Photo-induced reactions of sulfonated aluminum phthalocyanine with peptide

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Abstract The photosensitized oxidation mechanism of peptide in the presence of the ClAlPcS is studied by UV-Vis spectroscopic method. It is found that the oxidation of peptide is totally quenched in 1.1×10^{-2} mol/L NaN_3 solution, and the same reaction cannot proceed in the dark. $K_3Fe(CN)_6$ and L-Cys cannot quench the reaction. Therefore, the reaction mechanism is mainly Foote's type Ⅱ. This reaction process can be inferred as follows: (ⅰ) active oxygen can attack acylamide bond near by carbon atom; (ⅱ) carboxyl group departs from peptide; (ⅲ) I-benzazole cycle is destroyed when reaction is continued; (ⅳ) the peptide is completely damaged.

Keywords: sulfonated aluminum phthalocyanine, peptide, photosensitized oxidation.

PHOTOSENSITIZED oxidation was the process in which photosensitizer oxidized reaction medium under irradiation; it was the base of photodynamic therapy (PDT)[1]. Phthalocyanine is a very important photosensitizer, with molecular structure similar to that of porphyries, low toxin, high steadiness in light and heat, and stronger absorption in near infrared region (600—700 nm). It can selectively attach to cancer cell, so it can be potentially used in the therapy of cancer and in the restraint of human immunology virus (HIV), etc. Therefore, it is a fine photosensitizer[2]. Sulfonated aluminum phthalocyanine (ClAlPcS) does not aggregate in water solution[3]. It can oxidize L-tryptophane by type Ⅰ reaction mechanism[4]. Photosensitized oxidation of peptide with phthalocyanine has not been reported too much.

In this work, photosensitized oxidation dynamic of peptide with ClAlPcS was investigated and reaction mechanism was inferred.

1 Material and method

Absorption spectra were run on an HP8452A spectrophotometer. ^{1}H-NMR spectra were obtained on an ARX400 spectrometer, at 25℃, using DSS as inner standard.

ClAlPcS and peptide (Gly-L-Trp) were synthesized and purified following the method in refs. [3—5]. Their structures were confirmed by elemental analysis and spectrum method.

L-tryptophane (chromatographically pure) was provided by Shanghai Dongfeng Biochemistry Reagent Company. The other reagents were made in China, analysis pure. Redistilled water was used for preparing the sample solution. The light source was a 150 W Xe lamp. The wavelength shorter than 630 nm was cut off with a suitable filter.

The experiment was performed at 1 atmosphere. The mixture was stirred with a small magnetic bar. The change of absorption spectra at 280 nm was recorded at the interval of 5 min on an HP8452A spec-

trophotometer. The data of depression absorption peak were treated by exponential fitting method. This reaction was shown to be a first-order decay process.

Reaction rate constants were obtained with the corresponding absorption data using eq. (1),

$$\ln(A_t - A_e)/(A_0 - A_e) = kt, \quad (1)$$

where A_t is the absorbance measured at time t, A_e is that at equilibrium, and A_0 is that at the beginning of reaction, at 280 nm.

2 Result and discussion

(ⅰ) Kinetic analysis of photosensitized oxidation of peptide. According to Foote's law, photosensitized oxidation could differentiate between type Ⅰ and type Ⅱ[6]. Absorption spectrum remained unchanged when ClAlPcS solution was added to peptide solution. It reveals that there is no interaction between ClAlPcS and peptide in the dark.

Reaction in mixture could be initiated under irradiation, and the result is shown in fig. 1. Absorption peak gradually went down at 280 nm, but went up at 422 nm. There existed an equal light density point at 296 nm. It was found that the oxidation products decayed following the first-order kinetic. Good linearity ($r = 0.998\ 7$) is shown in fig. 2, so reaction rate constants could be obtained. The photooxidation mechanism was investigated by using NaN_3 as a probe for the generation of $^1O_2$[7]. Sodium azide is widely known for its ability to quench single oxygen. When NaN_3 was added to reaction solution, the reaction rates decreased as shown in fig. 3. When the concentration of NaN_3 was 1.1×10^{-2} mol/L, the photooxidation reaction was totally quenched.

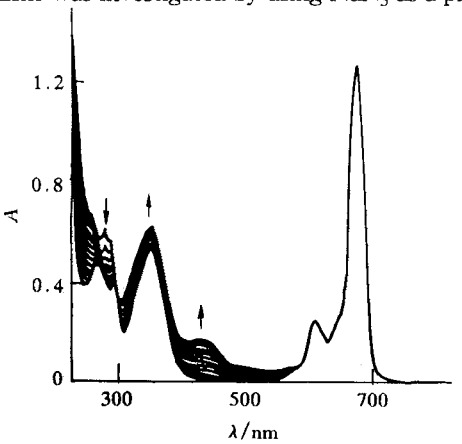

Fig. 1. Kinetic plot of photosensitized oxidation of ClAlPcS to peptide. ClAlPcS, 2×10^{-5} mol/L; peptide, 5×10^{-4} mol/L.

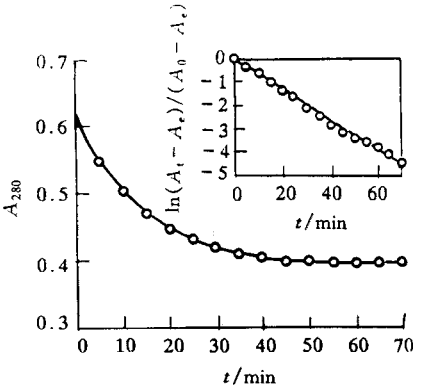

Fig. 2. Kinetic analysis of photosensitized oxidation of ClAlPcS to peptide.

The quenching ability of $K_3Fe(CN)_6$ or L-Cys in efficient in electron transfer reaction[8], but it is inefficient in this reaction.

Therefore, the Foote's type Ⅱ mechanism dominates in the reaction.

(ⅱ) Mechanism of photosensitized oxidation of peptide. One reason why absorption peaks appear at 422 nm was that new compound was produced. Conjugate of this new compound was larger than that of peptide. Photosensitized oxidation reaction was recorded by a high precision nuclear magnetic resonance spectrometer, and the spectrum is shown in fig. 4. There was an alkenyl structure at 8.1—8.2 ppm on NMR spectrum. This reaction process can be inferred as follows (fig. 5): ⅰ) active oxygen can attack acylamide bond near by carbon atom; ⅱ) carboxyl group departs from peptide; ⅲ) 1-benzazole cycle is

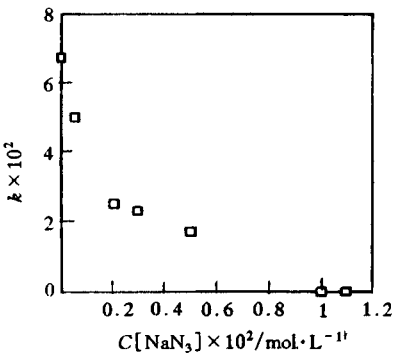

Fig. 3. Rate constants of peptide versus concentration of NaN_3 solution.

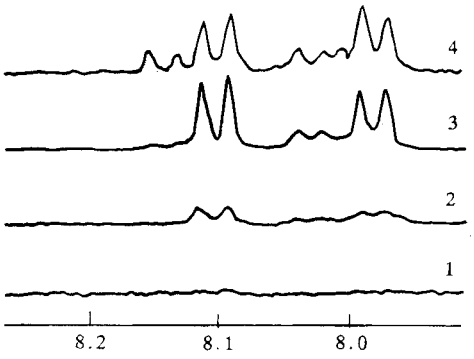

Fig. 4. ^{1}H-NMR spectrum of photosensitized oxidation of ClAlPcS to peptide (7.9—8.3 ppm). Irradiation time (min): 1, 0; 2, 20; 3, 40; 4, 70.

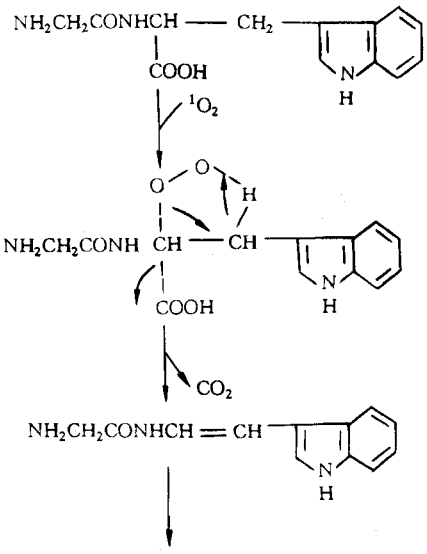

Fig. 5

destroyed when reaction is continued; (IV) the peptide is completely damaged.

References

1 Darwent, J. R., McCubbin, I., Phillips, D., Excited singlet and triplet state electrontransfer reaction of aluminum (Ⅲ)——Sulfonated phthalocyanine, *J. Chem. Soc. Faraday Trans.* 2, 1982, 18: 3347.

2 Spike, J. D., Phthalocyanines as photosensitizers in biological system and for the photodynamic therapy of tumors, *Photochem. Photobiol.*, 1986, 43(6): 691.

3 Dong Run'an, Qiu Yong, Zhang Fushi *et al.*, Study on the aggregation state of sulfonated phthalocyanine in physiological condition, *Journal of Tsinghua University* (*Sci. & Tech.*) (in Chinese), 1996, 36(6): 55.

4 Wagener, J. R., Hasrat, A., Langlais, R. *et al.*, Biological activation of phthalocyanines VI, photooxidation of L-tryptophan by selectively sulfonated gallium phthalocyanines: singlet oxygen yields and effect of aggregation, *Photochem. Photobiol.*, 1987, 45(5): 587.

5 Huang Weide, Chen Changqing, *Peptide Synthesis* (in Chinese), Beijing: Science Press, 1985.

6 Ferraudi, G., Arguello, G. A., Ali, H. *et al.*, Type Ⅰ and Ⅱ sensitized photooxidation of amino acid by phthalocyanines: a flush photochemical study, *Photochem. Photobiol.*, 1988, 47(5): 657.

7 Dearden, S. J., Kinetics of $O_2(^1\Delta g)$ photooxidation reaction in egg yolk lecith in vesicles, *J. Chem. Soc. Faraday Trans.* 1, 1986, 82: 1627.

8 Monroe, B. M., *Single Oxygen* (ed. Frimer, A. A.), Vol. 1, London: CRC Press, 1985.

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Genetic diversity of reared *N. miichthioides* population

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Abstract The genetic diversity of 30 reared *Nibea miichthioides* individuals was analyzed by random amplified polymorphic DNA (RAPD) with 20 random primers. The result showed that the genetic diversity of reared individuals was relatively low with 15.31% polymorphism and 0.031 9 of the average difference (AD). The result also indicated that RAPD is a useful way in genetic diversity analysis of fish population.

Keywords: *Nibea miichthioides*, genetic diversity, RAPD.

N. MIICHTHIOIDES has recently become a new kind of rearing species in southern China for its high