Single Molecule Spectroscopy of Mg Tetra-azaporphyrin in Solid Xenon

Alexander Starukhin 1, 2), Alexander Shulga 1, 2), Jerzy Sepiol 1, 3), Robert Kolos 1, 3), Alois Renn 1) and Urs P. Wild 1)

1) Physical Chemistry Laboratory,
Swiss Federal Institute of Technology
ETH-Hönggerberg, CH-8093 Zürich, Switzerland

2) Institute of Molecular and Atomic Physics
National Academy of Sciences
F. Scarina Av. 70, 220072 Minsk, Belarus

3) Institute of Physical Chemistry
Polish Academy of Sciences
Kasprzaka 44/52, 01-224 Warsaw, Poland

Correspondence to
Dr. Alois Renn
Physical Chemistry Laboratory,
Swiss Federal Institute of Technology
ETH-Hönggerberg,
CH-8093 Zürich, Switzerland
tel + 41-1-632 43 87
fax + 41-1-632 10 21
e-mail renn@phys.chem.ethz.ch

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Abstract

We report initial results on the spectroscopy of single Mg-tetra-azaporphyrin molecules in solid xenon. Samples were prepared following the conventional matrix-isolation procedure. Single molecules, detected by fluorescence microscopy, gave remarkably stable signals. Their fluorescence excitation spectra revealed sharp zero-phonon lines, with most represented linewidths around 50 MHz. This is the first study of single molecules of a tetrapyrrolic compound.

Introduction

Single molecule spectroscopy (SMS) at liquid helium temperature has revealed a wealth of information about processes, which could not be investigated by the averaging of data obtained for bulk molecular ensembles. In low temperature studies in various matrices single molecules belong mostly to the class of conjugated aromatic hydrocarbons (see for example [1-3]), but the importance of the investigations of biologically important species, based on fluorescent labels, is increasing [4]. Bio-molecules, e.g. peripheral light harvesting complexes with chlorophyll a and chlorophyll b, have recently been investigated at low and room temperatures [5-9]. These studies, however, were done for multi-chromophore systems.

The detection of single dibenzo-anthanthrene molecules in Xe and Kr matrices [10] gave the opportunity to broaden the choice of objects for SMS, taking profit from some unique properties of the atomic lattice such as chemical inertness, high ionisation potential and photophysical changes due to the heavy atom effect. Intersystem crossing (ISC) kinetics is, besides photostability, an important factor for single molecule detection. The S1-T1 crossing, often referred to as "triplet trap" in SMS, reduces the rate of S1-S0 photon emission under cw excitation, thus diminishing the chances for single molecule detection [1,2]. Ideally, species studied with SMS should be characterised by a low probability of S1 to T1 transition and/or fast return from T1 to the ground state. In Xe matrices one can count on the substantial increase of the radiative T1 -> S0 rate, which effect may be accompanied by a relatively slight enhancement of S1 -> T1 crossing [11,12]. ISC is regarded as an important relaxation channel for these compounds [13]. Based on these considerations, we used solid xenon
for the SMS of a substance belonging to the biologically distinguished porphyrin family. We chose Mg-tetraazaporphyrin (Mg-TAP) as a photochemically stable compound with a relatively high fluorescence quantum yield.

Experimental

Mg-TAP was synthesized according to the procedure described in Ref. [14]. The compound was purified on aluminum oxide by chromatographic method. Its identity and structure (Fig.1) were established with the aid of $^1$H NMR spectrum (Bruker WM-360).

Fig. 1. Fluorescence excitation (observed at 15 576 cm$^{-1}$, solid line) and fluorescence (excited at 27 027 cm$^{-1}$, dashed line) spectra of Mg-tetra-azaporphyrin in solid Xe at 5 K. Instrumental resolution 30 cm$^{-1}$.

The fluorescence of bulk samples was measured with a high resolution spectrofluorometric system [15]. Its excitation branch consisted of an Osram XBO 2.5 kW high-pressure xenon lamp combined with a Spex 1402 double monochromator. A sapphire substrate plate in a copper holder was attached to the "cold finger" of the liquid helium cryostat. Mg-TAP was evaporated at ~570 K inside the heated nozzle, in a stream of Xe gas (Linde, 4.0 grade). During the matrix deposition stage, the substrate temperature was kept around 55 K. A frontal excitation arrangement was used in all luminescence measurements. The emission light was analysed by another Spex 1402 monochromator and detected with a cooled Hamamatsu R2949 photomultiplier tube connected to a photon counting system.

The samples for SMS studies were prepared by direct solidification of gaseous Xe/Mg-TAP mixtures on the surface of the microscope mirror objective (N.A.=1; a modified version of the construction described in ref. [16]). The matrix deposition procedure was identical as for conventional spectroscopy measurements described above (except that the samples were made at least one order of magnitude thinner), with the sapphire plate replaced by the microscope objective. The presence of Mg-TAP in the matrix was checked by detecting its bulk fluorescence, excited with a cw dye laser. The Mg-TAP : Xe ratio was estimated to be less than $10^{-4}$. The objective was cooled down to ~2 K after the matrix deposition.

The optical set-up for the single molecule detection was similar to the one described in Ref. [10]. The beam from a cw Rhodamine 6G single mode dye laser (spectral bandwidth 2 MHz) was focused on the matrix surface near the objective axis (spot diameter ca. 50 μm). A video camera equipped with an image intensifier (Hamamatsu C2400-25) was used for the registration of resulting images, produced by individual fluorescing molecules. Schott RG 610 glass filters, placed between the cryostat window and the camera, absorbed the Rayleigh-scattered laser radiation and transmitted the Stokes-shifted fluorescence. The laser was scanned over 2 GHz in steps of 3.33 MHz; images corresponding to consecutive steps (with the integration time of 0.32 s) were stored. During the scans, particular single molecules in the field of view emitted the fluorescence, whenever their individual transition frequencies came into resonance with the excitation light. Further analysis of gathered data enabled to reproduce not only the spatial positions of individual molecules, but also their resonant frequencies and related line shapes.

Results and Discussion

The conventional spectra (dispersed fluorescence together with fluorescence excitation) for Mg-TAP/Xe sample deposited on a sapphire substrate are presented in Fig.1. A strong inhomogeneous broadening of all bands is visible (fwhm ~ 200 cm$^{-1}$). The vibrationless origin is located at about 17.11 x 10$^3$ cm$^{-1}$.

One video frame out of 600 images recorded while scanning the laser over 2 GHz range is shown in Fig.2. Several molecules were in resonance with the laser there; the positions of these appear as bright spots against the dark background. Analysis of signals coming from single molecules (i.e. from distinct sets of adjacent pixels on the
detector surface) as a function of laser frequency gave the individual fluorescence excitation spectra. The positions and widths of resonance lines were notably stable during the laser scans (i.e. on the time scale of seconds). The signal-to-noise ratio was typically higher then 10, with the laser beam intensity of about 0.1 W/cm².

Fig. 2. Fluorescence microscopy of single Mg-tetra-azaporphin molecules isolated in Xe. The traces of individual molecules reproduced as tiny clusters of illuminated CCD detector pixels. The image, accumulated for 0.32 s, corresponds to the excitation with a single-mode laser (2 MHz bandwidth) around 17 060 cm⁻¹.

Spectral profiles for 56 individual Mg-TAP molecules were analysed, as illustrated with the histogram in Fig. 3. Shapes of resonance lines were, for the majority of molecules, well reproduced by a Lorentzian fit (see insert of Fig. 3). The linewidths were distributed within the frequency range 20–130 MHz with a maximum around 50 ± 10 MHz. The fluorescence lifetime of Mg-TAP in solid Xe was not measured yet. In tetrahydrofuran, at room temperature, we obtained τ_f=4.5 ns; the dependence of the fluorescence lifetime on temperature is usually not significant [13].

Assuming that the τ_f value for Mg-TAP in Xe matrix is not much different from that in the solution (as observed for aromatic hydrocarbons [17,18]), the expected lifetime-limited zero-phonon linewidth should be ~35 MHz, which is not far from the low frequency histogram cut-off, at 20-30 MHz.

The ease of single Mg-TAP molecules detection implies a relatively high photon emission rate, ≥5x10⁶ s⁻¹ [2]. As already remarked, the intersystem crossing is important for this system, hence an estimation of the triplet decay rate was made. The value of 4.3 x 10⁶ s⁻¹, measured for Zn-TAP at 77 K in a diethyl ether / petroleum ether / iso-propanol (5:5:2) mixture [19], is expected to decrease ~2 times upon the substitution of Zn into Mg (based on the analogy with Zn-porphine and Mg-porphine). This would lead to a very low triplet decay rate of ~2 x 10⁵ s⁻¹, making the SMS of Mg-TAP impossible or extremely difficult. Can we expect the substantial increase of this rate in Xe?

Fig. 3. The distribution of Mg-tetra-azaporphin linewidths (fwhm) in solid Xe. Excitation with a single-mode laser (2 MHz bandwidth) around 17 060 cm⁻¹; laser intensity 0.1 W/cm². Inset shows an exemplary single molecule fluorescence excitation profile (thin curve), approximated with a Lorentzian function (bold).

Within the framework of the three-level model (S₀, S₁, T₁) the general requirement for high photon emission rate is \( \Sigma k_{23}^m/k_{31}^m < 20 \) [2], where \( k_{23}^m \) and \( k_{31}^m \) are ISC rates for S₁ → T₁ and T₁ → S₀ relaxation processes, respectively (Σ stands for the summation over m=x,y,z sublevels within the T₁ manifold). For Mg-TAP/Xe system, assuming the same fluorescence quantum yield, \( \Phi_f = 0.56 \), and the same fluorescence lifetime as measured in a tetrahydrofuran solution at 290K (see above), one obtains the fulfilment of \( \Sigma k_{23}^m/k_{31}^m < 20 \) condition for \( k_{31} > 10^7 \text{s}^{-1} \) (for simplicity, the rates for three triplet sublevels were assumed equal). This would imply that \( k_{31} \) is increased in Xe by 5 orders of magnitude. Such high a value seems surprising. Radziszewski et al. [12] reported the increase of \( k_{31} \) for the free base porphine, when moving from Ar to Xe, of more than 2 orders of magnitude [12]. In (dimethylamino)benzonitrile the 300-fold Xe-induced...
enhancement of the radiative $T_1 \rightarrow S_0$ rate was accompanied by only a \(<5\)-fold increase of nonradiative ISC processes ($S_1 \rightarrow T_1$ and $T_1 \rightarrow S_0$) \cite{11}. We are not aware of the existence of corresponding experimental data for porphyrin metalocomplexes.

**Conclusion**

Besides being one more host environment for the detection and spectroscopy of single molecules, solid Xe may influence the electronic relaxation of compounds studied by SMS, thus widening the choice of objects for these investigations. It would be very interesting to clarify the role of the triplet state for Mg-TAP/Xe, e.g. with the time-correlated photon counting technique \cite{20} or with the laser flash photolysis \cite{21}, which allows for the direct measurement of deactivation rates.

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