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Pheophorbide as Efficient Sensitizer for DNA Photocleavage. An Implication to Its Role in Photodynamic Cancer Therapy

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By use of pheophorbide a as sensitizer, DNA has been efficiently cleaved on visible light irradiation either in the presence or the absence of molecular oxygen. The possibility of DNA scission by pheophorbide a in photodynamic cancer therapy is indicated.

Pheophorbide a, a derivative of chlorophyll a (Fig. 1), has been attracting significant interest as highly potent sensitizer for photodynamic therapy of malignant tumors.[1] The advantages of pheophorbides over hematoporphyrins, the sensitizers widely explored for cancer phototherapy, involve higher selectivity for accumulation in or near the tumor cells, smaller side effect, and

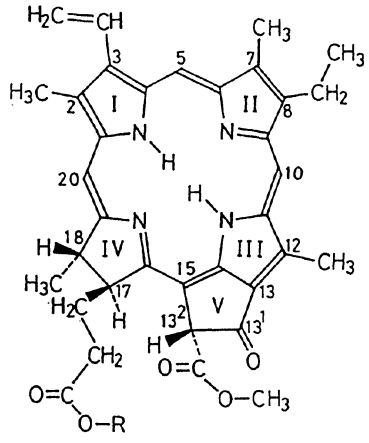

Fig. 1. Structures of pheophorbide a (R = H) and pheophytin a (R = phytyl): chlorophyll a is magnesium salt of pheophytin a.

superior photochemical properties. It has been believed that the photodynamic action is associated with degradation of cell membrane by singlet oxygen, from the analogy with the results on the therapy by hematoporphyrins.[2] The singlet oxygen is formed by energy transfer from the pheophorbide excited by laser-light to molecular oxygen.

This paper reports that pheophorbide promptly cleaves deoxynucleic acids (DNA) on irradiation of non-laser visible light. The possibility that photo-induced damage of DNA contributes to the photodynamic cancer therapy is indicated.

Pheophorbide a was prepared according to the literature [3] by acid treatment of chlorophyll a, extracted from Chlorella.[4] The crude specimen was purified by reversed-phase HPLC, and was finally converted to sodium salt to increase water-solubility. Visible light was irradiated from 500 W xenon lump at a distance 30 cm, using appropriate filter when necessary.

Figure 2 depicts electrophoresis patterns for the photocleavage of plasmid pBR 322 supercoiled DNA (form I) by pheophorbide a. Facile conversion of the form I DNA to the form II is clearly evidenced (lanes 2-4). In the absence of either photoirradiation or pheophorbide a, scission was

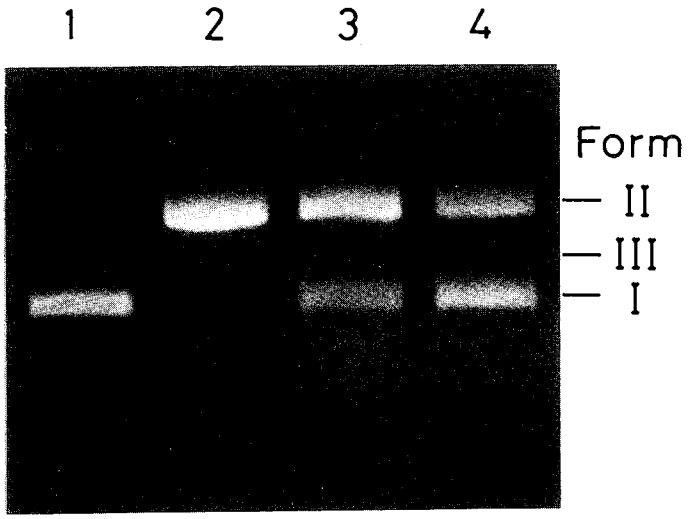

Fig. 2. Agarose gel electrophoresis patterns (stained by ethidium bromide) for the pheophorbide-induced photocleavage of pBR 322 plasmid DNA: lane 1, without photoirradiation; lane 2, in the absence of oxygen, irradiation 30 min; lane 3, in the absence of oxygen, irradiation 5 min; lane 4, under air, irradiation 5 min; $[\text{DNA base pair}]_0 = 3 \times 10^{-5}$ and $[\text{pheophorbide a}]_0 = 10^{-4}$ mol dm^{-3}.

virtually none. Complete conversion to the form II DNA was achieved in less than 30 min, and further irradiation produced the form III DNA. Action spectrum was consistent with excitation of the Q_Y band (650-690 nm) and the Soret band (around 400 nm) of pheophorbide a, and the cleavage rate monotonously increased with increase in the dye concentration.

Significantly, the photocleavage efficiently proceeded even in the absence of molecular oxygen (lanes 2 and 3), where oxygen was thoroughly removed by repeated (3 times) freeze-and-thaw cycles. The cleavage without oxygen is faster than the cleavage with it, by about 2 fold as estimated by densitometry.

Single-stranded homopolymers poly(dA) and poly(dT) were also photocleaved by pheophorbide a either with or without molecular oxygen. The cleavages were followed by ion-exchange HPLC (TOSOH DEAE-NPR column). Small oligomers (up to 5-mers) were dominantly produced, indicating that the scission took place preferentially near the 5'- and/or the 3'-terminal ends.

The oxygen-independent photocleavages by pheophorbide a are highly in contrast with requirement of molecular oxygen for the photocleavages by porphyrin derivatives.[5,6] Apparently the ring V in pheophorbide a (see Fig. 1) is responsible for the unique photochemistry. One of the plausible mechanisms involves photo-removal of the labile hydrogen on the C-13^2 carbon. The resultant radical of pheophorbide attacks DNA at either the ribose residue or the phosphate residue. The mechanism is supported by the fact that epimerization of pheophytin (phytyl ester of pheophorbide: Fig. 1) from form a to form a' (epimers with respect to the C-13^2 carbon) is largely accelerated by photoirradiation, as detected by HPLC.[7] The epimerization requires hydrogen removal from the C-13^2 carbon atom. Direct measurement of photo-induced epimerization of pheophorbide a has not been successful yet due to poor resolution of the epimers by HPLC.

The present results indicate that DNAs in tumor cells are cleaved when the cells are irradiated by laser-light in the presence of

pheophorbides for photodynamic therapy. This cleavage, which is obviously fatal for the tumor cells, can proceed simultaneously with the singlet oxygen-mediated degradation of cell membrane.[1] Precise evaluation of this possibility, of course, requires detailed investigation on the phototherapy itself. Pheophorbide can be also potent as catalytic site of photo-controllable artificial nuclease, which has been attracting significant interest.[8]

In conclusion, pheophorbide a effectively cleaves DNA on photo-irradiation. This finding sheds light on the mechanism of the photo-dynamic cancer therapy and should be useful for development of still more efficient photosensitizer for the therapy. Attachment of pheophorbide to sequence-recognizing moiety for preparation of artificial nuclease is now in progress.

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