

Hydrogen bonding in protein circular dichroism calculations[☆]

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Abstract

We present ab initio electronic structure calculations of *N*-methylacetamide (NMA) in solution. The solvent is modelled by a combination of explicitly defined water molecules and a continuum dielectric. This should describe both local and bulk solvent effects; in particular the effects of solute–solvent hydrogen bonds will be included. The influence of hydrogen bonding on calculations of the electronic circular dichroism (CD) spectra of proteins is explicitly incorporated through parameters derived from the ab initio calculations. We find no improvement on the accuracy of protein CD calculations compared with results from parameters for NMA in continuum solvent. We conclude that hydrogen bonding is probably adequately described through inter-amide electrostatic interactions that form the basis of the matrix method. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Circular dichroism; Protein; Hydrogen bonding; Ab initio

1. Introduction

Protein structure determination is an active area of research. In part, this interest arises because of the benefit to areas such as drug design. Furthermore, initiatives in structural genomics are fuelling developments in protein structure determination. Electronic circular dichroism (CD) spectroscopy, the differential absorption of left and right circularly polarized light, can provide low resolution structural information, as all the major elements of protein secondary structure have characteristic CD spectra in the far-UV. For example, an α -helical protein has a three band pattern. An intense positive band at 190 nm and a negative band at 208 nm arise from the exciton splitting of the amide $\pi_{\text{nb}}\pi^*$ (non-bonding π orbital to anti-

bonding π orbital) electronic transition. A further negative band arises at about 220 nm due to the amide $n\pi^*$ (oxygen lone pair orbital to π^* orbital) transition. The intensity of this band correlates with the helical content of the protein [1]. Recently, advances in time-resolved techniques on the microsecond [2] and nanosecond [3] time scales have caused a resurgence of interest in protein CD. These experiments can follow the early events in protein folding and coupled with the tractability of molecular dynamics simulations on the nanosecond time scale [4] highlight the need for accurate protein CD calculations. In this paper we report ab initio calculations on the electronic excited states of $\text{NMA}-(\text{H}_2\text{O})_2$ and work on incorporating the effects of hydrogen bonding into calculations of protein CD.

The interpretation of protein CD has largely been based on empirical methods [1]. However, to investigate the relationship between protein conformation and the associated spectra, ideally calculations from first principles are required. Several groups have reported calculations of protein CD that employ less

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empiricism. The dipole interaction model [5] considers individual atoms and the amide chromophore to be point dipole oscillators. In the presence of an electric field, these interact through mutually induced dipole moments. This model includes the amide $\pi_{nb}\pi^*$ transition described by empirically determined polarizabilities [6]. Several systems have been studied [6–9], including a number of proteins [10].

Another approach to calculating protein CD is the matrix method [11,12], where a Hamiltonian matrix is constructed assuming the protein consists of non-interacting chromophoric groups. This matrix is then diagonalized by a unitary transformation, which describes the transformation from a non-interacting regime to an interacting regime. Using this transformation, the necessary local properties are transformed and the CD of the protein computed. The accuracy of the method relies on the calculated elements of the Hamiltonian matrix. To date, the most accurate calculations neglect contributions from the protein side-chains [13–15]. Generally, *N*-methylacetamide (NMA) is used as a model of the protein backbone. Recently, there has been considerable progress in the parametrization of the charge distributions of NMA required to calculate protein CD [13–15].

Parameters derived from multi-reference configuration interaction calculations of NMA in the gas phase [16] showed an improved correlation between experimental and calculated spectra at 220 nm when tested on a set of 23 proteins [17]. Woody and Sreerama [13] reported parameters derived from a combination of CNDO/S calculations and experimental data. Comparison with the same 23 experimental spectra showed a large improvement in the correlation at 220 nm and a significant correlation at 190 nm. Our latest parameters [14,15] are based on ab initio calculations of NMA in solution [18]. In these calculations the solvent was modelled by a continuum dielectric. Including effects of solvation and introducing a more accurate representation of the charge distributions improved agreement with experiment. Significant correlation with experiment was obtained at the three key wavelengths of 190, 208, and 220 nm, with the correlation at 220 nm almost quantitative. Apart from the choice of bandwidth, no empirical parameters were used in these calculations. Although the results of this study are encouraging, the calculations are still inaccurate for a class of β -sheet

proteins whose CD spectra resemble that of a random coil. The reason for this discrepancy is unclear. The calculations make several assumptions, such as using the X-ray crystal structure and neglecting side-chains and non-chromophoric backbone groups. However, the inclusion of side-chain groups, using available parameters [13], did not improve agreement with experiment. Another feature that may not be described accurately in the matrix method is the hydrogen bonding between backbone chromophores. Calculations of formamide have shown that it is necessary to use a combination of explicit solvent and a continuum dielectric, in a so-called semi-continuum approach, to reproduce all the features of the electronic spectrum in water [19]. In this study, we derive parameters from calculations of NMA in water using a semi-continuum approach. The resulting parameters should reflect the effects of hydrogen bonding.

2. Ab initio calculations

As a consequence of its role as a model for the peptide bond, NMA has been extensively studied both experimentally [20–23] and theoretically [16,24–37]. The gas phase electronic spectrum has been determined with high-level ab initio methods [16,25,26]. The solution phase electronic structure has been studied using continuum solvent models [18]. Many studies of NMA with explicitly defined solvent have been reported [27–36]. These studies have generally been concerned with properties of the ground electronic state, such as minimum energy structures, hydrogen bond strengths and infrared spectroscopy. The inclusion of specific hydrogen bonds did not change the ground state charge distribution qualitatively compared to purely electrostatic models of solvation [36]. However, the sensitivity of the excited state charge distributions to specific hydrogen bonds has not been studied. Many early studies of minimum energy structures were limited by available computational resources to low levels of theory and small basis sets. In a more recent study [35], the conformations and stabilities of different methyl group orientations of $\text{NMA}-(\text{H}_2\text{O})_{n=1-3}$ complexes were investigated using density functional theory (DFT) and the 6-311++G-(d,p) basis set. The most stable $\text{NMA}-(\text{H}_2\text{O})_2$ complex was found to have two

Table 1
Optimized coordinates

Atom	<i>x</i> (Å)	<i>y</i> (Å)	<i>z</i> (Å)
C	1.443	−0.225	0.000
C	0.150	0.571	0.000
C	−2.281	0.507	0.000
N	−0.992	−0.160	0.000
O	0.131	1.812	0.000
H	1.263	−1.309	0.000
H	−0.939	−1.178	0.000
H	−3.073	−0.256	0.000
H	2.038	0.064	±0.879
H	−2.395	1.149	±0.889
O	−0.648	−3.132	0.000
H	1.905	2.413	0.000
H	3.008	3.447	0.000
O	2.879	2.492	0.000
H	−0.138	−3.458	±0.755

water molecules hydrogen bonding to the amide hydrogen. In this study, we are interested in complexes in which there are hydrogen bonds to both the amide hydrogen and the carbonyl oxygen. For NMA–(H₂O)₂ complexes in which one water molecule is hydrogen bonded to the carbonyl group and one to the amide hydrogen there are several conformations that lie close in energy. We have performed geometry optimizations of the NMA–(H₂O)₂ complex at the MP2 level using the valence double zeta basis set within the *C_s* point group using the GAUSSIAN94 [38] suite of programs. The true local minimum will only have approximate *C_s* symmetry. However, the advantages of retaining symmetry in the following calculations outweigh any error incurred. The results of our calculations agree with those reported in the DFT study, and the optimized co-ordinates are shown in Table 1. This structure is used in the subsequent calculations.

To determine the electronic structure of NMA in solution, the solvent is described by a combination of explicitly defined water and the complete-active-space self-consistent field coupled with multi-configurational perturbation theory approach within a self-consistent reaction field (CASSCF-SCRF/CASPT2-RF) [39–42]. This captures both local and bulk solvent effects, and reproduced all the features of the electronic spectrum of formamide [19].

In the CASSCF-SCRF approach, the response of reaction field is divided into nuclear and electronic

components. The relaxation times of the nuclear degrees of freedom are slow on the time-scale of an electronic excitation and are assumed constant. The electronic degrees of freedom will respond on this time-scale and consequently they are optimized for each electronic state. The NMA–(H₂O)₂ complex was placed in a cavity of radius 10.4 au, which was selected by considering the van der Waals radii [43]. Surrounding the cavity is a continuous dielectric, with a macroscopic dielectric constant $\epsilon = 80.0$ and a refractive index $\eta = 1.33$, which models water. An active space of 3*a'* and 3*a''*, denoted (3,3), was chosen. This includes the oxygen lone pairs (*n'* and *n*) and the π orbitals (π_b and π_{nb}) orbitals which are doubly occupied in the ground state. Placed outside the cavity is a repulsive potential [41,42] that describes the exchange interaction of the solvent and maintains the charge distribution within the cavity. The calculations used generally contracted basis sets of atomic natural orbitals (ANO) [44] with the contractions: C,N,O 4s3p1d and H 2s. Additional diffuse basis functions are not required because of the absence of Rydberg states. Following each CASSCF-SCRF calculation a CASPT2-RF calculation is performed. This accounts for the dynamic correlation. A level shift of 0.3 au was used in these calculations. These calculations were performed using the MOLCAS4 [45] suite of programs.

3. Calculation of CD spectra

The rotational strength represents the integrated intensity beneath a band in the CD spectra. For an electronic transition, $A \leftarrow 0$, the rotational strength can be expressed as the imaginary part of the product of the electronic and magnetic transition moments

$$R_{0A} = \text{Im}(\langle \psi_0 | \vec{\mu}_e | \psi_A \rangle \cdot \langle \psi_A | \vec{\mu}_m | \psi_0 \rangle) \quad (1)$$

For large systems, such as proteins, it is computationally too expensive to compute rotational strengths directly and an alternative approach is necessary. The matrix method considers the protein to consist of *M* chromophoric groups, with *n_i* electronic excitations within each group *i*. The total wave function is written:

$$\Psi_T = \sum_i^M \sum_a^{n_i} c_{ia} \Phi_{ia} \quad (2)$$

Table 2
Calculated transition energies (N/A = not available)

State	CASSCF(SCRF)/CASPT2-RF		Experiment	
	ΔE (eV)	f	ΔE (eV)	f
$n\pi^*$	6.20	~ 0	5.7–5.9	~ 0
$\pi_{nb}\pi^*$	6.81	0.35	6.67	0.30
$\pi_b\pi^*$	9.57	0.29	N/A	N/A
$n'\pi^*$	10.14	0.02	N/A	N/A

in which Φ_{ia} is a product of monomer wave functions

$$\Phi_{ia} = \phi_{10} \cdots \phi_{ia} \cdots \phi_{j0} \cdots \phi_{M0} \quad (3)$$

where ϕ_{ia} represents the wave function of chromophore i which has undergone an excitation $a \leftarrow 0$. A Hamiltonian matrix is formed by considering the electronic Hamiltonian

$$\hat{H} = \sum_{i=1}^M \hat{H}_i + \sum_{i=1}^{M-1} \sum_{j=i+1}^M \hat{V}_{ij} \quad (4)$$

Diagonal elements are given by the excitation energies of the electronically excited states. The off-diagonal elements describe interactions between different chromophoric groups and the mixing of excitations on the same chromophoric group due to the field of the molecule. Assuming purely electrostatic interactions, these elements typically have the form:

$$V_{i0a;j0b} = \sum_{s=1}^{N_s} \sum_{t=1}^{N_t} \frac{q_s q_t}{r_{st}} \quad (5)$$

in which N_s charges q_s and N_t charges q_t represent the charge distributions of permanent or transition densities on chromophores i and j , respectively. The unitary transformation that diagonalizes this matrix describes the transformation from the non-interacting regime to the interacting regime. The local electronic and magnetic moments can be transformed by the same matrix, and the rotational strengths for the protein evaluated.

As described in detail in our latest work [15], these charges were determined by evaluating the electrostatic potential for a given electron density on a grid of points and fitting charges to reproduce this potential. The density matrices produced from the calculations of NMA-(H₂O)₂ will contain coefficients of basis functions that are located on the water mole-

cules. The aim of this study is to determine charges that describe NMA but reflect the effects of hydrogen bonding on the electron distributions. To achieve this the coefficients of these basis functions are set to zero before evaluating properties such as the electrostatic potential. This will have a large effect on the properties of permanent electronic states. The effect on transition properties will be smaller since the electronic excitations are localized on NMA and hence these coefficients will be approximately zero. These calculations were performed using our modified version of the MOLPRO96 program package [46].

The parameters from the ab initio calculations were used to compute the CD of 29 proteins from their X-ray crystal structures. The proteins studied were cytochrome *c* (3cyt), hemoglobin (1hco), myoglobin (1mbn), bacteriorhodopsin (2brd), alcohol dehydrogenase (5adh), glutathione reductase (3grs), lactate dehydrogenase (6ldh), lysozyme (7lyz), papain (9pap), rhodanese (1rhd), subtilisin (1sbt), thermolysin (4tln), triose phosphate isomerase (1tim), flavodoxin (2fx2), carbonic anhydrase (1ca2), concanavalin A (3cna), λ -immunoglobulin (1rei), ribonuclease A (3rn3), ribonuclease S (2rns), erabutoxin (3ebx), plastocyanin (1plc), porin (3por), prealbumin (2pad), α -chymotrypsinogen (2cga), α -chymotrypsin II (5cha), elastase (3est), superoxide dismutase (2sod), trypsin inhibitor (4pti) and trypsin (3ptn). In all CD calculations bandwidths of 15.5 nm were used for all transitions.

4. Results and discussion

The electronic spectra of amides in the far-UV are dominated by the broad $\pi_{nb}\pi^*$ band. The results of the calculations, shown in Table 2, reflect this. The $\pi_{nb}\pi^*$ transition is computed to lie at 6.81 eV with an oscillator strength of 0.35. This represents a very small shift in energy arising from solvation. The red-shift observed in experiment is also small. Values of ~ 0.1 eV have been reported [20,21]. This is in contrast to other amides where larger red-shifts of up to ~ 0.5 eV are found [47]. The $n\pi^*$ transition is electronically forbidden and appears as a shoulder on the $\pi_{nb}\pi^*$ band. Consequently, the $n\pi^*$ band is more difficult to characterise experimentally. In gas-phase and non-polar solvents the amide $n\pi^*$ band appears at

Table 3
Permanent and transition dipole moments

State	With water			Water removed		
	μ_x	μ_y	μ_z	μ_x	μ_y	μ_z
<i>Permanent dipole moments (D)</i>						
GS	-1.45	-5.49	0.00	-1.41	-5.81	0.00
$n\pi^*$	-0.43	2.22	0.00	-0.71	2.70	0.00
$\pi_{nb}\pi^*$	-3.23	-4.97	0.00	-3.30	-5.33	0.00
<i>Transition dipole moments (D)</i>						
GS- $n\pi^*$	0.00	0.00	-0.13	0.00	0.00	-0.14
GS- $\pi_{nb}\pi^*$	2.39	2.79	0.00	2.37	2.79	0.00
$n\pi^*-\pi_{nb}\pi^*$	0.00	0.00	0.09	0.00	0.00	0.09

~5.5 eV [47]. However, in aqueous solution the amide $n\pi^*$ band is found to lie at ~5.7–5.9 eV [21]. This blue-shift is a result of the solute–solvent hydrogen bonds formed. The results of the current study find a large blue-shift for the $n\pi^*$ band. A transition energy of 6.20 eV is predicted, which is probably an over-estimation of the blue-shift. To model the blue-shift more accurately it may be necessary to consider more explicitly defined solvent molecules and many solvent configurations. Presently, this is too computationally demanding. The inclusion of explicit solvent has little effect on the transition energies of the $\pi_b\pi^*$ and $n'\pi^*$ bands. Computed transition energies are very close to those obtained with a purely continuum solvent model. Another feature of the electronic spectrum is the absence of Rydberg states, which are observed in gas-phase. In the solvent model employed here the Rydberg states are destabilized by the combined repulsive interaction of the solvent molecules and the repulsive potential used to model the exchange interaction of the solvent. The computed permanent and transition dipole moments for the ground, $n\pi^*$, $\pi_{nb}\pi^*$ states of NMA are shown

Table 4
Correlation between experimental and calculated CD spectra

Model ^a	$r_{[\lambda = 190 \text{ nm}]}$ ^b	$r_{[\lambda = 208 \text{ nm}]}$	$r_{[\lambda = 220 \text{ nm}]}$
CASSCF	0.84	0.73	0.90
CASSCF(HB)	0.71	0.76	0.88
CASSCF(HB/ $n\pi^*$)(2)	0.83	0.73	0.89
CASSCF(HB/ $n\pi^*$)(4)	0.72	0.72	0.88

^a For explanation of notation see text

^b Spearman rank correlation coefficient for 29 proteins.

in Table 3. The calculated dipole moment of the ground state is considerably larger than in gas phase. This shows that the electronic distribution is significantly polarized by the water molecules. The $n\pi^*$ and $\pi_{nb}\pi^*$ states also show an increase in dipole moments although not to the extent of the ground state.

Table 4 shows the Spearman rank correlation coefficient [48] at 190, 208, and 220 nm between the calculated and experimental CD spectra of 29 proteins. This data set includes a range of proteins from highly helical to predominantly β -sheet. The new parameters¹, denoted CASSCF(HB) have little effect on the correlation at 208 and 220 nm compared with previously reported results, denoted CASSCF [15]. However, at 190 nm the correlation deteriorates. This is a consequence of the increased oscillator strength, resulting in an over-estimation of the intensity of the $\pi_{nb}\pi^*$ band.

The large energy shift in the $n\pi^*$ transition energy suggests that it is this band which will be most sensitive to the hydrogen bonds. In order to isolate the contribution of hydrogen bonding to the $n\pi^*$ band, the new parameters for the $n\pi^*$ transition were combined with previous parameters for the ground and $\pi_{nb}\pi^*$ states, in a two state model, and the ground, $\pi_{nb}\pi^*$, $n'\pi^*$ and $\pi_b\pi^*$ states, in a four state model. The correlation at 220 nm remains virtually unchanged compared to the totally non-hydrogen bonding parameters with a little drop in correlation at 190 nm for the four state model. From these results it is concluded that the influence of hydrogen bonding on the charge

¹ The parameters can be obtained from the authors on request.

distributions of the permanent and transition electron densities does not have a large effect on protein CD. This is particularly true for the $n\pi^*$ band, for which the greatest effects might be anticipated.

5. Conclusions

In this study, calculations of NMA in aqueous solution have been presented in which the solvent is modelled by a combination of explicitly defined water molecules and a continuous dielectric. The results show a very small shift in energy for the broad $\pi_{nb}\pi^*$ band. In contrast, the $n\pi^*$ band undergoes a large blue-shift. Parameters derived from these calculations have been used to model the backbone amide chromophore in the computation of protein CD spectra. The resulting spectra show a similar accuracy to calculations based on parametrization of the amide chromophore in which there is no description of hydrogen bonding. This suggests that the effects of hydrogen bonding on protein CD spectra are adequately described by the electrostatic interactions included in the matrix method.

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