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Recent developments in the electronic spectroscopy of amides and α-helical polypeptides

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Abstract

Recent experimental and theoretical advances in understanding the electronic excited states of simple amides are reviewed. Polarized reflection spectroscopy of single crystals of *N*-acetylglycine shows that the direction of the first $\pi\pi^*$ (NV_1) transition dipole moment of a secondary amide differs by approximately 15° from that of a primary amide. Ab initio calculations on simple amides support this conclusion. Ab initio studies of di- and tri-amides demonstrate that several inter-amide charge-transfer (CT) transitions occur in the 150–175-nm region, between the NV_1 and NV_2 transitions. When the correct dipole transition moment direction for peptides is used in calculations of the circular dichroism of the α-helix, the results are much improved over those from earlier calculations that used the direction for primary amides. Studies that consider the mixing of the NV_1 transition with CT transitions are reviewed. These indicate that such mixing is likely to have a significant effect on the absorption and CD spectra of the α-helix and other types of peptide conformation. Nevertheless, the independent systems model gives a reasonable first approximation to the absorption and CD spectra of the α-helix.

Keywords: NV_1 dipole transition moment; Charge-transfer transition; Amide electronic structure; α-Helix; Circular dichroism; Absorption; Ab initio calculations; Matrix method; Exciton splitting

1. Introduction

The foundation of our current understanding of the circular dichroism of polypeptides and proteins was laid in 1956 by Moffitt's [1] brilliant application of exciton theory to the electronic spectrum of the α-helix.[2] Moffitt's work was extended by a number of groups in the 1960s and early 1970s to

[2] Moffitt used cyclic boundary conditions, appropriate only for molecules with at least one dimension very large compared to the wavelength of light, with the Rosenfeld [2] formulation of the rotational strength, which assumes dimensions small compared to the wavelength. This led him to miss an important term, called the helix term, which was subsequently corrected in collaboration with Fitts and Kirkwood [3], and it was concluded that cyclic boundary conditions cannot be used in helical systems. Several later studies [4–6] showed that cyclic boundary conditions can be used if the selection rules for light propagating along the helix axis are appropriately modified. Nevertheless, the role of exciton coupling among the amide groups of the α-helix, identified by Moffitt, remains a key element in all subsequent treatments of the optical properties of the α-helix and other polypeptide systems.

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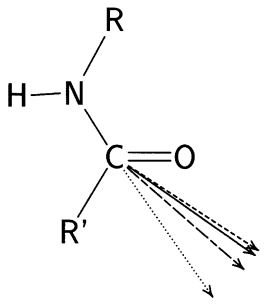

Fig. 1. NV_1 dipole transition moment directions in primary and secondary amides. Clark's [44] experimental directions for propionamide (——) and *N*-acetylglycine (·····); ab initio transition moment directions [58–62] for primary (-----) and secondary (— – — –) amides.

finite α-helices, β-sheets and other conformations, and by inclusion of the amide $n\pi^*$ transition [7–23]. Among the contributors to this effort, John Schellman and his co-workers [7–9] played a major role.

The standard interpretation of the far-UV CD of polypeptides and proteins considers two amide transitions, the $n\pi^*$ transition at longer wavelengths (~215–230 nm) and the first $\pi\pi^*$ (NV_1) transition at shorter wavelengths (~185–200 nm). The $n\pi^*$ transition is electrically forbidden and magnetically allowed. It acquires rotational strength by mixing with the NV_1 transition within the same amide by static-field mixing [7] and with the NV_1 transitions on nearby amides by dynamic coupling (Schellman's μ-m mechanism [24]). The $n\pi^*$ transition is responsible for the familiar long-wavelength negative CD bands at 222 nm for the α-helix and at 216–218 nm for the β-sheet.

The NV_1 transition is electrically allowed and magnetically forbidden. As Moffitt [1] pointed out, the interaction of the electric dipole transition moments among amides in a well-defined geometry gives rise to exciton splitting, which leads to a splitting of absorption bands and to closely spaced CD bands of opposing sign. Exciton splitting in the NV_1 band accounts for the negative CD band of the α-helix near 205 nm and the positive band near 190 nm, as well as the corresponding features in absorption: a shoulder near 205 nm and the main peak near 190 nm. Exciton splitting is less obvious in β-sheets [14,23,25]. It is not observable in absorption, but is responsible for the positive CD band near 195 nm and the negative band near 175 nm.

This model correctly reproduces the major features in the 190–250-nm region of the spectrum of the α-helix, β-sheet, the 3_{10}-helix, some types of β-turns, and the poly(Pro) I helix [26,27]. It is less successful for some β-turns, the poly(Pro) II helix, and unordered polypeptides. The model was applied to protein fragments by Madison and Schellman [9], and gave generally satisfactory results for α-helical and β-sheet regions, but poor results for unordered regions.

Recently, calculations have been reported for a number of whole proteins [28–37]. The results from studies of 20 or more proteins [34–36] showed a good correlation of the calculated and observed CD at 190 and 220 nm (correlation coefficients of ~0.9 or higher), and a weaker correlation near 205 nm (~0.75). Comparison of calculated and experimental spectra for individual proteins shows that the agreement is much better for proteins with substantial α-helix contents than for β-rich proteins. Calculated spectra for α-rich proteins frequently lack a distinct 205-nm band. The calculated spectra usually show only a shoulder near 205 nm and, if there is a discrete maximum, it is blue-shifted and its magnitude is underestimated. For β-rich proteins, theory generally gives a strong positive couplet centered near 190 nm, but experimental CD spectra for these proteins show no such feature.

What is the motivation for developing accurate computational models capable of reproducing the experimental CD spectra of model polypeptides and proteins? There is of course the intellectual goal of being able to account for such a subtle phenomenon as CD in such a complex molecule as a globular protein. In addition, however, there are solid practical reasons for pursuing this goal. Substantial progress has been made in the empirical analysis of protein secondary structure from CD spectra [38–41]. However, these analyses yield only the fractions of helix, sheet, etc. and, recently the number of α-helices and β-strands [39]. One cannot extract from the CD spectrum of a protein parameters such as ^3J coupling constants and nuclear Overhauser enhancements from NMR,

which provide relatively direct information about the torsional angles about specific bonds or the distance between a specific pair of atoms, respectively. However, if methods are developed to accurately calculate the CD spectrum of a protein from a set of atomic coordinates, CD will provide a very valuable method for screening hypothetical structures based upon homology modeling, threading and ab initio predictions of protein structure. Thus, theoretical CD calculations would become a useful part of the large-scale effort to determine protein structures from sequences derivable from the genome sequences.

2. New experimental results on amide electronic structure

Schellman and Becktel [42] presented a review of the status of the theory of the optical properties of polypeptides. Reviewing the available information about the amide NV_1 dipole transition moment direction, they noted that the reported directions spanned a range of 9°. Schellman and Becktel also pointed out that the linear dichroism data of Mandel and Holzwarth [43] permit an estimate of the transition moment direction in the α-helix. This calculation yields an NV_1 dipole transition moment direction that makes an angle of 66° with respect to the helix axis. This estimate, it was noted, is subject to error because the dichroic ratio can be affected by anisotropy of intensity transfer between transitions (hypo- or hyper-chromism).

Schellman and Becktel [42] contrasted the relatively good understanding of the $n\pi^*$ and NV_1 transitions in amides to the lack of information about higher excited states. They suggested that high-level quantum chemical calculations on amide excited states should soon be feasible and argued that it was the absence of such information that was impeding further progress on understanding polypeptide optical properties, not gaps in the fundamental theory.

Since 1995, two major developments, one experimental and the other theoretical, have dramatically improved our understanding not only of the transitions to higher excited states of the amide group, but also of the NV_1 transition.

Clark [44] determined the UV absorption spectrum and transition moment directions for single crystals of two amides, propanamide and *N*-acetylglycine (AcGly), by polarized reflection spectroscopy. Several important results emerged from this work. (1) The NV_1 transition moment direction in a primary amide (propanamide) agrees well with the earlier determination for myristamide by Peterson and Simpson [45] ($-35°$ for propanamide vs. $-41°$ for myristamide, measured relative to the carbonyl bond, with the C′–N bond making a positive angle). (2) The transition moment direction for the NV_1 transition in a secondary amide is substantially different from that in a primary amide: $-55°$ for AcGly. (3) The second amide $\pi\pi^*$ (NV_2) transition in AcGly is at 139 nm and its transition moment direction is $+61°$ or $+10°$. INDO/S [46] (intermediate neglect of differential overlap/spectroscopic) calculations suggest the former value. (4) A moderately intense band (oscillator strength, $f=0.17$) at 156 nm was attributed to the NV_1 transition of the carboxyl group. (5) Two weak features were assigned to Rydberg transitions, one inferred at 192 nm by analogy to a feature observed in the propanamide spectrum and attributed to the amide group, and one tentatively assigned to the carboxyl group, at 179 nm. Clark's NV_1 transition moment directions for primary and secondary amides are shown in Fig. 1.

Pajcini et al. [47] have studied single crystals of glycylglycine (GlyGly) by polarized near-resonance Raman spectroscopy. They found the NV_1 transition moment direction of the amide group to be $-46°$, and that for the carboxylate group to be within 5° of the O–O axis of the carboxylate. They also found evidence for a charge-transfer (CT) transition (carboxylate → amide) polarized nearly along the line connecting the carbonyl carbons of the amide and carboxylate groups. This CT transition was located at 197 nm in an aqueous solution of GlyGly [48], and a non-bonding orbital on the carboxylate was assigned as the donor orbital, with the π^* orbital of the amide as the acceptor.

Transition moment directions for a diamide, *N*-acetylglycinamide ($AcGlyNH_2$), have also been measured in single crystals by polarized Raman spectroscopy [49]. $AcGlyNH_2$ contains both a secondary and a primary amide, and because of the near degeneracy of the NV_1 transitions in the

two monomer units, there is strong exciton mixing between the transitions. Several combinations of transition moment directions, based on Clark's [44] experimental data and on Woody's INDO/S [50] calculations, were used to calculate the coupling energy using the point-dipole approximation and to derive the exciton mixing coefficients from the observed band splitting (0.41 eV). Pajcini and Asher found that the best fit resulted when the primary and secondary amide transition moment directions were taken to be $-25°$ and $-40°$, respectively. These values are close to those from INDO/S calculations on propanamide ($-25°$ or $-28°$) and AcGly ($-35°$ or $-36°$), respectively [44,51], but these latter values are for the isolated molecules, not experiencing a crystal field. Furthermore, as Pajcini and Asher point out, the point-dipole approximation may be inaccurate at the short inter-amide distance of a dipeptide (3–4 Å). Taking a more empirical approach, they estimated the coupling energy to be 0.17 eV by combining the observed splitting in $AcGlyNH_2$ in water with the difference in energy of the NV_1 transitions in the monomers in water. This value of the coupling energy is 45% larger than the largest value estimated by the point-dipole approximation. According to the authors, it also exceeds the value(s) calculated using transition monopoles, although they do not describe these calculations. The authors state 'The fact that U [the coupling energy] exceeds our calculated through–space interaction energies may indicate that through–bond coupling may…also be significant (in the form of mixing with CT transitions, for example).'

3. Ab initio theoretical results for amides

The theoretical side has also seen major developments since 1996. Previously, ab initio studies of formamide were reported [52–57], but these were limited by relatively small basis sets and/or limited treatment of correlation. Although the $n\pi^*$ transition energy was reproduced with reasonable accuracy, the NV_1 transition energy was generally overestimated by at least 1 eV. Calculations for simple amides using methods capable of reproducing experimental transition energies within a few tenths of an eV have been reported by Hirst and co-workers [58,59] using a multireference configuration interaction (MRCI) method, and by Besley and Hirst [60] and Serrano-Andrés and Fülscher [61,62] using CASPT2 (complete active space SCF and multi-configurational second order perturbation theory). A brief comparison of CASPT2 and time-dependent density functional theory calculations on a dipeptide, AcGlyNHMe, has also been reported [63].

CASPT2 calculations [61,62] give transition energies for both primary and secondary amides that agree within 0.2 eV of experimental values in the gas phase. The discrepancies are larger for the MRCI results [58,59] for the gas phase (0.6–0.8 eV), but less than 0.3 eV for water and cyclohexane solutions [60]. The calculations including a reaction field due to solvent [60] also reproduce the enhanced oscillator strength observed on transfer of *N*-methylacetamide from cyclohexane to water [64]. The NV_1 transition moment directions are calculated [58–61] to be approximately $-33\pm2°$ for primary amides and $-41\pm3°$ for secondary amides (Fig. 1). Thus, the larger angle for secondary amides relative to primary amides [44] is reproduced by the ab initio calculations. However, the absolute directions are too small by approximately 5° for primary amides and 15° for secondary amides.

Clark [44] determined the transition moment directions in single crystals, whereas the ab initio calculations have been performed on isolated molecules or, in one case [60], in solvents treated as continua. The effects of local electrostatic fields and of exciton mixing in the crystal have been treated at the semi-empirical level [51]. INDO/S [46] calculations for propanamide and for AcGly give NV_1 transition moments of $-25°$ and $-36°$, respectively. When the crystal field and exciton effects are taken into account, these directions rotate to $-35°$ and $-42°$, respectively. Thus, the corrected INDO/S transition moment directions are similar to those obtained by ab initio for the isolated molecules. This led Woody et al. [51] to suggest that INDO/S corrections should be applied to ab initio values for the isolated molecule to obtain theoretical transition moment directions for the crystal.

AcGly is more complex than the simple amides formamide, acetamide, etc. because it has a carboxyl group as well as a secondary amide. Four of the five bands observed by Clark [44] in the crystal spectrum of AcGly were assigned to local excitations between valence states in the amide or carboxyl groups. A fifth band at 179 nm was weak and was assigned as a Rydberg transition of the carboxyl group. Serrano-Andrés and Fülscher's ab initio calculations [62] on AcGly predicted an $n \rightarrow$ 3s transition for the carboxyl chromophore at an energy and with an oscillator strength compatible with this assignment. However, Rydberg transitions are generally strongly affected by the environment, undergoing strong blue-shifts and broadening on going from the gas phase to condensed phases [65], and are generally not observed in crystal or solution spectra, at least not in the normally accessible region. This is illustrated by the calculations of Besley and Hirst [60] that used a continuum model for treating solvent effects. Although Hirst et al. [58] found six Rydberg transitions in formamide in the gas phase below 10.0 eV, only one was found in water or cyclohexane [60]. In the case of *N*-methylacetamide, eight Rydberg transitions were reported below 7.5 eV in the gas phase [59], but none were found below 10 eV in water or cyclohexane [60].

The calculations of Serrano-Andrés and Fülscher [62] also gave several CT transitions in the AcGly spectrum that are close in energy to Clark's [44] 179-nm band, and suggested that one of these, a $\pi_{\text{amide}} \rightarrow \pi^*_{\text{carboxyl}}$ transition at 176 nm with an oscillator strength of 0.03, could be related to the 179-nm band. This same CT transition is also predicted by INDO/S semi-empirical calculations [44,51]. The transition is calculated at 185 nm in the isolated molecule, nearly degenerate with the intense NV_1 transition and with a similar polarization. This led Clark [44] to conclude that the transition would be unobservable. However, crystal-field and exciton effects are predicted [51] to blue-shift this transition to 176 nm and to red-shift the NV_1 transition, making resolution of the CT transition possible. Therefore, the CT assignment for the 179-nm band observed in AcGly crystals is at least as plausible as the Rydberg assignment.

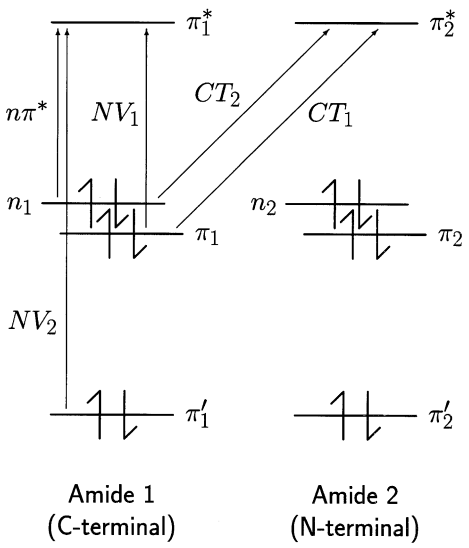

Fig. 2. Orbital diagram for a diamide showing locally excited $n\pi^*$ and $\pi\pi^*$ transitions and interamide CT transitions, CT_1 and CT_2.

Ab initio calculations have also been reported for several di- and tri-amides [62,63,66–69]. Linear combinations of the local excitations identifiable as $n\pi^*$, NV_1 and NV_2 were obtained, at wavelengths comparable to those predicted and observed in simple amides. In one study [66], various conformations of a diamide in the α_R- and β-regions of the Ramachandran map were considered, together with planar and other partially allowed conformations. The conformational variations had only minor effects on the $n\pi^*$ transition intensities. The splitting of the two NV_1 exciton components and their relative intensities varied with conformation. The transition energies for the $n\pi^*$ and NV_1 transitions showed modest variation, over a range of approximately 0.6 eV (approximately 20 nm in λ_{max}).

The most important result emerging from these ab initio studies of di- and tri-amides is the prediction of inter-amide CT transitions at energies approximately 1 eV above the NV_1 transition, in the 175–150-nm region. For a diamide, four types of inter-amide CT transitions can occur at relatively low energies—from each of the two highest filled orbitals (n and π) of either amide to the empty (π^*) orbital of the other amide (Fig. 2).

The π orbital of the amide has its largest electron density on the amide nitrogen, whereas the π^* orbital is concentrated on the carbonyl carbon. Consideration of the diamide structure shows that the smallest charge separation, and therefore the lowest energy CT state, arises from the π orbital of the C-terminal amide donating an electron to the π^* orbital of the N-terminal amide ($\pi_1 \rightarrow \pi_2^*$). The ordering of the $n_1 \rightarrow \pi_2^*$ and $n_2 \rightarrow \pi_1^*$ excited states is not obvious by this simple argument, but the $n_1 \rightarrow \pi_2^*$ generally is next in order of increasing energy [69] (the numbering of amides used in Refs. [66] and [69] is from the C-terminus, opposite to the usual convention).

Generally, only the two lowest CT transitions are discussed, labeled as CT_1 ($\pi_1 \rightarrow \pi_2^*$) and CT_2 ($n_1 \rightarrow \pi_2^*$). The conformational sensitivity of the CT_1 transition energy is comparable to that of the $n\pi^*$ and NV_1 transitions, but the CT_2 transition is substantially more sensitive: for some conformations, CT_2 is nearly degenerate with CT_1, whereas for others the gap is more than 1.5 eV [66,69]. The total intensity of the NV_1 transitions relative to that of the CT transitions is especially sensitive to conformation. For a tri-amide in the β-sheet conformation, the ratio of the total NV_1 intensity to the total CT intensity is 2.8, and for an octamide, the ratio is 3.7. By contrast, these ratios for the corresponding α-helical peptides are 2.0 and 1.6, respectively. (The octamide calculations were at the complete neglect of differential overlap (CNDO/S) [70] level, calibrated with respect to energy by comparisons with CASPT2 and experiment for di- and tri-amides.) The smaller ratio of the intensity of the NV_1 transition to those of the CT transitions in α-helical peptides as compared to β-sheet peptides is qualitatively consistent with the absorption spectra of synthetic polypeptides [71,72]. Although the calculations agree with the qualitative differences in $\varepsilon_{190}/\varepsilon_{165}$ between α-helical and β-sheet peptides, they do not reproduce the quantitative ratios. In fact, the observed ratio $\varepsilon_{190}/\varepsilon_{165}$ is less than 1 even for the β-sheet, whereas the calculated values are substantially greater than 1. It is not clear whether this is due to shortcomings of the CNDO/S method that has been, of necessity, used for the larger systems, or to the small size of the largest peptides treated relative to the experimental systems studied. It should also be noted that the 165-nm absorption appears to be superimposed on a strongly rising background [71,72] that may result from light scattering in the sample (a film) and/or from the tail of the absorption of the alkyl groups. Therefore, the experimental data may exaggerate the intensity of the 165-nm band relative to the 190-nm band.

Serrano-Andrés and Fülscher [66,69] did not comment on another feature of their calculations that reproduces a well-known qualitative difference in the absorption spectra of α-helices vis-á-vis β-sheets—the pronounced hypochromism of the NV_1 band in the α-helix [73] and the less marked hyperchromism of the β-sheet [74]. The calculated NV_1 oscillator strength per residue in the α-helical peptides is 10–20% less than that calculated [61] for the model secondary amide, *N*-methylacetamide, whereas the theoretical hyperchromism for β-strands ranges from 3 to 27%. If we discount the CNDO/S-derived values as being less accurate, the best values would be those for the trimer, −23% for the α-helix and +3% for the β-sheet. These values are less than those observed for the α-helix (−36% [73]), but are in qualitative agreement with the small hyperchromism of the β-sheet [74].

It is important to note that the inter-amide CT transitions must play an important role in the conservation of oscillator strength since there are no such transitions in the monomers. Tinoco [75] and Rhodes [76] developed the generally accepted theory of hypochromism, based upon coulombic coupling of transition moments in chromophores with non-overlapping charge distributions that do not undergo electron exchange. The substantial intensity of the inter-amide CT transitions in polypeptides requires that the Tinoco–Rhodes mechanism must be generalized to include inter-chromophoric CT.

4. Is the independent systems model valid for the α-helix?

Goldmann et al. [77] have recently reported a theoretical study of α-helical oligomers containing 5 and 15 amide groups. The object was to test the

validity of the independent systems model used in Tinoco's perturbation theory [78] and the matrix method of Bayley et al. [8]. The collective electronic oscillator (CEO) method [79,80] and the INDO/S [46] parameterization were used to calculate the electronic normal modes in the pentamer and the pentadecamer α-helix. Analysis of these normal modes showed substantial mixing of localized NV_1 excitations with CT excitations. There was no energy gap between NV_1-like normal modes and CT-like normal modes, and the normal modes gave rise to a single band in both oligomers. Some excitations were predominantly NV_1 in character, some predominantly CT, and others extensively mixed. In fact, the lowest energy normal modes for both the pentamer and the pentadecamer exhibited substantial CT character. In addition, this lowest energy mode in the pentadecamer contained in-phase NV_1 excitations that corresponded to Moffitt's [1] parallel-polarized exciton band. Although there was a suggestion of such a Moffitt-like parallel-polarized band in one of the pentamer normal modes, it was not the one at lowest energy and lacked the regularity of the pentadecamer normal mode.

Goldmann et al. [77] then calculated the off-diagonal elements for the mixing of NV_1 transitions localized on the individual amides from CEO normal modes for the pentamer, and applied these to an exciton calculation for the pentadecamer. Combining the eigenvalues and eigenvectors from this exciton treatment with the INDO/S transition energy and transition moment direction ($-36°$) for *N*-methylacetamide, Goldmann et al. calculated the absorption spectrum for the pentadecamer and compared it to that obtained by the CEO method. The two principal differences were: (1) the exciton calculations predicted the three strongest components of the NV_1 band to be blue shifted by 0.58–0.76 eV relative to their values from the CEO calculation; (2) the exciton splittings of the parallel and perpendicular bands, calculated from the energy difference between the parallel-polarized component and the strong component with the highest energy, was -0.09 eV from the exciton calculation and -0.27 eV from the CEO calculation. The CEO calculation gave much better agreement with experiment for the exciton splitting, which for long α-helices is observed to be ~ -0.56 eV [43]. With respect to the band position, the observed maximum is at ~ 188 nm, compared with ~ 205 nm from CEO and ~ 182 nm from the exciton calculations. Goldmann et al. point out that exciton calculations commonly overestimate the transition energy if they use the transition energy of the monomer in the diagonal elements, and that it is common to use a red-shifted NV_1 transition energy as the starting point in such calculations. As they suggested, mixing of the localized excited states with CT excited states accounts for at least part of the red shift in the CEO results relative to those from the exciton method. A factor that Goldmann et al. neglected, however, is the effect of the electrostatic environment of the α-helix upon the NV_1 transition energy. The dipole moment of the amide group increases in magnitude by $\sim 50\%$ upon NV_1 excitation [61], with only a small change in direction. The ground-state dipole moment of the amide is stabilized by its interactions with the groups to which it is hydrogen-bonded, so the NV_1 excited state will interact even more favorably, leading to a red-shift in the transition in the helix relative to that of the isolated molecule. Denisov [81] has estimated a red shift of 0.31 eV that, by itself, would bring the exciton results into agreement with experiment.

The major conclusion of Goldmann et al. [77] is that mixing of CT transitions with localized NV_1 excitations in the α-helix is sufficiently strong that 'the Frenkel exciton Hamiltonian cannot adequately describe the electronic excitations in the NV_1 band.' Recalling the substantial success of the exciton model in describing the absorption and CD spectra of the α-helix and other polypeptide conformations [26,27], this statement raises several questions.

Does the use of the semi-empirical INDO/S [46] parameterization bias the results? This parameterization is known to significantly underestimate the $n\pi^*$ transition energy [44,51]. Ab initio calculations on di- and tri-amides [66,68,69] place the lowest CT transitions approximately 1 eV above the NV_1 transitions. The absence of a gap between the NV_1 and CT transitions in the results of Goldmann et al. [77] suggests that the INDO/S parameters may lead to an underestimation of

the CT energies. This will exaggerate the extent of mixing of NV_1 and CT configurations. If the gap is really of the order of 1 eV, as suggested by the ab initio results, mixing will still occur but it will be far less extensive than expected from the calculations of Goldmann et al.

Are the limitations of the independent systems model solely responsible for the underestimation of the exciton splitting in the exciton calculations of Goldmann et al. [77]? First, it should be noted that even the CEO calculations give an exciton splitting that is nearly 50% too small. The exciton splitting in the α-helix is amazingly sensitive to the transition moment direction in the amide group. The exciton splitting in the infinite helix is given by [1]:

$$\Delta E = E_{\parallel} - E_{\perp} \quad (1)$$

$$E_{\parallel} = E_0 + 2\sum V_{0i} \quad (2)$$

$$E_{\perp} = E_0 + 2\sum V_{0i}\cos(2\pi i/P) \quad (3)$$

where E_0 is the energy of the unperturbed excited state, which is irrelevant for the splitting; V_{0i} is the energy of interaction of the transition density at residue 0 and that at residue i; and P is the number of amides per turn of the helix, ~3.6 for the α-helix. Table 1 shows the exciton splitting for the infinite α-helix calculated using NV_1 transition moments with θ varying from −40° to −55° at 5° intervals. The sign of the interaction energy between nearest neighbor amides along the chain is critical for determining the magnitude of the exciton splitting. For small values of θ, the nearest neighbor coupling energy is positive, opposite to that for the second and third nearest neighbors. Not only does a positive V_{0i} decrease the red-shift of the parallel exciton component, it also decreases the blue-shift of the perpendicular component because V_{01} is multiplied by $\cos(2\pi/P)$, which is negative for the α-helix. For values of θ in the neighborhood of −55° [44], the nearest neighbor interaction energy is negative and therefore reinforces the contributions from second and third nearest neighbors. The exciton splitting increases by nearly 50% as θ goes from −40° to −55°. The splitting for θ = −55° is −0.45 eV, in much better agreement with experiment (−0.56 eV) [43] than the value obtained with θ = −40° (−0.32 eV). Some of the residual discrepancy may be attributable to mixing of the NV_1 and CT transitions.

Table 1
Exciton splitting in the α-helix as a function of dipole transition moment direction

θ°[a]	α-Helix[b]
−40	−0.32
−45	−0.36
−50	−0.41
−55	−0.45
Experiment	−0.56[c]

Exciton splittings in eV.

[a] Angle of NV_1 dipole transition moment with respect to carbonyl bond direction, with the C′N bond defined as positive [44].

[b] Geometry calculated from standard amide geometry [84] with Ramachandran angles of Barlow and Thornton [83], (−62, −41), based upon an average over α-helices in proteins. The bond angle $NC_\alpha C'$ in the polypeptide chain was taken to be the tetrahedral angle. The monopole approximation was used. Monopole charges were placed above and below the plane of the amide, at distances determined by semi-empirical effective charges [22]. Charges calculated to reproduce the experimental [44] NV_1 dipole transition moment magnitude (3.0236 D) and the assumed direction.

[c] Mandel and Holzwarth [43] located the perpendicularly polarized NV_1 exciton band at 187.8 nm in poly(GluOMe) in hexafluoroisopropanol, and the parallel-polarized band at 205.4 nm. The negative sign results from the convention that the splitting is the energy of the parallel band minus that of the perpendicular band.

It is clear that the amides of a polypeptide chain are affected by through–bond interactions as well as by through–space electrostatic coupling. However, the extent to which through–bond effects modify the results of the independent systems model remains to be established. It seems likely that although mixing of the NV_1 excited configurations with CT configurations affects both the absorption and CD spectra of the α-helix, the exciton model still provides an appropriate first approximation for describing these spectra, and the effects of mixing with CT configurations can be described by perturbation theory.

5. Implications for α-helix CD

The new insights into the NV_1 transition moment directions and higher excited states in

amides have important implications for interpreting and predicting the CD spectra of peptides and polypeptides.

Use of the correct NV_1 transition moment direction for secondary amides [44] leads, as we have noted, to a much-improved estimate of the exciton splitting in the α-helix. Use of the direction for primary amides [45] in earlier calculations [9,12,82] made it difficult to reproduce the double minimum characteristic of the α-helix CD spectrum. Fig. 3 shows calculated CD spectra for the α-helix nonadecamer and decamer as the value of θ is varied from $-40°$ to $-55°$. For $\theta = -55°$, a discrete minimum is predicted at 205 nm, whereas $\theta = -40°$ yields only a negative shoulder in this region.

It is interesting to note that Clark's NV_1 transition moment direction [44], combined with the (φ, ψ) values for the average helical residue in a large number of proteins ($-62°$, $-41°$) [83] and the amide geometry of Ooi et al. [84], gives an angle of 70° with respect to the helix axis. This is in good agreement with the angle (66°) inferred by Schellman and Becktel [42] from the data of Mandel and Holzwarth [43]. By contrast, $\theta = -40°$, near the Peterson and Simpson [45] transition moment direction, gives an angle of 55° with respect to the helix axis. Thus the Clark transition moment direction is consistent with the absorption data for the α-helix without invoking significant anisotropy of hypochromism.

The more negative θ value for secondary amides also helps resolve another long-standing problem, the CD spectra of short α-helices. Earlier calculations [9,12,82] using the Peterson and Simpson [45] transition moment direction predicted that the CD spectra of short helices ($< \sim 10$ amides) would substantially differ from those for longer helices. Specifically, short helices would lack the negative 205-nm band, for example. Helices of five amides or less were expected to have dramatically different CD spectra (Fig. 4a). In the limit of the diamide in the α-helix conformation, the $\pi\pi^*$ couplet was predicted to be positive, in contrast to the negative couplet observed in long α-helices. The positive couplet resulted from the positive nearest-neighbor interaction energy. However, with Clark's transition moment direction [44], the nearest-neighbor interaction energy is negative. Therefore, with $\theta = -55°$, the long-wavelength component of the NV_1 transition is negative even in the dimer (Fig. 4b). Thus, the diamide CD spectrum has a weak negative feature in the 205-nm region, and the spectra of trimers, tetramers, etc. have negative maxima near 205 nm. This resolves the paradox of how proteins, with an average of approximately ten amides per helix [83], can give rise to CD spectra with the classic double minimum.

Until recently, it seemed inconceivable that CD calculations for helices containing three or four amides could be tested experimentally. However, an α-helix containing only four amides [85,86] has been demonstrated in a peptide based upon a Ca^{2+}-binding loop of calmodulin, in the presence of La^{3+}. The CD change induced by addition of La^{3+} to the apopeptide has the shape expected for an α-helix (Chin, Woody, Rohl and Baldwin, unpublished results), and the calculations using Clark's [44] dipole transition moment direction (Woody, unpublished results) are in good qualitative agreement with the results for short α-helices.

The first attempt [22] to include the effects of mixing of the $n\pi^*$ and NV_1 transitions with CT transitions led to the conclusion that such mixing should be negligible. However, the CT transitions considered were between hydrogen-bonded amides in the α-helix, rather than between nearest neighbors along the chain. In addition, the energies of the CT configurations were of the order of 10 eV because the stabilizing effect of the helix dipole on the charge-separated state were not considered.

We [87] have developed a new method called the Group–State Interaction (GSI) model for the calculation of CD spectra of polypeptides in the far UV that takes the through–bond effect into account and allows the consideration of CT between the chromophoric groups. Localized MOs [88] are assigned to the chromophoric groups and the alkane bridges between them. Chromophore orbitals [89] are generated by forming linear combinations of the localized MOs such that the portion of the Fock matrix corresponding to each group is diagonal. Locally excited configurations are generated by raising one electron from an occupied chromophore MO of one group into a

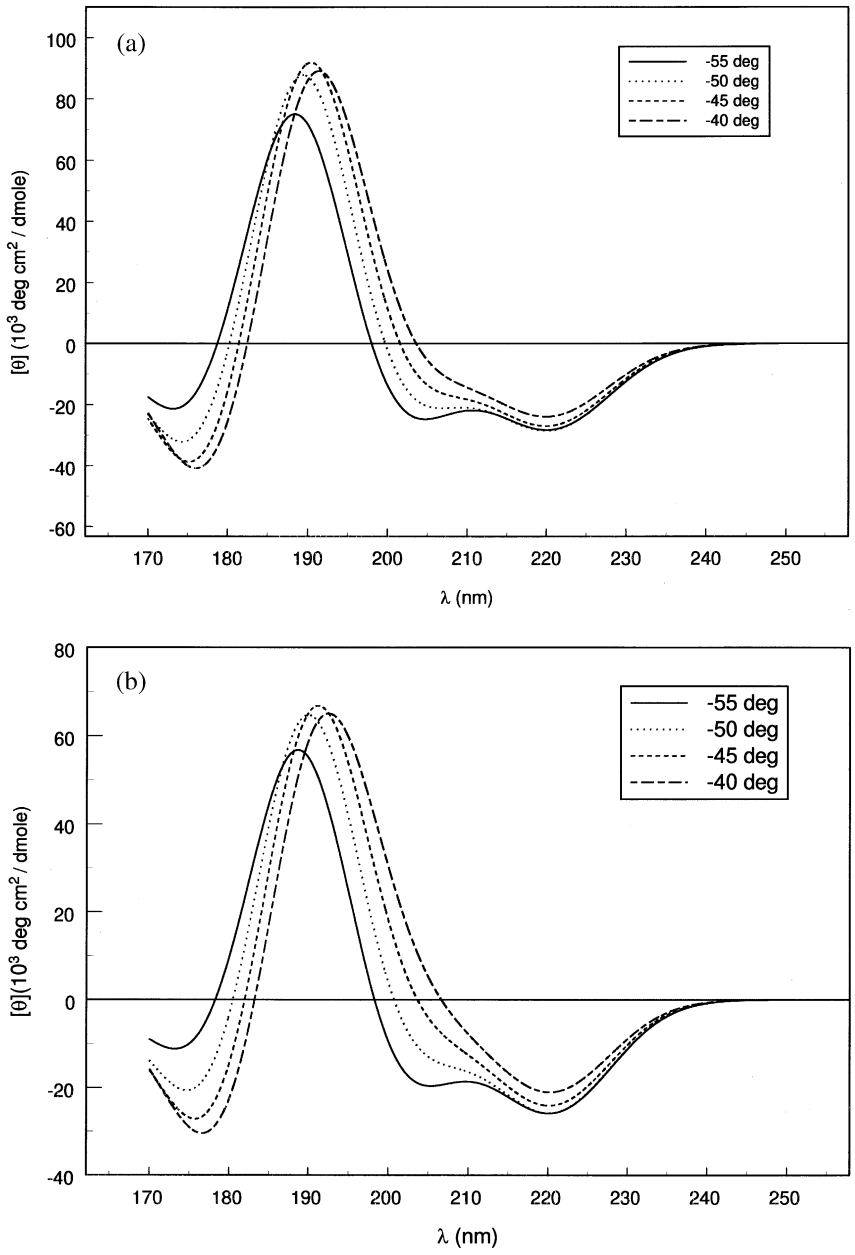

Fig. 3. CD spectra of α-helices calculated by the matrix method [8] with various NV_1 transition moment directions and other parameters as described in Ref. [34] and references cited therein: (a) nonadecamer; (b) decamer. θ = −55°, ——; θ = −50°, ·····; θ = −45°, -----; θ = −40°, — – — –.

virtual chromophore MO of the same group, and CT configurations by exciting an electron from an occupied chromophore MO of one group into a virtual chromophore MO of a neighboring group. A configuration interaction (CI) calculation is then performed using these locally excited and CT

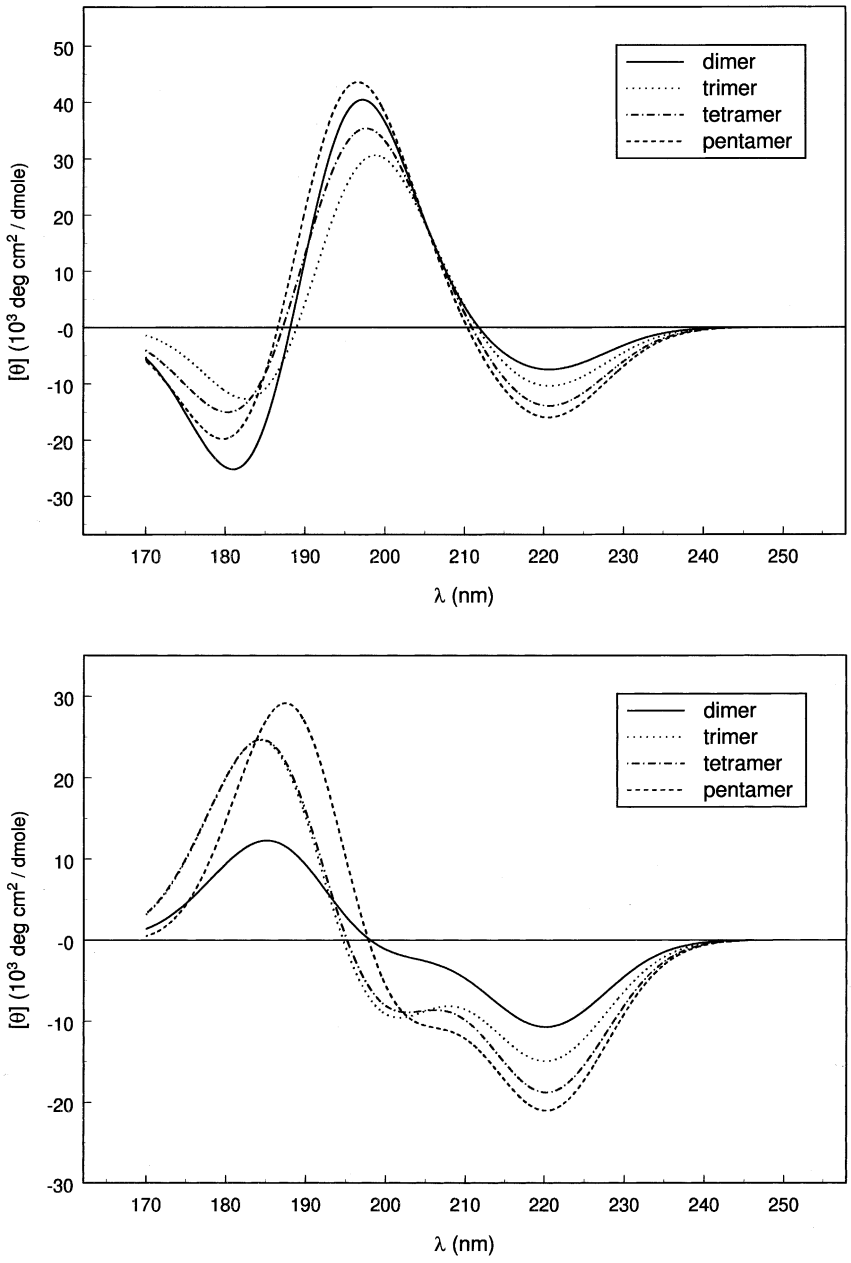

Fig. 4. CD spectra of short α-helices calculated by the matrix method [8] with two different NV_1 transition moment directions and other parameters as described in Ref. [34] and references cited therein for dimer,…, pentamer α-helices: (a) Peterson and Simpson direction [45], $\theta = -40°$; (b) Clark [44], $\theta = -55°$. Dimer, —; trimer, ••••••••; tetramer, ----; pentamer, — – — –.

configurations as the basis set after correcting the diagonal elements to experimental energies for the locally excited configurations and to ab initio values [66] for the CT configurations. From the eigenfunctions of the CI calculation, electric and magnetic dipole transition moments and rotational

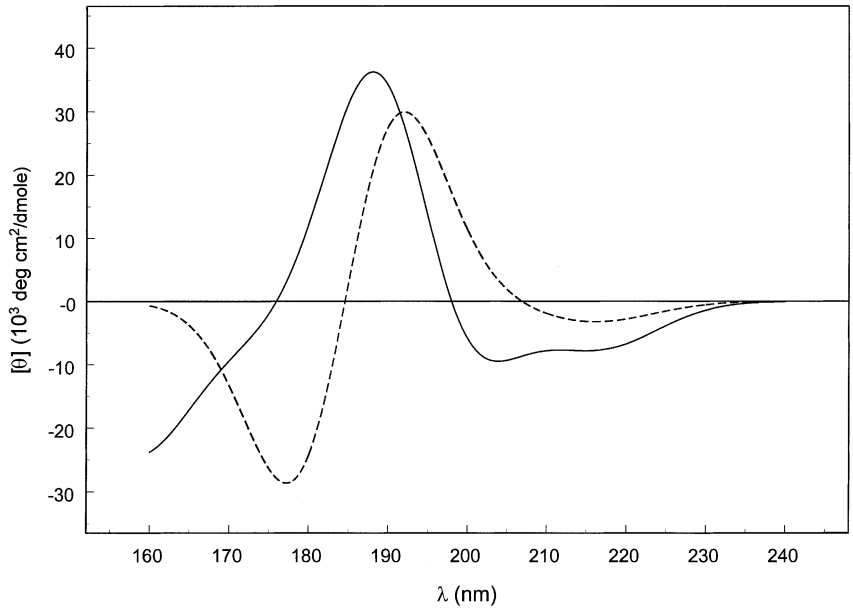

Fig. 5. CD spectrum of an α-helical nonamer, calculated by the GSI method [87]. The dashed curve shows the spectrum obtained when only locally excited basis functions were used; the solid curve shows the calculated spectrum including four CT configurations for each pair of adjacent peptide groups.

strengths are calculated, and CD curves are generated from the latter.

Calculations by the GSI method for α-helical $Ac(Ala)_{n-1}NHMe$, where $n=6$, 9, 18 and 36, showed highly encouraging results. Although the NV_1 transition moment direction for the locally excited NV_1 transition is $-23.5°$, a result of the CNDO/S [70] parameterization, the parallel exciton component of the NV_1 transition is the longest wavelength exciton component, even in short helices, and thus gives rise to a negative band near 205 nm. This contrasts with the results of calculations that omit the CT configurations and with matrix method calculations using NV_1 transition moment directions generated by CNDO/S. This is illustrated in Fig. 5, which compares the CD calculated for a nonamer, with and without incorporation of CT configurations. The calculated CD spectra also show improvement over earlier results below 190 nm, in that the predicted negative band is shifted from 175 to 180 nm from previous calculations to approximately 160 nm, where a negative band is observed for long α-helices [90]. Although the GSI method leads to marked improvement in the qualitative features of the spectra, it underpredicts the amplitudes, especially of the $n\pi^*$ transition. This is probably due to shortcomings of the CNDO/S parameterization, which we plan to replace with an ab initio basis. Calculations on diamides in the α-helix conformation show that inclusion of the CT transitions changes the sign of the NV_1 exciton couplet from positive to negative. Such a sign change is not obtained for diamides in either the P_{II} or β conformations, for which the couplets remain positive and negative, respectively. These results clearly demonstrate the importance of considering inter-peptide CT in predicting CD spectra. Since rotation of the NV_1 transition moment to more negative values and incorporation of CT transitions have the same effect, it is likely that Clark's [44] dipole transition moment direction, in combination with NV_1–CT mixing, will give a satisfactory treatment of the exciton splitting in the α-helix, in other regular secondary structures, and in proteins.

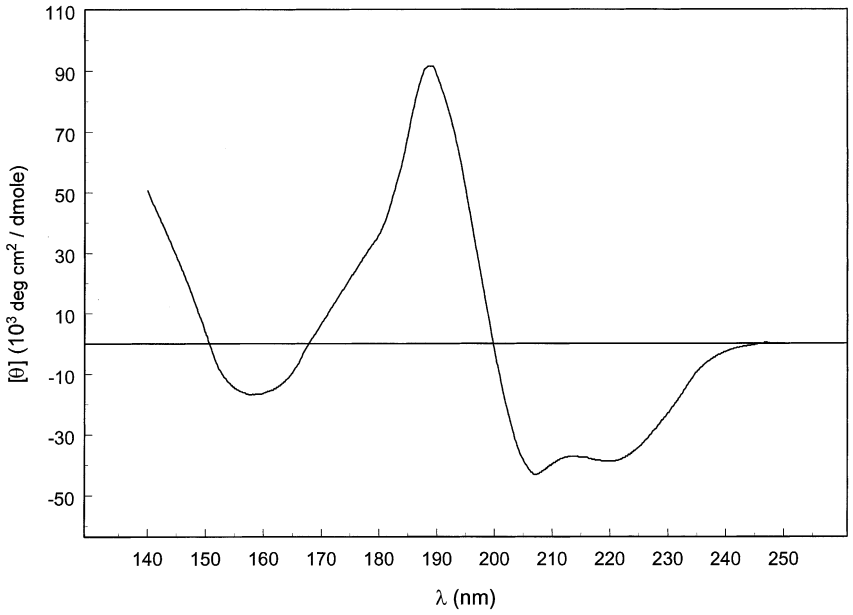

Fig. 6. Experimental CD spectrum of α-helical poly (GluOMe) in hexafluoroisopropanol, based upon the data of Johnson and Tinoco [90].

It should also be noted in Fig. 3 that Clark's [44] transition moment direction diminishes the amplitude of the negative lobe of the NV_1 exciton band calculated near 175 nm, relative to that predicted using the Peterson and Simpson [45] direction. A twofold reduction is seen in the nonadecamer calculations and a threefold reduction in the decamer results. Because a positive shoulder is seen in the 175-nm region of the experimental [90] α-helix spectrum (Fig. 6), not a negative band, the reduction in the predicted negative band represents an improvement in the calculations.

What is the origin of the 175-nm shoulder and the 160-nm negative band [90] in the α-helix CD spectrum? The positive band at the shortest observable wavelengths ($\sim$140 nm) can be assigned as the NV_2 band. Calculations (Woody, unpublished results) using Clark's [44] parameters for the NV_2 transition give a positive CD band near 140 nm for the α-helix. However, there has been no conclusive assignment of the features at 160 and 175 nm. Johnson and Tinoco [90] proposed that the 175-nm shoulder, which is not associated with any significant absorption, is a magnetically allowed $n\sigma^*$ transition. However, neither the crystal spectra [44] nor the ab initio studies of simple amides [58–61] indicate an $n\sigma^*$ transition in this region of the spectrum. The 160-nm CD band, which is associated with a strong absorption band polarized along the helix axis [91,92], has been assigned to a variety of transitions, as reviewed in Ref. [26]. Most of these assignments involve local amide transitions and are excluded by the demonstrated absence of such transitions in the spectrum of simple amides between the NV_1 and NV_2 transition.

Recent ab initio predictions of inter-peptide CT transitions in di- and tri-peptides at energies only slightly higher than the NV_1 transition [62,63,66–69] suggest an explanation for the two unassigned features in the CD spectrum of the α-helix. It is highly probable that the 175- and 160-nm transitions result from inter-amide CT transitions. A more detailed assignment requires further theoretical studies, but we suggest that the 175-nm transition is likely to be of $n \rightarrow \pi'^*$ origin (the prime indicates that the π'^* orbital is on a different amide than the n orbital) because it must have

significant magnetic dipole character, and the 160-nm transition is likely to be of $\pi \rightarrow \pi'^*$ origin because of its electric dipole character.

6. Summary

Our knowledge of the excited states of amides has improved dramatically since 1995. The determination of the NV_1 transition moment direction in secondary amides and the characterization of the amide NV_2 transition has led to marked improvements in the ability of the independent systems model to predict the CD spectra of α-helices and of whole proteins. In addition, we now know that inter-peptide CT transitions contribute significantly to the absorption spectra, and probably the CD spectra, of peptides and polypeptides. The challenge now is to develop extensions of the independent systems model, or new models, that can provide further improvements in the description of the absorption and CD spectra of oligo- and polypeptides, and of proteins.

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References

[1] W. Moffitt, Optical rotatory dispersion of helical polymers, J. Chem. Phys. 25 (1956) 458–478.
[2] L. Rosenfeld, Quantenmechanische Theorie der natürlichen optischen Aktivität von Flüssigkeiten und Gasen, Z. Physik 52 (1928) 161–174.
[3] W. Moffitt, D.D. Fitts, J.G. Kirkwood, Critique of the theory of optical activity of helical polymers, Proc. Natl. Acad. Sci. USA 43 (1957) 723–730.
[4] T. Ando, Microscopic description of optical rotation, Progr. Theoret. Phys. (Kyoto) 40 (1968) 471–485.
[5] F.M. Loxsom, Optical rotation of helical polymers—periodic boundary conditions, J. Chem. Phys. 51 (1969) 4899–4905.
[6] C.W. Deutsche, Exciton states and optical rotatory dispersion in helical polymers, J. Chem. Phys. 52 (1970) 3703–3714.
[7] J.A. Schellman, P.J. Oriel, Origin of the cotton effect of helical polypeptides, J. Chem. Phys. 37 (1962) 2114–2124.
[8] P.M. Bayley, E.B. Nielsen, J.A. Schellman, The rotatory properties of molecules containing two peptide groups: theory, J. Phys. Chem. 73 (1969) 228–243.
[9] V. Madison, J. Schellman, Optical activity of polypeptides and proteins, Biopolymers 11 (1972) 1041–1076.
[10] I. Tinoco, R.W. Woody, D.F. Bradley, Absorption and rotation of light by helical polymers: the effect of chain length, J. Chem. Phys. 30 (1963) 1317–1325.
[11] I. Tinoco, Circular dichroism and rotatory dispersion curves for helices, J. Am. Chem. Soc. 86 (1964) 297–298.
[12] R.W. Woody, I. Tinoco, Optical rotation of oriented helices. III. Calculation of the rotatory dispersion and circular dichroism of the alpha- and 3_{10}-helix, J. Chem. Phys. 46 (1967) 4927–4945.
[13] E.S. Pysh, The calculated ultraviolet optical properties of polypeptide β-configurations, Proc. Natl. Acad. Sci. USA 56 (1966) 825–832.
[14] E.S. Pysh, The calculated ultraviolet optical properties of poly-L-proline I and II, J. Mol. Biol. 23 (1967) 587–589.
[15] E.S. Pysh, Scalar spectral characteristics of one-dimensional solids, J. Chem. Phys. 52 (1970) 4723–4733.
[16] D.G. Barnes, W. Rhodes, Generalized susceptibility theory. II. Optical absorption properties of helical polypeptides, J. Chem. Phys. 48 (1968) 817–824.
[17] F.M. Loxsom, L. Tterlikkis, W. Rhodes, Non-perturbation method for optical properties of helical polymers, Biopolymers 10 (1971) 2405–2420.
[18] L. Tterlikkis, F.M. Loxsom, W. Rhodes, Theoretical optical properties of poly-L-proline, Biopolymers 12 (1973) 675–684.
[19] V. Zubkov, M.V. Vol'kenshtein, Rotatory dispersion of beta forms of polypeptides, Biopolymers 5 (1967) 465–472.
[20] V.A. Zubkov, M.V. Vol'kenshtein, Circular dichroism of the α-helical and β-forms of polypeptides, Mol. Biol. (Engl. transl. of Molekul. Biologiya) 4 (1970) 483–490.
[21] V.A. Zubkov, T.M. Birshtein, I.S. Milevskaya, M.V. Vol'kenshtein, Circular dichroism calculation for random coil polypeptide chains, Biopolymers 10 (1971) 2051–2061.
[22] R.W. Woody, Improved calculation of the $n\pi^*$ rotational strength in polypeptides, J. Chem. Phys. 49 (1968) 4797–4806.
[23] R.W. Woody, Optical properties of polypeptides in the β-conformation, Biopolymers 8 (1969) 669–683.

[24] J.A. Schellman, Symmetry rules for optical rotation, Accts. Chem. Res. 1 (1968) 144–151.
[25] K. Rosenheck, B. Sommer, Theory of the far-ultraviolet spectrum of polypeptides in the β-conformation, J. Chem. Phys. 46 (1967) 532–536.
[26] R.W. Woody, Optical rotatory properties of biopolymers, J. Polym. Sci. D Macromol. Rev. 12 (1977) 181–321.
[27] R.W. Woody, Theory of circular dichroism of proteins, in: G.D. Fasman (Ed.), Circular Dichroism and the Conformational Analysis of Biomolecules, Plenum Press, New York, 1996, pp. 25–67.
[28] M.C. Manning, R.W. Woody, Theoretical study of the contribution of aromatic side chains to the circular dichroism of basic bovine pancreatic trypsin inhibitor, Biochemistry 28 (1989) 8609–8613.
[29] I.B. Grishina, R.W. Woody, Contributions of tryptophan side chains to the circular dichroism of globular proteins: exciton couplets and coupled oscillators, Faraday Discuss 99 (1994) 245–262.
[30] G. Kurapkat, P. Krüger, A. Wollmer, et al., Calculations of the CD spectrum of bovine pancreatic ribonuclease, Biopolymers 41 (1997) 267–287.
[31] J.D. Hirst, Improving protein circular dichroism calculations in the far-ultraviolet through reparametrizing the amide chromophore, J. Chem. Phys. 109 (1998) 782–788.
[32] K.A. Bode, J. Applequist, Globular protein ultraviolet circular dichroic spectra. Calculation from crystal structures via the dipole interaction model, J. Am. Chem. Soc. 120 (1998) 10938–10946.
[33] T.M. Thompson, B.L. Mark, C.W. Gray, et al., Circular dichroism and electron microscopy of a core Y61F mutant of the F1 gene 5 single-stranded DNA binding protein and theoretical analysis of four Tyr→Phe substitutions, Biochemistry 37 (1998) 7463–7477.
[34] R.W. Woody, N. Sreerama, Comment on 'Improving protein circular dichroism calculations in the far-ultraviolet through reparametrizing the amide chromophore', J. Chem. Phys. 111 (1999) 2844–2845.
[35] J.D. Hirst, N.A. Besley, Response to ''Comment on 'Improving protein circular dichroism calculations in the far-ultraviolet through reparametrizing the amide chromophore''', J. Chem. Phys. 111 (1999) 2846–2847.
[36] N.A. Besley, J.D. Hirst, Theoretical studies toward quantitative protein circular dichroism predictions, J. Am. Chem. Soc. 121 (1999) 9636–9644.
[37] N. Sreerama, M.C. Manning, M.E. Powers, J.-X. Zhang, D.P. Goldenberg, R.W. Woody, Tyrosine, phenylalanine, and disulfide contributions to the circular dichroism of proteins: circular dichroism spectra of wild-type and mutant bovine pancreatic trypsin inhibitor, Biochemistry 38 (1999) 10814–10822.
[38] N.J. Greenfield, Methods to estimate the conformation of proteins and polypeptides from circular dichroism data, Anal. Biochem. 235 (1996) 1–10.
[39] N. Sreerama, S.Yu. Venyaminov, R.W. Woody, Estimation of the number of α-helical and β-strand segments in proteins using circular dichroism spectroscopy, Protein Sci. 8 (1999) 370–380.
[40] W.C. Johnson, Analyzing protein circular dichroism spectra for accurate secondary structures, Proteins: Struct. Func. Genet. 35 (1999) 307–312.
[41] N. Sreerama, R.W. Woody, Circular dichroism of peptides and proteins, in: N. Berova, K. Nakanishi, R.W. Woody (Eds.), Circular Dichroism: Principles and Applications, Wiley, New York, 2000, pp. 601–620.
[42] J.A. Schellman, W.J. Becktel, The optical activity of polypeptides, Biopolymers 22 (1983) 171–187.
[43] R. Mandel, G. Holzwarth, Circular dichroism of oriented helical polypeptides: the alpha-helix, J. Chem. Phys. 57 (1972) 3469–3477.
[44] L.B. Clark, Polarization assignments in the vacuum UV spectra of the primary amide, carboxyl, and peptide groups, J. Am. Chem. Soc. 117 (1995) 7974–7986.
[45] D.L. Peterson, W.T. Simpson, Polarized electronic absorption spectrum of amides with assignment of transitions, J. Am. Chem. Soc. 79 (1957) 2375–2382.
[46] J. Ridley, M. Zerner, Intermediate neglect of differential overlap technique for spectroscopy-pyrrole and azines, Theor. Chim. Acta (Berlin) 32 (1973) 111–134.
[47] V. Pajcini, X.G. Chen, R.W. Bormett, et al., Glycylglycine $\pi \rightarrow \pi^*$ and charge transfer transition moment orientations: near-resonance Raman single-crystal measurements, J. Am. Chem. Soc. 118 (1996) 9716–9726.
[48] X.G. Chen, P. Li, J.S.W. Holtz, et al., Resonance Raman examination of the electronic excited states of glycylglycine and other dipeptides: observation of a carboxylate→amide charge transfer transition, J. Am. Chem. Soc. 118 (1996) 9705–9715.
[49] V. Pajcini, S.A. Asher, Preresonance Raman single-crystal measurements of electronic transition moment orientations in *N*-acetylglycinamide, J. Am. Chem. Soc. 121 (1999) 10942–10954.
[50] R.W. Woody, Transition moment directions in amide crystals, Prog. Biophys. Mol. Biol. 65 (Suppl. 1) (1996) 57.
[51] R.W. Woody, G. Raabe, J. Fleischhauer, Transition moment directions in amide crystals, J. Phys. Chem. B 103 (1999) 8984–8991.
[52] H. Basch, M.B. Robin, N.A. Kuebler, Electronic states of the amide group, J. Chem. Phys. 47 (1967) 1201–1210.
[53] L.B. Harding, W.A. Goddard, Generalized valence bond description of valence states of formamide, J. Am. Chem. Soc. 97 (1975) 6300–6305.
[54] L.Z. Stenkamp, E.R. Davidson, Ab initio study of formamide, Theor. Chim. Acta 44 (1977) 405–419.
[55] L.E. Nitzsche, E.R. Davidson, Perturbation-theory calculation on $^1\pi\pi^*$ state of formamide, J. Chem. Phys. 68 (1978) 3103–3109.
[56] E. Oliveros, M. Riviere, C. Teichteil, P. Malrieu, CI (CIPSI) calculations of vertical ionization and excitation-energies of formamide molecule, Chem. Phys. Lett. 57 (1978) 220–223.

[57] A.L. Sobolewski, Ab initio study of the potential-energy functions relevant for hydrogen-transfer in formamide, its dimer and its complex with water, J. Photochem. Photobiol. 89 (1995) 89–97.

[58] J.D. Hirst, D.M. Hirst, C.L. Brooks, Ab initio calculations of the excited states of formamide, J. Phys. Chem. 100 (1996) 13487–13491.

[59] J.D. Hirst, D.M. Hirst, C.L. Brooks, Multireference configuration interaction calculations of electronic states of *N*-methylformamide, acetamide, and *N*-methylacetamide, J. Phys. Chem. A 101 (1997) 4821–4827.

[60] N.A. Besley, J.D. Hirst, Ab initio study of the effect of solvation on the electronic spectra of formamide and *N*-methylacetamide, J. Phys. Chem. A 102 (1998) 10791–10797.

[61] L. Serrano-Andrés, M.P. Fülscher, Theoretical study of the electronic spectroscopy of peptides. 1. The peptidic bond: primary, secondary, and tertiary amides, J. Am. Chem. Soc. 118 (1996) 12190–12199.

[62] L. Serrano-Andrés, M.P. Fülscher, Theoretical study of the electronic spectroscopy of peptides. 2. Glycine and *N*-acetylglycine, J. Am. Chem. Soc. 118 (1996) 12190–12199.

[63] D.J. Tozer, R.D. Amos, N.C. Handy, B.O. Roos, L. Serrano-Andrés, Does density functional theory contribute to the understanding of excited states of unsaturated organic compounds?, Mol. Phys. 97 (1999) 859–868.

[64] E.B. Nielsen, J.A. Schellman, Absorption spectra of simple amides and peptides, J. Phys. Chem. 71 (1967) 2297–2304.

[65] M.B. Robin, Higher Excited States of Polyatomic Molecules, vol. 1, Academic Press, New York, 1975, pp. 76–91.

[66] L. Serrano-Andrés, M.P. Fülscher, Theoretical study of the electronic spectroscopy of peptides. III. Charge-transfer transitions in polypeptides, J. Am. Chem. Soc. 120 (1998) 10912–10920.

[67] J.D. Hirst, B.J. Persson, Ab initio calculations of the vibrational and electronic spectra of diketopiperazine, J. Phys. Chem. A 102 (1998) 7519–7524.

[68] N.A. Besley, M.-J. Brienne, J.D. Hirst, Electronic spectra of a rigid cyclic diamide, J. Phys. Chem. B 104 (2000) 12371–12377.

[69] L. Serrano-Andrés, M.P. Fülscher, Charge transfer transitions in neutral and ionic polypeptides, J. Phys. Chem. B 105 (2001) 9323–9330.

[70] J. Del Bene, H.H. Jaffé, Use of the CNDO method in spectroscopy. I. Benzene, pyridine and the diazines, J. Chem. Phys. 48 (1968) 1807–1813.

[71] S. Onari, Vacuum ultraviolet absorption spectra of homo-polypeptides, J. Phys. Soc. Jpn 27 (1969) 269.

[72] C.R. McMillin, W.B. Rippon, A.G. Walton, Vacuum ultraviolet spectroscopy of poly-α-amino acids, Biopolymers 12 (1973) 589–597.

[73] I. Tinoco, A. Halpern, W.T. Simpson, The relation between conformation and light absorption in polypeptides and proteins, in: M.A. Stahman (Ed.), Polyamino Acids, Polypeptides and Proteins, University Wisconsin Press, Madison, 1962, pp. 147–157.

[74] K. Rosenheck, P. Doty, The far ultraviolet absorption spectra of polypeptide and protein solutions and their dependence on conformation, Proc. Natl. Acad. Sci. USA 47 (1961) 1775–1785.

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

[76] W. Rhodes, Hypochromism and other spectral properties of helical polynucleotides, J. Am. Chem. Soc. 83 (1961) 3609–3617.

[77] E. Goldmann, S.A. Asher, S. Mukamel, Electronic excitations of polyalanine; test of the independent chromophore approximation, Phys. Chem. Chem. Phys. 3 (2001) 2893–2903.

[78] I. Tinoco, Theoretical aspects of optical activity. Part two: Polymers, Adv. Chem. Phys. 4 (1962) 113–160.

[79] V. Chernyak, S. Mukamel, Size-consistent quasiparticle representation of nonlinear optical susceptibilities in many-electron systems, J. Chem. Phys. 104 (1996) 444–459.

[80] S. Tretiak, V. Chernyak, S. Mukamel, Collective electronic oscillators for nonlinear optical response of conjugated molecules, Chem. Phys. Lett. 259 (1996) 55–61.

[81] D.A. Denisov, Calculation of certain optical characteristics of polypeptides, Biophysics (Engl. transl. of Biofizika) 14 (1969) 11–16.

[82] M.C. Manning, R.W. Woody, Theoretical CD studies of polypeptide helices: examination of important electronic and geometric factors, Biopolymers 31 (1991) 569–586.

[83] D.J. Barlow, J.M. Thornton, Helix geometry in proteins, J. Mol. Biol. 201 (1988) 601–619.

[84] T. Ooi, R.A. Scott, G. Vanderkooi, H.A. Scheraga, Conformational analysis of macromolecules. IV. Helical structure of poly-L-alanine, poly-L-valine, poly-β-methyl-L-aspartate, poly-γ-methyl-L-glutamate, and poly-L-tyrosine, J. Chem. Phys. 46 (1967) 4410–4426.

[85] M. Siedlecka, G. Goch, A. Eichart, H. Sticht, A. Bierzynski, α-Helix nucleation by a calcium-binding loop, Proc. Natl. Acad. Sci. USA 96 (1999) 903–908.

[86] M.M. Lopez, D.-H. Chin, R.L. Baldwin, G.I. Makhatadze, The enthalpy of the alanine peptide helix measured by isothermal titration calorimetry using metal-binding to induce helix formation, Proc. Natl. Acad. Sci. USA 99 (2002) 1309–1313.

[87] A. Koslowski, R.W. Woody, The calculation of the circular dichroism of polypeptides in the far UV, Biophys. J. 76 (1999) A53.

[88] C. Edmiston, K. Ruedenberg, Localized atomic and molecular orbitals, Rev. Mod. Phys. 35 (1963) 457–473.
[89] J.D. Petke, Construction of chromophore orbitals, J. Chem. Phys. 93 (1990) 2561–2569.
[90] W.C. Johnson, I. Tinoco, Circular dichroism of polypeptide solutions in the vacuum ultraviolet, J. Am. Chem. Soc. 94 (1972) 4389–4390.
[91] R.K. Momii, D.W. Urry, Absorption spectra of polypeptide films in the vacuum ultraviolet, Macromolecules 1 (1968) 372–373.
[92] J.L. Bensing, E.S. Pysh, Polarized vacuum-ultraviolet absorption of poly (L-alanine), Macromolecules 4 (1971) 659–660.