Oxidative biomacromolecular damage from novel phthalocyanine

DONG Runan (董润安)[1,2], QIU Yong (邱 勇)[2], TIAN Shufang (田淑芳)[2], WANG Shengqi (王升启)[3] and SONG Xinqi (宋心琦)[2]

(1. State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China; 2. Department of Chemistry and Department of Chemical Engineering, Tsinghua University, Beijing 100084, China; 3. Institute of Radiation Medicine, Academy of Military Medical Sciences, Beijing 100850, China)

Received June 5, 1997

Abstract Photosensitized oxidation of the novel phthalocyanine modified by glycine [$ZnPc(NHCH_2COOH)_4$] in the presence of glucose oxidase or plasmid pBR322 DNA was investigated by using gel electrophoresis, capillary electrophoresis and the enzyme activity determination method. As a result, glucose oxidase was cleaved partially by photosensitizer, the cross-linkage between subunits occurred partially simultaneously and, moreover, enzyme was cleaved continually into pieces. Activity of glucose oxidase was 130 times lower than that of original within 12 h. DNA could be cleaved in the presence of photosensitizer $ZnPc(NHCH_2COOH)_4$. Super helix of DNA was depolymerized into nicked and linear DNA. Then, it was cleaved progressively into pieces. It was suggested that the novel photosensitizer could do apparent damage to biomacromolecules.

Keywords: **phthalocyanine, glucose oxidase, DNA, photosensitized oxidation.**

In recent years, phthalocyanines are widely used in photodynamic therapy (PDT) and light-induced electronic devices. They can be potentially used in the therapy of cancer and in the restraint of human immunology virus (HIV), etc.[1,2]. Photosensitized oxidation is a process in which photosensitizer oxidizes the reaction medium. It is the base of photodynamic therapy[3]. Phthalocyanines can selectively attack cancer cells, while it has low toxin to the normal tissues, high steadiness in light and heat and high toxin to cancer cells[4]. In order to study the relationship between the molecular structure and its properties, various kinds of phthalocyanines have been synthesized[5,6]. Authors synthesized a novel photosensitizer, $ZnPc(NHCH_2COOH)_4$[7]. It has strong absorption in the near infrared region. Its maximum absorption peak is at 750 nm. It is an excellent potential photosensitizer. Its structure is shown in fig.1. Partial enzyme system of cancer cells has stronger activity than that of normal cells[8]. DNA synthesis is vigorous in cancer cells[9]. If enzyme activity and rate of DNA synthesis can be reduced, the cancer cells will be controlled. Therefore, it is very important to study the effect of photosensitizer on enzyme activity and DNA molecules. Results of Gantchev et al. show that $AlPcS_4$ and $ZnPcS_4$ photosensitizer does severe and irreversible damage to the protein and causes destruction of the heme prosthetic groups, which results in the rapid inactivation of catalase[10]. Herein we report the photosensitized oxidation of glucose oxidase and pBR322

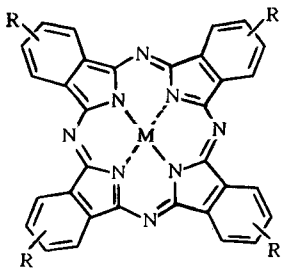

Fig. 1. Molecular structure of $ZnPc(NHCH_2COOH)_4$.

with the novel glycine-modified phthalocyanine, which is used as a model for investigation of damage of photosensitizer to biomacromolcules.

1 Materials and method

1.1 Materials

$ZnPc(NHCH_2COOH)_4$ and $ZnPcS_4$ were synthesized and purified following the method in references [7, 11].

Their structures were confirmed by elemental analysis and spectroscopic analysis method.

Glucose oxidase and peroxidase (horse radish), chromatographically pure, were obtained from Sigma. pBR 322DNA, chromatographically pure, was purchased from Promega. Agarose gel was provided by Bio Rad Company. Ethidium bromide and *o*-dianisidine, analytically pure, were provided by Fluka Company. Redistilled water was used for preparing the sample solution. All the other chemicals were of the highest analytical grade available.

1.2 Method

The photosensitized oxidation experiments were performed following the method in literature[1)], under 1.01×10^5 Pa. The mixture was stirred with a small magnetic bar. Solution glucose oxidase in phosphate buffer (pH 7.2) was irradiated in a standard spectroscopic 1 cm × 1 cm quartz cell in the presence of 5×10^{-4} mol/L $ZnPc(NHCH_2COOH)_4$ or $ZnPcS_4$. The red light source was a UV-IR-filtered Xe lamp ($\lambda > 630$ nm). The irradiation cell holder was a thermostat in a water jacket at 25℃. The intensity of irradation was tested to be 1.823 J/M·L·S using an illuminance instrument (ZDS-10, Lux Meter, Shanghai Jiading Xuelian Plant).

Determination of enzyme activity was performed following the method in literature[12] 1) on an HP8452A spectroscopic photometer. The data were treated by the multinomial fitting method. The initial velocity of reaction was obtained from fitting equation differentially.

Polyacrylamide gel electrophoresis was performed following the method in ref. [13] on a DYY-III23A type electrophoresis apparatus using 12% polyacrylamide with 4% stacking gel, etc. After gel electrophoresis, the gel was stained with Commassie brilliant blue and photographed.

Agarose gels electrophoresis was performed following the method in ref. [13] on a DYY-III31 type electrophoresis apparatus using 1% agarose gels under standard conditions with TAE (pH 8.0) as the buffer. After electrophoresis, gels were stained with ethidium bromide, and then visualized under UV light.

Capillary electrophoresis was performed on a Beckman P/ACE 5510 system under constant pressure, using diode-array detection and fused silica capillary (75 μm × 57 cm, Hebei Yongnian Silica Capillary Plant). The injection of all samples was controlled by pressure for 5 s[14].

2 Result and discussion

2.1 Photosensitized oxidation effect on enzyme activity

Photosensitiztion reaction of glucose oxidase was performed for different reaction times and under the same condition for $ZnPc(NHCH_2COOH)_4$ and $ZnPcS_4$, respectively. The sample and

1) Dong Runan, Photosensitized oxidation of profein, nucleic acid and related model compounds, Ph.D. Thesis of Tsinghua University, 1996.

reference were added to the enzyme activity determination system consisting of peroxidase (horse radish, 2.50 u/mL), *o*-dianisidine (1×10^{-3} mol/L) and glucose (50 mg/L). The initial velocity was obtained on a UV-Vis spectroscopic photometer as shown in table 1.

The inactivation of glucose oxidase under irradiation occurred at nearly similar rates for both photosensitizers. Glucose oxidase activities were reduced progressively along with time. It was 130 times lower than that of the original.

Table 1 Reaction initial velocity of photosensitized oxidation at different times[a)]

Time /h	Reaction initial velocity ($V_0\times10^4$/mol·L^{-1}·min^{-1})	
	$ZnPc(NHCH_2COOH)_4$	$ZnPcS_4$
0	4.16	4.16
2	2.83	2.92
4	1.09	1.01
6	0.72	0.83
8	0.55	0.52
10	0.37	0.33
12	0.30	0.32

a) Concentration of both $ZnPc(NHCH_2COOH)_4$ and $ZnPcS_4$ was 5×10^{-4} mol/L.

2.2 Damage from photosensitized oxidation to enzyme

To verify that photosensitization causes irreversible (covalent) protein cleavage and aggregation with the formation of intermolecularcross-links, we performed PAGE analysis under our conditions. A typical electrophoresis pattern with respect to the applied irradiation is shown in fig. 2 with $ZnPc(NHCH_2COOH)_4$ as photosensitizer. Nonirradiated glucose oxidase migrated as one protein band, but irradiated glucose oxidase migrated as two bands after 2 h. Bands increased progressively with time. It showed clearly that this product increased in quantity. Nevertheless, the number of bands was decreased considerably after irradiation for 10 h. Further irradiation for 12 h resulted in an almost complete disappearance of the above major bands. Shown in fig. 3 is the ease using $ZnPcS_4$ as photosensitizer. Protein cross-linkage increased very much when the reaction underwent for 2 h, but the activity enzyme descended in amount. It reduced continually for 6 h. Cross-linkage began to be cleaved after 8 h, which continued up to 10 h. Lastly, enzyme was cleaved into pieces 12 h later.

Capillary electrophoresis (CE) could analyse the precious structure of protein[15]. CE spectra of $ZnPc(NHCH_2COOH)_4$ solutions with added glucose oxidase under irradiation for different

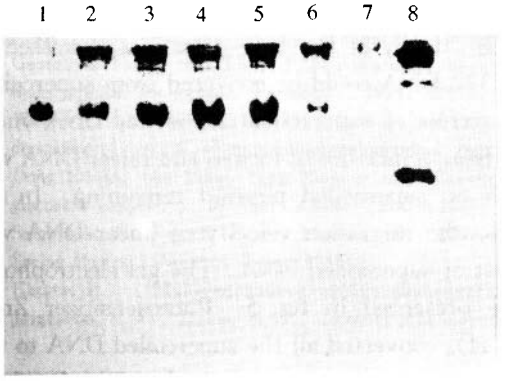

Fig. 2. Photosensitized oxidation of glucose oxidase (3.51 u/mL) with $ZnPc(NHCH_2COOH)_4$ (5×10^{-4} mol/L). Lane 1, GOD + $ZnPc(NHCH_2COOH)_4$; lanes 2—7, GOD + $ZnPc(NHCH_2COOH)_4$ under irradiation for 2, 4, 6, 8, 10 and 12 h; lane 8, marker.

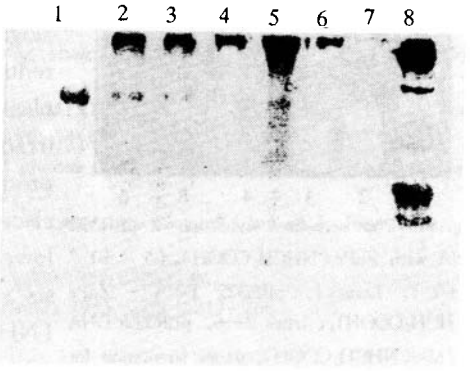

Fig. 3. Photosensitized oxidation of glucose oxidase (3.51 u/mL) with $ZnPS_4$ (5×10^{-4} mol/L). Lane 1, GOD + $ZnPcS_4$; lanes 2—7, GOD + $ZnPcS_4$ under irradiation for 2, 4, 6, 8, 10 and 12 h; lane 8, marker.

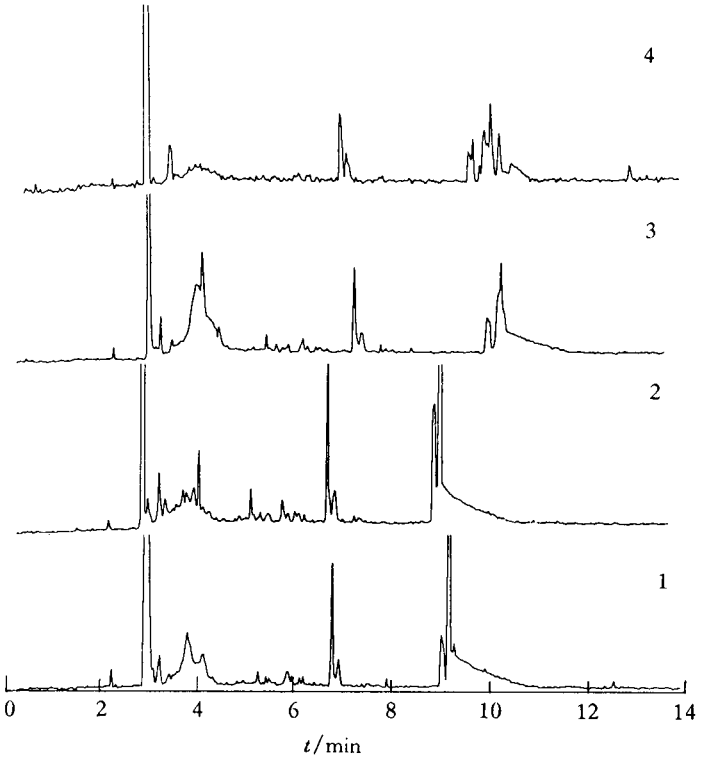

Fig. 4. Capillary electrophoresis analysis of photosensitized oxidation of glucose oxidase with ZnPc($NHCH_2COOH$)$_4$ (5×10^{-4} mol/L), under irradiation for 0 (1), 2 (2), 4 (3), 10 (4) h.

times are shown in figure 4.

Cross-linkage was increased after 6 h. It is obvious that seven minor peaks were formed on CE spectrum after 10 h. It is suggested that glucose oxidase was destroyed by photosensitizer.

Fig. 5 Photosensitized oxidation of pBR322 DNA with ZnPc($NHCH_2COOH$)$_4$ (5×10^{-4} mol/L). Lane 1, pBR322 DNA + ZnPc($NHCH_2COOH$)$_4$; lanes 2—6, pBR322 DNA + ZnPc($NHCH_2COOH$)$_4$ under irradiation for 1, 2, 3, 4 and 5 h.

2.3 Damage to DNA by photosensitized oxidation

DNA is the material base of gene. There will occur biological variation if any of the base pairs was increased or reduced. pBR322 DNA could be convered from supercoiled plasmid to a mixture of supercoiled and nicked DNA under irradiation. Then, a mixture of nicked and linear DNA was produced with no supercoiled plasmid remaining. In gel electrophoresis, the migration velocity of linear DNA was lower than that of supercoiled DNA. The gel electrophoresis results are presented in fig. 5. Photosensitizer ZnPc ($NHCH_2COOH$)$_4$ converted all the supercoiled DNA to the mixture of supercoiled and nicked DNA after 2 h. Furthermore, ZnPc ($NHCH_2COOH$)$_4$ converted nicked DNA cleanly to mixture of linear DNA and even smaller pieces after 3 h. Lastly, supercoiled DNA was all cleaved into pieces with the increase of time. The data indicate that the DNA was substantially damaged by photosensitizer.

3 Conclusion

Photosensitized oxidation of glucose oxidase or plasmid pBR322 DNA in the presence of novel phthalocyanine modified by glycine was investigated by using gel electrophoresis, capillary electrophoresis and enzyme acitvity determination method. As a result, the activity of glucose oxidase was decreased by approximately 130 times compared with that of the original when glucose oxidase was irradiated for 12 h. Early damage of glucose oxidase was partial cleavage, then, the cross-linkage between subunits occurred partially, and the enzyme was cleaved continually into pieces. DNA could be cleaved in the presence of ZnPc-$(NHCH_2COOH)_4$. Super helix of DNA was first depolymerized into nicked and linear DNA, which were cleaved progressively into pieces. This suggests that the novel photosensitizer could prominently damage biomacromolecules.

References

1 Dougherty, T.J., Photosensitizers: therapy and detection of malignant tumours, *Photochem. Photobiol.*, 1987, 45(2): 879.

2 Reddi, E., Jori, G., Steady-state time-resolved spectroscopic studies of photodynamic sensitizers: porphyrine and phthalocyanines, *Rev. Chem. Interm.*, 1988, 10: 241.

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

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

5 Davila, J., Harriman, A., Photosensitized oxidation of biomaterials and related model compounds, *Photochem. Photobiol.*, 1989, 50(1): 29.

6 Shopova, M., Gantchev, T., Comparison of the photosensitizing efficiencies of haematoporphyrin (HP) and its derivative (HPD) with that of free-base tetrasulphophthalocyanine ($TSPC-H_2$) in homogeneous and microheterogeneous media, *Photochem. Photobiol.*, B: *Biol.*, 1990, 6(1): 49.

7 Dong, R.A., Qiu, Y., Song, X. Q., Synthesis and spectroscopic properties of an amino acid modified phthalocyanine, *Chinese Chemical Letters*, 1996, 7(5): 399.

8 Chakravarty, P.K., Carl, P.L., Weber, M.J. et al., Plasmin-activated prodrugs for cancer chemotherapy: 1. Synthesis and biological activity of peptidylacivicin and peptidylphenylendiamine, *J. Med. Chem.*, 1983, 26: 633.

9 Fred, W.P., *Molecular Biology* (in Chinese), Beijing: Science Press, 1990.

10 Gantchev, T.G., van Lier, J. E., Catalase inactivation following photosensitization with tetrasulfonated metallophthalocyanines, *Photochem. Photobiol.*, 1995, 62(1): 123.

11 Weber, J.H., Busch, D.H., Complexes derived from strong field ligands (XIX)——Magnetic properties of transition metal derivation of 4,4′,4″,4‴-tetrasulfophthalocyanine, *Inorg. Chem.*, 1965, 4(4): 469.

12 Dong Runan, Qiu Yong, Song Xinqi et al., Kinetic properties of glucose oxidase reaction system and its application of glucose's analysis, *J. of Anal. Chem.* (in Chinese), 1996, 24(2): 135.

13 Sambrook, J., Fritsch, E.F., Manitial, T., *Molecular Cloning: A Laboratory Manual* (2nd ed.), New York: Cold Spring Harbor Laboratory Press, 1989.

14 Karger, B.L., High-performance capillary electrophoresis, *Nature*, 1989, 339(6226): 641.

15 Jorgenson, J.W., Lukes, K.D., Capillary zone electrophoresis, *Science*, 1983, 222(4621): 266.