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ARTICLE

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Circular dichroism spectra of β-peptides: sensitivity to molecular structure and effects of motional averaging

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Abstract Circular dichroism spectra of two β-peptides, i.e. peptides composed of β-amino acids, calculated using ensembles of configurations obtained by molecular dynamics simulation are presented. The calculations were based on 200 ns simulations of a β-heptapeptide in methanol at 298 K and 340 K and a 50 ns simulation of a β-hexapeptide in methanol at 340 K. In the simulations the peptides sampled both folded (helical) and unfolded structures. Trajectory structures with common backbone conformations were identified and grouped into clusters. The CD spectra were calculated for individual structures, based on peptide-group dipole transition moments obtained from semi-empirical molecular orbital theory and using the so-called matrix method. The single-structure spectra were then averaged over entire trajectories and over clusters of structures. Although certain features of the experimental CD spectra of the β-peptides are reproduced by the trajectory-average spectra, there exist clear differences between the two sets of spectra in both wavelength and peak intensities. The analysis of individual contributions to the average spectra shows that, in general, the interpretation of a CD signal in terms of a single structure is not possible. Moreover, there is a large variation in the CD spectra calculated for a set of individual structures that belong to the same cluster, even when a structurally tight clustering criterion is used. This indicates that the CD spectra of these peptides are very sensitive to small local structural differences.

Keywords Circular dichroism · Cluster analysis · Computer simulation · Molecular dynamics · Peptide folding

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Introduction

β-Peptides, i.e. peptides composed of β-amino acids, have recently been a subject of intense investigation using both experimental (Borman 1997; Gademann et al. 1999a, 1999b, 2000; Gellman 1998; Hamuro et al. 1999; Hintermann and Seebach 1997; Iverson 1997; Porter et al. 2000a, 2000b; Seebach et al. 1998b; Werder et al. 1999) and theoretical (Applequist et al. 1998; Applequist and Bode 2000; Bode and Applequist 1997; Chandrasekhar et al. 2001; Daura et al. 1997, 1998, 1999b, 2001; Möhle et al. 1999; Wu and Wang 1998, 1999) techniques. Structural studies performed on a variety of β-peptides have shown that, like peptides of α-amino acids, β-peptides may adopt stable secondary structures in solution (Abele et al. 1998; Appella et al. 1996, 1997, 1999; Chung et al. 1998; Gung et al. 1999; Krauthäuser et al. 1997; Seebach et al. 1996a, 1996b, 1997, 1998a, 1998c, 1999). The β-heptapeptide and β-hexapeptide shown in Fig. 1 were the first cases reported of β-peptides consisting of non-cyclic β-amino acids that fold into a left-handed 3_{14} helix (Seebach et al. 1996a) and right-handed 12/10 helix (Rueping et al. 2002; Seebach et al. 1997, 1998a), respectively. The structures of these peptides in methanol solution were determined by NMR spectroscopy and correlated with certain features of the corresponding CD spectra (Seebach et al. 2000a). To investigate the relation between the spectroscopic observables and the underlying ensemble of molecular

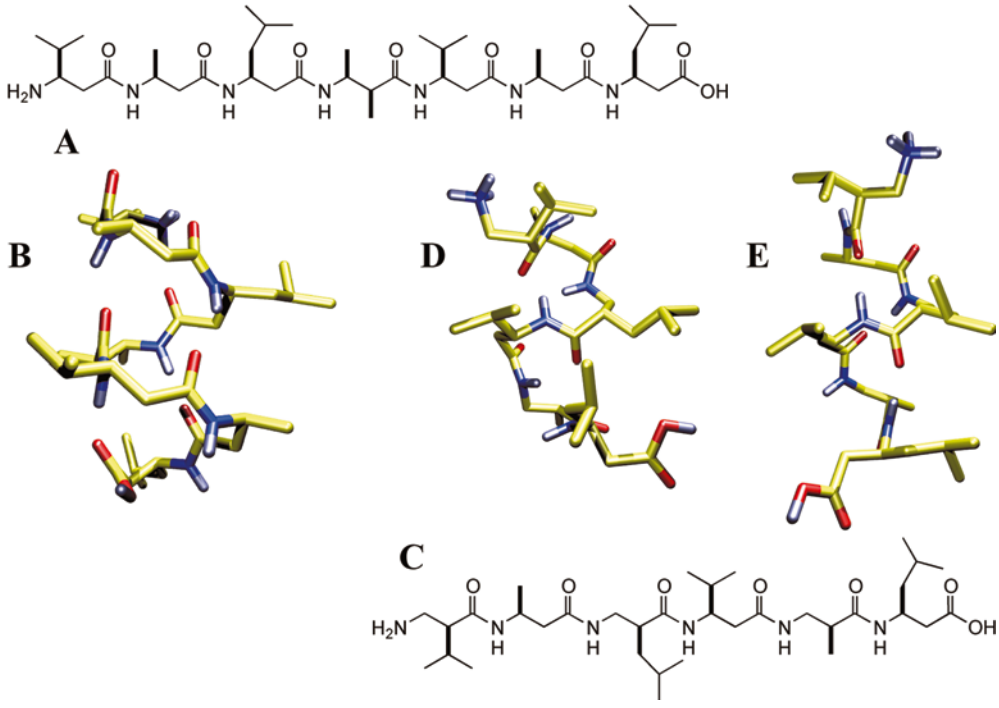


Fig. 1 **A** Structural formula of the β^3-heptapeptide. **B** Model structure derived from NMR data for the β-heptapeptide in methanol at 298 K (Seebach et al. 1996a). **C** Structural formula of the β^2,β^3-hexapeptide. **D** Model structure derived from NMR data for the β-hexapeptide in methanol at 298 K (Seebach et al. 1998a). **E** Model structure derived from NMR data of the β-hexapeptide in pyridine at 298 K (Seebach et al. 1997)

configurations, various molecular dynamics (MD) simulations of up to 200 ns length were performed for the β-heptapeptide (Daura et al. 1997, 1998) and the β-hexapeptide (Daura et al. 1999b) in methanol for temperatures ranging from 298 K to 360 K. These simulations showed a thermodynamic equilibrium between the folded conformation, i.e. a 3_{14} helix for the heptamer and a 12/10 helix for the hexamer, and a variety of unfolded conformations, as a function of temperature. The generated ensembles of configurations reproduce accurately the data derived from NMR measurements, namely interproton distances and J coupling constants. It was also shown that NMR-derived data are less sensitive to the underlying ensemble than commonly thought; in fact, the same data are compatible with various different distributions of configurations (Burgi et al. 2001; Daura et al. 1999a). Here, some of the previously generated trajectories (Daura et al. 1998, 1999b) are used to compute CD spectra for the two peptides.

The CD spectrum corresponding to a particular configuration of a molecule can be calculated with quantum mechanical methods. Small molecules can either be treated at the semi-empirical level (e.g. CNDO/S) (Del Bene and Jaffe 1968a, 1968b) or at the ab initio level (e.g. CIS, configuration interaction with single excitations) (Foresman et al. 1992). More approximate models are required for larger systems containing several chromophore groups. A popular approach is the dipole interaction model introduced by DeVoe and Applequist (Applequist 1973; DeVoe 1964, 1965). For polypeptides, proteins, and polynucleotides the perturbation theory of Tinoco (Tinoco 1960, 1962; Woody and Tinoco 1967) and the so-called matrix method of Bayley et al. (1969) are chiefly used. These methods are of approximate nature due to the independent chromophore assumption involved in generating the excited-state wave functions. Nevertheless, the experimental CD spectra of a small number of proteins have been reproduced successfully (Bode and Applequist 1998; Woody 1995, 1996). Most calculations reported in the literature are based on single structures, for example X-ray structures of proteins. In a few cases, however, calculations have been performed for structures from a simulation trajectory (Bringmann et al. 2001; Fleischhauer et al. 1994). These studies have shown that small structural changes in relative position and orientation of the chromophores may have sizeable effects on the calculated CD spectrum, particularly in the near-UV range of protein spectra. If the backbone is very flexible (as for peptides), a significant variation may also be observed for the far-UV CD.

The CD calculations presented here are based on 200 ns MD simulations of the β-heptapeptide H-β^3-HVal-β^3-HAla-β^3-HLeu-(S,S)-β^3-HAla(αMe)-β^3-HVal-β^3-HAla-β^3-HLeu-OH (Fig. 1A) in methanol at 298 K and 340 K, and a 50 ns simulation of the β-hexapeptide H-β^2-HVal-β^3-HAla-β^2-HLeu-β^3-HVal-β^2-HAla-β^3-HLeu-OH (Fig. 1C) in methanol at 340 K. In the simulations the peptides sampled both folded (helical) and unfolded structures. A computer program was used to identify trajectory structures with a common backbone conformation and group them into clusters of similar structure. The CD spectra were calculated for individual structures, based on peptide-group dipole transition moments obtained from semi-empirical molecular orbital theory and using the matrix method. The single-structure spectra were then averaged over entire trajectories and over clusters of structures. The resulting curves are compared to experimental spectra.

Our analysis focuses on the sensitivity of calculated single-structure CD spectra to small geometrical differences and of calculated average CD spectra to variations in the underlying ensemble of conformations. There is no agreement between calculated and experimental spectra for the β-heptapeptide and only fair agreement for the β-hexapeptide. Several shortcomings of the theoretical model may account for the differences between the calculated and observed spectra; these are discussed in some detail.

Methods

Molecular dynamics simulation

The MD simulations were performed using the GROMOS96 package (Scott et al. 1999; van Gunsteren et al. 1996) and the GROMOS96 43A1 force field (van Gunsteren et al. 1996). The dynamics of the two peptides in methanol were studied at 298 K (β-heptapeptide, 200 ns) and 340 K (β-heptapeptide, 200 ns; β-hexapeptide, 50 ns), at 1 atm pressure under periodic boundary conditions. The temperature and pressure were maintained by weak coupling to an external bath (Berendsen et al. 1984) with relaxation times of 0.1 ps and 0.5 ps, respectively. To generate the initial configuration of one of the systems, a 3_{14}-helical structure of the β-heptapeptide was placed in a periodic rectangular box with 962 methanol molecules. For the other system, a fully extended conformation of the β-hexapeptide and 1435 methanol molecules were placed in a periodic truncated-octahedron box. The shortest distance between the peptide and the box wall was initially 1.4 nm in both systems. The SHAKE algorithm (Ryckaert et al. 1977) was used to constrain all bond lengths to minimum-energy values. A twin-range cut-off of 0.8 nm/1.4 nm was used for all non-bonded interactions. Further details on the simulation setup for the two systems have been given elsewhere (Daura et al. 1997, 1998, 1999b).

Cluster analysis

A conformational clustering analysis was performed on sets of peptide structures taken at 10-ps intervals from the trajectories, using the backbone atom-positional root-mean-square difference (RMSD) as similarity criterion. For the β-heptapeptide the cluster analysis was performed over four sets of data, corresponding to the initial 10, 20, 50, and 200 ns of simulation. For the β-hexapeptide, only one set, covering the entire simulation (50 ns), was used. Two different groups of backbone atoms were considered for the translational and rotational superposition of structures and the calculation of RMSD values. In the first case ($N–C^{\beta}–C^{\alpha}–C$), all but the first and last residues of the peptide chains were used. In the second case (O–C–N–H), all the peptide groups were considered. The RMSD similarity cut-off or maximum cluster radius used in the clustering analysis was 0.1 nm for the β-heptapeptide and 0.08 nm for the β-hexapeptide. The value was chosen proportional to the number of residues and approximately equal to the maximum atom-positional RMSD between the various NMR model structures derived for each peptide. The clustering algorithm has been described elsewhere (Daura et al. 1999c). Important features of this algorithm are that it favours the most populated cluster and that it ensures a minimum atom-positional RMSD between centres of clusters equal to the RMSD similarity cut-off. It also results in many clusters having only one member. In general, these single-member clusters are not isolated in the RMSD space but lie at the border of other clusters. They result from the hard constraint that no member of a cluster may be further away from the central member than a specified distance.

Calculation of CD spectra

Circular dichroism (Woody 1996) is the difference in absorption of left and right circularly polarized light of given wavelength λ, as expressed by the extinction coefficients:

$$\Delta\varepsilon(\lambda) = \varepsilon_{\text{left}}(\lambda) - \varepsilon_{\text{right}}(\lambda) \tag{1}$$

The rotational strength of an electronic transition $0 \rightarrow a$ is related to the integrated intensity of a single band in the absorption spectrum:

$$R_{0a} \propto \int \frac{\Delta\varepsilon(\lambda)}{\lambda} d\lambda \tag{2}$$

and is given as the product of the electric and magnetic dipole transition moments:

$$R_{0a} = \text{Im}(\langle\psi_0|2\boldsymbol{\mu}_e|\psi_a\rangle\langle\psi_a|2\boldsymbol{\mu}_m|\psi_0\rangle) \tag{3}$$

For real-valued wavefunctions this will be a real number, as the electric dipole integral is real and the magnetic dipole integral is purely imaginary. While the calculation of rotational strengths is in principle straightforward, it appears impractical for all but the smallest molecules, given that the treatment of excited states ψ_a will require high-level wavefunctions including electron correlation. Even the use of semi-empirical treatments specifically designed for the calculation of electronic spectra appears out of reach for molecules as large as proteins or for sizeable collections of different geometries of medium-sized molecules, as in this study.

The independent chromophore approximation (Woody 1996) assumes non-overlapping charge distributions of all chromophores in the molecule, and treats the interaction between them either by perturbation theory (Tinoco 1962) or by exciton theory (Bayley et al. 1969). The latter, known as the matrix method, is well established for the calculation of CD spectra for protein structures. This approach uses a basis set of polymer (molecule) wavefunctions which are written as products of n monomer (chromophore) wavefunctions. Only single excitations within a monomer i are retained ($0 \rightarrow a$), neglecting double and higher excitations as well as charge-transfer excitations:

$$\Phi_{ia} = \phi_{10}\phi_{20}\ldots\phi_{ia}\ldots\phi_{n0} \tag{4}$$

The excited state wavefunction is linear-combined from this basis set, and the corresponding expansion coefficients are determined in a variational sense from the eigenvalue problem $\hat{H}\psi = E\psi$:

$$\psi_k = \sum_{i=1}^{n}\sum_{a=1}^{m_i} C_{k,ia}\Phi_{ia} \tag{5}$$

$$\hat{H} = \sum_{i=1}^{n}\hat{H}_i + \sum_{i\leq j}^{n}\hat{V}_{ij} \tag{6}$$

In matrix notation, the hamiltonian contains as diagonal matrix elements the energies e_{ia} of all m_i excitations in monomer i and as off-diagonal matrix elements the Coulomb interactions $V_{i0a,j0b}$ between monomer transition charge densities:

$$H_{kk} = \langle\psi_k|\hat{H}|\psi_k\rangle = \sum_{i=1}^{n}\sum_{a=1}^{m_i} C_{k,ia}^2\langle\phi_{ia}|\hat{H}_i|\phi_{ia}\rangle \tag{7}$$

$$H_{kl} = \langle\psi_k|\hat{H}|\psi_l\rangle = \sum_{i=1}^{n}\sum_{a=1}^{m_i}\sum_{j=1}^{n}\sum_{b=1}^{m_j} C_{k,ia}C_{l,jb}\langle\phi_{i0}\phi_{ia}|\hat{V}_{ij}|\phi_{j0}\phi_{jb}\rangle \tag{8}$$

$$h_{ia,ia} \equiv e_{ia} = \langle\phi_{ia}|\hat{H}_i|\phi_{ia}\rangle \tag{9}$$

$$h_{ia,jb} \equiv V_{i0a,j0b} = \langle\phi_{i0}\phi_{ia}|\hat{V}_{ij}|\phi_{j0}\phi_{jb}\rangle \tag{10}$$

The matrix $\mathbf{h}$ with elements $h_{ia,jb}$ is diagonalized, yielding the expansion coefficients $C_{k,ia}$ as eigenvectors and the composite excitation energies as eigenvalues. The rotational strengths are then readily evaluated from Eq. (3) using the expansion coefficients and

the transition dipole moments $\langle\phi_0|2\boldsymbol{\mu}_e|\phi_a\rangle$ and $\langle\phi_0|2\boldsymbol{\mu}_m|\phi_a\rangle$ of the monomers. For approximate wavefunctions, the rotational strength is origin-dependent in its original form (Eq. 3), but it may be rewritten in the commonly used dipole velocity form which removes this origin dependence (Woody 1996).

In practice, monomer excitation energies are parameters obtained either from calculation or experiment and should account for solvent effects, while monomer transition charge densities are represented as sets of monopoles, fitted to densities obtained from semi-empirical (Kurapkat et al. 1997; Woody and Sreerama 1999) or ab initio (Besley and Hirst 1999; Hirst 1998b) calculations. Finally, electric and magnetic transition dipole moments of the monomers are typically obtained from calculation, but orientations are sometimes taken from experiment (Woody and Sreerama 1999). Once all these parameters are derived, the polymer calculation involves no further quantum mechanical treatment; it is in fact limited to simple matrix algebra, rendering the calculation of CD spectra a computationally trivial task even for very large systems.

In the present study, we use the dipole velocity form of the rotational strength and a parameter set previously applied to CD calculations for bovine pancreatic ribonuclease (Kurapkat et al. 1997). The parameter set has been obtained from CNDO/S calculations on acetamide, augmented by experimental excitation energies that are poorly reproduced at the semi-empirical level of theory. Each of the amide fragments (peptide groups) of the hexa- and heptapeptide is represented by the planar amide fragment of acetamide, which is translated and rotated in a linear least-squares sense so as to best match the positions of the C, O, and N atoms on the peptide. The terminal amino and carboxylate groups are not included in the calculation since their motional flexibility will diminish their influence on the overall CD spectrum. The aliphatic side chains will not contribute to the CD signal either, which leaves the main chain amide fragments as the only chromophores explicitly treated in the calculation. For each of these monomers, three excitations are considered: $n\pi^*$ (from the oxygen lone pair, 220 nm), NV1, and NV2 (both $\pi\pi^*$, 190 and 140 nm). All calculations have been performed using the program MATMAC (Fleischhauer et al. 1991), which computes the molar ellipticity θ, a quantity proportional to $\Delta\epsilon$ (Woody 1996), for each transition and adds Gaussians to yield the final spectrum in the range 150–250 nm. Further technical details of the calculations may be found elsewhere (Kurapkat et al. 1997).

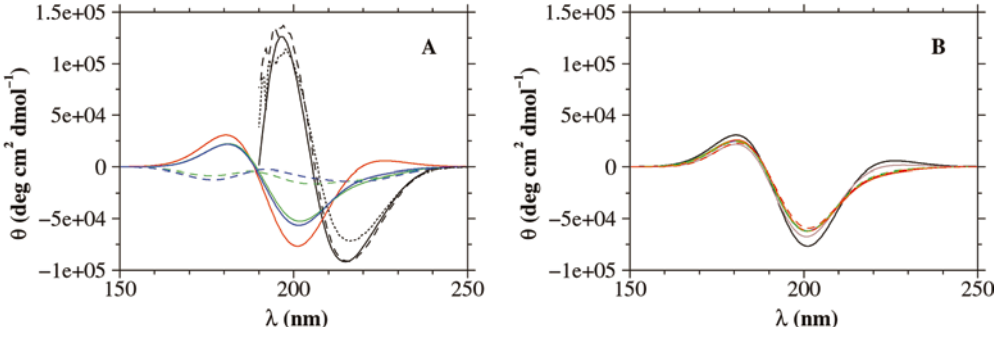
CD calculations have been performed for single structures obtained from NMR models, energy minimization, or MD simulation, clusters of structures obtained from MD simulation as described above, or sets of structures covering particular segments of the MD simulations. The experimental CD spectra were taken from the group of Seebach (Gademann et al. 1999c; Seebach et al. 1996a, 1997).

Fig. 2 **A** Experimental and calculated CD spectra of the β-heptapeptide in methanol. *Black solid*: experimental CD spectrum at 298 K (Seebach et al. 1996a); *black dashed and black dotted*: experimental CD spectra at 293 K and 333 K, respectively, from an independent measurement (Gademann et al. 1999c); *red*: CD spectrum calculated for the 3_{14}-helical NMR model structure (see Fig. 1B); *green solid*: average CD spectrum from the first 50 ns of the MD simulation at 298 K; *blue solid*: average CD spectrum from the entire 200 ns MD simulation at 298 K; *green dashed*: average CD spectrum from the first 50 ns of the MD simulation at 340 K; *blue dashed*: average CD spectrum from the entire 200 ns MD simulation at 340 K. **B** CD spectra of the 3_{14}-helical conformation of the β-heptapeptide calculated from the MD simulation in methanol at 340 K. *Black*: CD spectrum calculated for the NMR model structure (see Fig. 1B); *brown*: average CD spectrum of cluster number 1 (clustering based on the six O–C–N–H peptide groups, 134 structures or cluster members) from the initial 10 ns (1000 structures) of the MD simulation; *blue*: average CD spectrum of cluster number 1 (clustering based on the six O–C–N–H peptide groups, 983 members) from the initial 20 ns (2000 structures) of the MD simulation; *green solid*: average CD spectrum of cluster number 1 (clustering based on the six O–C–N–H peptide groups, 2084 members) from the initial 50 ns (5000 structures) of the MD simulation; *red solid*: average CD spectrum of cluster number 1 (clustering based on the six O–C–N–H peptide groups, 5630 members) from the entire 200 ns (20,000 structures) MD simulation; *green dashed*: average CD spectrum of cluster number 1 (clustering based on the N–C^{β}–C^{α}–C backbone atoms of residues 2 to 6, 2503 members) from the initial 50 ns of the MD simulation; *red dashed*: average CD spectrum of cluster number 1 (clustering based on the N–C^{β}–C^{α}–C backbone atoms of residues 2 to 6, 7175 members) from the entire 200 ns MD simulation. In all cases the clustering has been performed using an atom-positional RMSD similarity cut-off of 0.1 nm

Results

β^3-Heptapeptide

The experimental CD spectra collected for the β-heptapeptide at three different temperatures, 293, 298, and 333 K, the calculated CD spectrum for the 3_{14}-helical NMR model structure, and the average CD spectra, calculated over 50 ns and 200 ns time spans from the molecular dynamics trajectories at 298 K and 340 K, are shown in Fig. 2A. Clearly, the average calculated spectra do not reproduce the experimental curves. It is arguable whether the mismatch is indicating a shift towards shorter wavelengths of the calculated spectrum with respect to the experimental one or a signal inversion. The intensity of the peaks is smaller in the calculated average spectra and diminishes, almost vanishes, as a larger number of conformations enters the ensemble, that is, as the temperature is increased from 298 K to 340 K. The calculated CD spectrum, on the other hand, is similar whether averaged over 50 ns or 200 ns at any of the two temperatures. The calculated spectrum of the NMR model structure is very close to both calculated



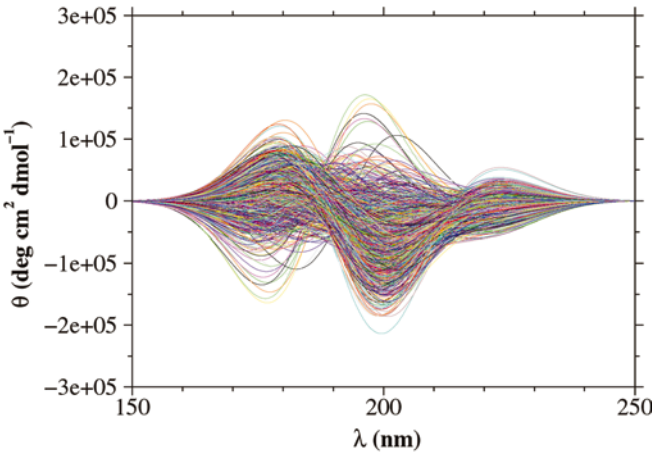


Fig. 3 Calculated CD spectra for the 2084 member structures of cluster number 1 (3_{14} helix) from the first 50 ns of the MD simulation of the β-heptapeptide in methanol at 340 K. The clustering is based on the six peptide groups (N, H, C, O atoms) of the molecule, using an atom-positional RMSD similarity cut-off of 0.1 nm

average spectra at 298 K. This is not surprising, since the 3_{14}-helical conformation is representative for 95% (200 ns) to 97% (first 50 ns) of the ensemble at this temperature. At 340 K, the population of the helix ranges from 35% (200 ns) to 50% (first 50 ns) and the inclusion of new conformations results in both a shift of the peaks and a reduction of the signal intensity. The reduction of peak intensities at higher temperature is also apparent in the experimental spectra, although to a much smaller extent.

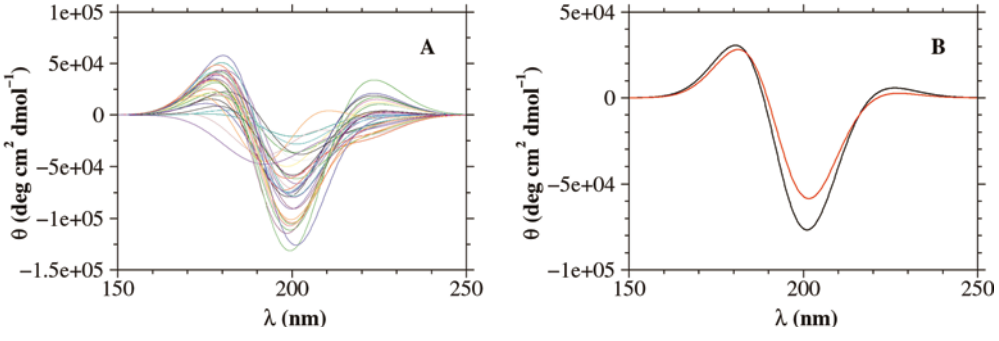
Figure 2B shows the dependence of the average calculated CD spectrum for a particular cluster (conformation) on the simulation time range over which the average has been calculated and on the backbone atoms used for the clustering. As expected, the average spectrum for cluster 1 (the most populated cluster, featuring the 3_{14} helix in all cases) is basically the same whether the clustering has been performed on the first 10, 20, 50, or 200 ns of the simulation at 340 K. It is also relatively insensitive to the selection of backbone atoms, N–C^{β}–C^{α}–C or O–C–N–H, for the clustering of structures. Similar observations have been made for other highly populated clusters at any of the two temperatures (results not shown). A plausible explanation for this would be that all the structures belonging to one cluster correspond to the same conformation and share the same basic CD spectrum. As a consequence, the average spectrum would be largely independent of the number of members of the cluster, which varies with simulation length. This explanation is simple but inappropriate, as Fig. 3 demonstrates that 2084 highly different single-structure CD spectra contribute to the average spectrum shown in Fig. 2B (green solid line). Note that all 2084 structures correspond to the same global fold and still give rise to very different calculated spectra. This surprising observation has been made for other highly populated clusters as well (results not shown).

In order to reduce the structural differences within a cluster even further, we have repeated the clustering procedure for structures obtained from the first 50 ns of the simulation at 340 K, now using an RMSD similarity cut-off of 0.03 nm for the O, C, N, and H atoms of the six peptide groups. The new cluster 1 contains 34 members, with CD spectra shown in Fig. 4A and backbone structures shown in Fig. 5A. The results point to an extreme sensitivity of the CD-spectrum calculations to the exact relative positions and orientations of the chromophores. Another example of this is given in Figs. 4B and 5B, which show the spectrum and backbone structure of the NMR model before and after energy minimization, respectively.

β^2,β^3-Hexapeptide

For this β-hexapeptide, two different NMR model structures were derived, based on data obtained for methanol (Fig. 1D) and pyridine (Fig. 1E) as solvent. Later, a molecular dynamics simulation study (Daura et al. 1999b) suggested that the model shown in Fig. 1E (12/10 helix) is also representative for the most populated conformation in methanol, while the model shown in Fig. 1D satisfies NOE constraints of both a 12/10

Fig. 4A, B Calculated CD spectra of structurally very similar individual structures of the β-heptapeptide. **A** Calculated CD spectra of the 34 members of cluster number 1 (3_{14} helix) from the first 50 ns of the simulation in methanol at 340 K (clustering based on the six O–C–N–H peptide groups using an atom-positional RMSD similarity cut-off of 0.03 nm). **B** *Black*: CD spectrum calculated for the NMR model structure (see Fig. 1B); *red*: CD spectrum calculated for the energy minimized NMR model structure (initial structure in the simulation at 298 K)



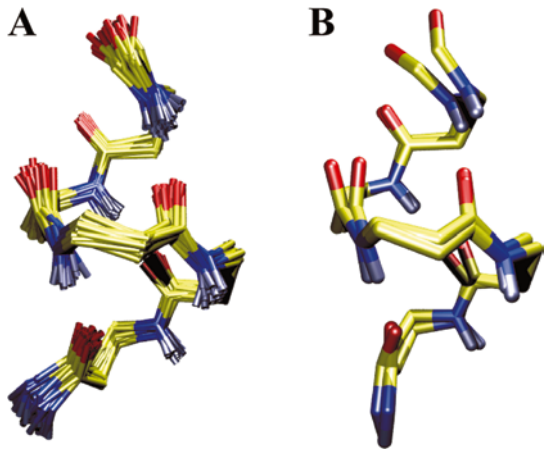


Fig. 5A, B Structures of the β-heptapeptide corresponding to the CD spectra in Fig. 4. **A** Superposition of the 34 members of cluster number 1 (3_{14} helix) from the first 50 ns of the simulation in methanol at 340 K (clustering based on the six O–C–N–H peptide groups using an atom-positional RMSD similarity cut-off of 0.03 nm); only the backbone atoms including the peptide groups (chromophores) are shown. **B** Superposition of the NMR model structure before and after steepest descent energy minimization within the GROMOS96 43A1 force field (van Gunsteren et al. 1996)

helix and a 3_{14} helix. Indeed, both folds are observed in the simulation.

The experimental CD spectrum of the β-hexapeptide at 298 K, the calculated CD spectra for the two model structures shown in Figs. 1D and 1E, and the average CD spectrum calculated over the 50 ns of the molecular dynamics trajectory at 340 K are shown in Fig. 6A. Unlike for the β-heptapeptide (see Fig. 2A), we observe a satisfactory agreement with experiment for the calculated position of the characteristic positive peak at around 200 nm. The calculated average CD spectrum for 340 K (blue line) is again characterized by low-intensity peaks, indicating sizeable contributions from non-12/10-helical conformations at elevated temperature.

Of the two calculated CD spectra corresponding to the NMR model structures of the peptide in methanol and pyridine, the one for the pyridine model (green line in Fig. 6A; see Fig. 1E) is clearly closer to the experimental spectrum for the peptide in methanol. If a single-structure CD spectrum was representative for the experimental spectrum, this result would support the previous conclusion that the 12/10 helix might be the most populated conformer in methanol and that the model shown in Fig. 1D was an artifact of the NMR structure calculation.

Figure 6B illustrates that the calculated CD spectrum of the central member structure of a cluster is not necessarily representative for the average spectrum of the cluster, and that two distinct structures may have similar spectra. For example, the central member structure of cluster 4 (green solid line), which does not conform to the 12/10-helical pattern, has a similar spectrum to that of the model 12/10 helix shown in Fig. 1E (Fig. 6A, green line). The observation that β-peptides with different folds may have very similar CD spectra has recently also been made by Seebach et al. (2000b), using NMR and CD spectroscopy, and Glättli et al. (2002), using the computational methods described here. On the other hand, the CD spectrum of the central member structure of cluster 4 is very different from the average spectrum of the same cluster (Fig. 6B, green solid line and green dashed line, respectively).

The variance in the calculated spectra for the member structures of a cluster is also remarkable in the case of the β-hexapeptide. The distribution of the CD spectra for cluster 1 (12/10 helix) is very similar (data not shown) to the one for the heptapeptide shown in Fig. 3. Averaging over the distribution results in the CD spectrum shown in Fig. 6B (red dashed line). Nevertheless, while the CD spectrum for a single structure within a cluster appears to be rather unpredictable (i.e. very sensitive to the exact coordinates), the average spectrum of a cluster seems to be characteristic for the conformation. Thus, the average spectrum corresponding to the 3_{14} helix has the same features whether calculated for the β-hexapeptide (Fig. 6B, blue dashed line) or the β-heptapeptide (Fig. 2A).

The influence of the fitting procedure on the calculated spectrum

For the calculation of CD spectra we use the independent chromophore approximation with theoretical data obtained for idealized geometries of peptide-bond units. This procedure requires the superposition of the idealized (planar) peptide-bond units onto the chromophore groups of the molecule. In particular, the MATMAC program performs a least-squares translational and rotational fit of the C, O, and N atoms of the parametrized amide fragment onto the corresponding atoms in the peptide.

However, the assumption of amide planarity introduces some arbitrariness into the fitting procedure. In practice, deviations from planarity can be quite substantial, and the details of the fitting procedure matter. We have tested the implications for the calculated CD spectra by comparing the results obtained with three alternative fitting procedures: (residue-number pointer in parenthesis): (1) projection of O(i) (preserving the C–O bond distance) onto the plane defined by $C^{\alpha}(i)$, C(i), and N($i+1$), followed by the fitting based on C(i), O(i), and N($i+1$) performed by MATMAC; (2) projection of O(i) (preserving the C–O bond distance) onto the plane defined by C(i), N($i+1$), and $C^{\beta}(i+1)$, followed by the fitting based on C(i), O(i), and N($i+1$) performed by MATMAC; (3) fitting of the idealized geometry of acetamide onto the $C^{\alpha}(i)$, C(i), O(i), N($i+1$), and H($i+1$) atoms of the peptide.

The single-structure and average spectra corresponding to cluster 1 of the β-heptapeptide at 340 K (same cluster as in Fig. 4A) have been calculated using

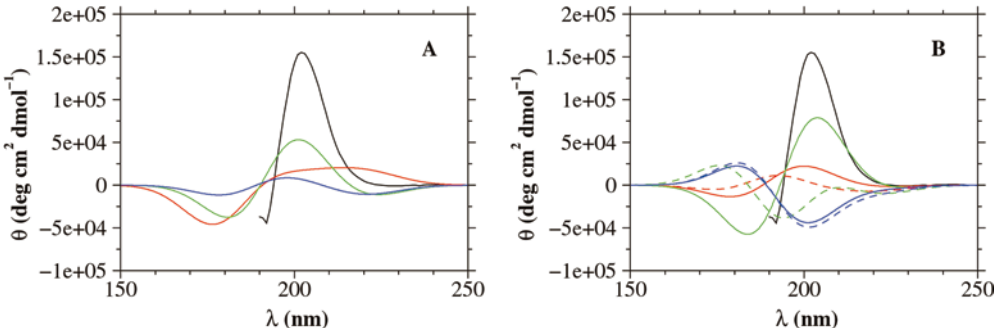


Fig. 6A, B Experimental and calculated CD spectra of the β-hexapeptide in methanol. **A** *Black*: experimental CD spectrum at 298 K (Seebach et al. 1998a); *red*: CD spectrum calculated for the model structure derived from NMR data in methanol (see Fig. 1D); *green*: CD spectrum calculated for the model structure derived from NMR data in pyridine (see Fig. 1E); *blue*: average CD spectrum from the entire 50 ns MD simulation at 340 K. **B** *Black*: experimental CD spectrum at 298 K; *red solid*: CD spectrum calculated for the central member structure of cluster number 1 (12/10 helix, from time point = 6.96 ns); *red dashed*: average CD spectrum of cluster number 1 (633 members); *green solid*: CD spectrum calculated for the central member structure of cluster number 4 (low free energy unfolded, i.e. non-12/10-helical structure, from time point = 1.78 ns); *green dashed*: average CD spectrum of cluster number 4 (181 members); *blue solid*: CD spectrum calculated for the central member structure of cluster number 14 (3_{14} helix, from time point = 25.01 ns); *blue dashed*: average CD spectrum of cluster number 14 (56 members). The clustering is based on the five peptide groups (N, H, C, O atoms) of the molecule, using an atom-positional RMSD similarity cut-off of 0.08 nm

each of the four different fitting schemes. The results are shown in Fig. 7A (average) and Figs. 7B–D (single structure). Clearly, the spectra of some of the individual structures vary with the fitting procedure. Compared to the differences between various structures within the same cluster, however, these effects appear rather small. The averaged spectra (Fig. 7A) are thus rather insensitive to the details of the fitting procedure.

Discussion

CD spectra of two β-peptides at two temperatures have been calculated based on molecular configurations obtained from molecular dynamics simulation. The CD spectra have been averaged over entire MD trajectories and over sets of similar structures (clusters in atom-positional RMSD space) for comparison to experimentally measured spectra. The trajectory-average spectrum for the β-hexapeptide at 340 K shows the characteristic positive peak at around 200 nm. In contrast, the trajectory-average spectra for the β-heptapeptide at 298 K and 340 K do not reproduce any of the features of the experimental spectra. In both cases, peak intensities are low compared to experimental values.

Surprisingly, the calculated single-structure CD spectra are extremely sensitive to small structural variations. Even the details of the geometric fitting procedure applied to superimpose the parametrized acetamide model with the peptide bonds have a notable effect on the spectrum. Consequently, the calculated CD spectrum of the central member structure of a cluster is not necessarily representative for the average spectrum of the cluster.

The unsatisfactory agreement between calculated and experimental spectra for the β-heptapeptide warrants some discussion of possible sources of error. The method of calculation is clearly very approximate, and, although successfully applied to protein crystal structures in the past, may fail in other cases. The principle shortcomings include the following. First, transition charge densities derived from semi-empirical CNDO/S calculations may be inaccurate, especially if the corresponding excitation energy is only poorly reproduced at the same level of theory (Hirst 1998a). This particularly applies to the $n\pi^*$ transition (CNDO/S: $\lambda = 340$ nm; experimentally: 220 nm). Second, the direction of the transition dipole moment vector appears to have a large influence on the resulting spectrum (Woody and Sreerama 1999), and differences between CNDO/S (for acetamide) and experiment (for *N*-acetylglycine) are sizeable (Clark 1995; Kurapkat et al. 1997). Third, the independent chromophore approximation does not allow for charge transfer between monomers, which may be inappropriate especially for NV1 excitations of poly-α-peptides (Goldmann et al. 2001). This problem should be less significant for β-peptides, which are characterized by a larger distance between chromophores. Fourth, the monomer parameters were derived for application to α-peptides in aqueous solution. A more specific parametrization for β-peptides and methanol as solvent is desirable. Fifth, the monomer parameters were derived for planar peptide units. However, thermal motion generates OCNH angles which deviate from planarity. While the amount of distortion may be exaggerated by the GROMOS force field in some cases, the uncertainty remains whether transition charge densities and dipole moment orientations, in particular, are transferable to non-planar peptide geometries. Note that non-planar amides will give rise to an intrinsic CD contribution reflecting their local chirality, while this contribution vanishes for planar amide units with orthogonal electric and magnetic transition dipole moment orientations (see Eq. 3). The question of parameter transferability should be distinguished from the

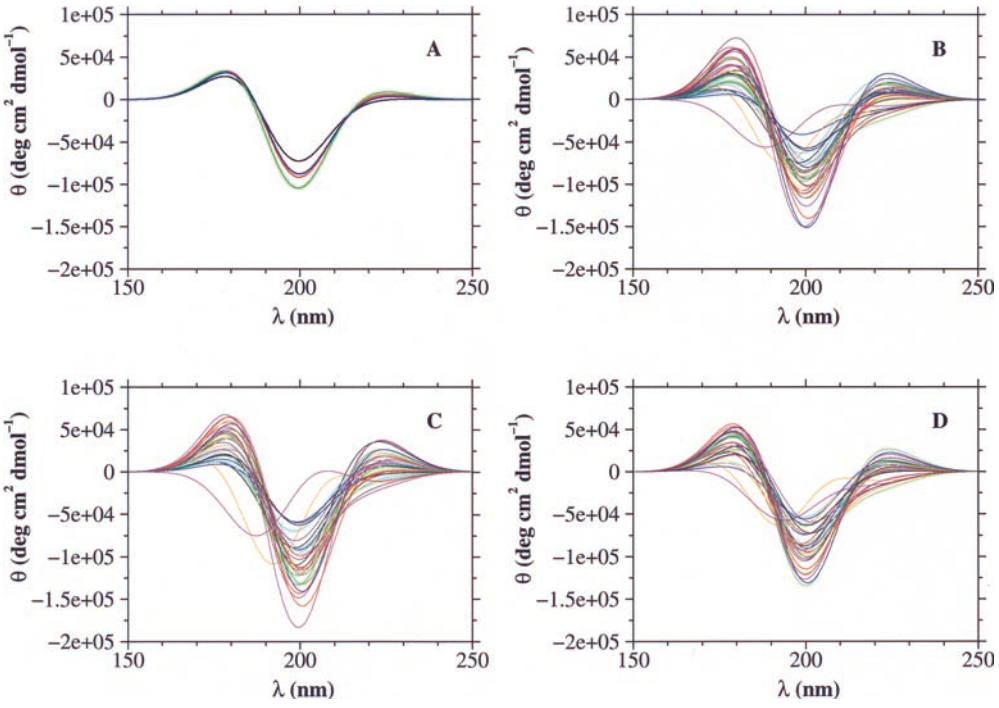


Fig. 7A–D Calculated CD spectra corresponding to cluster 1 (3_{14} helix) of the β-heptapeptide from the first 50 ns of MD simulation in methanol at 340 K. Clustering based on the six peptide groups (N, H, C, O atoms) of the molecule, using an atom-positional RMSD similarity cut-off of 0.03 nm. **A** Average spectra using different strategies to fit the parametrized chromophore onto the peptide groups (residue-number pointer in parentheses). *Black*: fitting based on C(i), O(i), and N($i+1$) (default in MATMAC); *red*: projection of O(i) onto the plane defined by $C^{\alpha}(i)$, C(i), and N($i+1$), followed by the fitting based on C(i), O(i), and N($i+1$) performed by MATMAC; *green*: projection of O(i) onto the plane defined by C(i), N($i+1$), and $C^{\beta}(i+1)$, followed by the fitting based on C(i), O(i), and N($i+1$) performed by MATMAC; *blue*: fitting based on $C^{\alpha}(i)$, C(i), O(i), N($i+1$), and H($i+1$). **B** Single-structure spectra of the 34 member structures of cluster 1 giving rise to the red-line average in **A**. **C** Single-structure spectra of the 34 member structures of cluster 1 giving rise to the green-line average in **A**. **D** Single-structure spectra of the 34 member structures of cluster 1 giving rise to the blue-line average in **A**

additional problem related to the influence of the geometric fitting procedure on the computed CD spectra. Finally, the MD trajectory may show an inaccurate distribution of the various folded and unfolded structures. While we cannot completely rule out this possibility, it seems unlikely that this is the major reason for the observed discrepancies between experimental and calculated CD spectra, as the NMR data (NOE intensities and J coupling constants) are reasonably reproduced by the simulations (Daura et al. 1999a; Peter et al. 2001).

Some of these shortcomings of the theoretical model have already been investigated in the literature: CD calculations with model parameters derived from ab initio wavefunctions at the MRCI (Multi-Reference Configuration Interaction) (Hirst 1998b) and CASSCF (Complete Active Space Self-Consistent Field) (Besley and Hirst 1999) levels of theory have been found to be more reliable for proteins. Woody and Sreerama (1999) have shown that experimental CD spectra are reproduced more accurately if the semi-empirical monomer model incorporates the experimental transition dipole orientation as well as refined monopole positions. Goldmann and collaborators (2001) have recently demonstrated improved accuracy in calculating the NV1 transition of polyalanine when using a method based on time-dependent Hartree–Fock theory which goes beyond the independent chromophore model.

The issues discussed above certainly warrant further investigation and provide several possibilities for improvement of the theoretical model. However, in this study we decided to use an established approximate procedure, since our major focus was to analyse the sensitivity of circular dichroism to molecular structure and to investigate the impact of thermal motion. Even though the semi-empirical CD model may not be accurate enough for quantitative predictions, we still feel that it has served the purpose of our investigation.

The results presented here suggest that CD spectra can be very sensitive to small structural variations. This implies that the spectrum of a flexible molecule can only be interpreted in terms of an average over conformations. Thus, the assignment of a particular conformer to a given spectrum may not generally be possible. This conclusion is not entirely unexpected: a particular conformer may be scarcely populated but have strongly coupled or so-called inherently chiral chromophores, causing a big Cotton effect and thus a large contribution to the CD spectrum. At the same time, this conformer may not be detectable by other experimental methods, especially by those covering longer time scales like NMR spectroscopy, and it may not be of any importance for the properties of the

corresponding compound (see the books by Berova et al. 2000 on CD spectroscopy, and Eliel and Wilen 1994 on stereochemistry).

References

Abele S, Guichard G, Seebach D (1998) (*S*)-β^3-Homolysine- and (*S*)-β^3-homoserine-containing β-peptides: CD spectra in aqueous solution. Helv Chim Acta 81:2141–2156

Appella DH, Christianson LA, Karle IL, Powell DR, Gellman SH (1996) β-Peptide foldamers: robust helix formation in a new family of β-amino acid oligomers. J Am Chem Soc 118:13071–13072

Appella DH, Christianson LA, Klein DA, Powell DR, Huang X, Barchi JJ, Gellman SH (1997) Residue-based control of helix shape in β-peptide oligomers. Nature 387:381–384

Appella DH, Barchi JJ Jr, Durell SR, Gellman SH (1999) Formation of short, stable helices in aqueous solution by β-amino acid hexamers. J Am Chem Soc 121:2309–2310

Applequist J (1973) Polarizability theory of optical rotation. J Chem Phys 58:4251–4259

Applequist J, Bode KA (2000) Fully extended poly(β-amino acid) chains: translational helices with unusual theoretical π-π^* absorption and circular dichroic spectra. J Phys Chem A 104:7129–7132

Applequist J, Bode KA, Appella DH, Christianson LA, Gellman SH (1998) Theoretical and experimental circular dichroic spectra of the novel helical foldamer poly[(1*R*,2*R*)-*trans*-2-aminocyclopentanecarboxylic acid]. J Am Chem Soc 120:4891–4892

Bayley PM, Nielsen EB, Schellman JA (1969) The rotatory properties of molecules containing 2 peptide groups: theory. J Phys Chem 73:228–243

Berendsen HJC, Postma JPM, van Gunsteren WF, DiNola A, Haak JR (1984) Molecular dynamics with coupling to an external bath. J Chem Phys 81:3684–3690

Berova N, Nakanishi K, Woody RW (2000) Circular dichroism: principles and application. Wiley-VCH, New York

Besley NA, Hirst JD (1999) Theoretical studies toward quantitative protein circular dichroism calculations. J Am Chem Soc 121:9636–9644

Bode KA, Applequist J (1997) Poly(β-amino acid) helices. Theoretical π-π^* absorption and circular dichroic spectra. Macromolecules 30:2144–2150

Bode KA, Applequist J (1998) Globular protein ultraviolet circular dichroic spectra. Calculation from crystal structures via the dipole interaction model. J Am Chem Soc 120:10938–10946

Borman S (1997) β-Peptides: nature improved? Chem Eng News 75:32–35

Bringmann G, Mühlbacher J, Repges C, Fleischhauer J (2001) MD-based CD calculations for the assignment of the absolute axial configuration of the naphthylisoquinoline alkaloid dioncophylline A. J Comput Chem 22:1273–1278

Burgi R, Pitera J, van Gunsteren WF (2001) Assessing the effect of conformational averaging on the measured values of observables. J Biomol NMR 19:305–320

Chandrasekhar J, Saunders M, Jorgensen WL (2001) Efficient exploration of conformational space using the stochastic search method: application to β-peptide oligomers. J Comput Chem 22:1646–1654

Chung YJ, Christianson LA, Stanger HE, Powell DR, Gellman SH (1998) A β-peptide reverse turn that promotes hairpin formation. J Am Chem Soc 120:10555–10556

Clark LB (1995) Polarization assignments in the vacuum UV spectra of the primary amide, carboxyl, and peptide groups. J Am Chem Soc 117:7974–7986

Daura X, van Gunsteren WF, Rigo D, Jaun B, Seebach D (1997) Studying the stability of a helical β-heptapeptide by molecular dynamics simulations. Chem Eur J 3:1410–1417

Daura X, Jaun B, Seebach D, van Gunsteren WF, Mark AE (1998) Reversible peptide folding in solution by molecular dynamics simulation. J Mol Biol 280:925–932

Daura X, Antes I, van Gunsteren WF, Thiel W, Mark AE (1999a) The effect of motional averaging on the calculation of NMR-derived structural properties. Proteins Struct Funct Genet 36:542–555

Daura X, Gademann K, Jaun B, Seebach D, van Gunsteren WF, Mark AE (1999b) Peptide folding: when simulation meets experiment. Angew Chem Int Ed 38:236–240

Daura X, van Gunsteren WF, Mark AE (1999c) Folding-unfolding thermodynamics of a β-heptapeptide from equilibrium simulations. Proteins Struct Funct Genet 34:269–280

Daura X, Gademann K, Schäfer H, Jaun B, Seebach D, van Gunsteren WF (2001) The β-peptide hairpin in solution: conformational study of a β-hexapeptide in methanol by NMR spectroscopy and MD simulation. J Am Chem Soc 123:2393–2404

Del Bene J, Jaffe HH (1968a) Use of CNDO method in spectroscopy. 2. 5-membered rings. J Chem Phys 48:4050

Del Bene J, Jaffe HH (1968b) Use of CNDO method in spectroscopy. 1. Benzene, pyridine and diazines. J Chem Phys 48:1807

DeVoe H (1964) Optical properties of molecular aggregates. 1. Classical model of electronic absorption + refraction. J Chem Phys 41:393–400

DeVoe H (1965) Optical properties of molecular aggregates. 2. Classical theory of refraction absorption and optical activity of solutions and crystals. J Chem Phys 43:3199–3208

Eliel EL, Wilen SH (1994) Stereochemistry of organic compounds. Wiley, New York

Fleischhauer J, Kramer B, Zobel E, Koslowski A (1991) MATMAC: matrix and Tinoco method Aachen. Aachen, Germany

Fleischhauer J, Grotzinger J, Kramer B, Krüger P, Wollmer A, Woody RW, Zobel E (1994) Calculation of the circular-dichroism spectrum of cyclo(L-Tyr- L-Tyr) based on a molecular-dynamics simulation. Biophys Chem 49:141–152

Foresman JB, Head-Gordon M, Pople JA, Frisch MJ (1992) Toward a systematic molecular-orbital theory for excited-states. J Phys Chem 96:135–149

Gademann K, Ernst M, Hoyer D, Seebach D (1999a) Synthesis and biological evaluation of a cyclo-β-tetrapeptide as a somatostatin analogue. Angew Chem Int Ed 38:1223–1226

Gademann K, Hintermann T, Schreiber JV (1999b) β-Peptides: twisting and turning. Curr Med Chem 6:905–925

Gademann K, Jaun B, Seebach D, Perozzo R, Scapozza L, Folkers G (1999c) Temperature-dependent NMR and CD spectra of β-peptides: on the thermal stability of β-peptide helices – is the folding process of β-peptides non-cooperative? Helv Chim Acta 82:1–11

Gademann K, Ernst M, Seebach D, Hoyer D (2000) The cyclo-β-tetrapeptide (β-HPhe-β-HThr-β-HLys-β-HTrp): synthesis, NMR structure in methanol solution, and affinity for human somatostatin receptors. Helv Chim Acta 83:16–33

Gellman SH (1998) Foldamers: a manifesto. Acc Chem Res 31:173–180

Glättli A, Daura X, Seebach D, Van Gunsteren WF (2002) Can one derive the conformational preference of a β-peptide from its CD spectrum? J Am Chem Soc 124:12972–12978

Goldmann E, Sanford AA, Mukamel S (2001) Electronic excitations of polyalanine; test of the independent chromophore approximation. Phys Chem Chem Phys 3:2893–2903

Gung BW, Zou D, Stalcup AM, Cottrell CE (1999) Characterization of a water-soluble, helical β-peptide. J Org Chem 64:2176–2177

Hamuro Y, Schneider JP, DeGrado WF (1999) De novo design of antibacterial β-peptides. J Am Chem Soc 121:12200–12201

Hintermann T, Seebach D (1997) The biological stability of β-peptides: no interactions between α- and β-peptidic structures? Chimia 51:244–247

Hirst JD (1998a) Improving protein circular dichroism calculations through better *ab initio* models of the amide chromophore. Enantiomer 3:215–220

Hirst JD (1998b) Improving protein circular dichroism calculations in the far-ultraviolet through reparametrizing the amide chromophore. J Chem Phys 109:782–788

Iverson BL (1997) Betas are brought into the fold. Nature 385:113–115

Krauthäuser S, Christianson LA, Powell DR, Gellman SH (1997) Antiparallel sheet formation in β-peptide foldamers: effects of β-amino acid substitution on conformational preference. J Am Chem Soc 119:11719–11720

Kurapkat G, Krüger P, Wollmer A, Fleischhauer J, Kramer B, Zobel E, Koslowski A, Botterweck H, Woody RW (1997) Calculations of the CD spectrum of bovine pancreatic ribonuclease. Biopolymers 41:267–287

Möhle K, Günther R, Thormann M, Sewald N, Hofmann HJ (1999) Basic conformers in β-peptides. Biopolymers 50:167–184

Peter C, Daura X, van Gunsteren WF (2001) Calculation of NMR-relaxation parameters for flexible molecules from molecular dynamics simulations. J Biomol NMR 20:297–310

Porter EA, Wang X, Lee H-S, Weisblum B, Gellman SH (2000a) Non-haemolytic β-amino-acid oligomers. Nature 405:298

Porter EA, Wang X, Lee H-S, Weisblum B, Gellman SH (2000b) Antibiotics – non-haemolytic β-amino-acid oligomers. Nature 404:565–565

Rueping M, Schreiber JV, Lelais G, Jaun B, Seebach D (2002) Mixed β^2/β^3-hexapeptides and β^2/β^3-nonapeptides folding to (*P*)-helices with alternating twelve- and ten-membered hydrogen-bonded rings. Helv Chim Acta 85:2577–2593

Ryckaert JP, Ciccotti G, Berendsen HJC (1977) Numerical integration of the Cartesian equations of motion of a system with constraints: molecular dynamics of *n*-alkanes. J Comput Phys 23:327–341

Scott WRP, Hünenberger PH, Tironi IG, Mark AE, Billeter SR, Fennen J, Torda AE, Huber T, Krüger P, van Gunsteren WF (1999) The GROMOS biomolecular simulation program package. J Phys Chem A 103:3596–3607

Seebach D, Ciceri PE, Overhand M, Jaun B, Rigo D, Oberer L, Hommel U, Amstutz R, Widmer H (1996a) Probing the helical secondary structure of short-chain β-peptides. Helv Chim Acta 79:2043–2066

Seebach D, Overhand M, Kühnle FNM, Martinoni B, Oberer L, Hommel U, Widmer H (1996b) β-Peptides: synthesis by Arndt-Eistert homologation with concomitant peptide coupling. Structure determination by NMR and CD spectroscopy and by X-ray crystallography. Helical secondary structure of a β-hexpeptide in solution and its stability towards pepsin. Helv Chim Acta 79:913–941

Seebach D, Gademann K, Schreiber JV, Matthews JL, Hintermann T, Jaun B, Oberer L, Hommel U, Widmer H (1997) 'Mixed' β-peptides: a unique helical secondary structure in solution. Helv Chim Acta 80:2033–2038

Seebach D, Abele S, Gademann K, Guichard G, Hintermann T, Jaun B, Matthews JL, Schreiber JV (1998a) β^2- and β^3-peptides with proteinaceous side chains: synthesis and solution structures of constitutional isomers, a novel helical secondary structure and the influence of solvation and hydrophobic interactions on folding. Helv Chim Acta 81:932–982

Seebach D, Abele S, Schreiber JV, Martinoni B, Nussbaum AK, Schild H, Schulz H, Hennecke H, Woessner R, Bitsch F (1998b) Biological and pharmacokinetic studies with β-peptides. Chimia 52:734–739

Seebach D, Abele S, Sifferlen T, Hänggi M, Gruner S, Seiler P (1998c) Preparation and structure of β-peptides consisting of geminally disubstituted $\beta^{2,2}$- and $\beta^{3,3}$-amino acids: a turn motif for β-peptides. Helv Chim Acta 81:2218–2243

Seebach D, Abele S, Gademann K, Jaun B (1999) Pleated sheets and turns of β-peptides with proteinogenic side chains. Angew Chem Int Ed 38:1595–1597

Seebach D, Schreiber JV, Abele S, Daura X, van Gunsteren WF (2000a) Structure and conformation of β-oligopeptide derivatives with simple proteinogenic side chains: circular dichroism and molecular dynamics investigations. Helv Chim Acta 83:34–57

Seebach D, Sifferlen T, Mathieu PA, Häne AM, Krell CM, Bierbaum DJ, Abele S (2000b) CD spectra in methanol of β-oligopeptides consisting of β-amino acids with functionalized side chains, with alternating configuration, and with geminal backbone substituents – fingerprints of new secondary structures? Helv Chim Acta 83:2849–2864

Tinoco I (1960) Optical and other electronic properties of polymers. J Chem Phys 33:1332–1338

Tinoco I (1962) Theoretical aspects of optical activity. 2. Polymers. Adv Chem Phys 4:113–160

van Gunsteren WF, Billeter SR, Eising AA, Hünenberger PH, Krüger P, Mark AE, Scott WRP, Tironi IG (1996) Biomolecular simulation: the GROMOS96 manual and user guide. Hochschulverlag, ETH Zürich/BIOMOS, Zürich/Groningen

Werder M, Hauser H, Abele S, Seebach D (1999) β-Peptides as inhibitors of small-intestinal cholesterol and fat absorption. Helv Chim Acta 82:1774–1783

Woody RW (1995) Absorption and circular dichroism. In: Sauer K (ed) Biochemical spectroscopy, vol 246. Academic Press, San Diego, pp 34–71

Woody RW (1996) Theory of circular dichroism of proteins. In: Fasman DG (ed) Circular dichroism and the conformational analysis of biomolecules. Plenum Press, New York, pp 25–67

Woody RW, Sreerama N (1999) Comment on "improving protein circular dichroism calculations in the far-ultraviolet through reparametrizing the amide chromophore", J Chem Phys 109:782 (1998). J Chem Phys 111:2844–2845

Woody RW, Tinoco I (1967) Optical rotation of oriented helices. 3. Calculation of rotatory dispersion and circular dichroism of α- and 3_{10}-helix. J Chem Phys 46:4927–

Wu YD, Wang DP (1998) Theoretical studies of β-peptide models. J Am Chem Soc 120:13485–13493

Wu YD, Wang DP (1999) Theoretical study on side-chain control of the 14-helix and the 10/12-helix of β-peptides. J Am Chem Soc 121:9352–9362