



Electronic spectroscopy of lumiflavin in superfluid helium nanodroplets

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ABSTRACT

We present the fluorescence excitation and dispersed emission spectra of lumiflavin doped into superfluid He nanodroplets. Both spectra show well resolved vibrational structure. The electronic origin transition at 21511 cm^{-1} is the strongest line in both spectra. Quantum chemical calculations with DFT and CASSCF methods support the assignment of S_1 to a $\pi\pi^*$ excited state. We obtain vibrational frequencies in the ground and lowest excited singlet state that can serve to test the validity of quantum chemical calculations. Multidimensional Franck–Condon factors are in good agreement with the intensities within the vibrational structure for S_0 and S_1 . The strongest progression forming mode has a frequency of 164 cm^{-1} in both states and is assigned to an in-plane bending mode of the whole flavin chromophore with a large amplitude on the two methyl groups at ring I.

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1. Introduction

Flavins are essential protein cofactors in all living cells. The most common examples are riboflavin (RF), flavin mononucleotide (FMN) and flavin adenosine dinucleotide (FAD). Flavins can exist in several protonation states and redox states, and are hence ubiquitous in enzymes catalyzing redox reactions. The first absorption band of flavins is in the range $\lambda < 470\text{ nm}$, and hence flavins absorb blue light. Nature has made use of this by employing flavins in photoreceptors for blue light. Three classes of protein domains with this function have been discovered and studied during the last decade [1]: LOV (light oxygen voltage) domains [2–4], BLUF (blue light sensing using flavin) domains [5,6], and cryptochromes [7,8]. The redox properties of the electronically excited state are used in photolyase [9,10] which repairs DNA damaged by cycloadditions between adjacent bases.

Whereas optical spectroscopy can be used to follow the various electronic states during the photocycles of these flavoproteins, the study of the dynamics inside these electronic states and the interaction with the protein require often techniques that provide more local and specific information. Vibrational spectroscopy is one of the most promising techniques for this purpose. Therefore, in addition to difference infrared (IR) and Raman spectroscopy on electronic ground state species also transient spectroscopy has been employed to follow the dynamics in the electronically excited singlet and triplet states [11]. In this context demand for a theoretical description is growing, not only for an assignment of various vibrational frequencies to normal modes, but also of the effect of solvent

and protein interaction on these modes and frequencies [12,13]. The reference state for most theoretical techniques is the isolated molecule in the gas phase.

So far, no vibrationally resolved gas phase spectrum of an electronic transition of a flavin has been published. The only high resolution spectrum that we could find is that of N_3 -undecyllumiflavin isolated in a n-decane Shpol'skii matrix at 4.2 K, published by Platenkamp et al. [14] in 1980. This study finds, on top of a broad background, several distinct sites with sharp lines. One of these sites with origin at 20956 cm^{-1} was studied by site selective excitation and fluorescence spectroscopy. The vibronic transition frequencies were compared to IR and Raman data, but no assignment was given. Although this study has been performed at high spectral resolution and at low temperature, a rigid alkane matrix is still a crude approximation of the non-interacting molecule in gas phase. The main aim of the present study was hence to obtain high resolution spectra of flavin under conditions that are as close as possible to those of the isolated molecule in the gas phase. This should establish whether the lowest excited singlet state has $\pi\pi^*$ character or, as some calculations predict, $n\pi^*$ character. As a result of our study we obtain vibrational frequencies in the ground and lowest excited singlet state that can serve to test the validity of quantum chemical calculations.

The essential optical chromophore common to riboflavin, FMN, and FAD is lumiflavin. In lumiflavin, the ribityl chain of riboflavin has been replaced by a methyl group. Further simplification by replacement of this methyl group through a hydrogen atom is not possible, since the resulting lumichrome molecule exists in a different tautomer. Ideally, one should study lumiflavin in a supersonic jet. However, at the temperature required to produce a substantial vapor pressure (ca. 1. mbar), lumiflavine will decompose

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very quickly. In recent years, spectroscopy in superfluid He nanodroplets has been developed as an alternative which in many respects simulates spectroscopy in the gas phase [15,16]. He droplets of several thousand atoms are produced by expansion of helium gas at high pressure (ca. 20 bar) through a nozzle cooled to ca. 10 K. By evaporation from the surface these helium droplets cool to a temperature of ca. 370 mK [15]. It has been shown that He in these droplets is in the superfluid phase. Molecules can be incorporated into these droplets by collision and are subsequently cooled to the same temperature as the droplet. The vapor pressure required scales inversely with the length of the pick-up cell and is typically 10^{-4} mbar, i.e. several orders of magnitude smaller than needed for supersonic jets. Of all possible solvents liquid helium has the smallest interaction energy with dissolved molecules. This results in small shifts of the electronic transition energies with respect to the gas phase, typically less than 1 % of the transition energy [16]. Hence, He droplets could be considered as a “vacuum with high thermal conductivity”. Fluorescence excitation spectra and dispersed emission spectra of large rigid organic molecules doped into He droplets show very sharp lines, even narrower than those observed in supersonic jets. Recent experiments have shown that the interaction of the molecule with the He environment is not always negligible, in particular when torsional motions are excited or substantial charge redistribution occurs upon electronic excitation. However, since other alternatives do not seem to exist, we decided to try superfluid helium droplets as a medium to study the spectra of lumiflavin under almost gaslike conditions.

2. Experiment and methods

Lumiflavin (LF) was purchased from Sigma–Aldrich corporation and used without further purification. The experimental setup for the embedding of molecules inside superfluid helium nanodroplets and their fluorescence and excitation spectroscopy was described previously [17,18]. Briefly, a continuous droplet beam was formed by supersonic expansion of pre-cooled helium at 12.5 K through a 5 μm nozzle at 20 bar stagnation pressure into a vacuum chamber. Under these conditions the average droplet has a diameter of 100 Å and contains ca. 2×10^4 helium atoms [15]. The droplets were doped with individual molecules by passing the droplet beam through a 2 cm long pick-up cell filled with LF and situated on the beam axis, heated to 210 °C. This temperature is well below the melting point of LF (325 °C [19]). The presence of the sample in the droplets was monitored with a mass spectrometer (Leybold). The mass spectra showed a strong peak at the mass of the LF parent ion (256 amu) with minor contributions at 213 and 44 amu, presumably from decomposition products or CO_2 . Vacuum conditions were optimized to avoid contamination of the droplets by background gas such as water or pump oil. Before each experiment the vacuum system was pumped for at least 12 h while the outer walls of the apparatus were heated to remove adsorbed water. During the experiment water was cryo-trapped by copper shields placed inside the whole flight tube with a small distance (ca. 1 cm) from the tube walls. These copper shields are cooled by connection to a liquid nitrogen reservoir. The mass spectrometer monitoring the droplet beam showed that neither water nor pump oil was transported by the beam. Since the path from the droplet source to the mass spectrometer is three times longer than the path to the LIF detection position, presence of water or pump oil inside the droplets can be excluded.

Laser induced fluorescence (LIF) was excited with a pulsed dye laser (Lambda Physics Scanmate 2E, bandwidth 0.1 cm^{-1}) pumped by the third harmonic of a Nd:YAG laser (SL803 Spectron). For excitation spectra, the emission was collected by a lens and detected with a cooled photomultiplier (R943-02, Hamamatsu), amplified

(SRS 445, Stanford Research Systems), integrated by a boxcar integrator (SRS 250, Stanford Research Systems), digitized by a 12 bit A/D converter (SRS 245, Stanford Research Systems) and transmitted to a computer. A Schott GG 495 longpass transmission filter was placed before the photomultiplier in order to suppress scattered light from the dye laser.

Fluorescence spectra of LF were excited by the radiation of an Ar^+ ion laser operating in multiline UV mode. The strongest lines are at 363.8 and 333.6 nm. Fluorescence was dispersed by a spectrograph (MS257, L.O.T. Oriel, $F = 3.9$) equipped with a CCD camera (1024 \times 255 pixel, DI 420A-BU2, Andor) Peltier cooled to -80 °C. Due to the low signal intensity the slit of the spectrograph was set to an effective spectral resolution of 15 cm^{-1} .

Quantum mechanical electronic structure calculations were performed with the GAMESS program package. For all calculations except those of the Hessian matrices for CASSCF wavefunctions the PCGAMESS implementation by A. Granovsky was used [20]. Since this version does not provide analytic second derivatives for CASSCF energies, the latter were calculated with the WinGAMESS implementation of the GAMESS-US version [21]. The 6–31G(d,p) basis set was used throughout. Excitation energies were calculated with the TD-DFT/B3LYP technique at the fixed ground state geometry optimized with DFT/B3LYP. The geometries of both electronic states S_0 and S_1 were optimized with a CAS(8,8) wavefunction. In all cases the Hessian matrix was calculated in order to verify that the stationary state is a minimum, and to obtain vibrational frequencies and normal coordinates.

3. Results and analysis

3.1. Excitation and emission spectra

The fluorescence excitation spectrum in the range 21400–23400 cm^{-1} is displayed in Fig. 1. The structural formula of lumiflavin indicating the atomic numbering scheme is shown as inset. All spectral features are assigned to He droplets containing a single lumiflavin molecule, for the following reasons: The mass spectrometer monitoring the droplet beam shows that lumiflavin is transported with the beam, but no water molecules. Water and pump oil is efficiently trapped by the liquid nitrogen cooled copper shields inside the flight tube. When water is intentionally added to the pickup cell, additional lines are seen in the spectrum, the first being 108 cm^{-1} red-shifted from the first line seen without water. When the partial pressure of the water is increased, only the new lines increase at the expense of the lines observed when no water is added to the pickup cell. If the spectrum would initially contain lines that are due to water clusters, these lines should increase when water is added.

The lowest-energy feature of the spectrum in Fig. 1 consists of a narrow line at 21511 cm^{-1} which is followed by a broader band of ca. 10 cm^{-1} width towards higher wavenumbers. The first narrow peak is by far the most intense line in the spectrum (note the axis break in Fig. 1). We assign it to the electronic origin transition 0_0^0 . All other bands in the spectrum show similar widths of ca. 10 cm^{-1} . The second band is observed at 164 cm^{-1} above the origin and assigned to a mode labeled 1_1^1 . This mode apparently forms progressions with the origin and with other modes. Some of these are indicated by combs (dotted lines) in Fig. 1. Although the first peak has a 3.5 times higher intensity than the second, the area under the first two bands, 0_0^0 and 1_1^1 , are of comparable size. We conclude that both transitions have similar Franck–Condon factors.

Fig. 2 provides a closer look on an expanded wavenumber scale on the origin band and the first two progression bands of the low frequency mode at 164 and 327 cm^{-1} , respectively. For each band two measurements are shown, one performed with very low laser

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