

Photophysical Properties of some Water soluble metal Phthalocyanines

Zhang Xianfu, Ma Jinshi, Xu Huijun

Institute of Photographic Chemistry, Academia Sinica, Beijing, 100101

ABSTRACT

The effect of sulfonation degree on aggregation and photophysical properties of metal phthalocyanines was studied. Absorption and fluorescence spectra showed that association was decreased with the increasing of the sulfonation degree in aqueous solution. Little change was observed for fluorescence quantum yields and lifetime; triplet-triplet absorption and triplet lifetime for phthalocyanines with different degree of sulfonation. However, the measurement of absolute yields of singlet oxygen in DMSO showed that the degree of sulfonation has a dramatic influence on the ability of photochemical generation of singlet oxygen. Irradiation of oxygenated aqueous solutions (PH10) of tryptophan containing 10 μ M sulfonated zinc phthalocyanine and/or specific quenchers of the possible reactive intermediates suggested that photooxidation of amino acid was largely proceeded by Type-II mechanism.

1. INTRODUCTION

Encouraging results have been achieved for Photodynamic Therapy of human tumors with hematoporphyrin derivative (HPD) in past twenty years¹. However, HPD is a complex mixture and can cause photosensitivity of skin to sunlight. Great effort has been made to develop new photosensitizers which absorb strongly in the red region (600-800 nm) in recent years. It should be stable, chemically defined, water soluble, non-cytotoxic in the dark and has a high yield of long lived triplet states for a new candidate to replace HpD. Phthalocyanines (MPCs) and its sulfonated derivatives (MSPCs) have attracted much attention² among new photosensitizers owing to its some advantages, such as good stability to heat and light, easy preparation, strong absorption in red region leading to increased light penetration into human tissue. It was reported that the degree of sulfonation of MSPCs has a dramatic effect³ on the photokilling of cancer in vivo. The reason for this effect is not clear at present time. In this paper, we report some photophysical parameters of selectively sulfonated metal phthalocyanines in order to elucidate the mechanism of photosensitization of phthalocyanines in PDT.

2. MATERIALS AND METHODS

Sulfonated metal phthalocyanines were prepared as literature⁴. All other reagents were analytical grade and used without

further purification. Absorption spectra were recorded on Hitachi-557 UV spectrometer. Fluorescence spectra were measured on Perking-Elmer LS-5 spectrofluorimeter with excitation at 610 nm. Transient absorption spectra measurements were performed with a Nd:TAG Q-switch laser(Quanta Ray DCR-2A-30,ex=355 nm,5 ns fwhm,2 mj) and with a pulsed xenon lamp(XF-300,Nissan Electric Co.,Japan,50 W) as a monitoring light source. Singlet lifetime was determined using single photon counting technique described elsewhere.

3.RESULTS AND DISCUSSION

3.1.Aggregation of sulfophthalocyanines in aqueous solution

It is well known tetrasulfonated phthalocyanines(H_2TSPC_c) and its metal derivatives($MeTSPC_c$;Me=Zn,Cu,Co,Ni,Fe) prepared by condensation of 4-sulfophthalic anhydride have a high tendency to form dimer which is non-photoactive upon excitation in aqueous solution. The addition of some organic solvents such as pyridine facilitate the monomerization of dimer. For sulfophthalocyanines ($MeSnPC_d$,n=1-3) synthesized by direct sulfonation of metal phthalocyanines with fuming sulfuric acid,although absorption spectra showed similar aggregation phenomena as that of $MeTSPC_c$,little tendency to aggregate was observed for MeS_4PC_d on the basis of the disappearance of absorption band of dimer in UV spectra shown in Fig.1. Perhaps this was attributed to the less symmetry of MeS_4PC_d than that of $MeTSPC_c$ caused by the simultaneous presence of sulfonic acid group at 3- and 4-position on different benzene ring of phthalocyanines. The measurement of fluorescence quantum yields of selectively sulfonated zinc and chloro aluminum phthalocyanines showed that the tendency of aggregation was decreased with the increasing of the degree of sulfonation,see Tab.1. It is important to point out that $AlSnPC(n=1-3)$ can form dimers in aqueous solution,this was never noticed by any other researchers. The determination of dimerization constant K using the method described by Monahan⁶ confirmed the relation between the tendency of association and the degree of sulfonation.

In order to investigate the mechanism of photosensitized oxidation of tryptophan by sulfophthalocyanines in micellar dispersed solutions,the interaction of phthalocyanines with micelles was evaluated by absorption and fluorescence spectra. The results showed all phthalocyanines with different degree of sulfonation can intercalated into CTAB and Triton-100 micelles,however,only phthalocyanines with low degree of sulfonation(n=1,2) can embed into SDS anion micelles owing to strong static repulsion between SDS and sulfonic anion.

Table 1,effect of degree of sulfonation on fluorescence quantum yield and dimerization constants

	n	1	2	3	4
ϕ_f	ZnSnPCd	3.9×10^{-3}	5.3×10^{-3}	2.4×10^{-2}	2.9×10^{-1}
	AlSnPCd	0.12	0.14	0.28	0.39
K	ZnSnPCd	-	$2.4 \times 10^{+6}$	$7.96 \times 10^{+5}$	-
	AlSnPCd	-	$3.18 \times 10^{+5}$	$3.54 \times 10^{+4}$	-

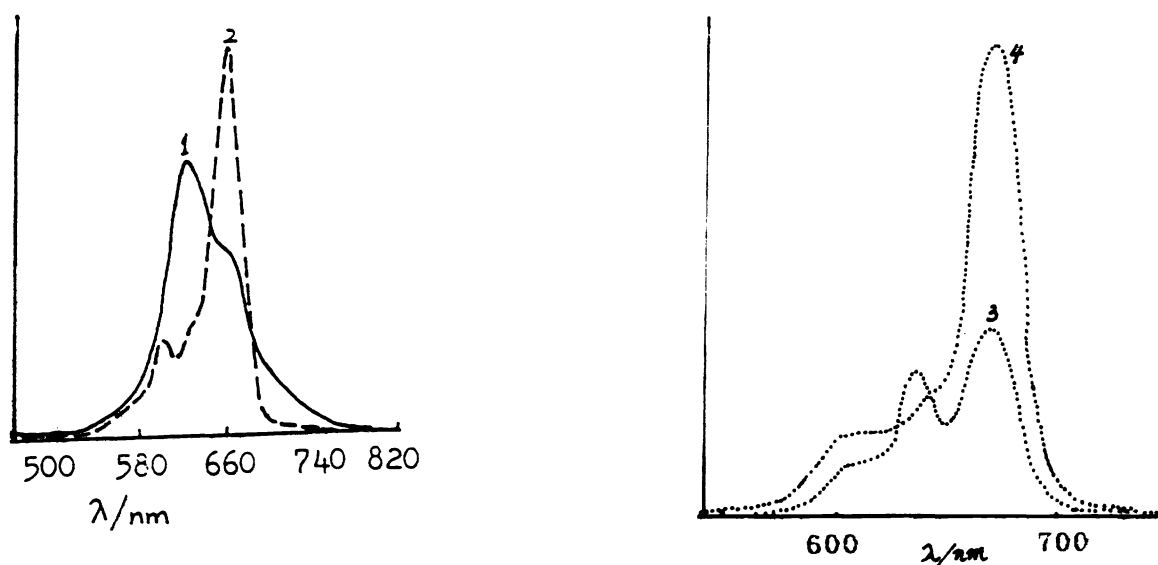


Fig.1. a, absorption spectra of 1-ZnS₂PCd and 2-ZnS₄PCd
 b, absorption spectra of 3-ALS₁PCd and 4-ALS₄PCd

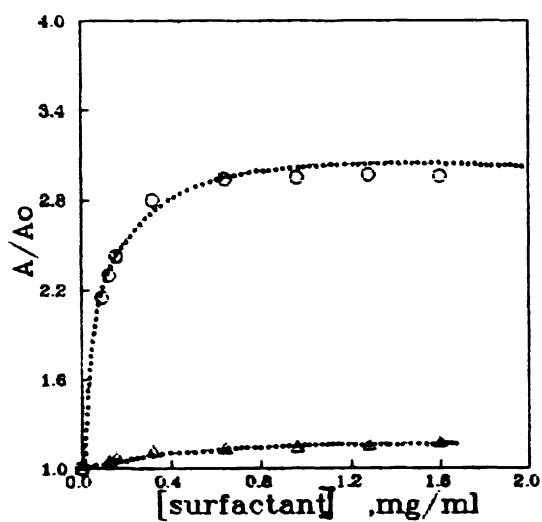


Fig.2.1

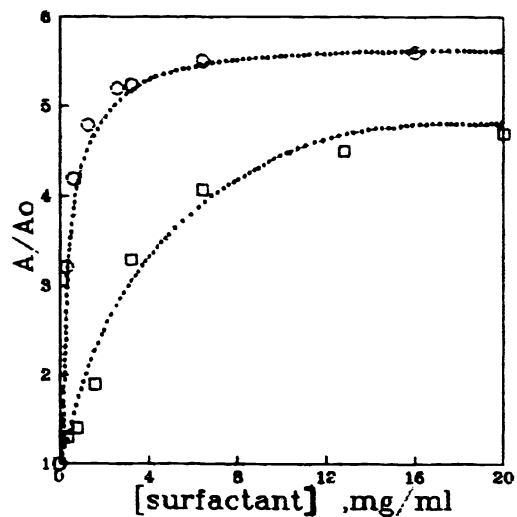


Fig.2.2

Fig.2. effect of the surfactant concentration on the absorbance at 670 nm of 5 μ M sulfonated phthalocyanines in 0.1 M Tris buffer at PH 10.

Fig.2.1, ZnS₁PCd(\circ) and ZnS₄PCd(Δ) at CTAB micelles
 Fig.2.2, ZnS₁PCd at Triton-100(\square) and SDS(\circ) micelles
 Fig.2.3, ZnS₄PCd at Triton-100(\square) and SDS(Δ) micelles

3.2. Photophysics

Some photophysical parameters are summarized in Table 2. In Table 2, some photophysical parameters of sulfonated phthalocyanines (MeSnPC)

Me	n	Φ_f		τ_f		τ_T	Φ_A	Kq(x10 ⁻⁹)
		DMF	W-P	DMF	W-P			
	0	0.30	-	3.9	-	300	0.20	-
	1	0.31	0.21	4.2	3.5	-	-	-
Zn	2	0.32	0.26	4.1	3.7	-	0.55	-
	3	0.31	0.27	4.3	3.7	-	0.21	-
	4	0.32	0.32	4.1	4.4	250	0.19	31
	0	-	-	-	-	500	-	-
	1	-	0.38	-	-	-	-	3.1
AlCl ₂	-	-	0.47	-	-	-	0.12	4.7
	4	-	0.64	-	5.0	500	0.067	6.6
	1	-	0.03	-	-	-	-	-
Sn	2	-	0.13	-	-	-	-	-
	4	-	0.17	-	4.4	-	-	37
GaCl ₄	-	-	0.41	-	4.8	-	-	7.9

W-P: Water-Pyridine (7:3, V/V)

disaggregated conditions, fluorescence quantum yields and lifetime is independent of degree of sulfonation and have almost the same value as that of the corresponding unsulfonated phthalocyanines. In the presence of dimerization, little fluorescence was observed in aqueous solution although singlet lifetime decreased slightly. Transient absorption spectra revealed that sulfonated phthalocyanines have the same T-T absorption band and triplet lifetime as that of unsulfonated phthalocyanines.

Using DPBF as trapper of singlet oxygen, the measurement of quantum yield of singlet oxygen was performed with irradiation at visible light in DMSO. The degree of sulfonation has a dramatic influence on , and the ability of photochemical generation of singlet oxygen was consistent with the activity of photokilling of tumor by phthalocyanines in PDT. However, in aqueous solution, H. Ali⁷ reported contrary results for selectively sulfonated GaClSnPC synthesized by condensation method.

Quenching of fluorescence of sulfophthalocyanines was carried out in H₂O-50%CH₃OH, using tryptophan as quencher at various concentrations. The relative fluorescence intensities at 685 nm were measured and Stern-Volmer relationship was obeyed. The quenching rate constants calculated showed that photoinduced electron transfer from tryptophan to sensitizers can take place and probably make a little contribution to Type-I reaction process.

3.3. Mechanism of Photosensitized Oxidation of Tryptophan

Oxygenated solutions (0.1M Tris buffer, PH10) of L-tryptophan containing 10 μ M ZnS₁PCd and/or specific quenchers of the possible reactive intermediates were irradiated with visible

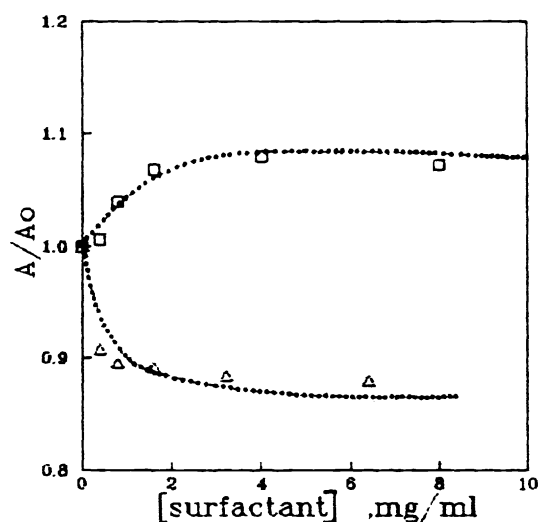


Fig.2.3

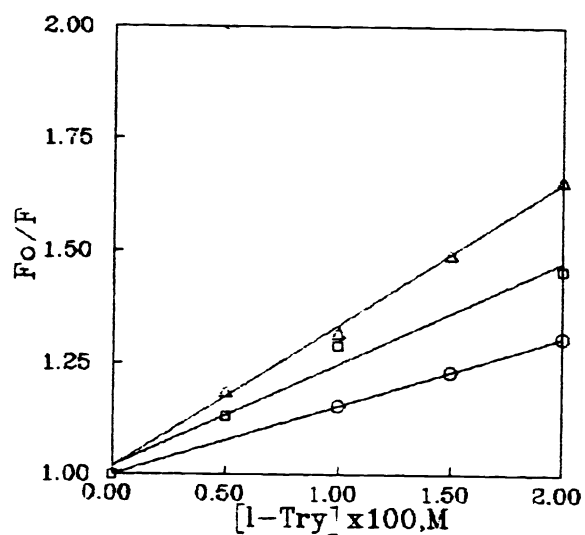


Fig.3.

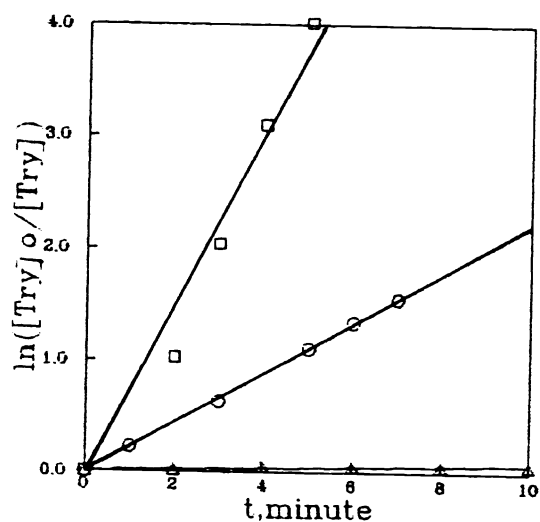


Fig.4

Fig.3. Stern-Volmer plot for the quenching of AlSnPCd(5 μ M in H_2O -50% CH_3OH) fluorescence by Tryptophan
 \circ , $n=1$ \square , $n=2$ \triangle , $n=4$

Fig.4. Time-course of l-Tryptophan photooxidation upon irradiation of 2 mM aminoacid in 0.1M Tris buffer at PH 10 in the presence and absence of 20 mg/ml SDS
 \triangle , in the absence of SDS. \square , D_2O . \circ , H_2O

light(>600 nm) in the presence of dispersed sodium dodecyl sulphate micelles. The rate of loss of tryptophan was monitored by the change of fluorescence intensity of tryptophan upon excitation at 294 nm. Irradiation caused a decrease of the aminoacid concentration which followed first order kinetics(Fig.4). The rate constants as deduced from the slope of the kinetic plots are summarized in Table 3. Apparently, NaN_3 , a

Table 3, relative rates for the ZnS_1PC -sensitized photooxidation of tryptophan in the presence of various additives

additive	none	D_2O	NaN_3 0.4mM	1mM	$\text{K}_3\text{Fe}(\text{CN})_6$ 0.22mM	1.1mM	cysteine 0.75mM	hydrquinone 0.3mM
relative rate	2.24	9.24	1.19	0.77	1.94	1.77	1.79	1.90

typical quencher of singlet oxygen had remarkable effect on the photooxidation rate, 50% inhibition occurred at 0.4mM N_3^- , whereas in typical Type-I process 50% inhibition is observed with azide concentration about 7.5mM⁸. It is indicated that tryptophan appeared to be photooxidized largely by a Type-II(O_2^1 -involving) mechanism. This conclusion was further supported by the 4-fold enhancement of the photooxidation rate when performing the irradiation in D_2O solution. The minor importance of Type-I process was evidenced by the weak quenching of the addition of radical scavenger, such as hydroquinone, fericyanide and cysteine.

4. REFERENCES

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