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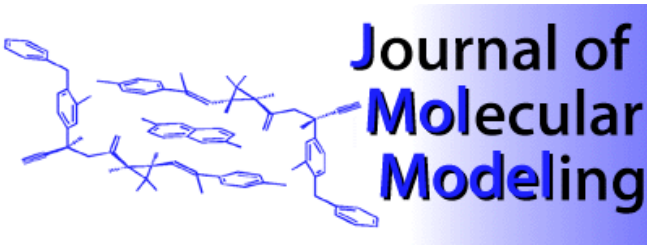

FULL PAPER

Comparison of a Monte Carlo Strategy with a Combined DG/MDSA Method for Structure Determination of Bicyclic Peptides

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Abstract The MC simulation program MOCCA and the combined methods of Distance Geometry and Molecular Dynamics are utilised for structural studies of four isomers of the bee venom toxin apamin. For the MC strategy the conformational space is reduced to torsional degrees of freedom. The study compares the efficiency of both simulation strategies for structure determination of bicyclic peptides and examines the limits of the Monte Carlo method. MOCCA shows a lower efficiency as compared to the combined methods of Distance Geometry and Molecular Dynamics for the structure determination of the bicyclic isomers of apamin.

Keywords Apamin, Distance Geometry, MOCCA, Monte Carlo Simulation, Molecular Dynamics

Abbreviations DG, Distance Geometry; MDSA, Molecular Dynamic and Simulated Annealing; MC, Monte Carlo; NOE, Nuclear Overhauser Effect; CVFF, Consistent Valence Forcefield; Sec, selenocysteine; RMSD, Root Mean Square Deviation;

Introduction

The Monte Carlo (MC) strategy is well established as an approach to simulations of model systems in science and engineering [1]. The method utilises stochastic steps in the calculation algorithm to solve a deterministic problem [2,3]. In chemistry, the simulation of deterministic molecular mechanics can also be performed by MC methods which randomly probe the geometry of the molecule [4]. For efficiency reasons the conformational space can be reduced to torsional degrees of freedom. In that case the stochastic step consists of random torsional changes [5-10]. As a consequence of the possibilities of the MC strategy, it has become an important approach to perform molecular simulations beside the deterministic Molecular Dynamics (MD) method [4]. In the case of small cyclic and open-chain molecules, several variations of the stochastic method have been proposed [7-10]. In these examples the distribution of the low energy conformations of monocycles and small molecules were calculated by MC methods after reducing the conformational space to torsions, and were then compared with determinis-

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tic simulations for efficiency. The test systems are usually no larger than penta or hexapeptides [6]. However, to our knowledge the MC strategy has not yet been applied for the conformational search of doubly restricted biopolymers such as bicyclic peptides containing two disulphide bridges. The bicyclic systems investigated by MC methods so far were small ring systems like heterobicyclic mimetics of single peptide residues or short peptides containing two prolines, where the problem is reduced to a proper parameterisation of the interaction potentials [11-14].

Disulphide bond formation in the oxidative folding of peptides and proteins is accompanied by restriction of the conformational space [15,16]. Apamin, a bee venom toxin, is an 18-membered peptide containing two disulphide bridges (Figure 1) [17]. Three different analogues of apamin were synthesised for folding studies, where two cysteines were rationally replaced by selenocysteines to produce the three possible isomers with two crossed (globular isomer: [Sec^{1},Sec^{11}]-apamin), parallel (ribbon isomer: [Sec^{3},Sec^{11}]-apamin) or consecutive disulphides (bead isomer: [Sec^{1},Sec^{3}]-apamin) (Figures 2-4) [18]. In the present study the preferred structures of the bicyclic isomers were calculated by two different methods. One computational approach was a combination of Distance Geometry, Molecular Dynamics Simulation and Simulated Annealing. The other was a MC-Simulation method where the conformational space is reduced to the torsional degrees of freedom. Although recently more sophisticated algorithms have been proposed [5,6], we chose the program MOCCA [19,20] developed and employed by the Bayer AG for the MC simulation, because MOCCA had been successfully applied to monocyclic and linear biopolymers [19,20]. The main goal of this study was to uncover limits of the Monte Carlo method in the case of the large bicyclic molecules and to determine which approach to the structure determination of these peptides is more efficient. Furthermore, significant differences in total energies of the final structures of the isomers were expected possibly to identify the preferred disulphide pattern of natural apamin.

Methods

The calculations were performed on Silicon Graphics O2 R5000, Silicon Graphics Power Challenge and Silicon Graphics Octane computers with the program MOCCA, an MC simulation program of the Bayer AG, [19,20] and the package of Molecular Simulations Inc. containing Insight II 97 [21], Discover [22] and NMRchitect [23], where the DG-II program of Havel [24,25] is implemented. All energy-based methods used a modified consistent valence force-field (cvff) [26] implemented with additional selenium parameters. The experimental, data were previously determined by NMR [18,27]. Therefore, distance constraints (NOE) and coupling constants were used as experimental restraints for both simulation types, although the restrictions imposed by NOE-restraints can reduce the efficiency of MC methods. Figure 5 shows the experimental long range NOE contacts (between

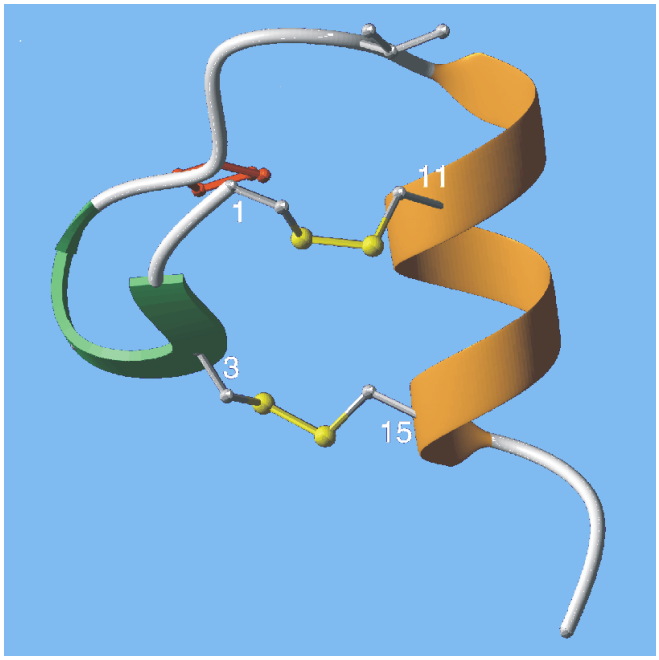

Figure 1 *Ribbon representation of the natural apamin analogue as calculated with DG/MDSA, the wild type apamin is crosslinked by two disulphide bridges of the cysteine residues 1, 11 and 3, 15*

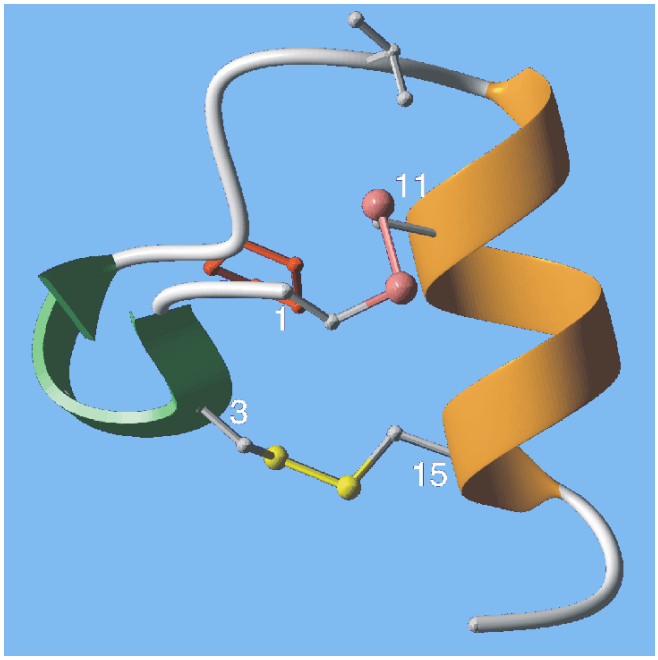

Figure 2 *Ribbon representation of the globular apamin analogue as calculated with DG/MDSA, the synthetic isomer is crosslinked by the disulphide bridge of the cysteine residues 3, 15 and by the diselenide bridge of the selenocysteine residues 1, 11*

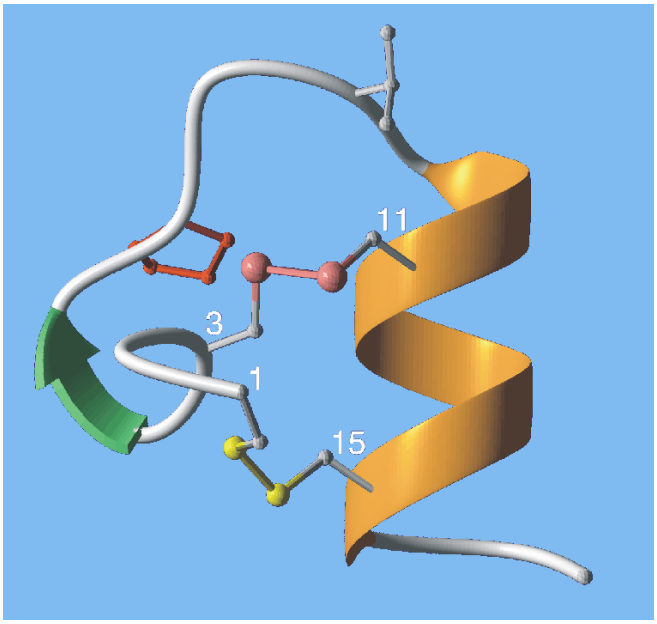

Figure 3 *Ribbon representation of the bead apamin analogue as calculated with DG/MDSA, the synthetic isomer has a consecutive bridge pattern formed by the disulphide bridge of the cysteine residues 11, 15 and by the diselenide bridge of the selenocysteine residues 1, 3*

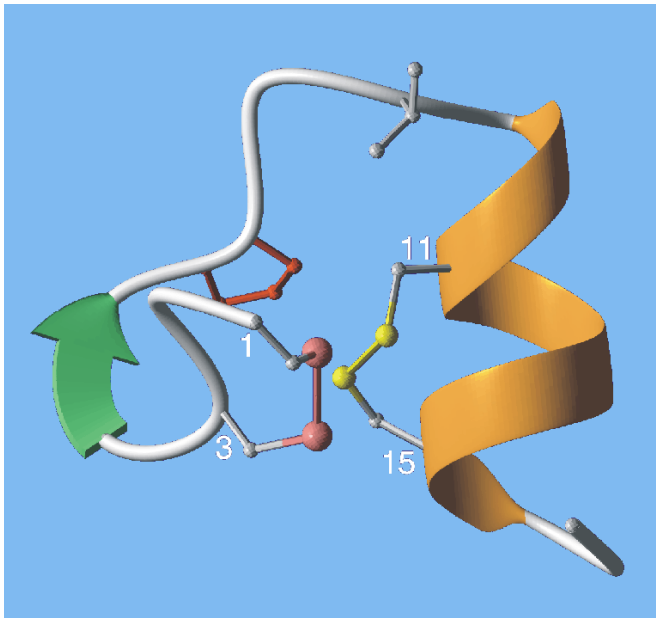

Figure 4 *Ribbon representation of the ribbon apamin analogue as calculated with DG/MDSA, the synthetic isomer contains a parallel bridge pattern formed by the disulphide bridge of the cysteine residues 1, 15 and by the diselenide bridge of the selenocysteine residues 3, 11*

residue i and j, |i-j| > 4) that are mainly responsible for the overall fold of the peptides.

Distance geometry

DG calculations were carried out *in vacuo*. All starting structures were generated by a minimisation followed by a short MDSA run. The resulting coordinates were used for the generation of the distance-bound matrices and triangle-bound smoothing was performed for all isomers. For every peptide 100 structures were generated by DG using the 19 chiral restrictions, the NOEs, four or five hydrogen bonds and 11 to 14 dihedral angle restraints. The force constant used for distance restraints was 50 kcal×mol^{-1} and was raised up to 100 kcal×mol^{-1} for hydrogen bonds during calculation. No constraints were included concerning the diselenide or disulphide bonds. The structures were first generated in four dimensions, optimised using the distance-driven dynamics method [24], reduced to three dimensions using the EMBED algorithm [24] and then optimised using a simulated annealing step *in vacuo* and 250 steps minimisation with the conjugate gradient algorithm maintaining the distance constraints [28].

Molecular dynamics

One structure from the DG derived family of most convergent and least violated structures was used for each apamin isomer to perform combined molecular dynamics-simulated annealing (MDSA) calculations *in vacuo* with ε = 80 in order to obtain correct dihedral angles of the disulphide and diselenide bridges.

The simplified MDSA model without explicit solvent treatment was analysed for the natural apamin isomer by comparing the resulting structures with those obtained from simulations in a water box. In both models the target temperature of 300 K was achieved during an equilibration period of 48 ps. Then the restrained molecular dynamics simulation was carried out over 125 ps and finally a free simulation over 100 ps followed. The temperature curve of the *vacuum* simulation showed a variation of 10 K around the target temperature, whereas an almost constant temperature level of 300 K could be observed for the box model (Figure 6). The backbone RMSDs for both methods fluctuated around 1.2 Å during the restraint MDSA. As expected, the fluctuations of the backbone were stronger for the *in vacuo* system. At the beginning of the free simulation the RMSD increased less for the explicit solvent model than for the peptide *in vacuo*. Additional free simulation of the box system up to 250 ps (data not shown) revealed a much slower increase of the RMSD compared to the peptide *in vacuo* but with a similar final level. This clearly indicates that, starting from the *in vacuo* structure, the resulting conformational backbone fluctuations in the two systems are comparable. Therefore, it is concluded that the introduction of an explicit solvent treatment does not result in significant changes of the backbone conformation for this bicyclic peptide. Since, independent of the method of calculation, the side-chain array and the dihedral angles of the disulphide and diselenide bridges were very similar, only *in*

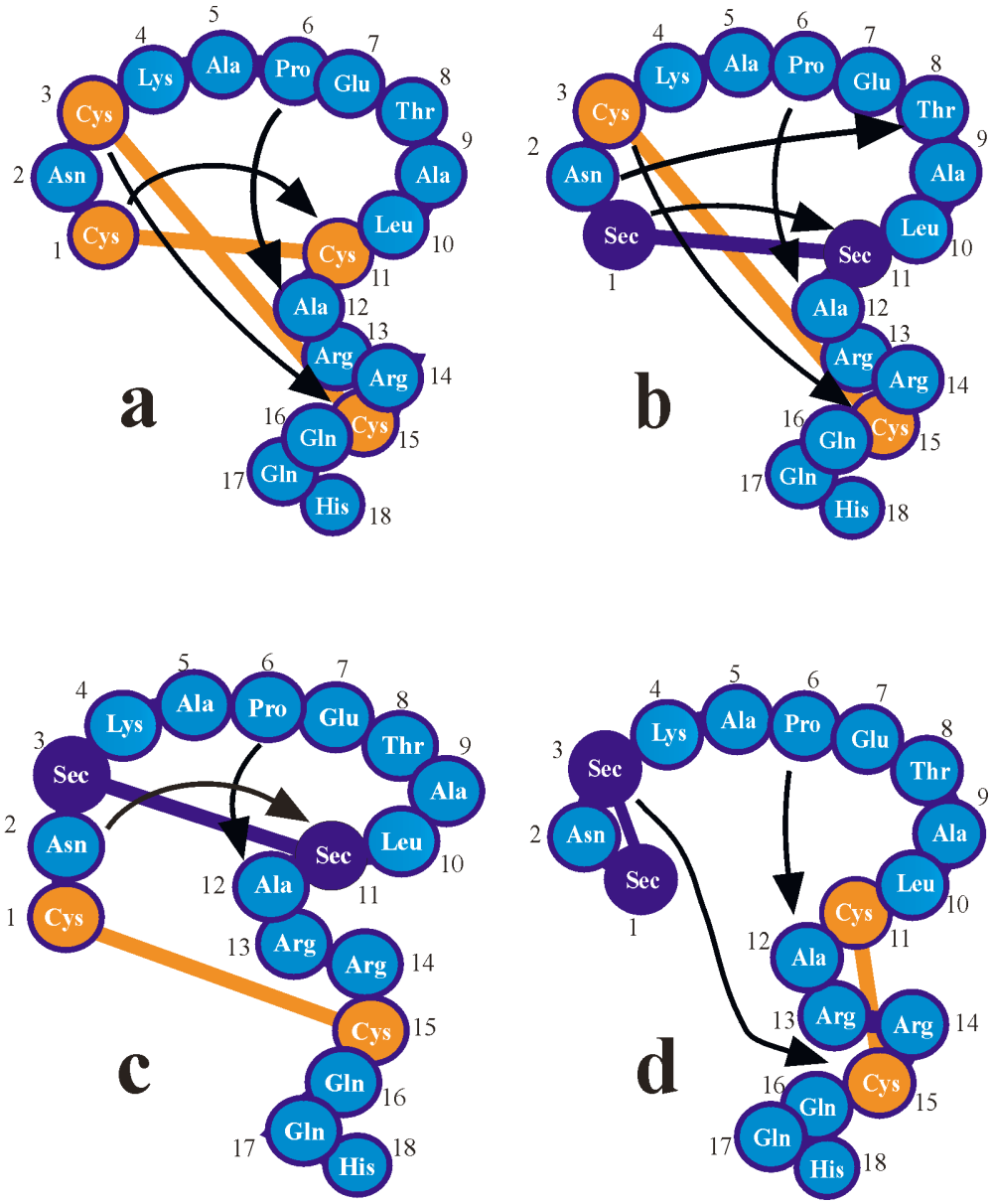

Figure 5 *Four different apamin isomers where the long range NOE contacts between residues i and j with |i-j| > 4 are represented by black arrows. In the top panels, very similar NOE contacts can be observed for the natural isomer (a) and the globular isomer (b). In the bottom panels the few NOE contacts of the ribbon (c) and the bead isomer (d) are shown. All four isomers show NOE contacts between proline 6 and alanine 12 that explain the similar backbone structure of the upper helix (see Figure 1-4)*

vacuo calculations were analysed for all apamin structures in the present work.

The MDSA protocol was as follows: A conjugate gradient minimisation (10^3 steps) with $\varepsilon = 80$ was the first run in order to obtain planar amide bonds. Subsequently NOE restraints were introduced and a 250 kcal mol^{-1} force was applied on the peptide bonds to keep them planar throughout the simulation. After one ps at 10 K the temperature was increased exponentially to a target temperature of 900 K during a 24 ps restrained molecular dynamics simulation. The time constant used was two ps, i.e. the difference between actual and final temperature was reduced by half every two ps. During the following 1000 ps 500 high temperature structures were saved (one every two ps). Subsequently each structure was cooled to 0 K within 9.5 ps using again a decreasing exponential curve with a time constant of 1 ps.

MC calculations

The MOCCA-generated ensembles comprised 500 representatives using the same starting structure as the DG/MDSA method. Each representative resulted from a simulated annealing procedure employing 2000 distinct Monte Carlo steps. A single Monte Carlo step consists of n random torsional steps $\Delta\varphi$ where n is the number of user-assigned rotatable bonds. Only single bonds between C-atoms of the chain or ring are rotatable. For all energy calculations different force-fields can be applied optionally, e.g. AMBER [29], cvff [26] and the Tripos force field [30]. In the case of apamin, the cvff force-field was used. Since the MC procedure is carried out in torsional space, all covalent bond lengths and angles are kept fixed and do not appear in the potential function:

box

in vacuo

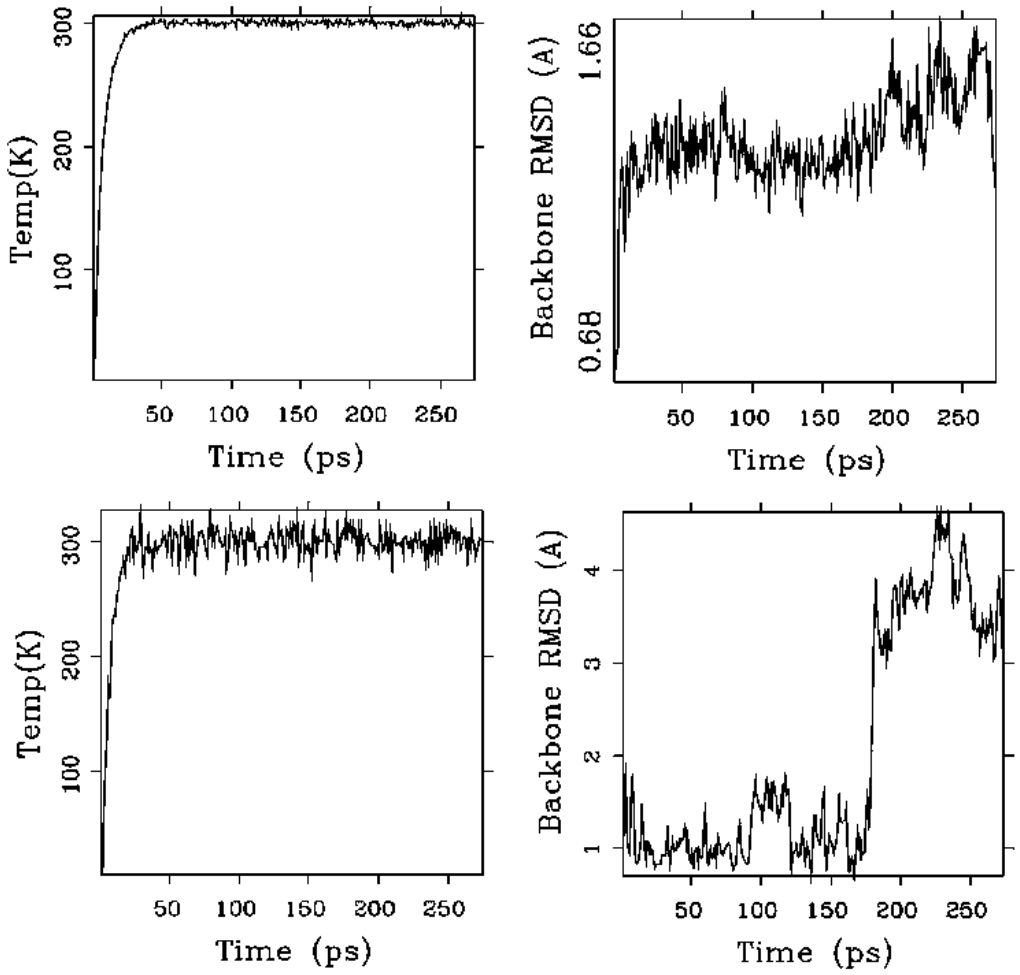

Figure 6 *The two upper graphs present the temperature and backbone RMSD curve of the explicit solvent model for the complete simulation. The two lower graphs show the temperature and backbone RMSD of the simulation using* in vacuo *conditions*

$$E_{pot} = E_{torsions} + E_{v.d.Waals} + E_{electrostatics} + (E_{penalty\ functions}) \quad (1)$$

Penalty functions can be potential functions according to experimental data. For the apamin simulation these were terms for the distance constraints according to the NOE-values and dihedral angle constraints according to ^{3}J-coupling constants. The temperature profile ranged from T_{start} = 2000 K to T_{final} = 300 K which turned out to yield a sufficient acceptance rate. The temperature of the system was decreased exponentially during the course of the MC simulation. If open chain molecules are calculated, the random walk is entirely unrestricted ($0° < \Delta\varphi < 180°$). However, restrictions in Dj can be user-defined. Cyclic systems are broken at a user-defined bond in the ring. Furthermore, the torsional variation is scaled down with increasing MC steps and according to a "through-bond" distance relative to the ring-cleavage site. The higher the distance between broken bond and randomly selected torsion bond and the higher the number of MC steps already performed, the smaller the allowed changes in torsional angles (Figure 7) [19].

The final structures were obtained by a minimisation using Discover [22] to separate families of structures. Every second representative of the whole ensemble was taken for a cluster graph analysis.

Results and discussion

In Table 1 the results are listed for the best structures obtained by both methods. The structures were chosen according to their backbone convergence, the low NOE violations and the correct secondary structure as observed by NMR. Figures 8-11 show the superposition of one representative of the DG/MDSA and all selected MOCCA structures.

The comparison of the two methods shows that the number of acceptable structures is significantly lower in the case of

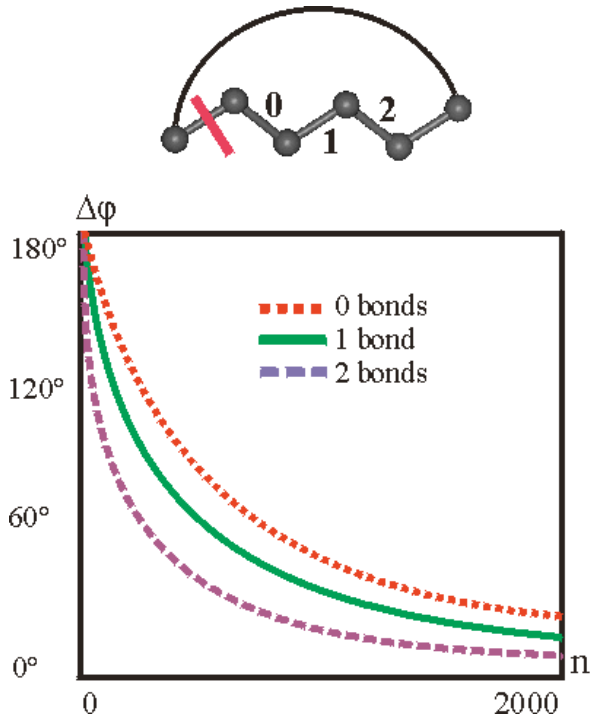

Figure 7 *The graph shows the scaling of torsional changes Δφ by the MOCCA program depending on the distance from the broken bond and the number* n *of MC steps already performed. In the cartoon of the molecule the red bar indicates the broken bond and the numbers represent the distance of the single bonds from this break. The allowed range Δφ of the torsion angles for single bonds decreases depending on their distance to the broken bond*

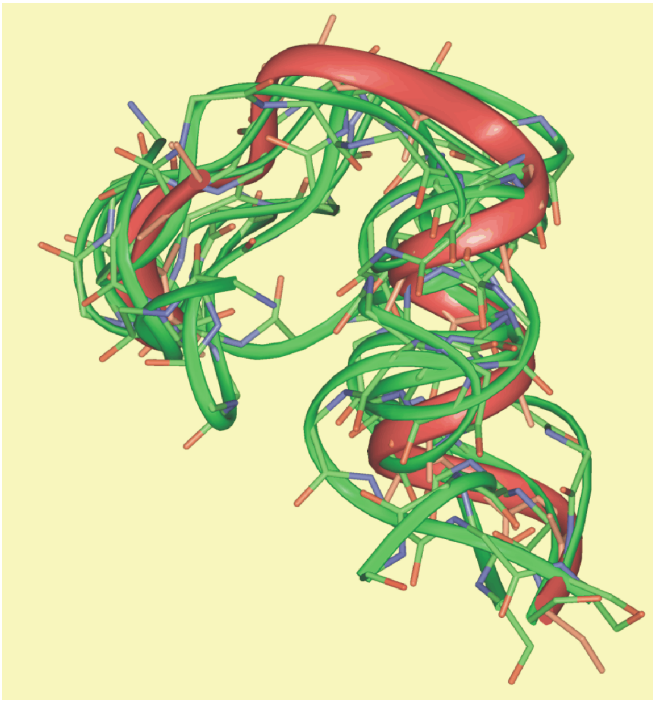

Figure 8 *The backbones of the MOCCA-calculated structures of the natural apamin analogue (green) are superimposed on one representative backbone (red) of the DG/MDSA ensemble*

MOCCA than in the case of the combined DG/MDSA. Only the MOCCA generated ensemble of the bead apamin isomer includes more than 10 representatives, whereas for every isomer more than 40 convergent low-energy structures are present in the ensemble derived from the DG/MDSA calculation, which were reduced to 20 for the comparison of the two methods. The MOCCA ensembles are not as convergent as the trajectories of the MDSA. Additionally, the MC method generated more than 60% badly folded conformations for every isomer, e.g. left handed α-helices. The worst result was obtained for the natural apamin, where only five structures met the selection criteria (Figure 8). As the remaining families of structures are small, the results represent only trends of the MOCCA simulation of each apamin isomer.

The quality of the selected structures were assessed for every isomer and for each simulation method by the program Procheck-NMR [31]. Except for the MOCCA simulation of the natural isomer (Figure 8), where only 73.3% of the residues are in allowed and favoured regions of the Ramachandran plot, more than 92% of the residues of the other isomers are in allowed and favoured regions (Figures 9-11). According to Ramachandran plots, the quality of structures determined by MOCCA calculations is worse than in the case of DG/MDSA.

The mean distance violations of all ensembles are in an acceptable range independent of the method of calculation. In comparison to the DG/MDSA, the values for maximum distance and mean distance violations of the MOCCA simulations are always higher. This was expected for an ensemble of independent structures. The mean RMSD values of the backbone coordinates for the MC simulation are significantly higher than for the other method. The RMSD values of the DG/MDSA ensembles do not differ significantly, in contrast to the MOCCA derived ensembles, where the RMSD values increase dramatically from the globular to the bead and ribbon up to the natural isomer.

In terms of energy values no correlation was found between the two strategies (Table 1). Moreover, the variations in the total energies within the MOCCA ensembles are too high to allow any conclusions in terms of preferred conformations. Although the lowest energy was expected for the natural apamin isomer, this was the molecule with the highest value. Also for the DG/MDSA method no significant low energy conformers of the apamin isomers could be extracted.

For the MC calculation one ensemble of 500 structures required 10-14 days on a SGI origin, i.e. up to five times more computational time than for the combined DG/MDSA methods. Thus, larger structure ensembles were not investigated due to computational costs. Moreover, according to

Table 1 *Structural data of selected structures of MOCCA and DG/MDSA (cursive letters) calculations according to the criteria of secondary structure, backbone convergence and NOE violation*

Isomer	**Natural Apamin**	**Globular Apamin-Analogue**	**Bead Apamin-Analogue**	**Ribbon Apamin-Analogue**
number of structures	5	8	15	6
	20	*20*	*20*	*20*
total restraints	87	95	73	87
H-bonds [a]	5	5	4	4
dihedrals	14	13	11	13
max. distance violation (Å)	0.80	0.85	0.83	0.49
	0.11	*0.40*	*0.22*	*0.16*
mean distance violation (Å) [b]	0.1±0.16	0.06±0.08	0.06±0.10	0.05±0.37
	0.1±0.001	*0.03±0.001*	*0.03±0.001*	*0.02±0.001*
mean RMSD values (Å)	3.53±0.64	1.35±0.59	1.68±0.53	1.92±0.21
	0.50±0.22	*0.31±0.11*	*0.53±0.21*	*0.25±0.11*
$E_{Coulomb}$ (kcal mol^{-1}) [c]	4.04±0.48	-1.34±0.19	-1.37±0.08	-0.05±0.11
	3.44±0.27	*-1.29±0.09*	*-1.32±0.11*	*2.41±0.14*
$E_{out\ of\ plane}$ (kcal mol^{-1}) [c]	8.54±7.96	4.85±4.94	2.42±0.79	3.03±1.06
	1.34±0.09	*11.52±0.22*	*1.69±0.19*	*2.20±0.34*
E_{vdW} (kcal mol^{-1}) [c]	182.91±103.83	127.75±69.01	110.41±12.05	89.17±11.27
	59.51±2.96	*77.11±3.74*	*69.75±6.95*	*59.84±2.94*
$E_{bond+theta+phi}$ (kcal mol^{-1}) [c]	382.68±39.55	271.59±42.72	235.54±14.80	233.00±10.52
	175.21±2.61	*176.54±3.31*	*174.80±4.52*	*168.14±2.24*
E_{tot} (kcal mol^{-1}) [c]	578.12±225.31	402.85±197.75	347.01±51.40	325.50±21.67
	239.43±2.81	*263.88±3.52*	*244.76±6.04*	*232.89±2.59*
Ramachandran-plot regions [d]:				
allowed+favoured	73.3%	92.5%	93.3%	95.5%
	100%	*100%*	*100%*	*100%*
generously allowed	13.3%	5.0%	3.6%	2.2%
	0%	*0%*	*0%*	*0%*
forbidden	13.3%	2.5%	3.1%	2.2%
	0%	*0%*	*0%*	*0%*

[a] H-bonds include only NH-O distances
[b] average NOE-violation per NOE
[c] in case of CVFF force-field average energies have no "cut-off".
[d] calculated by the program "Procheck" [31]

published examples [19,20] and to the well defined tertiary structure of apamin [32], it was expected that an ensemble of 500 should be sufficient for a complete statistical analysis. Increasing the start temperature to 3000 K did not improve the results.

Conclusions

In the case of apamin and its Sec-isomers the MOCCA simulation method generated only a small number of useful structures. No correlation of the two methods could be observed. Due to the small ensembles and to the low differences of the total energies, none of the methods was useful for estimating the stability of the structures. Probably the breaking and fusing of bonds of the bicyclic systems of the apamin isomers lead to an unpredictable bias of the MC algorithm. This is supported by the fact that the MC method generated almost two times more useful structures for the separated ring system of the bead apamin than in case of the bicyclic isomers. Considering the complexity of the test molecules and the intrinsic problems of MC methods [5,6] the results are not as bad as it might seem. In spite of the NOE restraints and the

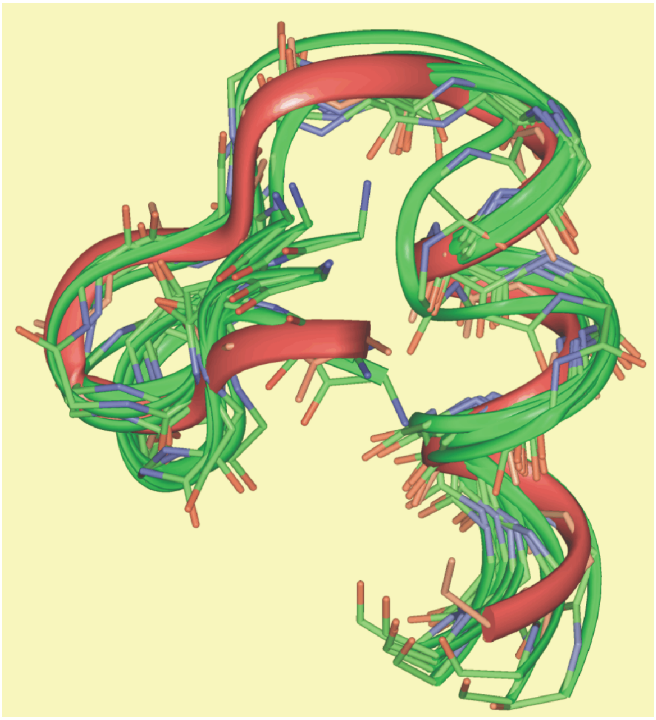

Figure 9 *The backbones of the MOCCA-calculated structures of the globular apamin analogue (green) are superimposed on one representative backbone (red) of the DG/MDSA ensemble*

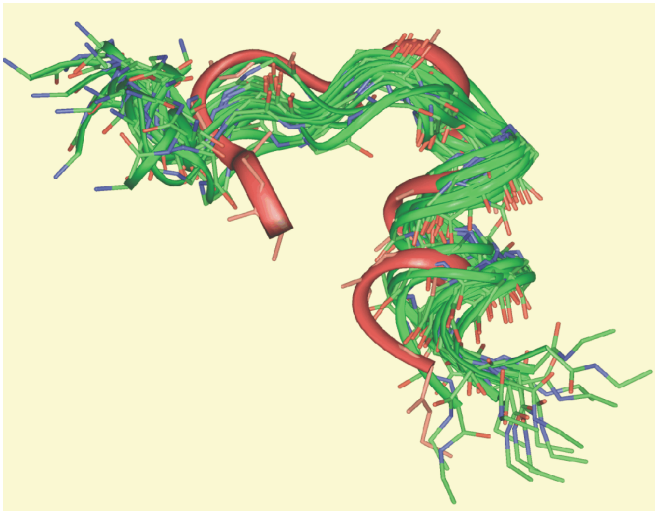

Figure 10 *The backbones of the MOCCA-calculated structures of the bead apamin analogue (green) are superimposed on one representative backbone (red) of the DG/MDSA ensemble*

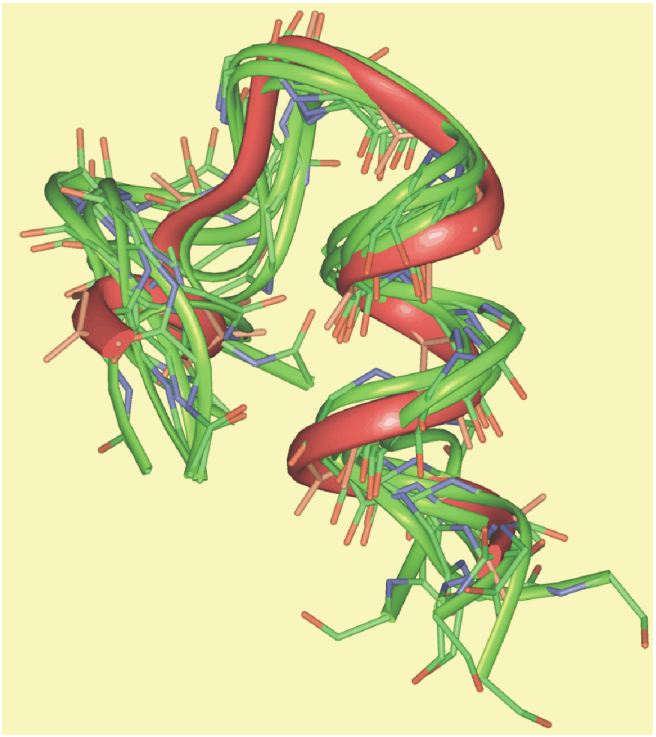

Figure 11 *The backbones of the MOCCA-calculated structures of the ribbon apamin analogue (green) are superimposed on one representative backbone (red) of the DG/MDSA ensemble*

breaking and fusing of two ring systems, a few correct structures are found among the relatively small number of total calculated structures. However, the advantage of the MOCCA simulation strategy is the fast ensemble generation of independent structures for macrocycles and linear biopolymers, which is more effective than the combined DG/MDSA methods [19,20].

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Supplementary Material Available Cartesian Coordinates for the best MDSA structures as calculated by DISCOVER [22] and the best MC structures as calculated by MOCCA [19,20] are available in PDB format.

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