Fluorescence Spectroscopy of Jet-Cooled Tryptophan Peptides

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Abstract: Laser desorption has been used to seed several tryptophan di- and tripeptides into a supersonic expansion. Moderately high resolution fluorescence excitation spectra have been taken which complement previous photoionization studies and aid in the identification of different ground-state conformations. Dispersed fluorescence spectra of individual conformers have also been recorded. These spectra reveal that in certain conformers strong perturbations to the indole chromophore exist which result in very broad and red-shifted emission from the excited electronic state. Other conformers, in contrast, show structured, resonance fluorescence as do many simpler indole derivatives under similar conditions. The existence of conformers with broad emission spectra appears to require the presence of an intramolecular hydrogen bond in the peptide backbone, which is postulated to result in a large backbone dipole moment.

As one of the few aromatic amino acids, tryptophan plays a fundamental role in the near-ultraviolet spectroscopy of peptides and proteins. Of particular interest is the sensitivity of the emission from an excited tryptophyl residue to its local environment. Recent investigations from this laboratory have employed the tryptophyl chromophore to study the electronic spectroscopy of several di- and tripeptides in a supersonic molecular beam.[1] These spectra clearly revealed that a number of stable ground-state peptide conformers were present; however, since only photoionization excitation spectra were recorded, no conclusions could be drawn concerning their emissive properties. The present study directly addresses this question by characterizing the excited-state emission spectra of individual conformers of several different small tryptophan-containing peptides.

The work on these small peptides expands on earlier spectroscopic investigations by Rizzo et al. on jet-cooled tryptophan.[2,3] In tryptophan, several ground-state conformers were also identified and were determined to be noninterconverting on the time scale of the excited-state lifetime. The majority of these conformers were found to display strong, structured resonance fluorescence, which essentially mirrored their excitation spectra. However, the dispersed fluorescence spectrum of one particular conformer was found to contain a very substantial broad and red-shifted component.

The different types of emission exhibited by the different isolated tryptophan conformers is reminiscent of the behavior of many simpler indole derivatives in solvents of varying polarity. In polar solvents, the emission from these excited indole derivatives is substantially red-shifted from the emission observed in a nonpolar medium. Several mechanisms have been suggested to account for this effect. One ascribes the red-shifted fluorescence to an exciplex formed between the excited-state indole and a polar solvent molecule.[4] Others are based on the fact that the lowest electronic absorption band of indole is actually composed of two nearly degenerate transitions to the excited L_b and L_a states. Following excitation of the L_b state in a polar solvent, solvent-solute relaxation may invert the L_a and L_b levels, eventually leading to emission from an excited L_a state.[5] This emission should exhibit a large Stokes shift due to the reoriented solvent dipoles. An alternative explanation proposes that, in a polar solvent, emission actually occurs from an excited electronic state having substantial charge-transfer character and parentage in the usual L_a state.[6]

In the absence of a polar solvent, the red-shifted emission seen in one of isolated tryptophan conformers requires an alternative explanation. By comparison to the fluorescence spectra of several jet-cooled tryptophan derivatives, Rizzo et al. proposed a model based on the formation of an intramolecular exciplex in the one suitable tryptophan conformer.[3] This exciplex was believed to arise from an interaction between the excited indole ring system and the charge distribution on the amino acid backbone that resulted following proton transfer and zwitterion formation in the excited electronic state. In parallel with our work on the fluorescence spectroscopy of tryptophan peptides, we have also extended the studies on tryptophan derivatives to include, in particular, various tryptophan amides.[7] From these studies, it appears that, instead of relying on excited-state zwitterion formation, the conformer responsible for the exciplex-like emission contains an intramolecular hydrogen bond between the carboxylic acid proton and the amine nitrogen.

Interestingly, the analogous conformation of the amino acid glycine has recently been identified from its microwave spectrum where it was found to have a large dipole moment, exceeding 4.5 D.[8] Thus, the broad red-shifted emission may arise from a dipole-induced perturbation to the indole π electron system which originates on a particular hydrogen-bonded conformation of the amino acid backbone.

By studying the fluorescence spectra of the different conformers of Trp-Gly, Gly-Trp, and Trp-Gly-Gly, the ideas formulated for tryptophan and its derivatives can be tested on more complex systems. The hydrogen-bonded backbone model has some interesting implications for the di- and tripeptides. While an amino acid backbone contains only one hydrogen bond donor, the carboxylic acid group, a dipeptide backbone also includes an amide group that may function as a second potential hydrogen bond donor. Although a hydrogen bond with the acid proton would seem to correlate most naturally to the glycine conformer, an amide hydrogen-bonded species is structurally more similar, as illustrated in Figure 1, and could reasonably be expected to have a similarly large backbone dipole moment. To isolate the effects from these different species, derivatives of Trp-Gly and Gly-Trp that lack the carboxylic acid group, and hence can only hydrogen bond with an amide proton, have also been investigated.

Experimental Section

As in our earlier multiphoton ionization work,[1] the tryptophan peptides were seeded into a supersonic expansion of helium by using a laser vaporization technique. Thermal vaporization in a conventional oven could not be used since these compounds decompose at temperatures lower than those required to produce sufficient vapor pressure of the parent compound. The laser desorption source was built around a pulsed valve operated with gas pulse durations of approximately 800 μs and a backing

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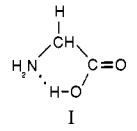

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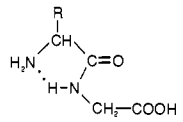

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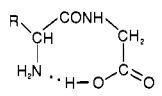

III

Figure 1. Comparison of the structure of the hydrogen-bonded conformer of glycine,[8] I, with possible hydrogen-bonded structures of the backbone of a dipeptide. Two schematic structures are feasible for a dipeptide such as Trp-Gly, R = CH_2-indole, and are illustrated by II and III. Structure II contains an amide–amine hydrogen bond, while in III a carboxylic acid–amine hydrogen bond is present.

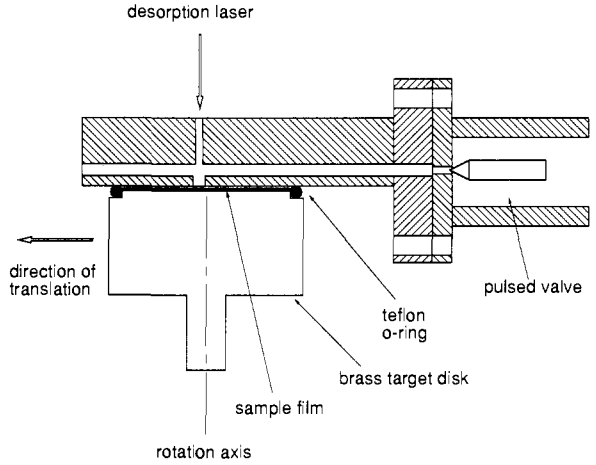

Figure 2. Detailed view of the laser desorption nozzle. The pulsed valve discharges He into a 2-mm-diameter 2-in.-long gas channel which is crossed at right angles by a 1-mm-diameter channel through which the desorption laser is focused. The laser is incident on a thin (0.01-mm) sample film which has been evaporated from a methanol solution onto a brass target disk. This disk is sealed against the nozzle body with a Teflon O-ring and is simultaneously translated and rotated so that each desorption event occurs from a fresh area of the film.

pressure of 11 atm of He. A pulsed excimer pumped dye laser[9] was synchronized with the pulsed valve. The visible (530-nm) output of the dye laser was focused into the source and onto a thin sample film from which desorption into the carrier gas occurred. Sample films were deposited onto a brass target by evaporation of methanol solutions containing the desired peptide. Typical desorption conditions employed a laser pulse energy of 0.1 mJ in a pulse width of approximately 10 ns, focused onto the sample film by a 25-cm $f/1$ lens to yield an intensity on the order of 5×10^7 W/cm^2. Such conditions were found to readily vaporize these thermally sensitive compounds with minimal fragmentation. The basic design of the nozzle is illustrated in Figure 2. A more detailed description can be found in ref 1.

Once seeded with peptide, the He carrier pulse expanded supersonically from the desorption nozzle into a vacuum chamber pumped by a 4000 L/s oil vapor booster pump. The free expansion was crossed 3-cm downstream of the nozzle by the frequency-doubled output of a tunable, Nd:YAG-pumped, pulsed dye laser. Both the total and dispersed fluorescence could be monitored simultaneously from the resonantly excited molecules. The total fluorescence was collected by an $f/1.0$ lens and focused through a variable slit onto a photomultiplier tube. Dispersed fluorescence was also by collected by an $f/1.0$ lens and focused onto the entrance slit of a 1-m monochromator. Details of the fluorescence collection optics and data acquisition electronics have been discussed previously.[10]

(9) The excimer pumped dye laser was used only for convenience, and the doubled output of a Nd:YAG laser has proven equally effective for the desorption process.

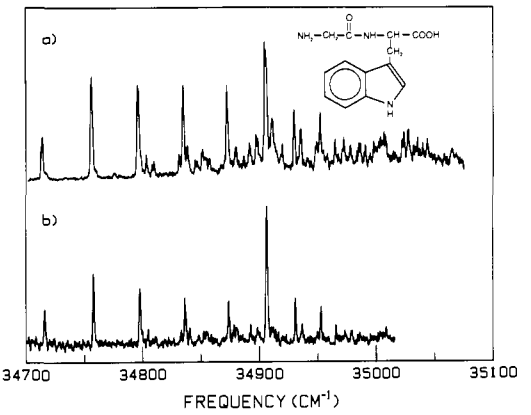

Figure 3. Excitation spectra of Gly-Trp: (a) mass selected resonant two-photon ionization spectrum from ref 1; (b) fluorescence excitation spectrum from the current work. The fluorescence excitation spectrum was taken through a monochromator tuned to 33 030 cm^{-1} and having a spectral band-pass of 100 cm^{-1}.

Initial attempts to study the fluorescence properties of these peptides were aimed at measuring fluorescence excitation spectra by collecting the total undispersed emission and monitoring its intensity as a function of the incident laser frequency. These experiments were made difficult by a significant component of scattered laser light which could mask many of the weaker features in the spectrum. Since scattering could only be seen when the desorption laser was in use, it must have originated from the vaporization process. The most reasonable explanation is that the desorption laser not only vaporizes neutral parent species but additionally removes larger particles from the film surface that also become entrained in the He carrier gas pulse. This particulate matter efficiently scatters the excitation laser, thereby obscuring weaker features of the intrinsic monomer fluorescence. Previous work on the peptides using ionization techniques was completely insensitive to the presence of these particles since mass spectrometric detection was employed. Since some velocity slip between the desorbed peptides and the larger particles was present, careful adjustment of the timing of the excitation laser to a 1-μs observation window could minimize particulate scattering but very seldom to an acceptable level.

For this reason, an alternative approach to measure fluorescence excitation spectra was devised. By filtering the combination of laser scatter and monomer fluorescence through a monochromator prior to detection, it was possible to greatly reduce the scattering interference. Of course, any resonance emission coincident with the laser frequency was also lost. This effect will skew an excitation spectrum by the probability that the excited feature has for fluorescing in the wavelength region selected by the monochromator. Attempts can be made to minimize this effect by using large spectral band-passes on the monochromator. The three tryptophan peptides used in this study were all commercially available. The two dipeptide derivatives, tryptophanmethylamide and glycyltryptamine, were synthesized from the corresponding *Z*-protected amino acid nitrophenyl ester and the appropriate amine.[11] The *Z* protecting group was then removed by catalytic-transfer hydrogenation from cyclohexene.[12]

Results

Although many indole derivatives have been shown to have rich vibronic structures in their first electronic transitions,[13,14] the present investigation has focused on only the lowest 600 cm^{-1} of the analogous transition in the tryptophan peptides. In this wavelength region, most rigid dipole derivatives show only a very

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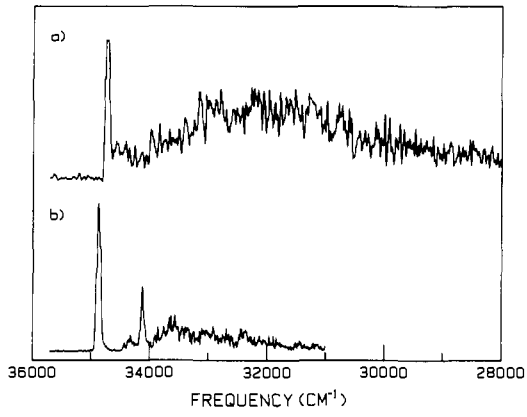

Figure 4. Dispersed fluorescence spectra of Gly-Trp obtained by exciting the features at (a) 34 758 cm^{-1} and (b) 34 907 cm^{-1}. Both spectra were recorded with a resolution of 75 cm^{-1}. The sharp resonance line in (a) is composed entirely of scattered laser light while in (b) the resonance line is primarily molecular fluorescence with less than a 10% contribution from scattered light.

strong origin transition and possibly several much weaker vibronic transitions. The electronic spectra of the various tryptophan peptides are, in contrast, very complex and congested due to the presence of multiple ground-state conformations and the introduction of very low frequency vibrational or torsional modes in the peptide backbone.

Gly-Trp. In Figure 3, the fluorescence excitation spectrum and the resonant two-photon ionization spectrum (from ref 1) of the dipeptide Gly-Trp are compared. The fluorescence excitation scan was made by filtering the total emission through a monochromator having a band-pass of 100 cm^{-1} centered at 33 030 cm^{-1}. Features from two conformers previously identified in the photoionization work are clearly visible in the fluorescence excitation spectrum. Five members of a 40-cm^{-1} vibrational progression beginning at 34 716 cm^{-1} are associated with one conformer, and the peak at 34 907 cm^{-1} originates on a second ground-state conformer. In the previous study, it was necessary to resort to power saturation techniques to distinguish between the two conformers; however, the much larger discrepancies in intensity seen with fluorescence excitation now make that assignment obvious. At frequencies greater than 34 907 cm^{-1}, many of the features seen in the photoionization spectrum are absent, yet two fairly strong features at 34 931 and 34 953 cm^{-1} are still present. Although not shown, poorer quality excitation spectra can also be taken without the use of the filtering monochromator. These spectra, which show considerable scattered light contamination, are qualitatively similar to Figure 3, particularly with regard to the relative intensities of the peaks that correspond to different conformers. This then indicates, in comparison with the photoionization spectrum, that the overall fluorescence quantum yields of the two conformers must be fairly similar.

The upper spectrum in Figure 4 displays the dispersed emission observed upon exciting the second member in the 40-cm^{-1} progression, at 34 758 cm^{-1}, of the conformer having the lower enegy transition. This spectrum was taken with the slits of the monochromator adjusted for 75-cm^{-1} resolution. The spectrum shows a sharp peak at the exciting frequency and a broad, structureless band peaking nearly 3000 cm^{-1} to the red. The sharp resonant peak was found to be composed entirely of scattered laser light, implying that the excited-state fluorescence from this particular conformer is completely structureless and dramatically red-shifted. In contrast, the lower section of Figure 4 shows the dispersed emission spectrum that arises from excitation of the strongest feature in the excitation spectrum, at 34 907 cm^{-1}, which belongs to a second conformer, under identical experimental conditions. In this case, the spectrum shows strong resonance emission, with the feature coincident with the exciting line dominated by resonance fluorescence. The contribution of scattered light to this feature is less than 10%. Fluorescence and scatter are readily distinguished by tuning the exciting laser off of the resonant

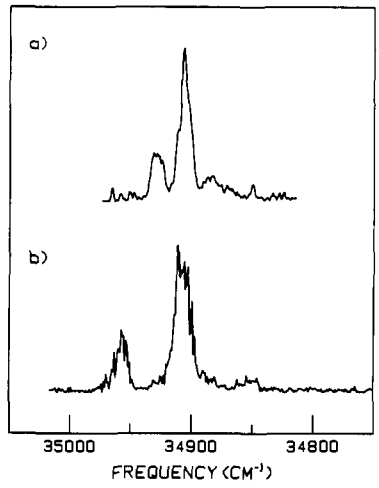

Figure 5. Higher resolution dispersed fluorescence spectra of Gly-Trp obtained by exciting the features at (a) 34 931 cm^{-1} and (b) 34 953 cm^{-1}. The spectrum in (a) was taken with 10-cm^{-1} resolution and in (b) with 12-cm^{-1} resolution. In both cases, the strongest emission is red-shifted from the resonance line and corresponds to a $v'' = 1$ to $v' = 1$ vibronic transition, thus confirming the assignment of the 34 931 cm^{-1} feature as a 29-cm^{-1} vibration and the 34 953-cm^{-1} feature as a 51-cm^{-1} vibration both built off the origin transition at 34 907 cm^{-1} for this conformer.

transition. A second sharp peak is seen shifted approximately 760 cm^{-1} from the excitation frequency, corresponding to a transition terminating on an excited vibrational level in the ground electronic state which is localized on the rigid indole system and best characterized as a ring-breathing mode.[15] Very little emission is seen shifted more than 3000 cm^{-1} from the excitation line, and at this spectral resolution, the entire spectrum looks very similar to that of indole itself.[14] Thus, while excitation spectra seem to indicate that the fluorescence quantum yields for the two different conformers are similar, the distribution of the emission as a function of wavelength is very different.

Dispersed fluorescence from the two peaks in the excitation spectrum at 34 931 and 34 953 cm^{-1} has also been observed with 75-cm^{-1} resolution and found to be dominated in both cases by resonance emission. Higher resolution spectra allow unambiguous assignments to be made for these two peaks. The upper trace in Figure 5 shows the dispersed emission from the 34 931-cm^{-1} feature taken with 10-cm^{-1} resolution, and the lower trace shows the analogous results for the 34 953-cm^{-1} peak. In both cases, excitation is coincident with the higher frequency feature in the spectrum, while the strongest emission is shifted 22 and 49 cm^{-1} to the red. This behavior suggests that the 34 931- and 34 953-cm^{-1} peaks arise from excitation of 24- and 46-cm^{-1} excited-state vibrations of the conformer having its origin transition at 34 907 cm^{-1}. The strong peaks in the emission spectra then occur for transitions characterized by no change in vibrational quantum numbers and must terminate on the corresponding ground-state fundamental vibrations having frequencies of approximately 22 and 49 cm^{-1}.

Trp-Gly. Figure 6 compares the photoionization spectrum and the fluorescence excitation spectrum of the dipeptide Trp-Gly. The fluorescence excitation scan was made by filtering the total emission through a monochromator tuned to 32 790 cm^{-1} and having a spectral band-pass of 250 cm^{-1}. All features seen in the photoionization spectrum are reproduced in the fluorescence excitation spectrum. The most noticeable difference between the two occurs for the intensities of two peaks at 34 948 and 34 973 cm^{-1}, which appear much stronger in the fluorescence detected spectrum. Power saturation techniques could not be used to identify the different conformer contributions to the photoionization spectrum, so conclusions could only be drawn by attempts

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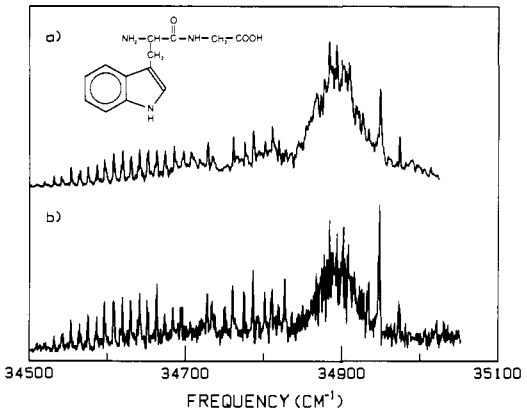

Figure 6. Excitation spectra of Trp-Gly: (a) mass-selected resonant two-photon ionization spectrum from ref 1; (b) fluorescence excitation spectrum from the current work. The fluorescence excitation spectrum was taken through a monochromator tuned to 32 790 cm^{-1} and having a spectral band-pass of 250 cm^{-1}.

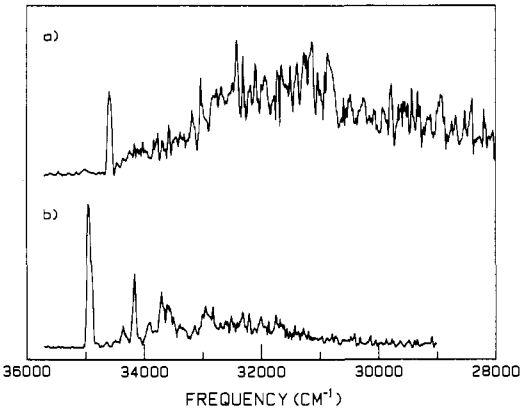

Figure 7. Dispersed fluorescence spectra of Trp-Gly obtained by exciting the features at (a) 34 587 cm^{-1} and (b) 34 948 cm^{-1}. Both spectra were recorded with a resolution of 75 cm^{-1}. The sharp resonance line in (a) is composed entirely of scattered laser light, while in (b) the resonance line is dominated by molecular fluorescence.

at spectral assignment. At lower frequencies in the spectrum, a long series of peaks separated by 11 cm^{-1} was attributed to a vibrational progression of a single ground-state conformer. The broad feature at 34 900 cm^{-1} was believed to arise from the overlapped origin transitions of a number of different conformers. By utilizing fluorescence detection, we now have the first piece of direct evidence for yet another Trp-Gly conformer, which appears to have appreciably different emission properties from the rest.

The dispersed fluorescence spectra in Figure 7 display the different radiative properties of two different Trp-Gly conformers. The upper spectrum is observed following excitation at 34 587 cm^{-1}, resonant with one of the members of the extended 11-cm^{-1} vibrational progression associated with the conformer exhibiting the lowest transition frequency. Fluorescence from this species contains virtually no resonant component (the sharp peak at the excitation line is dominated by scatter) but is very broad with a maximum shifted to the red by approximately 3000 cm^{-1}. In contrast to this behavior is the emission from the species responsible for the peak at 34 948 cm^{-1} in the excitation spectrum, as shown in the lower trace of Figure 7. Here the spectrum is dominated by very strong resonance emission, coincident with the excitation frequency. Also present are several weaker, but sharp, vibronic features, corresponding to transitions terminating on excited ring vibrational levels in the ground electronic state. A 760-cm^{-1} vibronic peak is particularly noticeable, just as in Figure 4. Both scans in Figure 7 were obtained at a spectral resolution of 75 cm^{-1}.

The above results clearly identify two distinct conformers in the Trp-Gly excitation spectrum. However, other features are also seen, such as the broad absorption peak centered near 34 900

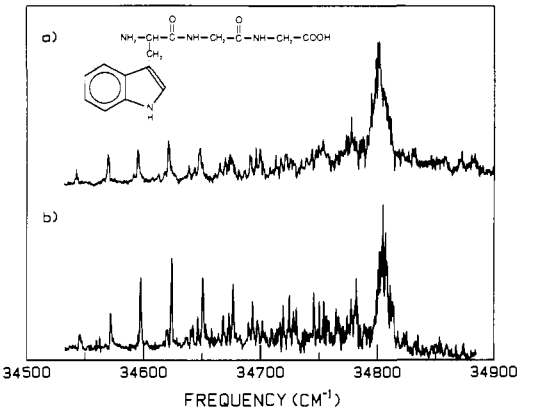

Figure 8. Excitation spectra of Trp-Gly-Gly: (a) mass-selected resonant two-photon ionization spectrum from ref 1; (b) fluorescence excitation spectrum from the current work. The fluorescence excitation spectrum was taken through a monochromator tuned to 33 060 cm^{-1} and having a spectral band-pass of 250 cm^{-1}.

cm^{-1} and a group of sharp peaks occurring between 34 720 and 34 840 cm^{-1} that do not appear to be part of the 11-cm^{-1} progression of the previously mentioned conformer. Dispersed emission from all of these features has been found to be very broad and red-shifted, with no resonance component, and therefore quite similar to the upper trace of Figure 7.

At low resolution, 75 cm^{-1}, the dispersed emission from the peak at 34 973 cm^{-1}, which was also noted to have an increased intensity in the fluorescence detected excitation spectrum, appears to be very similar to the lower trace in Figure 7 and therefore is composed primarily of sharp resonance emission. With 15-cm^{-1} spectral resolution, the dominant component of the resonance fluorescence is actually found to be shifted 25 cm^{-1} to the red of the excitation line, similar to the behavior of the resonant emitting conformer of Gly-Trp illustrated in Figure 5. The interpretation of the 34 973-cm^{-1} line in the excitation spectrum is then that this feature corresponds to an excited 25-cm^{-1} vibronic transition built off the stronger 34 948-cm^{-1} origin transition of the resonantly emitting conformer. The ground-state analogue of this vibrational mode also appears to have a vibrational frequency of 25 cm^{-1}. Just as with Gly-Trp, only one conformer is found in the supersonic expansion, which shows strong resonance emission.

Trp-Gly-Gly. Figure 8 compares the photoionization and fluorescence excitation spectra of the tripeptide Trp-Gly-Gly. Fluorescence excitation was obtained by monitoring a 250-cm^{-1} band-pass of the total fluorescence, centered at 33 060 cm^{-1}. No dramatic differences between the two spectra are apparent. Both contain a 10-cm^{-1} broad, unresolved peak at 34 800 cm^{-1} and a series of sharp, resolved features to the red. The sharp structure has previously been assigned to one specific ground-state conformer and can easily be explained in terms of several long vibrational progressions in a 27-cm^{-1} mode, built off an origin transition at 34 546 cm^{-1} and in combination with three fundamental vibrations having frequencies of 97, 102, and 147 cm^{-1}.

Following excitation of the broad peak at 34 800 cm^{-1}, the fluorescence spectrum, shown as the lower curve in Figure 9, is observed with a spectral resolution of 75 cm^{-1}. The emission is very broad with a maximum approximately 3000 cm^{-1} from the excitation frequency. In this spectrum, laser scatter has fortuitously been eliminated, allowing a direct observation of the complete lack of resonance emission from this particular conformer. The upper trace in Figure 9 displays the emission that is observed from excitation of the third member of the main 27-cm^{-1} vibrational progression of the conformer showing the structured excitation spectrum. Also taken with 75-cm^{-1} resolution, this spectrum again shows only broad, red-shifted emission. The sharp peak coincident with the excitation frequency is composed entirely of scatter. Similar fluorescence spectra are observed following excitation of other peaks in the vibrational progression. Much like Trp-Gly, the fluorescence spectra of Trp-Gly-Gly, from both a broad unstructured feature and from a vibrationally active

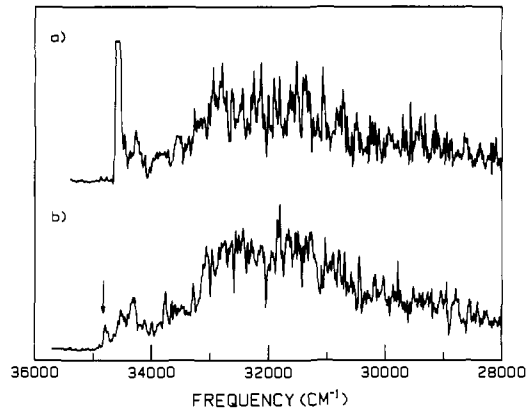

Figure 9. Dispersed fluorescence spectra of Trp-Gly-Gly obtained by exciting the features at (a) 34 598 cm^{-1} and (b) 34 804 cm^{-1}. Both spectra were recorded with 75-cm^{-1} resolution. The sharp resonance line in (a) is composed entirely of scattered laser light. In spectrum (b), this scatter has been fortuitously minimized, marked with an arrow, and therefore places an upper bound on the intensity of the resonance emission. Even this small feature was measured to consist primarily of scattered light.

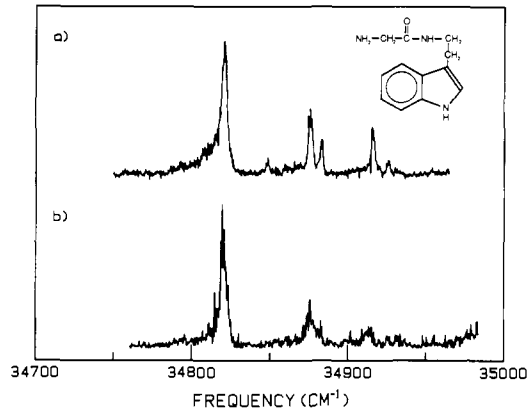

Figure 10. Excitation spectra of glycyltryptamine: (a) mass-selected resonant two-photon ionization spectrum; (b) fluorescence excitation spectrum. The photoionization spectrum was recorded under conditions similar to those used in ref 1, detecting only the parent ion mass. The fluorescence excitation spectrum was taken through a monochromator tuned to 33 700 cm^{-1} and having a spectral band-pass of 100 cm^{-1}.

conformer having a lower transition frequency, are composed entirely of red-shifted emission. Unlike the dipeptide, Trp-Gly-Gly shows no features in its excitation spectrum that, when excited, yield sharp resonance emission. In Trp-Gly, only one conformer with this property was found, and it appears that in the larger tripeptide the analogue of this conformer is not formed under the conditions found in the supersonic expansion.

Glycyltryptamine. Glycyltryptamine is a derivative of the dipeptide Gly-Trp where the carboxylic acid group has been replaced by a hydrogen atom. Analysis of its optical spectrum should allow conclusions to be drawn on how the acid group affects both the conformer distribution and the excited-state emission properties. Work on several tryptophan derivatives suggests that hydrogen bond formation plays a key role in determining whether an isolated molecule will exhibit red-shifted emission.[7] Since dipeptides have two potential hydrogen bond donors, the acid and amide protons, glycyltryptamine will still be able to form an intramolecular hydrogen bond, but only between the amide and the amine group.

Figure 10 displays the resonant two-photon ionization spectrum of glycyltryptamine and compares it to a fluorescence excitation spectrum. The photoionization spectrum was taken with a linear time-of-flight mass spectrometer detecting only the parent ion, under similar conditions to the other photoionization spectra described in ref 1. Fluorescence excitation was done with the monochromator adjusted for a spectral band-pass of 100 cm^{-1}

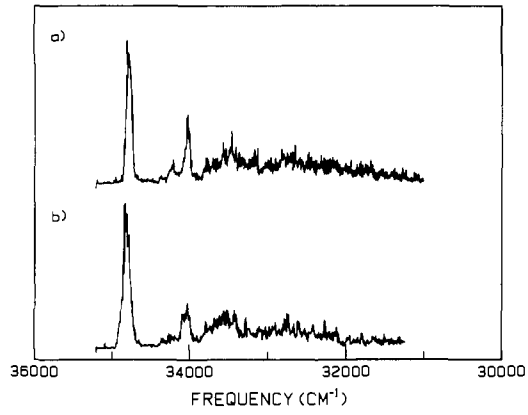

Figure 11. Dispersed fluorescence spectra of glycyltryptamine obtained by exciting the features at (a) 34 820 cm^{-1} and (b) 34 876 cm^{-1}. Spectrum (a) was recorded with a 75-cm^{-1} resolution, while spectrum (b) was taken at a resolution of 100 cm^{-1}. In both cases, the sharp resonance line is dominated by molecular fluorescence.

centered at 33 700 cm^{-1}. The spectra in Figure 10 are considerably different than the excitation spectrum of Gly-Trp, Figure 3. The spectrum of this derivative is much simpler than that of the dipeptide and appears to be composed of a strong origin transition, at 34 820 cm^{-1}, with several weaker vibronic transitions. Power saturation studies support this assignment. No obvious vibrational progressions are evident, in contrast to the one Gly-Trp conformer which showed a long progression in a 40-cm^{-1} mode.

To determine the effect of the missing carboxylic group on the exciplex-like interaction seen in the dipeptide, dispersed fluorescence spectra have been taken following excitation of the strong 34 820-cm^{-1} feature as well as of the weaker peak at 34 876 cm^{-1}. These spectra, shown in Figure 11, both display strong resonance fluorescence, and no evidence is found for any of the broad red-shifted emission seen in one of the Gly-Trp conformers. The strongest line in both spectra is dominated by resonance fluorescence. Both spectra also display a sharp feature shifted 760 cm^{-1} from the excitation line, characteristic of a vibrational mode localized on the rigid indole ring.[15] The carboxylic acid group in Gly-Trp thus seems to be an essential element in promoting the red-shifted emission from the electronically excited indole.

Tryptophanmethylamide. An analogous derivative can be made from the dipeptide Trp-Gly by removal of the carboxylic acid group to form tryptophanmethylamide. Unlike all of the molecules mentioned thus far, it is possible to vaporize tryptophanmethylamide in a conventional oven without thermal decomposition. For this reason, a heated continuous wave (cw) nozzle with a 100-μm pinhole and 4 atm of He backing pressure was used to form the supersonic expansion, rather than the pulsed laser desorption source. An advantage of this oven source is that laser scatter from ablated particles is not present, allowing fluorescence excitation spectra to be taken directly, rather than relying on a monochromator to filter out the interfering scatter.

In Figure 12, two fluorescence excitation spectra of tryptophanmethylamide are displayed. The spectra were recorded simultaneously on two separate photomultiplier tubes, but with each detecting different types of emission. The upper trace was recorded by collecting the unfiltered total fluorescence emitted by the resonantly excited molecules as a function of excitation frequency, while the lower spectrum was made by filtering the emission through a monochromator with a 150-cm^{-1} band-pass tuned to and scanning synchronously with the laser frequency. Thus, the upper spectrum is insensitive to the wavelength region where the excited molecules fluoresce, while in the lower trace only resonance emission (and weak laser scatter) is detected. The total fluorescence detected excitation spectrum is expected to be the same as what would be observed in a photoionization experiment if the quantum yield remains constant among different conformations and at different levels of vibrational excitation, and this has also been experimentally verified.

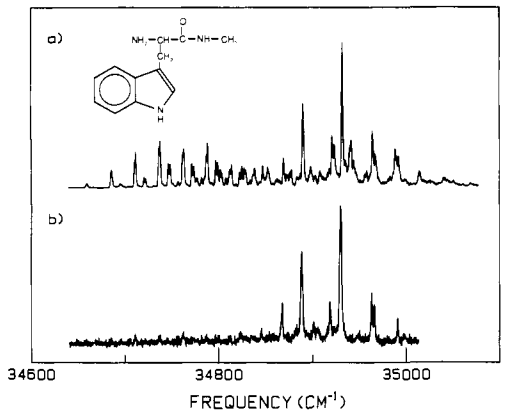

Figure 12. Fluorescence excitation spectra of tryptophanmethylamide monitoring (a) total undispersed fluorescence and (b) resonance fluorescence. Spectrum (b) was recorded by detecting fluorescence through a monochromator with a 150-cm^{-1} band-pass which was scanned synchronously with the frequency of the exciting laser.

In contrast to glycyltryptamine, the spectrum of this dipeptide derivative is quite complex, showing many characteristics of the spectrum of the parent compound. The spectrum originates at 34 660 cm^{-1} and is followed by a long progression in a 26-cm^{-1} vibrational mode. Several other progressions appear in combination with this 26-cm^{-1} mode; one that is particularly noticeable is built off of a 36-cm^{-1} fundamental. Between 34 850 and 35 000 cm^{-1}, several intense peaks are seen that do not seem to be associated with the previously mentioned progression, suggesting that these features are associated with different ground-state conformers.

By examining the lower trace in Figure 12, it is possible to determine the fluorescence characteristics of the various conformers, since in this spectrum only resonantly emitting species are detected. The spectrum illustrates that none of the peaks associated with the 26-cm^{-1} progressions show appreciable resonance fluorescence while many of the stronger peaks near 34 900 cm^{-1} do. Thus, even without the carboxylic acid group, many conformers of this Trp-Gly derivative are present in the expansion and these different conformers exhibit strikingly different fluorescence properties. This behavior is very similar to what was seen in Trp-Gly itself. A more detailed discussion of the tryptophanmethylamide spectrum can be found in ref 7.

Discussion

The fluorescence spectra of the various tryptophan peptides demonstrate the sensitivity of the excited indole chromophore to the perturbations that arise from different conformations of the peptide chain. This type of conformational sensitivity has previously been seen in the fluorescent properties of tryptophan.[3] Several observations from the present study suggest that the perturbations in these peptides are significantly larger. In both the amino acid and the peptides, the different conformers can be classified according to whether their emission is predominantly resonant in character or predominantly broad and red-shifted. For the peptides, this distinction is very clear-cut since the two types of behaviors are mutually exclusive. In tryptophan, though, even the broad emitting conformer displays a significant component of resonance emission in its fluorescence spectrum, indicating that some of the emission originates from an excited level possessing a reasonably large Franck–Condon overlap with the lower ground-state vibrational levels.

A second indicator of the perturbation to the excited electronic state seems to be the extended low-frequency vibrational progressions found in several of the peptide conformers. In all cases, conformers showing these progressions also exhibit broad fluorescence spectra, although the converse is not true. In tryptophan, the single conformer which displayed the large component of broad emission was also characterized by a 26-cm^{-1} vibrational progression having at least four members in its excitation spectrum. In contrast, one conformer of Trp-Gly is found with an 18-member

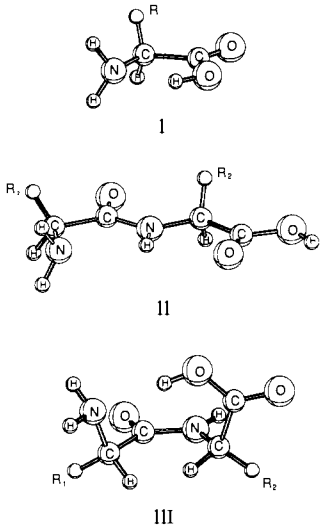

Figure 13. Illustration of the possible hydrogen-bonded backbone structures for I, an amino acid, and II and III, dipeptides. Structure I corresponds to the ab initio structure of glycine, R = H, from ref 16. In tryptophan, R = CH_2-indole. Structures II and III are only suggested as possible structures for an amide hydrogen-bonded and a carboxylic acid hydrogen-bonded dipeptide backbone, respectively, and do not correspond to any experimental or theoretical determinations. The figures are drawn using standard bond lengths and bond angles, and only the dihedral angles have been varied. For Trp-Gly R_1 = CH_2-indole and R_2 = H, for Gly-Trp R_1 = H and R_2 = CH_2-indole, and for Gly-Gly R_1 = R_2 = H.

progression in an 11-cm^{-1} vibrational mode, and this same conformer shows exclusively broad emission from its excited electronic state. Formally, a vibrational progression indicates only that the excited-state surface to which the transition occurs is substantially displaced (or has a different curvature) from the ground-state surface and has no direct bearing on whether the emission is sharp or broad. The frequencies of the vibrational modes observed to form progressions clearly indicate that their composition must include substantial motion of atoms that are not part of the rigid indole ring, and yet their appearance in the excitation spectrum demonstrates that they are coupled to the electronic transition. It then follows that, upon excitation of the indole chromophore in a progression forming conformer, substantial reorientation of the backbone is required to reach the minimum energy configuration.

In order to better understand the origins of the side-chain perturbation to the excited indole chromophore, we have recently studied several tryptophan derivatives, to determine what substituents are required to produce the broad exciplex-like emission.[7] As has previously been reported, this type of emission can be eliminated by removal of either the amine or the carboxylic acid group.[3] In addition, we find that either acylation of the amine or esterification of the carboxylic acid produces the same results. However, replacement of the carboxylic acid with an amide group does not eliminate the red-shifted emission but instead appears to enhance it.[7] Similar fluorescent properties are observed in the monomethylamide, but in the dimethylamide, the red-shifted emission is completely absent. These observations favor a mechanism where the perturbation arises from a particular backbone conformation containing an intramolecular hydrogen bond between the amine and either the amide or carboxylic acid proton.

An insight into the nature of the backbone conformation of gas-phase amino acids has recently been provided by Suenram and Lovas from their work on the microwave spectroscopy of glycine.[8] Two distinct conformers were identified by their different rotational constants, and geometries were determined in conjunction with ab initio calculations.[16] Of particular interest is

(16) Schäfer, L.; Sellers, H. L.; Lovas, F. J.; Suenram, R. D. *J. Am. Chem. Soc.* **1980**, *102*, 6566–6568.

the higher energy conformer, shown as the ball-and-stick structure I in Figure 13, which is estimated to lie approximately 490 cm^{-1} above the more stable form. This conformer was found to have a dipole moment of approximately 4.5 D (compared to 1.0 D for the lower energy form), and its structure was consistent with the presence of an intramolecular hydrogen bond between the amine and the carboxylic acid. Such a structure had been predicted in several theoretical papers as one of the possible stable conformations for the neutral form of glycine.[16,17] When applied to our tryptophan results, it suggests that the red-shifted emission results from the perturbation of a large dipole moment on the glycine-like backbone with the π electrons of the indole ring system. The dipole moment of the side chain is very dependent on its conformation, most likely reaching a maximum in a hydrogen-bonded geometry similar to that found for glycine.

Unfortunately there have been no analogous gas-phase experimental studies on the dipeptide Gly-Gly, which could be used as a model for the peptide backbone in the tryptophan dipeptides we have studied. One would expect the conformational space of a dipeptide to be much more complex than that of a single amino acid, simply from considerations of the number of internal degrees of freedom. A recent ab initio study has addressed the question of the conformation of the neutral form of Gly-Gly and reports to find six metastable conformations within 4 kcal/mol of the minimum energy structure.[18] With bond lengths and bond angles fixed, the Gly-Gly conformations were described by six dihedral angles, four of which were varied in the search for local minima on the potential energy surface. Interestingly, three of the reported metastable structures contained a cis peptide bond.

If we transfer the ideas of hydrogen bonding over to the dipeptide systems, we again find things are more complex than for a single amino acid. Using the terminal amine group as the primary hydrogen-bond acceptor, in analogy with the experimental structure of the glycine conformer, there now exist two potential hydrogen-bond donors: the amide and the carboxylic acid groups. Schematic representations of these conformations are shown in Figure 1 by structures II and III. Attempts to picture these conformers in three dimensions resulted in the ball-and-stick figures, II and III, displayed in Figure 13. In drawing structures II and III, bond angles and bond lengths were fixed at standard values, and the six dihedral angles were varied by hand until a suitable structure having a reasonable hydrogen-bonded geometry was found. Attempts at visualizing structure III, the head-to-tail hydrogen-bonded conformer, were best made with the peptide bond having a cis linkage, a structure not unreasonable in terms of the ab initio calculations. Unfortunately, neither structure II nor III appears as one of the minimum energy structures in ref 18, since in those calculations the carboxylic acid was constrained to the trans configuration, eliminating participation of that proton in a hydrogen bond, and the dihedral angle describing the orientation of the amine group was also fixed at a value precluding the nitrogen lone pair from interacting with the amide proton. Strutures II and III are thus shown only to illustrate possible side-chain conformations and have not been determined to represent local minima on the conformational potential energy surface.

Though not intended to represent actual structures, geometries like II and III may be useful in understanding the fluorescence spectra of the tryptophan peptides and their derivatives. In a structure like III, the backbone perturbation to the indole chromophore might be expected to be roughly equivalent in the two isomers Trp-Gly and Gly-Trp. In this case, both amino acid residues are involved in forming the hydrogen bond, and the dipole moment associated with this particular orientation and charge distribution of the peptide chain should probably exert equivalent perturbations to the indole group in either molecule. However, in structure II, the amide–amine hydrogen-bonded structure, this may not be the case. For the indole group in Trp-Gly, structure II looks very similar to the high dipole side-chain conformation found in glycine, suspected to be the backbone structure of the broad emitting conformer of tryptophan. In the Gly-Trp isomer, the hydrogen-bonding interactions now occur exclusively on the glycine residue, and the perturbation to the indole chromophore on the tryptophan residue might be expected to be quite different.

The results from the two dipeptide derivatives, tryptophanmethylamide and glycyltryptamine, lend support to this hypothesis. In both cases, the derivatives differ from their parent dipeptides by the replacement of the carboxylic acid group with a hydrogen. This substitution clearly eliminates the formation of any conformers resembling structure III. With tryptophanmethylamide, the Trp-Gly derivative, Figure 12 demonstrates that at least one conformer has retained the red-shifted emission seen in the parent compound. Although not shown, a dispersed fluorescence spectra from the broad emitting features in the progression-forming conformer shows very little resonance emission, unlike the case of the amino acid tryptophan. Assuming an amide hydrogen-bonded species is responsible for these features, then one would conclude that the perturbation to the indole electrons is even greater in this species than in the structurally similar amino acid conformation that is stabilized by the carboxylic acid–amine hydrogen bond. The derivative of Gly-Trp, glycyltryptamine, appears to be quite different from the Trp-Gly derivative in that this compound seems to exist primarily as a single conformer in the expansion. Also, the emission from this species is primarily resonance fluorescence, indicating little perturbation to the excited indole ring.

In Gly-Trp, it appears that the carboxylic acid proton is required for the molecule to adapt a conformation where the excited electronic state will show broad, red-shifted emission. This conclusion is also backed up by some preliminary work on the methyl ester derivative of Gly-Trp, which is found to have a very similar excitation spectrum to glycyltryptamine and also exhibits primarily resonance emission. In the context of Figure 13, this would then imply that structure II, which may be present for Gly-Trp in the supersonic expansion, does not give rise to broad emission. Of course many other structures which lack hydrogen bonds are also feasible. Trp-Gly, in contrast, most likely will show broad emission from conformers having the backbone geometry of structure II, in analogy with tryptophan, tryptophanamide, and tryptophanmethylamide. The importance of the hydrogen-bond donor is emphasized by the fact that only resonance emission is seen from excited tryptophandimethylamide and tryptophanmethyl ester.

These hypotheses might be further tested by studying dipeptide derivatives having methylated amide groups, thereby eliminating structure II type conformations. A structure III type conformer of Trp-Gly should also give rise to broad emission, as it appears to do in Gly-Trp, and a second broad emitting backbone conformer may explain the fact that several different features in the excitation spectrum fail to show any resonance emission.

Most of our attention has so far been focused on the effect of the backbone conformation on the fluorescent properties of the indole chromophore, and little has been said regarding the mechanism of the perturbation. Solution-phase studies on indole derivatives lend an interesting perspective to this question. In solution, the L_b transition frequency of many indoles is fairly insensitive to solvent polarity, while the L_a transition is strongly perturbed. Indole emission is also strongly influenced by solvent polarity, becoming broad and red-shifted in polar solvents. Most explanations for this effect rely on solvent reorganization to stabilize a fluorescent state of L_a parentage, possibly containing significant charge-transfer character.[6] Thus, excitation is believed to occur initially to an L_b-type excited state which is very strongly coupled to an L_a-type state through the solvent coordinates. In the isolated peptides, we also find very little difference in the electronic transition frequency for the excitation step among the different conformers but find very large differences in their fluorescence properties. The peptide backbone then appears to perturb the indole chromophore in much the same manner as does a solvent environment, with the postulated high dipole moment backbone conformation corresponding to a polar solvent. Broad

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and red-shifted emission may then also occur through a L_a-type state which is stabilized by a large backbone dipole moment and is strongly coupled to the initially excited L_b levels through the peptide backbone coordinates.

Summary

Fluorescence spectroscopy has revealed that two distinct types of conformers are present in the isolated dipeptides Trp-Gly and Gly-Trp in a supersonic expansion. One type of conformer exhibits sharp resonance fluorescence from the excited indole chromophore of the tryptophyl residue, while the other type of conformer shows only broad fluorescence with a maximum shifted approximately 3000 cm^{-1} from the excitation frequency. In analogy with work on several tryptophan derivatives, the existence of a broad emitting conformer appears to require the formation of an intramolecular hydrogen bond in the peptide backbone. In these dipeptides, the terminal amine may hydrogen bond to either the carboxylic acid or the amide proton and work on derivatives of the two peptides has been used to isolate the contribution from the amide hydrogen-bonded species. Here it is found that only the Trp-Gly derivative exhibits the exciplex-like fluorescence. These results appear to be consistent with a dipole-induced perturbation to the indole chromophore which originates from a high dipole, hydrogen-bonded conformation of the peptide backbone.

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Registry No. Gly-Trp, 2390-74-1; Trp-Gly, 7360-09-0; Trp-Gly-Gly, 20762-31-6; Trp-NHMe, 53708-63-7; glycyltryptamine, 122902-82-3.

Dissymmetry Effects in μ-Oxo Diiron(III) Species: Structures and Spectroscopic Properties of $[N5FeOFeX_3]^+$ (X = Cl, Br) and Implications for Oxo-Bridged Dinuclear Iron Proteins

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Abstract: We report the first nonbiological unsymmetrical μ-oxo diiron(III) complexes: $[(N5)FeOFeX_3]X$, where N5 is *N*-(hdyroxyethyl)-*N*,*N*',*N*'-tris(2-benzimidazolylmethyl)-1,2-diaminoethane and X is Cl (compound I) or Br (compound II). X-ray crystallographic studies reveal structures in which octahedral and tetrahedral iron atoms are bridged by the oxo group with an Fe–O–Fe angle of 149.5 (3)° for I and 150.6 (4)° for II. A small but significant difference in Fe–O separations is observed in which the longer distances are associated with N5Fe–O [1.784 (4) Å in I, 1.797 (9) Å in II] and shorter distances with X_3Fe–O [1.751 (4) Å in I, 1.726 (9) Å in II]. Unusually strong antiferromagnetic coupling is observed for I [J = −122 (1) cm^{-1}] and a more typical value for II [J = −106 (1) cm^{-1}]. The nonequivalent iron atoms exhibit distinct Mössbauer spectroscopic parameters for I at 293 K (δ = 0.23, ΔE_q = 1.15 mm/s and δ = 0.34, ΔE_q = 1.36 mm/s), whereas II at 77 K gives an even better resolved pair of doublets (δ = 0.31, ΔE_q = 1.41 mm/s for $FeOBr_3$ and δ = 0.41, ΔE_q = 1.22 mm/s for N5FeO). The resonance Raman spectrum of I shows a band at 850 cm^{-1} (806 cm^{-1} for ^{18}O) assigned to the Fe–O–Fe asymmetric stretch, ν_{as}. Contrary to the behavior of symmetrical dinuclear species, the ν_{as} mode in I and II has more than 2 times the Raman intensity of the symmetric Fe–O–Fe stretch. A stable one-electron-reduced derivative of I is produced by cyclic voltammetry of the compound in acetonitrile. Many properties of the asymmetric μ-oxo diiron(III) complexes are similar to those observed for dinuclear iron proteins (hemerythrin, ribonucleotide reductase, purple acid phosphatase, methane monooxygenase); a comparable inequivalence of the two iron atoms is inferred for several of these proteins.

μ-Oxo diiron(III) complexes have long been of considerable interest for their distinctive magnetic and spectroscopic properties[1] and relevance to non-heme diiron proteins.[2] Whereas almost all of the many synthetic systems are symmetrical and remarkably unreactive species, in the biological systems the diiron μ-oxo motif is unsymmetrical, as inferred from ^{57}Fe Mössbauer spectroscopy, and mediates or is in the vicinity of a variety of biochemical activities. These include oxygen transport (hemerythrin[2,3]), hydrolysis (purple acid phosphatase[2,4]), reduction (ribonucleotide reductase[2,5]), and oxidation (methane monooxygenase[2,6]).

Magnetic susceptibility measurements are a primary means for the identification of the μ-oxo diiron(III) motif in metalloproteins.[2] Singly bridged μ-oxo diiron(III) complexes show substantial coupling, for which, with the possible exception of some porphyrin and phthalocyanine complexes, the values of J ($H = -2JS_1S_2$) lie in the remarkably narrow range of −85 to −110 cm^{-1} regardless of the Fe–O–Fe angle and of the nature and number of donor

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