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Can One Derive the Conformational Preference of a β-Peptide from Its CD Spectrum?

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Abstract: CD spectroscopy is often used to elucidate the secondary structure of peptides built from non-natural amino acids such as β-amino acids. The interpretation of such CD spectra is not always unambiguous. Here, we present a case where two β-hexapeptides, a dimethyl-β-hexapeptide indicated as DM-BHP (**A**) and its nonmethylated analogue indicated as BHP (**B**), exhibit similar CD spectra, whereas they are expected to differ in secondary structure. The structural properties of both peptides were studied by molecular dynamics simulation, and from the resulting trajectories, the corresponding CD spectra were calculated. Starting from a fully extended conformation, BHP is observed to form a 3_{14}-helix, while DM-BHP remains unfolded. However, even though these two peptides hardly share any conformations, their calculated CD spectra are alike and show the same features as the experimentally measured ones. Our results imply that a particular CD pattern can be induced by spatially different structures, which makes it difficult to derive the conformational preference of a peptide from its CD spectrum alone. To gain more insight into the relationship between the preferred conformation of a peptide and its CD spectrum, more accurate methods to calculate the CD spectrum for a given conformation are required.

1. Introduction

Circular dichroism (CD) is a chiroptical method that is used to study the conformational properties of a wide range of compounds, from small chiral molecules to macromolecules such as proteins or polymers. Especially in the field of proteins, CD spectroscopy has become, in combination with NMR spectroscopy, a widely used experimental technique for structure determination.[1] As the CD is very sensitive to protein conformation, it is used for monitoring folding/unfolding processes in globular proteins and for detection and characterization of structural changes upon site-directed mutagenesis. The basis of understanding the CD spectra of proteins, in particular in the far-UV region (below 250 nm), is provided by CD measurements of model polypeptides under various conditions and a detailed characterization thereof. The analysis of the secondary structure of a protein by CD spectroscopy is commonly based on the fitting of the spectrum to that of a combination of isolated secondary structure elements or of a set of proteins with known X-ray or NMR structures.[2]

Recently, CD spectroscopy in the far-UV region has been used to elucidate the secondary structures of so-called foldamers,[3] (non-natural) oligomeric compounds with a strong tendency to adopt specific, three-dimensional conformations. Prominent examples are oligomers of β-, γ-, or even δ-amino acids.[4−7] The groups of Seebach and Gellmann have been synthesizing and investigating numerous short-chain β-peptides (from homologues of α-amino acids and from cyclic β-amino acids, respectively) which are found by NMR, CD spectroscopy, and molecular dynamics (MD) simulations to adopt helical (3_{14}-,[8−11] 2.5_{12}-,[12] and 12/10-helices[13,14]) or hairpin[15,16] conformations in solution. On the basis of NMR and CD spectroscopic investigations of several β-hexa- and β-hepta-peptides, Seebach

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and co-workers could assign a distinct CD pattern to the *M*-3_{14}-helical conformation (peak near 200 nm, zero crossing between 205 and 210 nm, trough between 215 and 220 nm).[17] However, this pattern does not seem to be unique: β-peptides that should not, on the basis of steric considerations, be able to adopt a 3_{14}-helix show CD spectra similar to those of *M*-3_{14}-helical peptides.[18] Furthermore, the CD spectrum exhibited by a β-hexapeptide built of *trans*-2-aminocyclohexane-carboxylic acid differs from the previous ones, even though the crystal structure was reported to be a *M*-3_{14}-helix.[19] For oligomers for which structural information from NMR or X-ray is lacking, the CD spectrum can only be taken as a *hint* that a chiral secondary structure is present, since an unambiguous assignment of a CD spectrum to a specific secondary structure is currently impossible. Although there are examples of stable peptide conformations in solution for which the CD spectrum can be assigned to a single conformation, in many cases, the solution state of a peptide is characterized by an ensemble of conformers with different contents of secondary structures. In these cases, the observed CD spectra obviously represent an average of the contributions from different conformers.

There is clearly a need for a better understanding of the relationship between the protein or peptide conformation and its CD spectrum. Theoretical methods to calculate the CD spectrum of peptides and proteins could be helpful for gaining insight into this relationship and may validate the interpretation of CD experiments, especially for new classes of oligomers. The quantity describing the circular dichroism is the rotational strength which is proportional to the area of the CD band.[1] It can be calculated from the electric and magnetic transition dipole moments between the ground and the excited states. When given the size of a protein or peptide, a direct quantum-mechanical calculation of the optical rotatory strengths is not feasible. Therefore, most of the theoretical methods for CD spectrum calculations make use of either the so-called matrix method[20] or the dipole interaction method.[21−23] In the latter method, the CD spectrum calculation is based on classical physics, considering individual atoms and the amide chromophore as point dipole oscillators. The method has been applied to the CD spectrum calculations of single model structures of a number of systems,[23,24] in particular β-peptides.[25−27] The matrix method, however, is based on quantum-mechanical theory and assumes that the charge distributions of the various chromophores of the protein do not overlap, that is, that there is no charge transfer between them. The interaction between these monomers is then computed using a set of parameters describing the different chromophores. The parameters are derived from either semiempirical[28,29] or ab initio[30,31] calculations of small model chromophores such as *N*-methyl-acetamide or acetamide for the amide chromophore. Although this method suffers from a number of deficiencies,[32−34] it is the current state of the art and has been applied to calculate CD spectra of polypeptides[35−38] and of several globular proteins from a single structure, typically obtained from X-ray diffraction.[28−31] Recently, the CD spectra for a β-hexapeptide and a β-heptapeptide have been calculated from molecular dynamic trajectories using the matrix method, with the aim to investigate its sensitivity to molecular structure and the effects of motional averaging.[34]

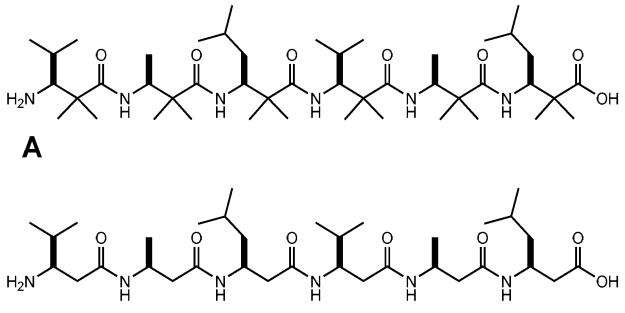

Figure 1. Molecular formulas of the two β-hexapeptides studied. Peptide A, denoted as DM-BHP, differs from peptide B, denoted as BHP, by having two methyl groups attached to the C_α atoms. Note that in the simulations both endgroups were protonated in line with experimental data.

Here, we present a case which illustrates both the difficulty of a reliable interpretation of experimental CD spectra of peptides and the need for an accurate theoretical method to calculate the CD spectrum given the molecular structure. We studied two β-hexapeptides which only differ in the substitution at the C_α atoms: DM-BHP (dimethyl-β-hexapeptide, peptide **A** in Figure 1) is built from geminally dimethyl-substituted $\beta^{2,2,3}$-amino acids [sequence: H-(β-HVal(Me_2)-β-HAla(Me_2)-β-HLeu-(Me_2))$_2$], whereas BHP (β-hexapeptide, peptide **B** in Figure 1) consists of β^3-amino acid residues without substituents at the C_α atoms [sequence: H-(β-HVal-β-HAla-β-HLeu)$_2$]. The latter is experimentally known to fold into a 3_{14}-helix, as confirmed by NMR measurements.[8,9] C_α-Methyl groups would have to occupy an axial position in a 3_{14}-helix of DM-BHP (**A**), which is sterically impossible.[9,18] Despite that, DM-BHP exhibits a CD spectrum that is very similar to that of BHP, showing the pattern reported to be typical for β-peptides forming a 3_{14}-helix (Figure 2). So far, there are no NMR data available for the methylated peptide that could give more information about its secondary structure.

Certainly, these facts raise some questions: First, which conformations does the geminally dimethylated peptide (DM-BHP) predominantly adopt? Second, which conformations other

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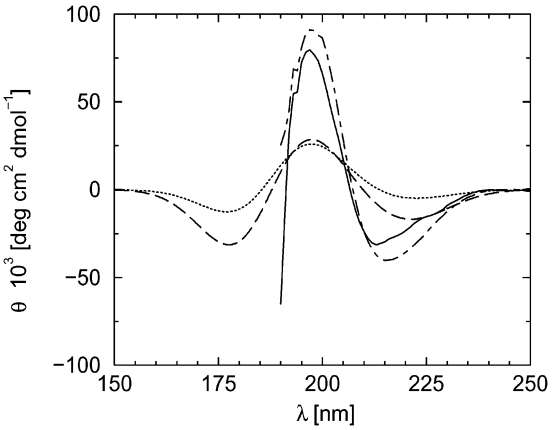

Figure 2. Experimental and calculated CD spectra of the two β-hexapeptides in methanol. Solid line: experimental CD spectrum of DM-BHP (**A**).[18] Dashed−dotted: experimental CD spectrum of BHP (**B**).[18] Dotted: average CD spectrum of DM-BHP from the MD simulation (100 ns trajectory). Dashed: average CD spectrum of BHP from the MD simulation (100 ns trajectory). Both the experimental and the calculated CD spectra are at room temperature (298 K).

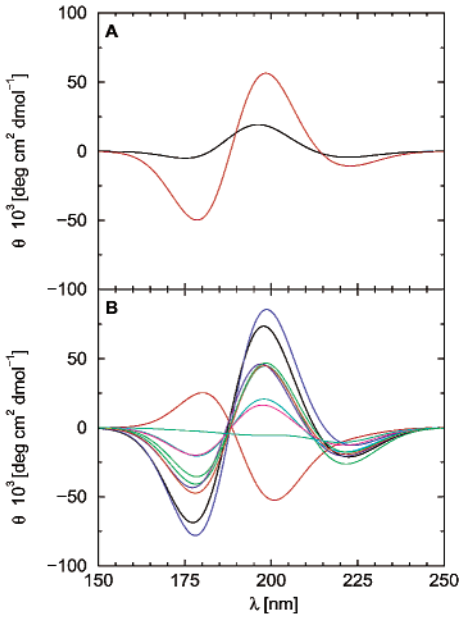

Figure 3. CD spectra averaged over the members of the clusters for which the central structures are shown in Figure 4. Panel A: CD spectra of DM-BHP (**A**); (black) averaged over the 7458 members of cluster 1, (red) averaged over the 1294 members of cluster 2. Panel B: CD spectra of BHP (**B**); (black) averaged over the 2045 members of cluster 1, (red) averaged over the 1807 members of cluster 2, (green) averaged over the 1446 members of cluster 3, (blue) averaged over the 676 members of cluster 4, (violet) averaged over the 460 members of cluster 5, (cyan) averaged over the 445 members of cluster 6, (magenta) averaged over the 255 members of cluster 7, (orange) averaged over the 194 members of cluster 8, (turquoise) averaged over the 171 members of cluster 9, (olive) averaged over the 157 members of cluster 10.

than a 3_{14}-helix give rise to a similar CD spectrum? And third, can the CD spectra of β-peptides be really assigned to a single, specific secondary structure or do they rather represent an average over different conformers, may they be folded or unfolded? In an attempt to answer these questions, the two peptides, DM-BHP (**A**) and BHP (**B**), were investigated by MD simulations using the GROMOS package[39] and the GROMOS 43A1 force field,[39,40] in a way analogous to former MD studies on β-peptides.[41,14,16] The initial conformation was in both cases chosen to be fully extended. From the resulting MD trajectories, the CD spectra of both peptides were then calculated using the above-mentioned matrix method.

2. Results and Discussion

We performed for each peptide in a box with 1462 or 1463 methanol molecules a 100-ns simulation at constant temperature (298 K) and at constant pressure (1 atm). The resulting trajectories were analyzed using the cluster algorithm described by Daura et al.[42] (see also Computational Methods section). The CD spectra of peptide structures taken every 10 ps were calculated with the matrix method implemented by Fleischhauer and co-workers in MATMAC.[43] In this section, the results of the clustering analysis and the CD spectrum calculations are presented and discussed.

Figure 2 shows the mean CD spectrum for each peptide, averaged over 10 000 spectra of single structures extracted from the MD trajectory, together with the experimentally measured ones. Although the intensities of the calculated spectra are much lower, they show the same characteristics as the experimental ones: The calculated spectra of both peptides show a maximum at 197 nm, which corresponds to that of the experimentally observed peaks. DM-BHP exhibits a weak negative Cotton effect at about 223 nm, whereas BHP shows a slightly stronger one at 221 nm. However, both minima as well as the zero crossing are slightly red-shifted compared to the case of the experimental spectra, where for DM-BHP a negative Cotton effect is observed at 213 nm and for BHP at 215 nm. Experimentally, a zero crossing is observed for both peptides around 207 nm, whereas the theoretical spectra show a zero crossing at around 213 nm for DM-BHP and at 210 nm for BHP. As stated before, the intensities of the calculated spectra are much weaker but reproduce qualitatively the experimental difference in the CD spectra between the two β-peptides: DM-BHP shows a weaker negative Cotton effect and a less intensive peak than BHP. The reduced intensity might be an effect of averaging over a large number of structures. Even though the mean CD spectrum calculated from a long MD trajectory reproduces qualitatively well the experimentally observed CD pattern for both DM-BHP and BHP, the individual trajectory structures show a wide variation in their spectra as already observed for other β-peptides.[34]

Figure 3 serves as an indication of how much the spectra of distinct conformers can differ. Here, for each cluster or conformation, the mean CD spectrum averaged over all structures belonging to the same cluster is displayed. The corresponding central member structures of each cluster considered, representing the dominant conformations, are shown in Figure 4. For DM-BHP, the first two clusters already represent more than 87% of the total population, and for BHP, more conformational variation is observed: the first 10 clusters

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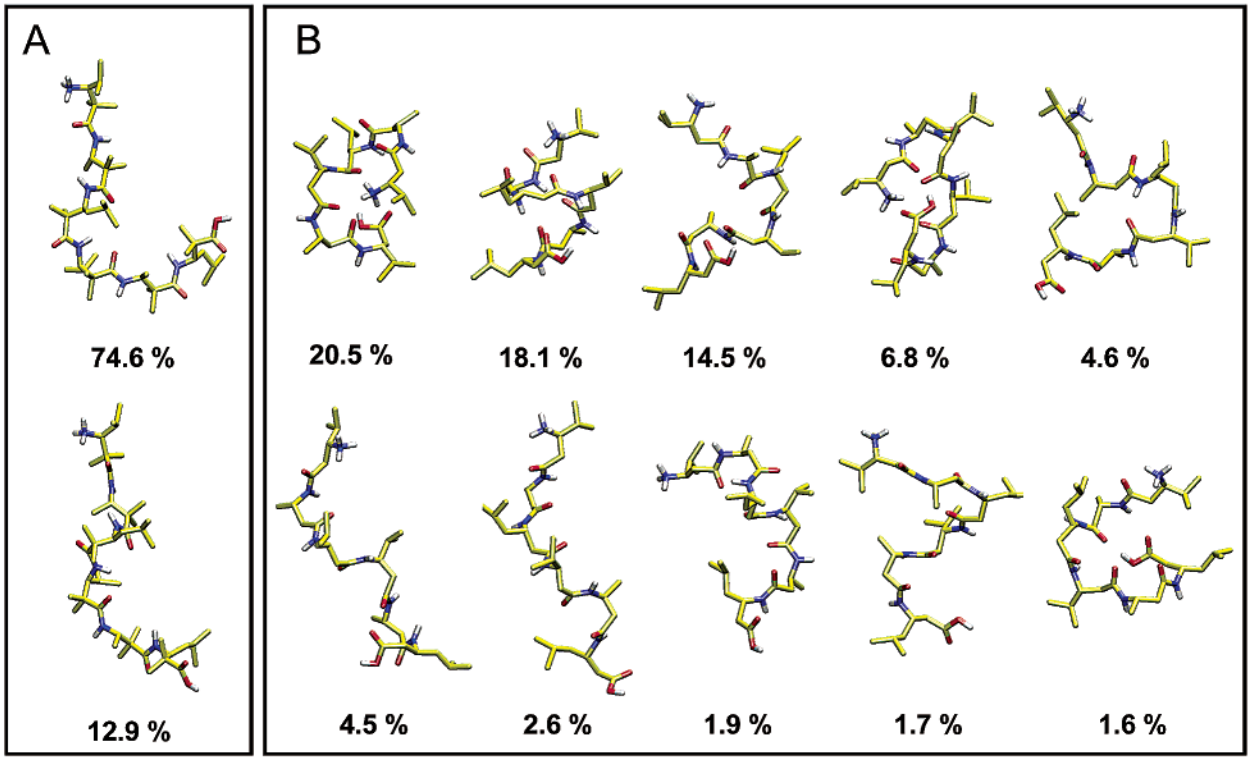

Figure 4. Panel A: Central structures of clusters 1 and 2 of DM-BHP (**A**) at 298 K. Cluster 1 and 2 represent more than 87% of the total population. The first three most populated clusters of a total of 16 clusters represent over 90% of the total population, the first 6 clusters over 98%. Panel B: Central member structures of cluster 1−10 of BHP (**B**) at 298 K. Clusters 1−10 represent more than 76%, cluster 1−24 more than 90%, and cluster 1−55 more than 98% of the total population.

Table 1. Occurrence of Intramolecular Hydrogen Bonds[a]

			hydrogen bond occurrence (%)	
	donor[b]	acceptor[b]	0.25 nm/135°[c]	0.3 nm/135°[d]
DM-BHP (**A**)	NH(4)	O(2)	7.8	9.7
	NH(4)	O(4)	2.9	2.9
BHP (**B**)	NH(1)	O(3)	7.7	8.4
	NH(1)	O(4)	7.7	8.3
	NH(1)	O(5)	9.7	10.4
	NH(2)	O(4)	17.7	18.0
	NH(3)	O(5)	13.5	13.8
	NH(4)	O(6)	8.2	9.0
	NH(5)	O(3)	2.3	2.7
	OH(6)	O(3)	3.0	3.1

[a] Only hydrogen bonds occurring in more than 2% of the analyzed conformations have been considered. [b] The residue sequence numbers of the atoms are indicated in parentheses. [c] In the first case, a hydrogen bond is considered to exist when the donor-hydrogen-acceptor angle is larger than 135° and the hydrogen-acceptor distance is smaller than 0.25 nm. [d] The second criterion enlarges the maximally allowed distance to 0.3 nm.

represent more than 76% of the total population. This indicates that the double methylation of the C_α atoms severely restricts the conformational space of the peptide. The clustering as well as the hydrogen bond analysis (Table 1) reveals that during the whole simulation DM-BHP does not adopt any defined secondary structure, although its CD spectrum would suggest a helical conformation to be the dominant one. BHP, on the other hand, folds into the expected *M*-3_{14}-helix within the first half of the simulation and, thereafter, repeatedly unfolds and folds to this helical secondary structure. The helical conformation represents 18% of the total population, being the second most populated cluster. The central member structure of this cluster only reproduces the two central hydrogen bonds of the four present in a complete 3_{14}-helix, because in the clustering algorithm the N- and C-terminal residues were excluded in view of their high mobility. Interestingly, as shown in Figure 3B (red line), the mean CD spectrum of the helical conformation (cluster 2) does not show the experimentally observed CD pattern of a trough at 215 nm and a peak at about 200 nm (Figure 2) but shows a peak at 180 nm and a trough at 201 nm, whereas the clusters corresponding to unfolded conformations exhibit CD spectra with a pattern similar to the experimental one. These findings, along with the fact that DM-BHP exhibits a similar CD spectrum, even though it does not adopt a defined secondary structure, would imply that the unfolded rather than the folded conformers determine the shape of the CD spectra.

Seebach et al.[17] concluded from their CD spectroscopic investigations on various β-peptides that each type of intramolecular hydrogen bond, classified as hydrogen-bonded rings of 8, 10, 12, and 14 members, contributes to the CD spectrum in a specific way. In particular, 14-membered rings, the structural elements of the 3_{14}-helix, make a contribution to negative and positive Cotton effects at about 215 and 200 nm, respectively. Therefore, we monitored the occurrence of intramolecular hydrogen bonds. For every intramolecular hydrogen bond that is present in more than 2% of the sampled trajectory structures, the occurrence is indicated in Table 1. Two hydrogen bond definitions were used: (i) the standard GROMOS definition, where a hydrogen bond is considered to exist when the donor−hydrogen−acceptor angle is larger than 135° and the hydrogen−acceptor distance is smaller than 0.25 nm, and (ii) a slightly more generous definition with the maximum hydrogen−acceptor distance lengthened to 0.3 nm. DM-BHP (**A**) does only show two hydrogen bonds, one forming an eight-membered ring between the residues four and two and another one forming an

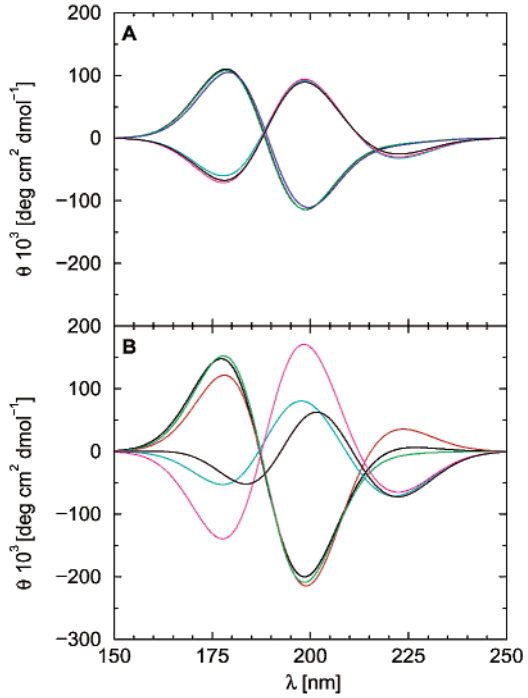

Figure 5. CD spectra for individual structures that show either a very pronounced negative Cotton effect or a Cotton effect near the experimentally observed wavelength (around 215 nm). Panel A: spectra of structures of DM-BHP (**A**); (black) structure a in Figure 6A at 750 ps, (green) structure b in Figure 6A at 45150 ps, (blue) structure c in Figure 6A at 57 700 ps, (cyan) structure d in Figure 6A 83 200 ps, (magenta) structure e in Figure 6A at 85 920 ps, (maroon) structure f in Figure 6A at 95 020 ps. Panel B: spectra of structures of BHP (**B**); (black) structure a in Figure 6B at 32 200 ps, (red) structure b in Figure 6B at 45 460 ps, (green) structure c in Figure 6B at 59 120 ps, (cyan) structure d in Figure 6B at 38 150 ps, (magenta) structure e in Figure 6B at 38 900 ps, (maroon) structure f in Figure 6B at 72 050 ps.

intraresidue six-membered ring. BHP (**B**) forms four 14-membered hydrogen-bonded rings (NH(1)−O(3), NH(2)−O(4), NH(3)−O(5), NH(4)−O(6)), where the two central ones occur more often than the terminal ones, reflecting the mobility of the peptide tails. In addition, single (terminal) hydrogen bonds forming 16- (OH(6)−O(3)), 18- (NH(1)−O(4)), and 22-membered (NH(1)−O(5)) rings are observed, and a single eight-membered hydrogen-bonded ring (NH(5)−O(3)) with low occurrence was detected. From this hydrogen-bond analysis, one would predict, on the basis of the conclusions drawn from experiment,[17] that BHP would exhibit a CD spectrum with the characteristic pattern of that of an *M*-3_{14}-helix, whereas the introduction of two geminal methyl groups in the α-position of the residues would lead to a breakdown of the observed Cotton effect. However, this is not the case as both experiment and simulation demonstrate. Therefore, it seems questionable whether the presence of intramolecular hydrogen bonds is a sufficient criterion for a β-peptide to exhibit Cotton effects of either sign. After all, it is mainly the relative orientation of the amide chromophores in the peptide and not the presence of hydrogen bonds that determines the chiroptical properties of a peptide in the far-UV region. Certainly, a specific pattern of intramolecular hydrogen bonds gives rise to a specific relative orientation of the chromophores and consequently to a characteristic CD spectrum. However, in small and rather flexible peptides, as those investigated here, certain chromophore orientations that would

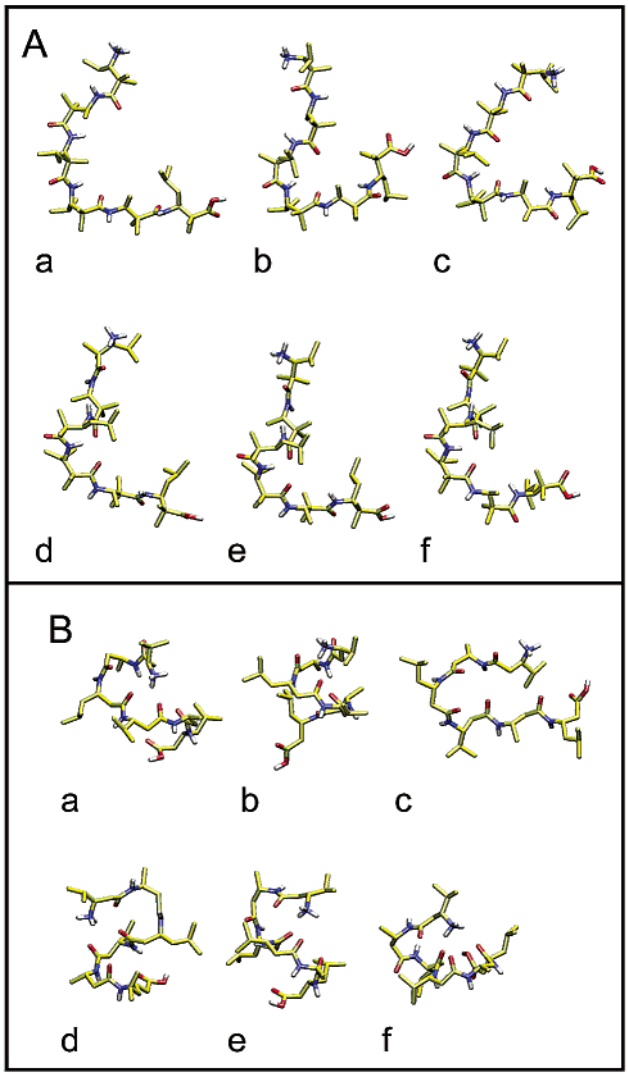

Figure 6. Structures of the two peptides corresponding to the CD spectra in Figure 5. Panel A: structures from the trajectory of DM-BHP (**A**). Structure a at 750 ps; structure b at 45 150 ps; structure c at 57 700 ps; structure d at 83 200 ps; structure e at 85 920 ps; structure f at 95 020 ps. Panel B: structures from the trajectory of BHP (**B**). Structure a at 32 200 ps; structure b at 44 560 ps; structure c at 59 120 ps; structure d at 31 850 ps; structure e at 39 800 ps; structure f at 72 050 ps.

give rise to a Cotton effect might also occur in unfolded conformers.

So, what are the structural characteristics that give rise to a strong negative Cotton effect? In an attempt to find an answer to this question, CD spectra of individual trajectory structures exhibiting either a particularly strong Cotton effect or a reasonable Cotton effect in the range of the experimentally measured and theoretically calculated wavelengths (between 210 and 225 nm) have been selected, and their structures compared with each other. Figure 5 shows the CD spectra of six conformers of each peptide, the corresponding structures being displayed in Figure 6. First of all, it is remarkable that the strongest negative Cotton effects (≥10 000 deg cm^2 dmol^{-1} for DM-BHP (**A**) and (≥ 20 000 deg cm^2 dmol^{-1} for BHP (**B**)) are observed around 200 nm and not around 215 nm. Structures a to c shown in panel A of Figure 6 (DM-BHP), which give rise to CD spectra with a peak at about 176 nm and a trough around 200 nm, are all characterized by a kink at the fourth residue but do not show any hydrogen bonding. On the other hand,

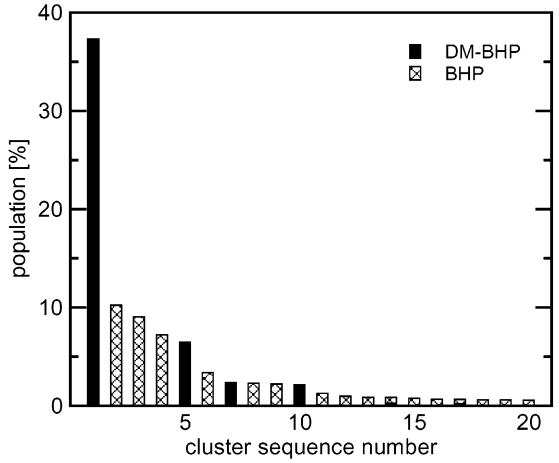

Figure 7. Clustering of the merged or combined trajectories of DM-BHP (**A**) and BHP (**B**). The plot shows the population in percentage per cluster and the portion of structures per cluster that belongs to the trajectory of DM-BHP (black) and to the trajectory of BHP (squared).

structures d to f in panel A of Figure 6, which give rise to a weak negative Cotton effect around 222 nm and a peak at about 198 nm, all have a more or less distorted hydrogen bond between residues four and two, forming an eight-membered ring (structure d, hydrogen−acceptor distance = 0.17 nm, donor−hydrogen−acceptor angle = 152°; structure e, H−A distance = 0.19 nm, D−H−A angle = 133°; structure f, H−A distance = 0.20 nm, D−H−A angle = 136°). In the case of BHP (Figures 5 and 6, panel B), three very different structures exhibit a similar CD spectrum with a trough around 200 nm and a peak at about 176 nm. Structure a forms an 18-membered hydrogen-bonded ring involving the N-terminus (NH(1)−O(4), H−A distance = 0.2 nm, D−H−A angle = 150°), whereas structure b represents a partly unwound 3_{14}-helix with two hydrogen bonds forming 14-membered rings (NH(1)−O(3) and NH(2)−O(4)). Structure c, however, does not show any intramolecular hydrogen bonding at all. Of the three conformers that exhibit a negative Cotton effect around 222 nm and a peak in the region of 200 nm, structures e and f show a hydrogen bond forming a 14-membered ring at the N-terminus (NH(1)−O(4)), whereas structure d does not show any hydrogen bonding. These examples basically show that similar CD spectra do not necessarily imply similar structures. A given CD pattern can be generated by different structures. However, the calculated CD spectra should be interpreted cautiously, since the method to calculate the CD spectrum from a given structure makes use of a series of approximations and suffers from some deficiencies,[32,34] which will not be commented here.

The results presented show that both peptides exhibit a similar CD spectrum, even though one (BHP) adopts an *M*-3_{14}-helix and the other (DM-BHP) remains practically unfolded hardly forming any hydrogen bonds. The conclusion that it is the unfolded state that mainly contributes to the observed CD spectra is very tempting but cannot presently be claimed with certainty. However, it is clear that although a single structure must lead to a unique CD spectrum, the inverse is not true. A given CD spectrum can be produced by spatially very different structures. In that context, it is of interest to analyze the similarity of the conformational space visited by both peptides, which produce very similar CD spectra. To that end, an additional cluster analysis of the combined trajectories of DM-BHP and BHP was performed using the same similarity criterion as in the case of the individual trajectories. A total of 138 clusters were found, and the first 7 most populated clusters represent more than 75% of the total population. The percentage population of the first 20 clusters (≥ 90% of the total population) and the portion of structures originating from one (DM-BHP) or the other (BHP) trajectory of structures are shown in Figure 7. Strikingly, almost none of the clusters comprehend structures from both trajectories; they either hold exclusively structures of DM-BHP or of BHP. Only cluster 14 (2% populated) and 17 (1% populated) contain structures from both trajectories. This means that the two peptides do not populate at all the same conformational space. Yet, they exhibit a similar CD spectrum, as shown by experiment and simulation (Figure 2).

3. Conclusion

The CD spectra of two β-hexapeptides, called DM-BHP (**A**) and BHP (**B**), have been calculated from molecular dynamics trajectories at room temperature and ambient pressure. As in experiment, both show the CD pattern previously assigned to *M*-3_{14} helices, even though DM-BHP remains unfolded and BHP reversibly folds into a 3_{14}-helical conformation, as illustrated by the cluster analysis and the occurrence of intramolecular hydrogen bonds. We also observed that for BHP the CD spectra of the unfolded conformers exhibit the experimental pattern commonly assigned to a helical conformation, whereas the helical conformer exhibits a CD spectrum different from the experimental one. This would imply that also the unfolded conformations contribute to the CD spectrum, and it questions whether the presence of intramolecular hydrogen bonds forming 8-, 10-, 12-, or 14-membered (hydrogen-bonded) rings is a necessary condition to induce Cotton effects. Particular constellations of relative chromophore orientations that do not result from intramolecular hydrogen bonding could also give rise to Cotton effects. This would explain to some extent the contribution of the unfolded conformers to the CD spectrum of a peptide. However, this assumption should be validated by further investigations using improved theoretical methods. Furthermore, inspection of CD spectra of individual structures that either show strong Cotton effects or Cotton effects in the range of the experimentally measured wavelengths reveals that spatially different structures can exhibit very similar CD spectra (see Figures 5 and 6).

These findings, together with the fact that the two peptides practically do not populate a common conformational space (Figure 7), suggest that for small peptides the same CD spectrum can be exhibited by different ensembles of conformers. Certainly, this complicates the interpretation of CD spectra and may easily lead to false conclusions. Furthermore, it implies that the conformational preference of a peptide cannot be unambiguously derived from CD measurements. However, the method applied here to calculate the CD spectra is an approximate, but established, procedure with a number of shortcomings repeatedly addressed in the literature.[31−34] To gain a deeper understanding of the relationship between peptide conformation and its CD spectrum, a more accurate method to calculate the latter is required. On the other hand, continued experimental studies of the chiroptical behavior of various (non-natural) peptides in combination with other spectroscopic techniques are also necessary to eventually develop, in a joint effort of theory and

experiment, a standardized method for the conformational analysis of non-natural oligomers such as β-peptides.

Finally, a second finding of general interest is the observation that two β-peptides that only differ in the methylation of their C_α atoms adopt completely different ensembles of conformers in solution. This demonstrates the sensitivity of solution structures of peptides to details of their composition.

4. Computational Methods

4.1. Molecular Model. The β-peptides were modeled using the GROMOS96 biomolecular force field, parameter set 43A1,[39,40] as described by Daura et al.[41] Methanol was modeled using the standard GROMOS96 set of solvents[39,40] as a rigid three-point model, whose properties agree well with the experimentally measured ones.[44]

4.2. Simulations. Two 100-ns MD simulations at 298 K and 1 atm were performed for DM-BHP and BHP. Periodic boundary conditions were applied. In both cases, the initial structure was extended (all backbone dihedral angles were set to 180°). DM-BHP was solvated with 1462 methanol molecules, and BHP, with 1463 methanol molecules, both in a truncated octahedron. The initial minimum distance between peptide atoms and the square walls of the truncated octahedron was chosen to be 1.4 nm. After relaxation of the systems using steepest descent energy minimization, the MD simulations were started by taking the initial velocities from a Maxwellian distribution at 298 K. Solvent and solute were independently weakly coupled to a temperature bath with a relaxation time of 0.1 ps.[45] The pressure was calculated with a molecular virial and held constant at 1 atm using the weak coupling method[45] with a relaxation time of 0.5 ps and an isothermal compressibility of $4.575\ 10^{-4}$ (kJ mol^{-1} nm^{-3})$^{-1}$. Bond lengths were constrained using the SHAKE algorithm[46] with a geometric tolerance of 10^{-4}. The equations of motion were integrated using the leapfrog algorithm and a time step of 2 fs. The interaction between atoms in so-called charge groups[39] was calculated according to a spherical twin-range cutoff scheme: short-range van der Waals and electrostatic interactions were evaluated at every time step by using a charge-group pair list that was generated with a short-range cutoff radius of 0.8 nm between the centers of geometry of the peptide charge groups and the oxygen atoms of the methanol solvent molecules. Longer-range van der Waals and electrostatic interactions, between pairs at a distance longer than 0.8 nm and shorter than a long-range cutoff of 1.4 nm, were evaluated every fifth time step, at which point the pair list was also updated and were kept unchanged between these updates. The systems were equilibrated for 2 ns, and the following 100 ns were used for analysis saving configurations every 0.5 ps.

4.3. Analysis. A cluster analysis was performed using the structures every 0.01 ns as described by Daura et al.[42] To that end, the atom-positional root-mean-square deviation (RMSD) using the backbone atoms of residues two to five was calculated for every pair of structures. For each trajectory structure, the number of structures (neighbors) with an RMSD $\leq$ 0.09 nm was determined. The structure with the highest number of (structural) neighbors was then taken as the central member of the cluster of similar structures forming a conformation. After removing the structures belonging to this first, most populated cluster from the pool of structures, the procedure was repeated to find the second cluster and so on. The CD spectra of the β-peptides were obtained via the calculation of the rotational strength using the matrix method[20] with parameters from CNDO/S calculations on acetamide as described by Kurapkat et al.[28] and Krüger et al.[34] This method considers the peptide or protein to consist of a number of independent chromophore groups, that is, with nonoverlapping charge distributions. As in the present cases, the side chains are all aliphatic and, therefore, do not contribute to the CD signal. The only chromophores considered were the peptide groups. The terminal amide and carboxylate groups were not included in the calculation, since their influence on the overall CD spectrum is small because of their high flexibility. For each of these backbone chromophores, three peptide transitions were considered: $n\pi^*$ at 220 nm, $\pi\pi^*$ (NV_1) at 190 nm, and $\pi\pi^*$ (NV_2) at 140 nm. All the calculations have been performed using the program MATMAC developed by Fleischhauer et al.[43] Mean CD spectra were obtained by averaging over the CD spectra of either the entire 100-ns MD-trajectory or of all members of a particular cluster.

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