

The isolation of porphyrins in the gas phase using pulsed-laser desorption

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Introduction

Porphyrins have a diverse role in nature and exhibit widely different properties depending on their environment^[1]. For example, the porphyrin unit in heme is responsible for both coordinating oxygen in hemoglobin and myoglobin and facilitating electron transport in cytochrome c, and the reduced-porphyrin unit in chlorophyll is the active site for photochemical-light harvesting. In each case, the physical and chemical properties (i.e. oxygen affinity, redox activity or photosensitivity) of the basic porphyrin unit (see figure 1) are precisely tailored by covalent addition of side groups, coordination of various metal ions (four different examples are observed in nature: Fe, Mg, Ni, Co), or reduction of one or more carbon-carbon double bonds. In addition, the secondary structure of a host protein leads to specific intermolecular forces, and the tertiary structure creates either a hydrophobic or hydrophilic environment for the porphyrin group.



Figure 1. The structure of the porphyrin group (or tetrapyrrole unit, C₁₆H₁₄N₄).

In natural and synthetic metalloporphyrins, the tetrapyrrole ring is commonly distorted from a planar geometry due to the size of the metal ion relative to the strain-free distances between the central nitrogen atoms, and steric effects from the presence of substituents located on β - and meso-carbons. The equilibrium structure can be described as a linear superposition of displacements along the 3N-6 normal coordinates corresponding to the molecular-vibrational eigenfunctions. However, there are only 6 normal modes in porphyrin with restoring forces that are suitably low to make contributions to the overall conformation in the ground state^[2]. Each of these modes lead to an out-of-plane deformation of the ring and they are referred to as dome, saddle, wave (two orthogonal modes), ruffle, and propeller. There has been considerable

interest in quantifying their individual contributions to classify the nonplanar distortions in natural porphyrins (in particular, see Shelnutz's work in ref. [2]). This has been driven by observations that the structures of tetrapyrroles in various classes of proteins are conserved between different species. It is anticipated that ring distortions influence the biological activity and that the flexibility of the tetrapyrrole group could also be used as a mechanism to regulate processes in living organisms.

We have begun an investigation into the properties of isolated porphyrins using molecular-beam methods. This will build on much earlier work from Jortner's laboratory^[3] and complement recent studies using ultrafast spectroscopy^[4]. The conformational landscape of either metalloporphyrins or free-base porphyrins with large substituents have a number of energy minima. These conformational states are trapped behind the potential barriers on the energy surface in a cold-molecular beam. We hope to be able to identify the individual structures, and selectively measure their properties. Gas-phase methods have already provided valuable data on important biomolecules; notable examples include carbohydrates^[5], amino acids and small peptides^[6], and nucleobases^[7]. In each case, the absence of solvent or a host has given insight into the intrinsic properties of the molecular subunit.

Experiment

The experiments are carried out in a high vacuum apparatus. In previous work, the organic molecules were vaporized by heating a solid pellet of graphite and porphyrin placed in a supersonic expansion of an inert gas. This arrangement did not lead to sufficient cooling of molecules in the molecular beam. Currently, the organic component is desorbed from the surface of a rotating disk using the fundamental of a pulsed-Nd:YAG laser at ~ 5 mJ/pulse, focused to a 1 mm spot size (see figure 2). The disk is formed from a 3:1 mixture of graphite and porphyrin using a 40 mm die and a 50 ton hydraulic press. The thickness of the disk is only 2 mm and it is positioned immediately below the exit of a commercial solenoid valve (Series 9, Parker Hannifin). The opening of the valve (for short pulse lengths of ~ 200 μ s) is synchronized with the desorption laser and the vaporized porphyrins are seeded into a supersonic expansion as the backing gas, Ar at 2 atm, expands into the vacuum chamber. A stepper motor is used to rotate the disk and re-refresh the surface area in front of the valve.

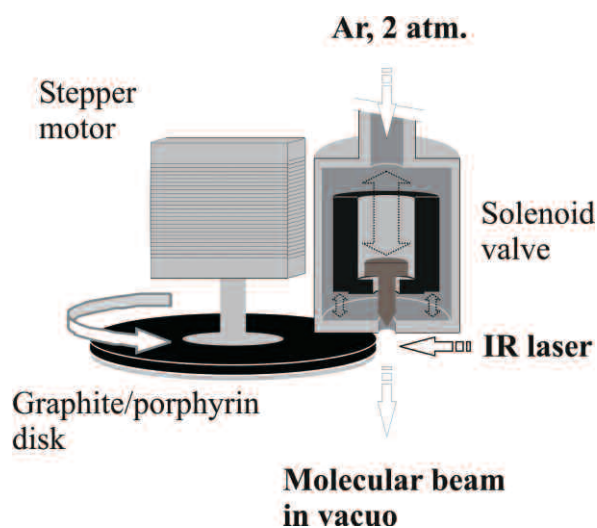


Figure 2. Pulsed-laser desorption of porphyrins. The apparatus has been constructed by modifying a commercial pulsed-solenoid valve (the electrical coil is embedded into the casing and is not shown). The low-C steel barrel containing a plastic poppet is drawn back from the valve exit by the solenoid. The restoring force is provided by springs (shown as double-headed arrows). See main text for further details.

The centerline of the expanding gas is extracted using a 2 mm-skimmer located 20 mm downstream of the valve exit. The molecules remaining in the expansion have cold rotational and vibrational temperatures (~ 10 K) with a narrow distribution of speeds. The molecular beam is then intersected by a UV laser (266 nm, pulsed Nd:YAG pumped-dye laser) in the extraction region of a Wiley-McClaren time-of-flight mass spectrometer (R M Jordan),

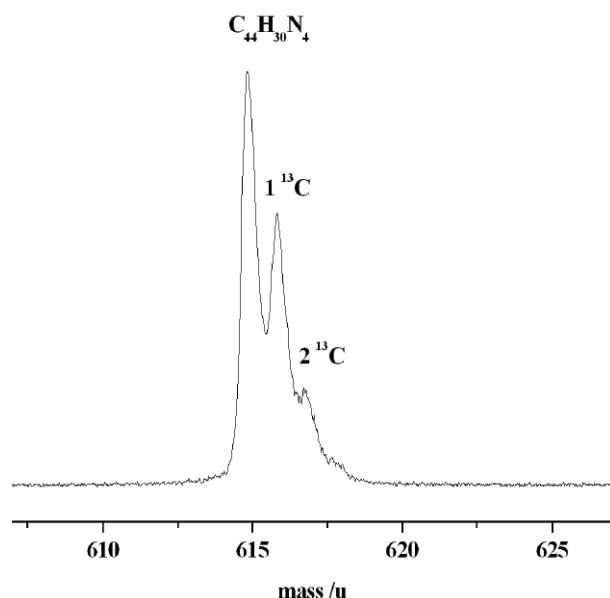


Figure 3. UV photoionization time-of-flight mass spectrum of meso-tetraphenylporphyrin (recorded using the original apparatus involving thermal evaporation of the substrate). The presence of 1, 2 and 3 isotopic substitutions of ^{13}C is clearly resolved.

and the ion-signal is measured with a matched pair of microchannel plates. A typical time-of-flight spectrum for a substituted porphyrin is shown in figure 3. This example has a molecular mass of 615 au and consists of the basic porphyrin unit (see figure 1) with 4 phenyl groups attached to the methane-bridging groups between each pair of pyrrole units.

With the addition of a 2nd visible-laser wavelength overlapping the UV-laser beam it is possible to probe the excited-electronic states (with vibration resolution) in the porphyrin. In this case, the power of the UV laser is reduced from around 10 mJ/pulse (used to measure non-resonant ionization, as in fig. 3) to a value less than 1mJ/pulse. The resulting signal in the mass spectrum is then due to two colour (1+1), resonant-enhanced multiphoton ionization (REMPI).

Results and discussion

Initial data obtained using the present method of pulsed-laser desorption is shown in figure 4. The wavelength region for observation of an ion signal via a 1+1 resonant process is greatly reduced from the range measured in the photoabsorption spectrum for the Q_x band of meso-tetraphenylporphyrin under conditions of much higher temperature (see ref.^[8] for details). In the latter case, there is a pair of overlapping peaks covering a broad range of wavelengths from approx. 570 nm to 670 nm, with a minimum at 640 nm. However, there is only a single, narrow resonance observed in the present spectrum and this actually appears at 640 nm. The REMPI signal in figure 4 is very weak and the observed transition represents the vibrational origin of S_1 (1B_3) \leftarrow S_0 (1A); in the D_2 point group. Although this process is formally allowed, the oscillator strength is small as a result of configuration interaction between the highest-occupied-molecular orbitals, b_1 and a , and the lowest-unoccupied molecular orbitals, b_3 and b_2 . For the observed band (Q_x), the resulting transition-dipole moment can be calculated as

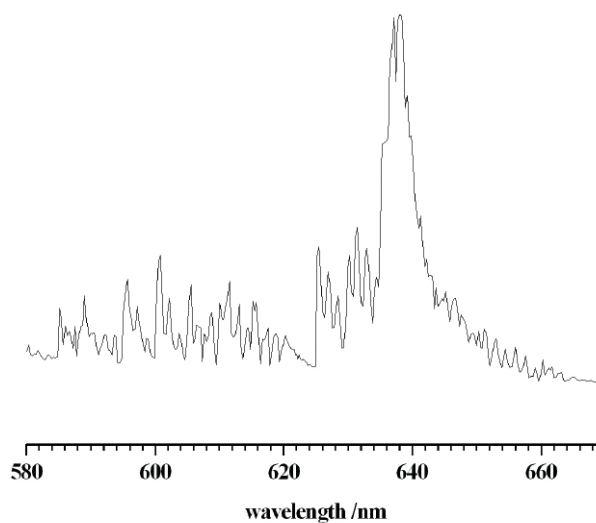


Figure 4. (1+1) resonance-enhanced multiphoton ionization spectrum of meso-tetraphenylporphyrin. The wavelength of the laser used in the initial excitation step is given on the abscissa. The ionization wavelength is fixed at 266 nm.

the difference between the two possible orbital transitions with the same symmetry as the x component of the dipole-moment operator; in this case, $b_2 \leftarrow b_1$ and $b_3 \leftarrow a$ (see ref.^[9] for details). Although the orbital transitions are symmetry allowed, they produce an overall matrix element that is nearly zero. This transition can become much stronger through vibrational coupling in the ground and excited states of the porphyrin, and this is the reason that a much stronger absorption is observed at higher temperatures (as in ref.^[8]). In this case, it is necessary that vibrational modes with the same symmetry couple to both the ground and excited electronic states to maintain a symmetric product with the dipole-moment operator, and the stronger regions observed to either side of 640 nm in the spectrum from ref.^[8] correspond to excitation from molecules with initial out-of-plane vibrations in the tetrapyrrole ring. However, in the cold molecular beams produced by pulsed-laser desorption these vibrational modes are absent.

The linewidth of the remaining resonance at 640 nm in figure 4 is due to the presence of (unresolved) low frequency torsional modes of the phenyl groups.

Conclusions

We have developed a pulsed-laser desorption apparatus to study isolated porphyrins. We have demonstrated that we can obtain cold molecules where the out-of-plane normal modes for the tetrapyrrole ring are at the zero-point level. We did not reproduce the very sharp feature for the band origin observed by Jortner and co-workers in their laser-induced fluorescence spectrum of meso-tetraphenylporphyrin (see ref.^[3]). In our case, the transition is broadened by the presence of low frequency torsional modes for the side groups attached to the tetrapyrrole unit in the isolated porphyrins. Nevertheless, our setup is well suited to investigate the electronic structure and dynamics of the central-ring system, and we are beginning a new series of experiments to investigate the properties of different porphyrins relevant in nature.

Acknowledgements

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