# GENERATION OF REACTIVE OXYGEN SPECIES BY PHOTOEXCITED PHEOPHORBIDE a AND ITS EFFECTS ON ERYTHROCYTE MEMBRANES

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Electron spin resonance (ESR) and human-erythrocyte ghosts were employed to elucidate the phototoxic effects of pheophorbide a (PPa), which is a causal substance of dietary photosensitization in animals. Since PPa had two maximum light absorbances at 404 and 664 nm, two light-sources, which contained light wavelengths of around 404 nm and around 664 nm, respectively, were used for irradiation.

ESR examination combined with 2,2,6,6-tetramethyl-4-piperidone proved that PPa excited with light containing either 404 nm or 664 nm generated signlet oxygen ($^1O_2$) at physiological pH 7.4, and it combined with 5,5-dimethyl-1-pyrroline-N-oxide demonstrated that only PPa excited with light containing 404 nm generated superoxide anions ($O_2^-$) at the same pH.

The effects of photoexcited PPa on biomembranes were next studied in resealed ghost-PPa complexes for which the ghosts had previously been prepared from human erythrocytes. Irradiation of the complexes was separately carried out with two light sources. The efflux rates of trapped D-glucose-6-phosphate from resealed ghosts were measured as a marker of an increase in membrane permeability. Activities of glyceraldehyde-3-phosphate dehydrogenase were measured to detect the effects of photoexcited PPa on membrane enzymes. The effects of photoexcited PPa on the structural integrity of membrane proteins were observed as changes in the electrophoretic patterns of sodium dodecyl sulfate-gel electrophoresis. The results indicated that exposing the complexes to light caused an increase of membrane permeability, the inactivation of enzymes and the denaturation of proteins. Examination with $^1O_2$ quenchers and $OH^\cdot$ (from $O_2^-$) scavengers proved that when the complexes were exposed to the light containing 404 nm at pH 7.4, the simultaneous addition of the $^1O_2$ quencher and the $OH^\cdot$ scavenger gave protective effects whereas, when the complexes were exposed to the light containing 664 nm at pH 7.4, only the addition of the $^1O_2$ quencher was effective, suggesting that both $^1O_2$ and $OH^\cdot$ were responsible for the induction of membrane damage at 404 nm, and that only $^1O_2$ was responsible for it at 664 nm. From these results it was concluded that the effects of photoexcited PPa on biomembranes were due to oxidative stress caused by reactive oxygen species.