMR and theoretical methods) should be applied together to study the molecular structure of AmB and its derivatives in different environments. Further experimental data and theoretical simulations of the AmB channel are needed to understand whether the antibiotic structure in the membrane and in the channel is similar to that in solution or in the solid state, or rather whether it is close to some conformers postulated by us and based on our theoretical conformational analysis.

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