Conformational change in the thiazole and oxazoline containing cyclic octapeptides, the patellamides. Part 2. Solvent dependent conformational change †

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Solvent dependent conformational change of the thiazole and oxazoline containing cyclic peptides, the patellamides, is examined by a combination of experimental and theoretical methods. A mechanism for the simultaneous formation of two type-II β-turns in the patellamides is proposed based on molecular dynamics and NOE restrained molecular dynamics studies as well as literature evidence. The effect of the solvent and desymmetrisation of the patellamides is crucial, with symmetrical patellamides in polar solvents giving the open type-I conformation, whereas symmetrical patellamides in non-polar solvents and asymmetrical patellamides in both polar and non-polar solvents give rise to the folded type-II conformation.

Introduction

The unusual thiazole and oxazoline containing cyclic octapeptide patellamides (**1**–**6**, Scheme 1, Table 1), isolated from the ascidian (seasquirt) *Lissoclinum patella*, have been much investigated due to their interesting biological activity, such as potent cytotoxicity and the reduction of multi-drug resistance of certain types of lymphoblasts.[1] They can be divided into two naturally occurring families: symmetrical patellamides where $R^1 = R^3$ and $R^2 = R^4$ (**1**, **2**) and asymmetrical patellamides where $R^1 \neq R^3$ and R^2 may equal R^4 (**3**–**6**) and $R^1 = CH_2Ph$. The patellamides exist in one of two conformations depending on the side-chains present in the molecule, a 'square' conformation (I) and a 'figure of eight' conformation (II) (Scheme 1).[2]

† Electronic supplementary information (ESI) available: further calculational details. See http://www.rsc.org/suppdata/p2/b2/b201824c/

Direct NOE and CD evidence for the solution state conformations of various natural patellamides and a number of synthetic analogues are known (Table 1), and from the available data a clear trend emerges. The symmetric compounds such as patellamide A (**2**) and the synthetic patellamide analogue **7** with $R^1 = R^3$; $R^2 = R^4$ all adopt a square conformation (I) in methanolic solution at room temperature. This can be contrasted with patellamides B (**3**), C (**4**) and E (**6**)[2–4] and the synthetic analogues **8**–**10** ($R^1 \neq R^3$; R^2 may equal R^4) which all take up the closed 'figure of eight' conformation (II) in both polar and non-polar solvents. The effect of the side-chains in these compounds on their conformation is corroborated by the X-ray diffraction crystal structures of the symmetrical ascidiacyclamide (**1**) and its synthetic analogue **7** which both adopt the open structure (I). The X-ray crystal structure of the asymmetric variant, patellamide D (**5**), is in the closed conformation (II).

Table 1 Structures and published solution and crystalline state conformations of natural and synthetic patellamides **1**–**10**

Compound	R^1	R^2	R^3	R^4	R^5	R^6	Conformation	Method	Solvent	Ref.
1 Ascidiacyclamide	*i*-Pr	*s*-Bu	*i*-Pr	*s*-Bu	Me	Me	I	Crystal	C_6H_6	6
							I	NMR	$CDCl_3$	6
2 Patellamide A	*i*-Pr	*s*-Bu	*i*-Pr	*s*-Bu	H	Me	I	Crystal	MeOH	12
							I	CD (299 K)	MeOH	4
							II	CD (199 K)	MeOH	4
							I	CD	MeOH	This study
							II	NMR-MD	$CDCl_3$	This study
3 Patellamide B	CH_2Ph	CH_2CHMe_2	Me	*s*-Bu	Me	Me	II	NMR-MD	$CDCl_3$	2
							II	CD	MeOH	4
4 Patellamide C	CH_2Ph	*s*-Bu	Me	*i*-Pr	Me	Me	II	NMR-MD	$CDCl_3$	2
							II	CD	MeOH	This study
5 Patellamide D	CH_2Ph	*i*-Pr	Me	*i*-Pr	Me	Me	II	Crystal, MD	MeOH	13
6 Patellamide E	CH_2Ph	*i*-Pr	*i*-Pr	*s*-Bu	Me	Me	II	CD	MeOH	4
7[a]	*s*-Bu	*i*-Pr	*s*-Bu	*i*-Pr	H	H	I	CD, NMR	MeOH	14
8[a]	*i*-Pr	H	*i*-Pr	*s*-Bu	Me	Me	II	NMR-MD	DMSO	3
9[a]	*i*-Pr	CH_2CHMe_2	*i*-Pr	*s*-Bu	Me	Me	I	Crystal	C_6H_6	3
							I/II	NMR-MD	DMSO-d_6	3
10[a]	*i*-Pr	CH_2Ph	*i*-Pr	*s*-Bu	Me	Me	II	NMR-MD	DMSO-d_6	3
11[b]	CH_2Ph	*s*-Bu	*i*-Pr	*s*-Bu	Me	Me				

[a] Denotes synthetic analogue. [b] Used in theoretical calculations only.

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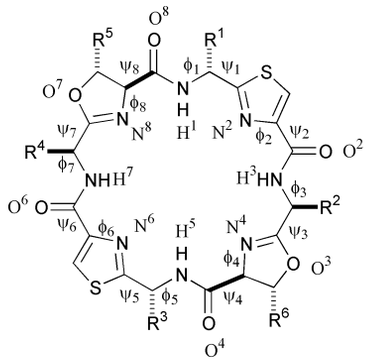
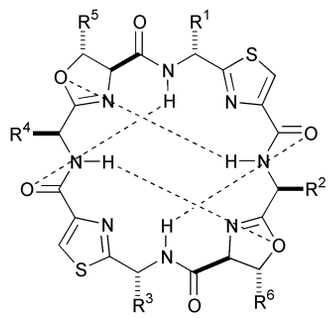
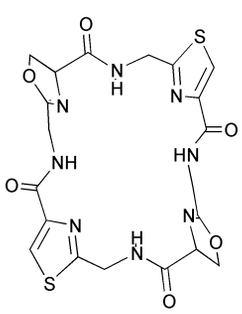




H-bonds monitored during molecular dynamics.



Square (I)

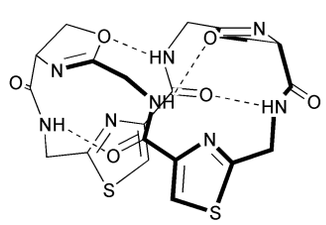


Figure of eight (II)

Scheme 1 The chemical structure of the patellamides, the two conformations and the hydrogen bonds monitored during molecular dynamics simulations.

Studies by Doi *et al.* show that disturbing the symmetry at R^2 also alters the conformation.[3] Changing R^2 from *s*-Bu (**1**) in conformation (I) to H (**8**) results in the closed conformation (II) whereas altering it to CH_2CHMe_2 (**9**) gives the open conformation (I) in the crystalline state, but results in a hybrid I/II conformation in DMSO-d_6 solution. Changing the *s*-Bu at R^2 for CH_2Ph (**10**) results in the closed conformation (II) in solution.

Variable temperature circular dichroism studies[4] and our own molecular mechanics calculations suggest that the closed conformation (II) is favoured by enthalpic (ΔH) contributions to the free energy of the molecule. This raises the important question as to why the 'square' or 'open' conformation (I) exists almost exclusively at room temperature for symmetrical **1** (R^1 = *i*-Pr) and the pseudosymmetrical patellamide A (**2**, R^1 = *i*-Pr), whereas the 'figure of eight' or 'closed' (II) conformation is dominant for **3**–**6** ($R^1 = CH_2Ph$). Evidence is presented below for the importance of solvent polarity on the conformational state and a mechanism is proposed for the interconversion of conformation I to II.

Results and discussion

It is obvious from the three dimensional structure of the open (I) and closed (II) form of the patellamide structure that large changes in ϕ and ψ, the backbone torsion angles, are involved in their interchange (Table 2), with the largest changes being observed in ψ_3 and ψ_7. The process is driven by the formation of two unusual type-II β turns[5] (residues 3–4 and 7–8) in the closed conformation (II). These turns are stabilised in (Table 3) by the formation of two new C=O to N–H hydrogen bonds (O^2–H^5 and O^6–H^1) and two new oxazoline O to N–H hydrogen bonds (O^3–H^7 and O^7–H^3), which are not part of normal peptidic β-turns (Scheme 1). This conformation is not static and dynamics simulations show that at any given moment the four hydrogen bonds are not present simultaneously; one may be formed as another is broken. This is consistent with studies by Ishida *et al.* which show that all N–H protons are exposed to solvent for **3** and **4** in the conformation II.[3]

Table 2 ϕ and ψ torsional angle values (deg) and changes in ϕ and ψ for conformations I and II

	1 (square, I)		**5** (figure of eight, II)		I ⟶ II	
Residue	ϕ	ψ	ϕ	ψ	$\Delta\phi$	$\Delta\psi$
1	134.5	47.0	83.8	−12.4	50.7	59.4
2	179.0	−8.0	179.1	−4.0	−0.1	−4.0
3	−127.0	−21.2	−76.4	121.1	−50.6	−142.3
4	113.7	18.8	101.5	−14.3	12.2	33.1
5	134.5	47.0	91.0	−11.1	43.5	58.1
6	179.0	−8.0	179.5	−4.9	−0.5	−3.1
7	−127.0	−21.2	−80.2	113.6	−46.8	−134.8
8	113.7	18.8	102.0	−1.4	11.7	20.2

Table 3 Type-II β turn in the figure of eight conformation (II) for compound **5**[5]

	ϕ_i/deg	ψ_i/deg	ϕ_{i+1}/deg	ψ_{i+1}/deg
Ideal	−60.0	120.0	80.0	0.0
Residue 3–4	−76.4	121.1	101.5	−14.3
Residue 7–8	−80.2	113.6	102.0	−1.4

Little information is available relating to the conformations of the symmetrical patellamides in non-polar solvents except for the early work by Ishida *et al.*[6] in which H_N–N–C_α–H_α NMR coupling constants measured for ascidiacyclamide (**1**) in $CDCl_3$ solution gave torsional angles consistent with the crystal structure of the type-I conformation. However, our measurement of the same coupling constants for patellamide C (**4**), which exists in the closed conformation (II) in $CDCl_3$ solution,[2] gave similar values to those obtained for **1**, suggesting that these coupling constants are not a good indicator of conformation. This ambiguity may result from a combination of two factors: a rapid averaging of the torsional angles, and the fact the coupling constants are on a steep part of the Karplus curve. Furthermore, it is not possible to distinguish between the open (I) and closed (II) form of the patellamides using CD spectra in $CHCl_3$ due to solvent interference. It was therefore decided to investigate the conformations of the pseudosymmetrical patellamide A (**2**) and the asymmetrical patellamide C (**4**) in pure $CDCl_3$ by NOE restrained molecular dynamics with the conformations present in MeOH being determined by circular dichroism.

The conformations of the open (I) and closed (II) forms of the patellamides each give a characteristic circular dichroism (CD) spectrum with positive maxima at 211 and 205 nm for the first form and 250 nm for the second.[7] Therefore, the presence of a positive maximum at 250 nm for patellamide C can be used to indicate the presence of the closed conformation (II) and its absence for patellamide A suggests the presence of the open conformation (I).

NOE restrained molecular dynamics has been used previously to determine the solution conformation of **3**, and **4**[2] and the synthetic analogues **8**–**10**.[3] During the course of this study the structure of **4** in $CDCl_3$ was obtained from approximately 60 NOEs and our calculation of the conformation is in

good agreement with that calculated previously (backbone RMSD 0.20 ± 0.09 Å).[2]

Patellamide A (**2**) is nearly symmetrical and only approximately 30 NOEs could be obtained due to chemical shift degeneracy. This degeneracy can prove to be a major problem in restrained molecular dynamics calculations as if all possible restraints are applied many violations will occur and this will lead to strained and distorted structures. Therefore, to solve this problem we appropriated the technique of ambiguous distance restraints used with biopolymers for the calculation of dimer structures and in automated structure calculation protocols. Thus, for each possible assignment a function based on the $1/r^6$ sum average of the two possible distances was used as the restraining function during X-PLOR molecular dynamics calculation.[8] As an example, the strong restraint from $3H_\alpha$ to NH^5 was set as $3H_\alpha$ to NH^5 *or* $3H_\alpha$ to NH^1 as the shifts of NH^5 and NH^1 are isochronous in the ^1H NMR spectrum. This ambiguous restraint allows both assignments to contribute in an $1/r^6$ sum average, but at the end of the calculation it is clear that this restraint is satisfied by a short distance between $3H_\alpha$ to NH^5 with the distance $3H_\alpha$ to NH^1 contributing little to the restraining energy. A total of 52 low energy structures were calculated from 100 structures in which ϕ and ψ had been randomised with 8 *C*=O to 1 *N*–H being set as an 'elastic bond' to relieve strain in the ring. All of these structures were refined and a cluster of 22 structures with the same backbone geometry and a low target energy function were chosen as representative of the solution structure (Fig. 1, backbone RMSD 0.22 ± 0.14 Å,

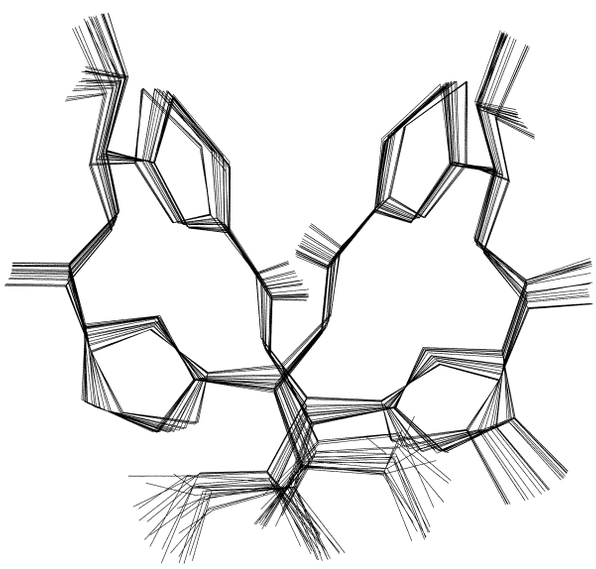


Fig. 1 Ensemble of the 22 minimum energy structures of patellamide A (**2**) in $CDCl_3$ (backbones only shown).

E_{Total} = 11.8 kcal mol^{-1}). The final conformation is closed (II), and the agreement with our calculated structure for **4** is good (Fig. 2, backbone RMSD = 0.34 Å).

These results indicate that both symmetrical and asymmetrical patellamides can adopt the closed conformation (II) in non-polar solvents. To test the effect of solvent polarity on the structure of the patellamides we carried out molecular mechanics minimisations[9] of ascidiacyclamide (**1**), and a non-natural ascidiacyclamide variant **11** in which $R^1 = CH_2Ph$. These were used to represent the symmetrical and asymmetrical patellamides respectively, changing R^1 from *i*-Pr to CH_2Ph whilst the rest of the molecule remained constant. Energies were calculated for each structure in both the open (I) and closed states (II) using a GB/SA continuum treatment of solvation in MacroModel.[10] The solvent parameters for H_2O and $CHCl_3$ were used. This system provided a pair of solvents that emphasise the effect of changing from a non-polar aprotic solvent to a polar protic solvent and uses a force field parametrisation which is readily available.[10] The results show that the folding from the open (I) to closed (II) form was highly favoured enthalpically in all cases. In H_2O $\Delta E_{I \longrightarrow II}$ was −12 kJ mol^{-1} for **1**, −10 kJ mol^{-1} for **11**, whereas in $CHCl_3$ the figures were −58 and −66 kJ mol^{-1} respectively. This indicates that, within the bounds of error, purely based on enthalpic terms including electrostatic and van der Waals effects the closed form is always favoured. This is consistent with the observations of Freeman *et al.*[4] who observed by CD that at 'low temperature' (173 K) in methanol patellamide A has a closed conformation (II) but that at 'high temperature' (298 K) it is open (II).

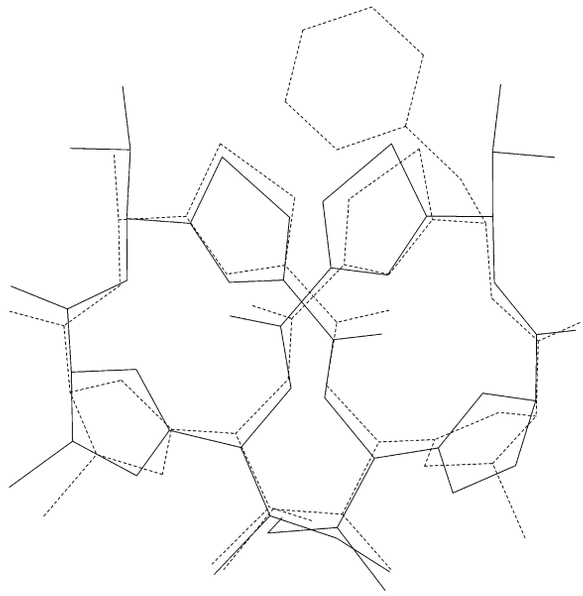


Fig. 2 Overlay of the minimum energy structure of patellamide A (**2**, line) and the minimum energy structure of patellamide C (**4**, dotted line) in $CDCl_3$ (backbones only shown).

To investigate the process by which the open and closed forms interconvert, molecular dynamics (MD) simulations were carried out on **1** and **11** at 300 K in H_2O and $CHCl_3$. The same GB/SA treatment of solvation as described above for the determination of enthalpy was used. The starting point for each MD simulation was a type-I conformation selected at random from the output of a previous 10 ps run. Six MD simulations were conducted for each system with a 1 fs time-steps for 200 ps and 1000 structures were sampled. For these structures the O^2–H^5, O^6–H^1, O^3–H^7 and O^7–H^3 distances were used to monitor the conversion of the structure from the open (I) to closed form (II) (Fig. 3). It was assumed that if these distances fell below 2.5 Å a hydrogen bond was able to form. Fig. 3 gives a good qualitative description of the evolution of the interconversion process with time. The results for **1** and **11** in $CHCl_3$ agree with the experimental observation that both symmetric and asymmetric patellamides will fold to give the closed conformation (II) in non-polar solvents. In polar solvents ascidiacyclamide (**1**) is expected to remain in the open conformation (I) (*cf.* **2** in MeOH, Table 1) which is confirmed by the aqueous theoretical simulation. The results of the MD simulations for **11** in H_2O however are more ambiguous as both open and closed forms are observed, whereas CD studies of **4** in MeOH show it is in the closed form (II). However, a number of factors must be considered, as the distance–time graph for **11** in H_2O (Fig. 3) shows that the monitored distances show significant excursions to near hydrogen bonding lengths during many of the simulations. Furthermore, the solvent system in the simulation used has a much higher dielectric (78.5) than the polar solvents used in the experimental studies which resulted in the closed (II) conformation (MeOH, 32.7; DMSO, 46.7). It could be postulated that this may tend to over emphasise the open conformation (I) in the simulations.

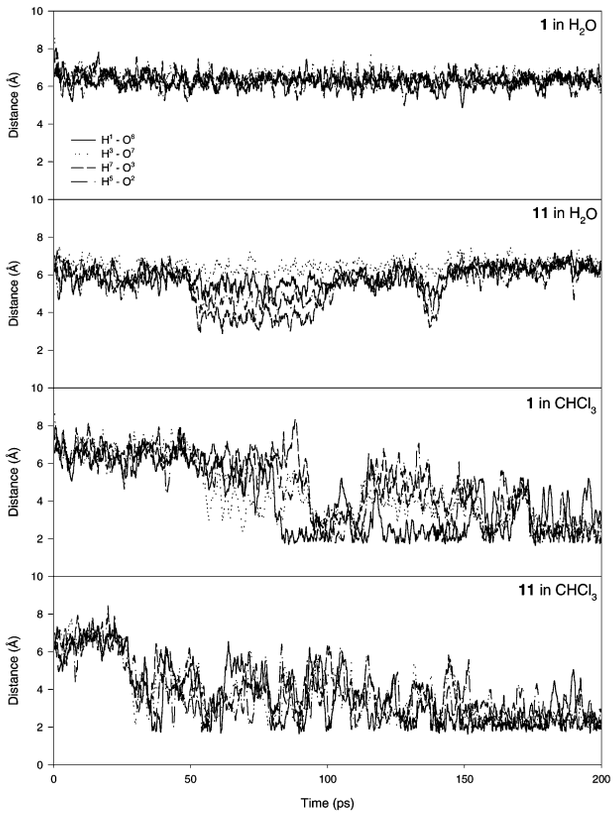


Fig. 3 Monitoring four key H-bonding distance during molecular dynamics simulations of ascidiacyclamide (**1**) and **11** (R^1 = CH_2Ph, R^2 = *s*-Bu, R^3 = *i*-Pr, R^4 = *s*-Bu) in H_2O and $CHCl_3$ at 300 K.

The MD simulations for **1** and **11** in $CHCl_3$ were used to construct a qualitative picture of how the folding from the open (I) to closed conformations (II) occurs; the observed pathway is different from the empirical model proposed by Ishida *et al.*[2] Fig. 4 shows the process broken up into four distinct steps, although in the MD simulations adjacent steps may occur in a concerted manner. The first step in almost all simulations in

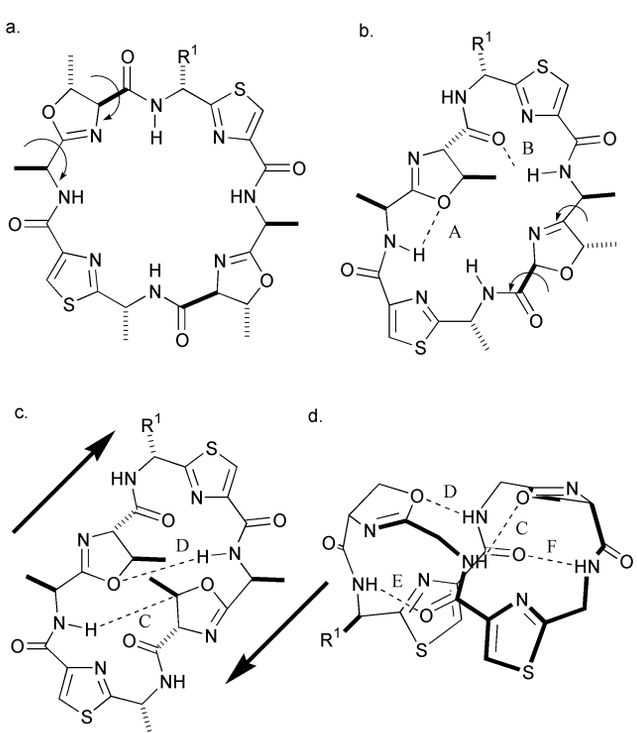


Fig. 4 A proposed mechanism for the folding process I ⟶ II derived from molecular dynamics simulations.

which folding occurred was the rotation about one oxazoline ring as in Fig. 4a. This event was monitored using the distances O^3–H^3 and O^7–H^7 which are roughly 4 Å apart in the open conformation (I) and only 2.5–3.0 Å upon rotation of the oxazoline ring (Fig. 5). Initially it was thought that this rotation

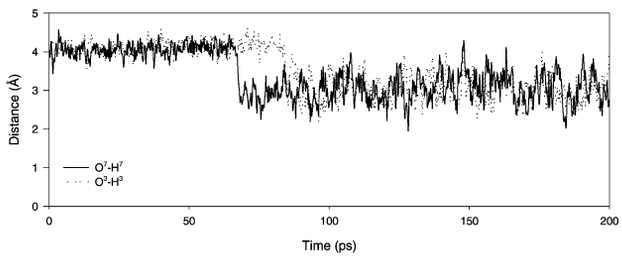


Fig. 5 Monitoring O^3–H^3 and O^7–H^7 distances during a molecular dynamics simulation of **11** in $CHCl_3$ at 300 K.

was facilitated by the presence of a symmetry disturbing group at R^1, but in both **1** and **11** in $CHCl_3$ the first rotation could occur at either oxazoline ring. This rotation may facilitate the formation of a hydrogen bond from the oxazoline oxygen to the adjacent NH (H-bond A in Fig. 4b) and a stabilising hydrogen bond B (Fig. 4b). The next step is the rotation of the second oxazoline ring, which is in some cases simultaneous with the rotation of the first oxazoline ring, giving rise to the structure in Fig. 4c in which distances C and D are of the order of 3.7 Å. In order to go to the closed conformation (II) shown in Fig. 4d, the two halves of the molecule need to experience 'shear' as indicated by the arrows to bring C and D into hydrogen bonding distance. This is corroborated by the observation that lengths of C and D are inversely related to the length of O^8–H^3 (H-bond B in Fig. 4b) and O^4–H^7. The 'shear' process simultaneously brings about the formation of hydrogen bonds E and F, thus completing the formation of two type-II β-turns.

Conclusions

It is clear from these studies that asymmetry in the structure of patellamides favours conformational change to the 'closed' conformation II. The molecular dynamics simulations predict the experimentally observed conformation correctly except in the case of the asymmetrical variant **11** in polar solvents. This may be due to the large increase in dielectric in going from MeOH, in which the CD studies are conducted, to H_2O, in which the molecular dynamics simulations are performed. This may be solved in future studies by developing a continuum model of MeOH. Separate simulations of **11** in H_2O starting from conformation II do not show the expected change back to conformation I, suggesting that the use of explicit solvent molecules might be desirable in future simulations.

This study gives an indication of the design features of this intriguing natural product allowing it to achieve such a fold. Certain motifs from this molecule may be used to study protein folding. Planned studies include this, as well as investigation of the above process by ultrafast spectroscopic measurements using temperature jumps and stopped-flow methods.

Experimental

Calculation of structures

30 NOE restraints were derived from the T-ROESY spectrum (T_{mix} = 400 ms) and classified as weak (1.8–5.0 Å), medium (1.8–3.5 Å) or strong (1.8–2.5 Å). ROE's were quantified by contour counting. Dihedral restraints were included for the four H_N–N–C_α angles. Restrained molecular dynamics calculations were carried out with X-PLOR 3.851[8] with a force field with repulsive non-bonded terms. No dielectric term was included in the calculations to take account of solvent effects.

ab initio Simulated annealing calculations (YASAP 3.0: 120 ps total time simulated annealing from 2000 to 100 K, 200 steps minimisation) were used to calculate structures from 100 starting conformations with randomised ϕ and ψ torsion angles using an elastic bond between 8 *C*=O and 1 *N*–H to relieve strain. From this ensemble 52 structures were refined using a simulated annealing with slow cooling protocol (600 ps, cooling from 1500 to 100 K, 4000 steps of minimisation). The 22 lowest energy structures from the ensemble were selected to represent the final structure. The overlay and display of structures was achieved using Molmol.[11]

Molecular dynamics

Molecular dynamics simulations were performed at 300 K over a 200 ps time period using the MM2* force field within Macromodel v6.5.[9] Starting structures were selected at random from the output of previous 10 ps equilibration runs. The simulations were performed in solvent (H_2O and $CHCl_3$) described by the GB/SA continuum model.[10] The update of the non-bonded interactions occurring within the system was performed with a period of 1 fs and cut-off distances were fixed at 12 and 7 Å for electrostatic and van der Waal's interactions, respectively. These parameters allowed for a higher degree of accuracy in the monitoring of any event that involved rapid and/or large conformational changes. The simulations in each solvent were repeated 6 times using different starting structures in an attempt to reduce the possibility that the initial geometry selected might bias the course of the simulation.

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