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Amplified Vibrational Circular Dichroism as a Probe of Local Biomolecular Structure

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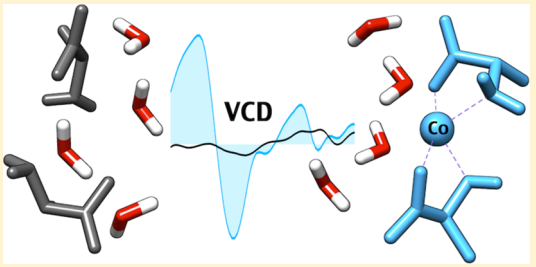
Supporting Information

ABSTRACT: We show that the VCD signal intensities of amino acids and oligopeptides can be enhanced by up to 2 orders of magnitude by coupling them to a paramagnetic metal ion. If the redox state of the metal ion is changed from paramagnetic to diamagnetic the VCD amplification vanishes completely. From this observation and from complementary quantum-chemical calculations we conclude that the observed VCD amplification finds its origin in vibronic coupling with low-lying electronic states. We find that the enhancement factor is strongly mode dependent and that it is determined by the distance between the oscillator and the paramagnetic metal ion. This localized character of the VCD amplification provides a unique tool to specifically probe the local structure surrounding a paramagnetic ion and to zoom in on such local structure within larger biomolecular systems.



INTRODUCTION

Chirality plays an essential role in the functionality of biomolecular systems. A key step in understanding the functionality of such systems relies on the determination of their stereochemistry, conformation, and structural heterogeneity. Vibrational circular dichroism (VCD),[1] the differential absorption of left- and right-handed circularly polarized infrared light, has become one of the most powerful spectroscopic methods to determine the absolute configuration and conformational distribution of chiral molecules in solution,[2−12] in particular with recent developments that address both experimental[13−18] and theoretical[19−21] issues, thereby improving the sensitivity, specificity, and selectivity of VCD. As yet, however, the inherent small signal intensities have seriously impeded extensive application of this spectroscopic technique. In particular, VCD spectra of important systems, such as amino acids, peptides, and proteins, are difficult to obtain under biologically relevant conditions. Although in recent years impressive progress has been made in VCD instrumentation and analysis,[22−32] one is commonly forced to work with highly concentrated samples to reach acceptable signal intensities, but this is often not possible due to low solubility and aggregation. One further issue that should be addressed in order to investigate large biologically active systems such as proteins and enzymes, is spectral congestion. The functionality of biomolecules is generally associated with a spatially restricted region, but in the VCD, all parts of the molecule contribute with comparable amplitude, so the contribution of the functional part is difficult to observe. Spectroscopic studies of biomolecular functionality thus ideally would be able to zoom in on such active sites, but as yet this has been hard to realize.[33] In the present study, we employ a paramagnetic transition-metal auxiliary to address both the issue of increasing the sensitivity of VCD and its application for probing local structure.

The peak intensities in a VCD spectrum are proportional to the rotational strength given by the imaginary part of the inner product of the electronic and magnetic transition-dipole moment vectors. Within a vibronic coupling approach, it can be shown that when a molecule is in an electronic state $|\psi_0\rangle$, the electronic part of the magnetic transition dipole moment of a transition between the $\nu = 0$ and $\nu = 1$ levels of a vibrational mode is given to first order by[34]

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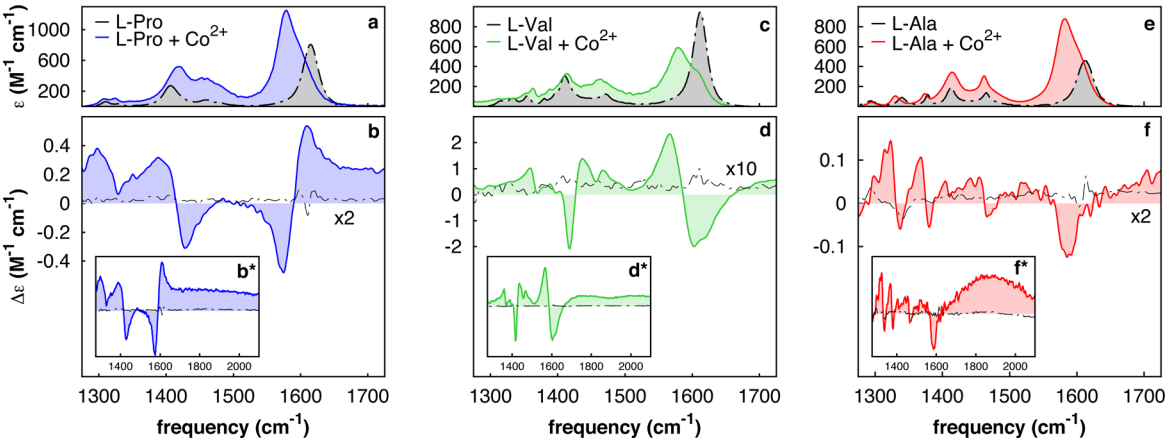

Figure 1. IR absorption and VCD spectra of the amino acids L-proline (a, b), L-valine (c, d), and L-alanine (e, f) in D_2O (black dashed lines) and of the complexes formed by complexation with cobalt: $Co^{II}(Pro)_2(D_2O)_2$ (a, b), $Co^{II}(Val)_2(D_2O)_2$ (c, d), and $Co^{II}(Ala)_2(D_2O)_2$ (e, f) (solid filled lines). The samples were prepared in D_2O ($c \approx 25$ mM). The VCD spectra of the amino acids and of the complexes were averaged with 4320 scans (1 h) at a resolution of 4 cm^{-1}. The VCD spectra of the amino acids (dashed lines in b, d, and f) have been scaled for better comparison with the enhanced VCD spectra of the complexes. Insets b*, d*, and f* display an extension (up to 2100 cm^{-1}) of the spectra depicted in b, d, and f, respectively.

$$\langle \Psi_f | \vec{\mu}^{e}_{mag} | \Psi_i \rangle = \langle \chi_{\nu=0} | \sum_{n \neq 0} \frac{\langle \psi_0 | \vec{\mu}^{e}_{mag} | \psi_n \rangle}{E_n - E_0} (\langle \psi_n | T_{nucl} | \psi_0 \rangle - \langle \psi_0 | T_{nucl} | \psi_n \rangle) | \chi_{\nu=1} \rangle \quad (1)$$

where $|\chi_{\nu=0}\rangle$ and $|\chi_{\nu=1}\rangle$ are the nuclear wave functions of the $\nu = 0$ and $\nu = 1$ states in the electronic ground state $|\psi_0\rangle$, T_{nucl} is the nuclear kinetic energy operator, $\vec{\mu}^{e}_{mag}$ is the electronic contribution to the magnetic transition dipole moment, and $|\psi_0\rangle$ and $|\psi_n\rangle$ are the Born−Oppenheimer electronic wave functions for the ground state and the nth electronically excited state, with energies E_0 and E_n, respectively. Theory thus leads one to expect that in systems with low-lying electronic states an enhancement of VCD signal intensities might occur compared with analogous systems in which such low-lying electronic states are absent. Such enhanced VCD signals have indeed been observed, starting with studies on the CH-stretching region of (−)-sparteine transition-metal complexes.[35] It is, however, only in the studies of Nafie et al.[36] on the same system that a full explanation in terms of vibronic coupling was provided and that the pertaining theoretical expressions for the VCD intensities were developed.[37] In these particular transition-metal complexes, the low-lying excited states are intrinsically already present. Conceptually, one should also be able to enhance VCD signal intensities by modulating the energies of the electronically excited-state manifold in such a way that a manifold is created with low-lying electronically excited states. We recently confirmed the validity of such an approach in a study in which electrochemical reduction was used to "create" the required low-lying electronically excited states, leading to an order-of-magnitude amplification of VCD signals.[15]

Since the initial studies on (−)-sparteine transition-metal complexes, a number of other studies have been reported in which analogous intensity enhancements in open-shell transition metal complexes were observed.[36,38−43] However, practically all of these studies concerned complexes with rigid, nonbiologically active ligands, dissolved under nonphysiologically relevant conditions. Here, we employ the manifold of low-lying electronically excited states provided by transition metal ions to induce enhanced VCD intensities in flexible biomolecular systems in aqueous solutions. We perform VCD studies on amino acids to determine to what extent vibrational circular dichroism can be enhanced in such systems. We show that amplification factors of more than 2 orders of magnitude can be obtained, bringing vibrational differential absorption on an equal footing with electronic differential absorption. Subsequently, we investigate how structural parameters influence this amplification. To this purpose, we perform VCD studies on di- and tripeptides and show that VCD enhancement is strongly dependent on the distance of the oscillator from the amplifying center. This spatial sensitivity of the VCD amplification thus provides excellent opportunities for its use as a structural tool for bioinorganic systems.

RESULTS

Cobalt Ions and Amino Acids. In Figure 1a,b, we show infrared absorption and VCD spectra of monomeric L-proline (black dashed lines). The IR spectrum shows a number of readily identifiable bands, the strongest one occurring at 1600 cm^{-1}, which is assigned to the carboxylate stretching mode. The VCD spectrum, on the other hand, shows bands with extremely small intensities that are hardly discernible from the noise of the measurement, typically $\Delta A \approx 5 \times 10^{-6}$ OD. In order to enhance these differential absorptions, we have altered our system in such way that L-proline is actively bound to a paramagnetic metal ion, thereby creating a model of a binding pocket where proline and water molecules can adopt multiple configurations around the metal. To this purpose, the hexacoordinated octahedral $Co^{II}(Pro)_2(D_2O)_2$ complex was synthesized by adding Co^{2+} ions to a solution of L-proline in a molar ratio of 2:1 (see Supporting Information). The IR and VCD spectra of this complex are shown in Figure 1a,b as blue solid lines. The IR spectrum confirms that L-proline is now incorporated into the $Co^{II}(Pro)_2(D_2O)_2$ complex, since a splitting is observed for the carboxylate stretching mode as a consequence of exciton coupling between the two proline moieties. Figure 1a,b shows that complexation has a minor effect on the intensities of the bands in the IR spectrum but leads to spectacular enhancements, by more than 1 order of magnitude, of the intensities of VCD bands. Apart from vibrational bands, the spectra in Figure 1 also show broad bands (specially for the alanine complex) that can be assigned to

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transitions to the lower-lying electronic states, thereby providing evidence for the presence of low-lying electronically excited states, in agreement with previous experiments.[19,35,36,41]

In principle, an increased number of conformations could lead to signal cancellation by oppositely signed VCD contributions. Thus, cancellation can become less when the number of contributing conformers becomes less due to coordination to the metal ion. To confirm that the observed enhancement is due to the presence of low-lying electronic states provided by the paramagnetic, high-spin Co^{2+} ion, we compare the VCD spectra of the proline–Co complex with Co in two distinct redox states: Co^{II} having low-lying electronically excited states and diamagnetic Co^{III} having well-separated electronic states. The diamagnetic Co^{III}–proline complex was obtained by oxidation of the Co^{II}–proline complex following a previously reported procedure, which leaves the geometry around the complex virtually unchanged.[44] In Figure 2 we

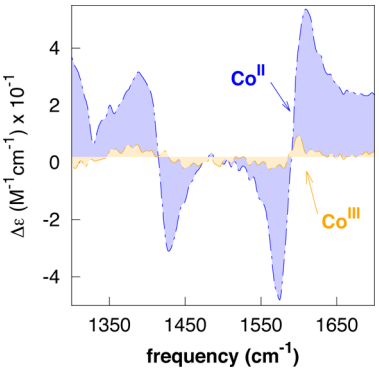

Figure 2. Experimental VCD spectra of $Co^{II}(Pro)_2(D_2O)_2$ (in blue) and $Co^{III}(Pro)_2(D_2O)_2$ (in orange) complexes.

compare the VCD spectra of these Co^{II}– and Co^{III}–proline complexes. The spectra clearly show that upon oxidation, the VCD signals of complexed L-proline reduce to the same magnitude as for uncomplexed L-proline. This observation is direct proof that the VCD amplification is due to vibronic coupling to low-lying electronically excited states. The absence of unpaired electrons in the d-orbitals of the diamagnetic complex removes the low-lying states from the system, and the VCD signals are consequently no longer amplified. We have found similar amplification effects for other amino acids. Typical examples (L-alanine and L-valine) are shown in Figure 1c–1f. It is worth notice that the VCD bands of L-valine are enhanced by more than 2 orders of magnitude by complexation to Co^{2+} ions. The intensity enhancement of a vibrational transition is usually characterized by its anisotropy ratio $g = \Delta\varepsilon/\varepsilon$, which generally is in the range of 10^{-4}–10^{-5}. Here, we find, for the complexed amino acids, ratios that range from 1×10^{-3} to 6.5×10^{-3} and that thus promptly qualify as enhanced VCD. We notice in particular that the latter value is one of the largest molecular vibrational anisotropy ratios reported so far. Similar g factors can only be found in previously reported studies on heme proteins by Marcott,[38] Bormett,[39] and Teraoka.[45]

The $Co^{II}(\text{amino acid})_2(D_2O)_2$ complexes can in principle adopt various binding configurations. To determine the binding configuration(s) that are actually present in our experiments, we have performed density functional theory (DFT) calculations and simulated the experimental IR and VCD spectra of each of the complexes. For each of the complexes, we find four isomers that differ in the arrangement of the amino acid pairs with respect to the cobalt ion (trans–trans, trans–cis, cis–trans, and cis–cis). Optimized molecular structures and associated energies are given in the Supporting Information. For the proline and the valine complexes, we find that the trans–cis isomer is the one of lowest energy, in agreement with previous calculations on the proline complex.[44] Moreover, the relative energies of the other isomers are such that under our experimental conditions one does not expect them to be present in significant amounts. For the alanine complex, on the other hand, the trans–cis and cis–trans isomers are of similar energies, and one may expect that the experimental spectra contain contributions from both. Comparison of the predicted IR and VCD spectra of the various isomers with the experimentally observed spectra confirms these conclusions.

In Figure 3, we show such a comparison for the trans–cis isomer of the proline and valine complexes as well as for the

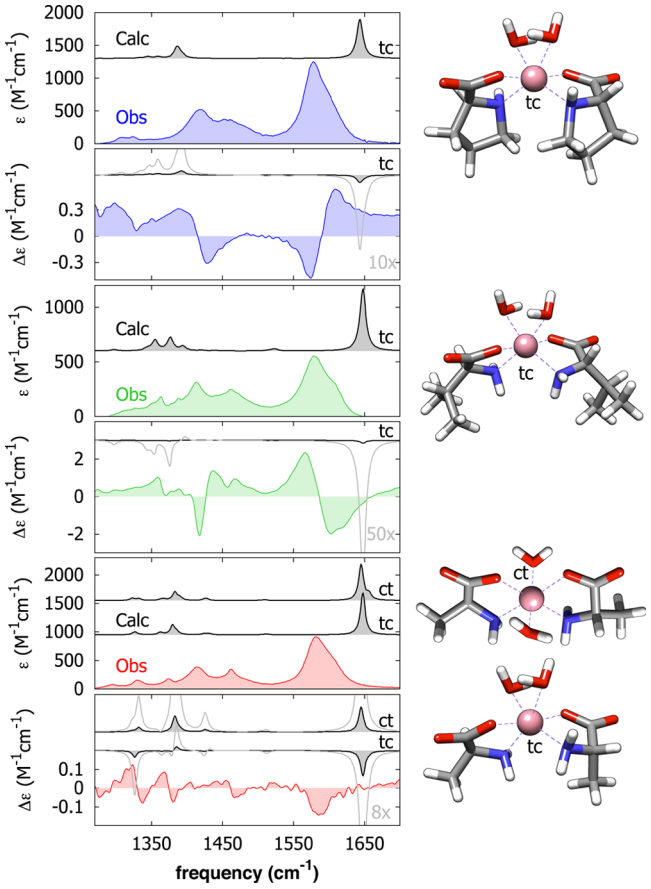

Figure 3. Experimental and calculated IR and VCD spectra of $Co^{II}(Pro)_2(D_2O)_2$ (top panels), $Co^{II}(Val)_2(D_2O)_2$ (center panels), and $Co^{II}(Ala)_2(D_2O)_2$ (lower panels). The calculated spectra (in black), corresponding to the isomer(s) energy minima for each of the complexes have been scaled (in gray) to better show the VCD peaks with weak intensities. DFT optimized structures of the trans–cis (and cis–trans for the alanine complex) isomers are displayed next to the spectra.

trans–cis and cis–trans isomers of the alanine complex, while in the Supporting Information the predicted spectra for other isomers are reported. In the derivation of eq 1, it has been assumed that vibronic energies (that is, electronic plus vibrational energy) can be replaced by electronic excitation energies. For the systems studied in the present work, in which

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the lower electronically excited states have energies comparable to vibrational energies, this clearly is not the case. Equation 1 thus allows us to assess qualitatively the role of the various electronic excited states, but a correct description would require an extension of the theory including correction terms that account for such level of vibronic detail as has been derived by Nafie.[37] One may thus anticipate that compared with the usual results obtained for other molecules, including transition metal complexes without any low-lying electronic states, the calculations will not achieve yet a similar satisfactory degree of agreement, as is indeed observed.[36,46] Nevertheless, for the proline and valine complexes comparison of the experimental spectrum with the predicted spectra for the four isomers favors an assignment to the trans−cis isomer. For the alanine complex, the assignment to a single isomer is much less clear-cut. In fact, from the structure observed in the 1300−1450 cm^{-1} region, one would tend to conclude that the experimental spectrum has contributions from more than one isomer. This observation is in agreement with the calculations that predict similar energies for the trans−cis and the cis−trans isomers.

The experimental VCD spectra of the monomeric and complexed species (Figure 1) show that complexation leads to enhancements of the spectrum by a factor of roughly 8, 10, and 50 for alanine, proline, and valine, respectively.[47] Importantly, when we compare the *absolute* intensities of the bands in the experimental and predicted VCD spectra, as opposed to the comparison of the *relative* intensities that we have done so far, we find that the experimental spectra are enhanced with respect to the predicted spectra by similar factors. This observation thus fully supports the idea that vibronic coupling with low-lying electronic states is at the basis of both enhancements.

Figures 1 and 3 demonstrate that the three amino acid complexes display rather different enhancements. In order to rationalize this behavior, we have performed TD-DFT calculations of the excitation energies and magnetic transition-dipole moments to the lower electronically excited states (see Supporting Information). Interestingly, we find that the excitation energy of the lowest excited state in the valine complex is markedly lower than the excitation energy in the other two amino acid complexes. For the valine complex, one therefore expects larger vibronic couplings and thus larger enhancements of VCD bands, as is indeed observed in the experiments. As yet, it would appear that there is no simple explanation for the differences in excitation energy. It might, however, be a worthwhile subject for further study.

Cobalt Ions and Peptides. Because nearly one-third of all biomolecules contain transition-metal ions, it is interesting to explore the applicability of the observed VCD enhancement to larger biomolecular systems.[48] One particular example, which has been previously reported, involves the coordination of an azide to the heme group of hemoglobin, leading to strong VCD enhancements of the antisymmetric NN stretch.[39] The conformational details of metal-binding pockets of peptides and proteins in solution are often unknown due to the lack of suitable local probes that can assess site-specific geometry. We can locally probe the binding geometry of such systems using the intensity and shape of the amplified VCD signals. Since these signals are highly sensitive to molecular conformation, they can highlight important structural features of the binding sites that may not be detectable with other techniques. A requirement is of course the presence of a transition-metal ion with low-lying electronically excited states. It may be argued that for those systems that lack a suitable transition metal, a metal substitution may distort the binding pocket. However, it has been shown that replacing Zn^{2+} for Co^{2+} often leads to only small changes in the conformation and activity of a protein.[49] In particular, it was shown that carbonic anhydrase retains its enzymatic activity when the native Zn^{2+} ion is replaced by Co^{2+}.[50] As a first step in this direction, we show in the following that one can retrieve the coordination geometries of larger molecular systems (di- and tripeptides) on the basis of the intensity and shape of the enhanced VCD spectral signatures.

Figure 4 displays IR (a) and VCD (b) spectra of bare Val-Val (black line) and Val-Val complexed to Co^{2+} (green line). Figure

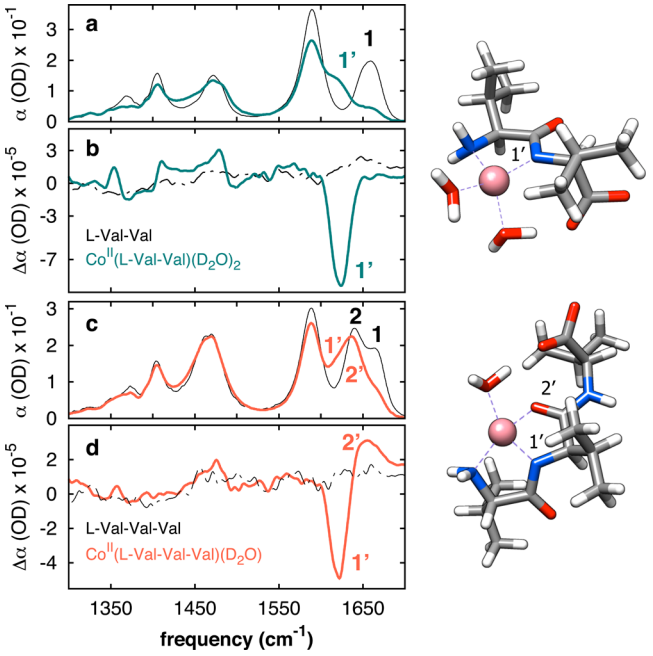

Figure 4. FTIR (a) and VCD (b) spectra of L-Val-Val (black lines) and Co^{II}(L-Val-Val)$(D_2O)_2$ (green lines) and FTIR (c) and VCD (d) of L-Val-Val-Val (black lines) and Co^{II}(L-Val-Val-Val)(D_2O) (red lines). The numbered IR bands in panels a and c correspond to the VCD bands in panels b and d, respectively. The numbered moieties in the molecular structures of Co^{2+} bound valine dipeptide (high-spin) and Co^{2+} bound valine tripeptide (high-spin) correspond to the numbered peaks in the VCD spectra.

4a shows that upon complexation the band at 1650 cm^{-1} disappears while a new band comes up at 1610 cm^{-1}. This red shift can be explained by the deprotonation of the amide nitrogen and the subsequent coordination of this nitrogen atom to the metal.[51] From the IR spectrum, one can conclude that the carboxylate group (1580 cm^{-1}) does not participate in the binding since no shift is observed for its C═O-stretch vibration. The binding thus occurs through the deprotonated amide nitrogen, the basic N-terminus and two water molecules forming a distorted tetrahedral (high-spin, see Supporting Information) configuration (Figure 4, top right).

Comparison of the signal intensities of the salient bands in the VCD spectra of the bare and complexed dipeptide (Figure 4b) readily leads to the conclusion that in the complex VCD bands are significantly amplified. Interestingly, however, we find that this amplification is strongly mode dependent. This holds in particular for the amide I mode, which in the complex is enhanced by at least an order of magnitude more than the other modes. The VCD spectra thus give evidence for selective amplification of vibrational modes and thereby demonstrate the

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potential of the method to zoom in on local details within a much larger, complex molecular system.

To investigate the spatial range of the ion-induced enhancement, we performed VCD measurements on a tripeptide (Val-Val-Val). In this case, two amide groups of the backbone can coordinate to the metal ion. In Figure 4c,d, we show IR and VCD spectra of the unbound tripeptide (black lines) and the Co^{2+} bound tripeptide (red lines), respectively. In the amide I frequency range (1650 cm^{-1}), two amide I modes labeled 1 and 2 can be observed. Mode 1 is at a higher frequency and is assigned to the amide group closer to the N terminus of the tripeptide.[52] From the IR spectra, it can be concluded that the two amide moieties do not participate equally in the binding. Upon coordination, the amide I band 1 changes into band 1′, and is thus red-shifted by approximately 40 cm^{-1}, while amide I band 2′ is only shifted by 3 cm^{-1} from band 2. We thus conclude that the amide moiety 1′ is strongly bound to Co^{2+} (as in the dipeptide), while the non-deprotonated amide functionality 2′ is only weakly bound. Previous studies[53] on Cu^{II}-tripeptides have shown that for pH values between 7 and 9 these systems adopt a configuration in which one of the amide groups has a deprotonated nitrogen that is strongly bound to the metal, while the other non-deprotonated amide group is weakly bound through the amide oxygen (see Figure 4, lower right). For the Co^{II}-tripeptide considered here, detailed information on the conformation of the binding pocket can be obtained from the analysis of the VCD spectra and in particular from the intensities of the two amide I bands. The two red-shifted amide I bands 1′ and 2′ give rise to a negative and a positive band, respectively, in the VCD spectra. It is striking to observe that the intensity amplification of these two bands is markedly different, with a much larger enhancement for **1**′ than for **2**′ (Figure 4d). This observation confirms that **1** has a much stronger interaction with the metal ion, and thus corresponds to the C═O-stretch vibration of the anionic amido moiety bound directly to Co^{2+} via its nitrogen atom, while **2** corresponds to the C═O-stretch vibration of the weaker bound neutral amide group, in agreement with the frequency shifts of these bands and with conclusions drawn previously for the Cu^{II} tripeptides.[53]

CONCLUSIONS

The present study has demonstrated that the signal intensities in a VCD spectrum can be enhanced up to 2 orders of magnitude provided there is an electronic manifold with low-lying electronic states that can be coupled to the molecule of interest. We have shown that VCD spectra of amino acids and peptides in water, which in the past have been notoriously difficult to obtain, become readily accessible with unprecedented signal-to-noise ratios by coupling the amino acid or peptide to an open-shell transition metal ion. Our studies on di- and tripeptides demonstrate unambiguously that the enhancement of the VCD signal intensities is strongly localized, paving the way for its use as a probe of local structure in larger biomolecules. Our experimental results demonstrate the necessity to implement correction terms to obtain agreement between calculated and measured enhanced VCD for systems with low-lying electronically excited states. Many biomolecular systems have open-shell transition metal ions as part of their structure. Due to their low-lying electronically excited states, these ions will amplify the VCD signals of surrounding functional groups. The same strategy can be employed to study biomolecules containing closed-shell metals such as zinc-containing biological complexes, simply by substituting Zn^{II} for Co^{II}. VCD can therefore be used as a highly sensitive and site-specific structural probe to determine conformational details of binding pockets in biological systems.

EXPERIMENTAL SECTION

Experimental Methods. All samples were prepared in deuterium oxide (D_2O) with concentrations ranging from 5 to 40 mM. The solutions were prepared under inert conditions and inserted in sealed infrared cells with 3 mm thick CaF_2 windows separated by a 50 μm Teflon spacer. Fourier-transform infrared (FTIR) and VCD spectra (with spectral resolution of 2 and 4 cm^{-1}, respectively) were obtained with a Bruker Vertex 70 spectrometer in combination with a PMA 50 module. The photoelastic modulator (PEM) was set to a center frequency of 1500 cm^{-1} for quarter-wave retardation. All spectra were corrected by the spectra of dry D_2O. Room temperature magnetic susceptibility measurements were performed on packed solid samples with a Sherwood Scientific MK 1 magnetic susceptibility balance.

Theoretical Methods. Density functional theory (DFT) calculations were carried out with Gaussian 09.[54] Ground-state geometry optimizations and harmonic vibrational frequencies were computed using the B3LYP hybrid functional, which includes the Becke three-parameter exchange[55] and the Lee, Yang, and Parr correlation functionals.[56] Based on the work of He et al.,[36] we have chosen the LANL2DZ effective core potential (ECP) for Co, whereas the 6-31+G(d) basis set was used for all other elements. In principle other basis sets for the metal might improve the description of the electronic structure, but we have not further investigated this aspect. A combined implicit−explicit solvent model has been employed, including two water molecules in addition to a polarizable continuum model (PCM). Excitation energies and electronic magnetic transition-dipole moments were calculated using time-dependent DFT.

ASSOCIATED CONTENT

Supporting Information

A detailed description of the syntheses and DFT results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(12) It should be noted that NMR is intrinsically not sensitive to the absolute configuration of a chiral molecule and requires chiral additives. In addition, if the exchange between conformations is faster than the NMR time scale (1 ms), the peaks of the different conformers coalesce in the observed NMR spectrum, and the structural information about the individual conformations is lost. In the vibrational spectrum, the difference between the resonance frequencies is much larger than that in the NMR spectrum, corresponding to a time scale of a few picoseconds. Hence, any exchange taking place more slowly than this time scale is not averaged out in the VCD spectrum, and the exchanging conformers appear as separate peaks or sets of peaks. In many cases paramagnetic NMR is impossible because of too fast nuclear spin relaxation behavior, caused by the paramagnetic metal ion, leading to extremely broad and hence often useless NMR spectra. Only in cases where the lines remain relatively sharp, paramagnetic NMR is useful. VCD is not limited to such cases.
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