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Magnetic Circular Dichroism Spectrum of Plastocyanin by Calculation

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The magnetic circular dichroism (MCD) spectrum of the metalloenzyme plastocyanin is calculated using time-dependent density functional theory. The calculation reproduces most of the main features of the experimental spectrum. The calculated spectrum is analyzed to elucidate the contributions to the spectrum and the source of the MCD intensity. As expected, the temperature-dependent MCD intensity is found to be dominant. More surprisingly, contributions from the ground-state mixing with excited states are found to be significant.

In a magnetic circular dichroism (MCD)[1,2] experiment, the difference in the absorption of left and right circularly polarized light in the presence of a magnetic field is measured as a function of the energy of the incident light. A MCD spectrum forms a useful complement to an absorption spectrum because MCD is an assigned quantity and generally has intensity patterns different from those of the standard absorbance. MCD spectroscopy has found application in many areas of chemistry.[2] It has been found to be particularly useful in the study of metalloenzymes.[3,4] If a molecule has a degenerate ground state, then its MCD spectrum will be temperature-dependent.[1,2] The intensity of the temperature-dependent component of MCD increases as the temperature decreases. This component, often referred to as a *C* term, is also dependent on the strength of the spin−orbit coupling present in the molecule. At low temperatures, the MCD spectrum of a metalloenzyme will be dominated by excitations associated with the metal center because as a heavier atom its spin−orbit coupling constant will be much greater than that of the other atoms present.[5] It is therefore often possible to gain significant insight into the electronic structure of the active site of a metalloenzyme through MCD spectroscopy when the corresponding absorption spectrum is more difficult to interpret as a result of the presence of many overlapping bands.

One particular example where MCD spectroscopy has been very useful is the electronic structure of plastocyanin. Plastocyanin, a blue-copper protein, plays an important role in electron transfer during photosynthesis. Blue-copper proteins have several unusual properties that have been studied intensely in recent years.[6,7] MCD spectroscopy has played a significant role in this work, in particular in the assignment of the absorption spectrum of plastocyanin.

A drawback of MCD spectroscopy is that the spectra produced are often difficult to interpret. Some kind of calculation is generally necessary to aid in the interpretation. In recent years, methodologies have been developed for the calculation of temperature-dependent and -independent MCD spectra from first principles.[8−16] In our laboratory, we have focused on the calculation of MCD spectra using density functional theory (DFT) and, in particular, time-dependent DFT (TDDFT).[14−16] Our approach to calculating MCD spectra takes advantage of the efficiency of TDDFT and can be applied to quite large molecules. In this paper, we present the first calculation of the MCD spectrum of a metalloenzyme, that of plastocyanin.

We model the metal center of plastocyanin with the 52-atom complex shown in Figure 1. This model includes parts or all of the four amino acids that ligate the Cu atom, Cys84, Met92, His37, and His87, and is intermediate in size between the PCU-S and PCU-L models of plastocyanin used in studies

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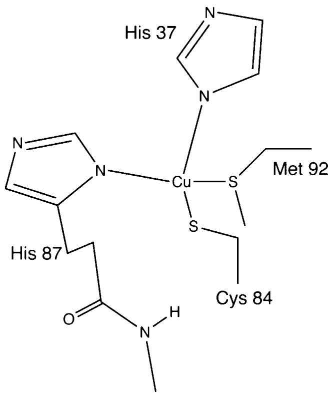

Figure 1. Model structure of plastocyanin.

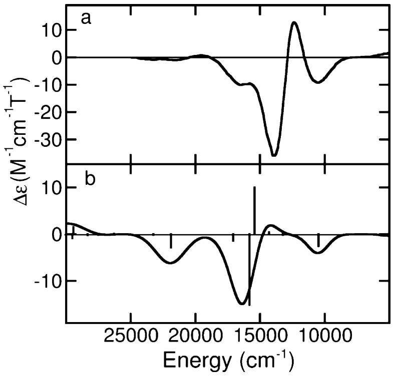

Figure 2. MCD spectrum of plastocyanin: (a) experimental;[5] (b) calculated. The energies and intensities of individual excitations as derived by calculation are indicated by bars.

of its electron paramagnetic resonance spectrum.[17]

The experimental MCD spectrum of plastocyanin measured at a temperature of 4.5 K in a magnetic field of 4 T[6] is plotted in Figure 2a. All of the peaks below 15 000 cm^{-1} are assigned as d−d transitions, while those above 15 000 cm^{-1} are assigned as charge-transfer (CT) transitions.[6]

We calculate MCD spectra by first evaluating the unperturbed excitation energies and intensities and then perturbing these quantities by a magnetic field and spin−orbit coupling.[14,15] Both temperature-dependent and -independent contributions to the MCD intensity are evaluated. The methodology avoids sum-overstates approaches and includes contributions nonlinear in the magnetic field to the temperature-dependent MCD. Both of these features are very important when attempting to calculate the low-temperature spectrum of a molecule of this size.

The calculated MCD spectrum of plastocyanin is plotted in Figure 2b. The spectrum reproduces all of the qualitative features observed experimentally. It includes most of the major peaks of the experimental spectrum with the correct sign and similar relative intensity. Previous calculations of the absorption spectrum of plastocyanin using TDDFT have found some disagreement in assignments between the calculated values and experiment.[6] It is believed that the disagreement arises because the electronic structure of plastocyanin calculated with DFT is too covalent. Simulated absorption spectra obtained from TDDFT calculations do reproduce the qualitative form of the absorption spectrum.[17] Attempts at calculating the **g** tensor of plastocyanin with DFT have not been completely satisfactory.[17−19] With the present model, the bonding of plastocyanin is still too covalent though less covalent than that obtained with the 33-atom model[6] (see the Supporting Information). However, despite these deficiencies, we are able to produce a reasonable simulated MCD spectrum. It is apparent that first-principles calculations may be able to reproduce the MCD spectra of metalloenzymes with sufficient accuracy to prove useful in their interpretation even for more difficult cases like plastocyanin.

Table 1. Parameters of the Calculated MCD Spectrum[a]

E (cm^{-1})	C (au, ×1000)	% C	C/D	% C_{ex}
5 100	−0.33	105	−0.07	−70
10 500	−2.5	97	−0.15	−11
14 300	0.43	98	0.018	406
15 400	10	98	0.15	142
15 800	−15	98	−0.23	88
17 100	−1.4	99	−0.07	107
21 900	−2.88	99	−0.0045	−92
29 400	1.5	98	0.07	11
29 500	−0.84	100	−0.11	68

[a] See the text for definitions of C, % C, C/D, and % C_{ex}, and see the Supporting Information for further details.

Experimentally, eight bands are observed. Our calculations find 16 excitations between 5000 and 30 000 cm^{-1}, a range that corresponds to the lowest six experimentally observed bands. Of these 16 excitations, 9 are predicted to produce significant MCD intensities. Some parameters associated with these 9 excitations are presented in Table 1.

The second column in Table 1 lists the C term parameter associated with each excitation. The MCD intensity produced by each excitation is proportional to C. For the most part, the values correspond to what might be inferred from Figure 2. The exception is the two excitations near 15 500 cm^{-1}. The C term parameters of these two excitations are an order of magnitude greater than all of the others. However, the overall MCD intensity in this region does not dominate the spectrum as much as it might because the two oppositely signed bands cancel each other to a large extent.

The third column in Table 1 lists the percentage of the MCD intensity of the given band that is temperature-dependent. As might be expected, our results indicate that almost all of the observed MCD intensity of plastocyanin is temperature-dependent at 4.5 K and 4 T.

The ratio C/D of an excitation can often provide insight into the nature of that excitation. D is the dipole strength,[1] a quantity that is closely related to the oscillator strength. The MCD intensity is proportional to D. The ratio C/D is independent of the absorption intensity. This quantity therefore gives an indication of the strength of spin−orbit coupling between states. The C/D ratio has been used to assign the bands of the MCD spectrum as either d−d (C/D ∼ 0.1) or CT (C/D ∼ 0.01) transitions.[4] The negative band

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near 17 000 cm^{-1} in the experimental MCD spectrum corresponds to a very intense band in the experimental absorption spectrum. Because the MCD intensity of this band is only intermediate in strength, the C/D ratio for this band is relatively small, indicating that it corresponds to a CT excitation. Lower-energy bands contribute less intensity to the absorption spectrum but significant intensity to the MCD spectrum, leading to larger C/D ratios. These bands therefore correspond to d−d excitations.[5] This behavior is reproduced in the calculated spectrum. The band near 22 000 cm^{-1} shows significant negative MCD intensity but has a very large value of D, giving a final C/D ratio that is relatively small. Lower-energy excitations are calculated to have larger C/D ratios.

Temperature-dependent MCD can be interpreted in terms of the mixing of states induced by spin−orbit coupling. Neese and Solomon[12] have shown that the overall temperature dependence of the MCD intensity can be divided into contributions from mixing of the excited state of a transition with other excited states, mixing of other excited states with the ground state of a transition, and mixing between the two states involved in a transition. It is normally assumed that the first contribution is the largest. In our calculations, it is straightforward to divide the MCD intensity into contributions from mixing of other excited states with the upper state of a transition and from mixing of all other states (including the upper state) with the ground state.[14,15] Further details can be found in the Supporting Information. We have performed this analysis and list the results in Table 1 under the column labeled % C_{ex}. % C_{ex} is defined as the proportion of the temperature-dependent MCD intensity due to mixing of other excited states, with the final state expressed as a percentage. The rest of the temperature-dependent MCD intensity arises from mixing of excited states with the ground state. Note that, because the contributions to the MCD intensity are assigned quantities, then the two parts may be opposite in sign, which can lead to values of % C_{ex} that are greater than 100% or less than 0%. In several cases, the expected situation, where $50 < \% C_{ex} < 150$, occurs. In particular, the two near-degenerate excitations with much larger values of C couple strongly with each other. In many cases, however, the mixing of states with the ground state provides a significant, often dominant, contribution to the MCD intensity. This is in contrast to what is usually expected, though we do note a recent study of the Cu$_A$ site where it was found that the MCD intensity of the lowest-energy band in the spectrum was dominated by mixing between excited states and the ground state.[20]

In summary, we have calculated for the first time the MCD spectrum of a metalloenzyme molecule from first principles. The calculated spectrum reproduces many of the qualitative features of the experimental spectrum. The calculations offer significant scope for analysis. MCD spectra can often be difficult to interpret, especially when molecules as complicated as metalloenzymes are involved. Calculations of this type should make the interpretation of these spectra more straightforward.

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Supporting Information Available: Full structural parameters of the model system, computational details of the calculations, parameters quantifying how covalent the calculated results are, calculated parameters associated with all excitations, and further details about the parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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