LASER EXCITED FLUORESCENCE LIFETIMES OF HEMATOPORPHYRIN IX AS A FUNCTION OF SOLUTION pH: IMPLICATION IN PHOTODYNAMIC THERAPY

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I. INTRODUCTION

The combination of a harmless chemical photoactivated with safe visible electromagnetic radiation in order to eradicate tumors with a photosensitization reaction, offers much hope for the treatment of a large variety of neoplastic diseases. The origin for the selective biodistribution of photosensitizer toward tumor tissue is not yet clearly understood. The pH of many rapidly growing tumors is often found to be significantly lower than that of normal tissue in the same individual. Recent volumetric titration of hematoporphyrin IX (HP) have yielded the distribution diagrams of five HP ionic species, having net charges of +2, +1, 0, -1 and -2. However, only three ionic species may be associated with the steady state absorbance and fluorescence spectra. Further, fluorescence decay measurements carried out in aqueous solutions at physiological pH generally show two lifetimes; a 15 nsec component normally ascribed to a monomeric species and a 3.8 nsec component usually assigned to a dimeric species. Since both monomeric and dimeric species, as well as different ionic species, may play a role in the selective biodistribution of drugs toward tumor tissue, it was deemed important to evaluate the fluorescence lifetimes of HP as a function of both pH and concentration. These measurements were carried out in aqueous solutions as well as in the presence of a surfactant, sodium dodecyl sulfate (SDS).

II. MATERIAL AND METHODS

Hematoporphyrin IX dihydrochloride was obtained from Porphyrin Products, Logan, UT, USA (Lot No. 111684) and was used without further purification. The neutral form of hematoporphyrin IX was obtained from the "Museum National d'Histoire Naturelle de Paris, Laboratoire de Biophysique" and was purified as reported by Vever-Bizet et al. and Dellinger et al. Sodium dodecyl sulfate salt, Baker Analyzed Reagent grade lot No. 527220

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was purchased from J. T. Baker Chemical Co., Phillipsburg, N. J. Stock solution were prepared by dissolving 1 mg HP into a 25 ml volumetric flask, using 0.010 molar HCl as the solvent. Complete solubility could be achieved by placing the solution flask in an ultrasonic bath for few minutes. From this stock solution, 2 ml aliquots were diluted to a final volume of 5.00 ml. The dilution was done with buffered solutions adjusted to the desired pH. The HP concentration range studied was between 0.5 μM and 30 μM. The final SDS concentration was 0.4% W/V when present in solution. The solution pH was obtained with the use of an Orion Research Model 811 microprocessor pH/millivolt meter equipped with an Orion Ross type model 8103 combination electrode. A two-points calibration was carried out daily prior to the start of any measurement. The solutions were bubbled with nitrogen just prior to each run, in order to be oxygen free. All measurements were carried out on freshly prepared hematoporphyrin solutions. Fluorescence decay curves were obtained from two independent systems, which were both single photon counting instruments. On the first, a 575 nm laser excitation beam was used, the details of which have been reported previously by Szabo10. The fluorescence emission was detected at 610 and 660 nm. The second system consisted of a 400 nm pulsed excitation source having a 50 nm band pass, which has been previously described by Sun et al.11. The fluorescence emission was detected using a low-pass filter at 590 nm.

Steady state fluorescence spectra were measured using a 75 W xenon lamp as excitation source (PTI A-1010), stabilized with a PTI LP8200X feedback control unit. The 404 nm excitation wavelength was obtained using an interference filter that has a 17 nm band pass. The excitation radiation was directed on the sample at a 90° angle from the emission monochromator, a Jarrell-Ash, 0.27 meter monochromator (Monospec – 27,100 grooves/mm grating blazed at 600 nm). The entrance slit of the monochromator was 25 μm. The entire fluorescence spectrum, from 500 to 800 nm, was recorded via an intensified photodiode array having 1024 diodes (Princeton Instruments, Inc. Model No. DMCP 700G). The exposure time of the analyzing light was typically of the order of 6 sec, during which 200 scans of the detector were integrated.

Absorbance measurements were carried out on Shimadzu-UV 160 double beam recording spectrophotometer. All quartz cuvettes used were cleaned in 3M nitric acid overnight prior to any series of measurements. Before any specific measurement was done, the cuvettes were further washed with ethanol and distilled water, and then rinsed with the solution to be analyzed.

III. RESULTS AND DISCUSSION

Except at very low pH values, three lifetimes were obtained in aqueous solution of HP. The solutions studied were adjusted to pH values that correspond to regions in which the steady state absorbance and fluorescence spectra reveals the presence of distinct monomeric species. A study between 0.5 μM and 30 μM in HP shows that the concentration of the solution does not affect markedly the monomeric lifetime values. Thus from the results in table 1A, the monomeric species of HP in aqueous solutions is characterized by a fluorescence lifetime of 5.8 nsec at pH 0.5, 7.6 nsec at pH 3 and 15.2 nsec at pH 8. In addition, a shorter lived (3.8 nsec fluorescent species is detected at high pH, probably reflecting the dimeric form of HP, as has been suggested in the literature7,12. A very short-lived species (~0.2 nsec) can be detected at pH higher than 2.5, possibly arising form higher aggregates of Hp. The pH dependant relative proportions of the longer lived monomeric species are in good agreement with recent results obtained via volumetric titration5.