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QUANTUM EFFICIENCY OF PHOTOGENERATION OF SINGLET OXYGEN FROM THE CLUSTER TYPE OF BINUCLEAR IRON-SULFUR CENTER [2Fe-2S]*

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Abstract — Photosensitization *via* the singlet oxygen ($^1O_2$) mechanism by the binuclear iron-sulfur center, denoted as [2Fe-2S], was investigated, using a highly purified ferredoxin (Fd) preparation from spinach leaves. Since the apoprotein of Fd contains a good number of amino acid residues that are readily reactive with $^1O_2$ and thus interfere with the detection of $^1O_2$ generated from [2Fe-2S], we attempted to deprive the $^1O_2$-sensitive residues of their $^1O_2$-scavenging capacity as much as possible by treating Fd with rose bengal plus 550 nm monochromatic light and thereby photooxidatively degrading these residues. The photochemically modified Fd was found to keep the structural integrity of its Fe-S group virtually unaffected by the treatment. By employing chemical trap method for $^1O_2$ measurement and examining the kinetic effects of azide and deuterium oxide on the reactions of $^1O_2$ with various trap compounds, we were able to demonstrate that [2Fe-2S] indeed acts as a photosensitizer *via* $^1O_2$. Further, the minimum quantum yield of $^1O_2$ production by [2Fe-2S] was estimated to be 0.0047.

INTRODUCTION

Iron-sulfur centers, containing iron and acid-labile sulfur in equal portion, play an important role in membrane-bound electron transport systems of cells. In thylakoid membranes where light-driven electron transfer occurs, five centers have been documented, *viz* the Rieske center associated with the cytochrome b/f complex, the center of soluble ferredoxin, and three centers (F_X, F_A and F_B) involved in electron transport activity of photosystem I (PS I). Among these, the former two have the cluster-type of binuclear center denoted as [2Fe-2S], and the centers in PS I are of the form of [4Fe-4S].[1]

An interesting feature of Fe-S centers is the observation that, apart from their fundamental function as electron carriers, they act as endogenous photosensitizers in near UV to blue light, causing photooxidative damage to various cellular components. Although this extraordinary role of Fe-S centers in cells under high light condition has primarily been deduced from the action spectra of singlet oxygen ($^1O_2$) generation from thylakoid and mitochondria membranes,[2,3] as compared with those from the membranes with the centers destroyed by a mercurial treatment, a series of our studies has consistently provided results corroborating this concept.[4-10]

We felt that it would be timely to undertake an investigation so as to provide a rather concrete information on photodynamic sensitization by Fe-S clusters *via* $^1O_2$: in the present study, as a first step, photogeneration of $^1O_2$ from the binuclear Fe-S center in chloroplast ferredoxin was focused. We herein report that the blue light-absorbing chromophore of ferredoxin indeed promotes photodynamic reactions *via* type II mechanism and that the minimun quantum yield of $^1O_2$ generation by [2Fe-2S] is 0.0047.

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* Dedicated to Prof. Pill-Soon Song on the occasion of his 60th birthday.

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Abbreviations : DEAE, diethylaminoethyl; D_2O, deuterium oxide; F_A, Fe-S_A; F_B, Fe-S_B; F_X, Fe-S_X; Fd, ferredoxin; PM-Fd, photochemically modified ferredoxin; PS I, photosystem I; Φ^1O_2, quantum yield of singlet oxygen; RB, rose bengal; RNO, N,N-dimethyl-4-nitrosoaniline; $^1O_2$, singlet oxygen; TBARS, thiobarbituric acid-reactive substance; Tris, 2-amino-hydroxymethyl-1,3-propandiol.

MATERIALS AND METHODS

Preparation of ferredoxin (Fd). Fd was prepared from spinach leaves, as described by Buchanan and Arnon[11] with a modification. Instead of two ion exchange chromatographic steps, we employed three steps: the first step using a DEAE-cellulose column (2.5 x 10 cm) preequilibrated and washed with 0.15 *M* Tris-HCl buffer (pH 7.3) containing 80 m*M* NaCl and then eluted with 0.3 *M* Tris-HCl buffer (pH 7.3) containing 0.55 *M* NaCl; the second step using a DEAE-cellulose column (3 x 20 cm) preequilibrated and eluted with 0.15 *M* Tris-HCl buffer (pH 7.3) containing 0.15 *M* NaCl; and the third step using a DEAE-Sepharose CL 6B column (3 x 20 cm) preequilibrated and eluted with 0.15 *M* Tris-HCl buffer (pH 7.3) containing 0.2 *M* NaCl. The final preparations of ferredoxin showed the absorbance ratio between 420 nm and 270 nm (A_{420}/A_{270}) 0.46 - 0.48 as well as a single protein band on a polyacrylamide gel when subjected to electrophoresis, indicating that the protein was highly purified and structually intact.[11,12]

Preparation of photochemically modified ferredoxin (PM-Fd). Fd was briefly incubated with rose bengal (RB) in the dark and then irradiated with 550 nm monochromatic light (350 W/m^2) obtained from a 3 kW Xe-Arc lamp (Osram, Germany). During irradiation, samples were gently bubbled with air. PM-Fd, some of whose amino acid residues were supposedly photooxidized but whose Fe-S moiety remained intact, was separated from the irradiated Fd samples by using a DEAE-Sepharose CL 6B column (2 x 13 cm) preequilibrated with 0.15 *M* Tris-HCl (pH 7.3)/0.2 *M* NaCl buffer. In order to remove RB from the photolyzed samples prior to applying the anion exchange chromatography, the samples was passed through a Sephadex G-25 column (1 x 5 cm).

Preparation of PM-Fd entrapped liposomes and measurement of membrane lipid peroxidation. Liposomes were prepared from L-α-phosphatidyl choline following the procedure of New[13] in the presence of either bovine serum albumin as a reference or PM-Fd. Special care was taken to prevent oxidative reactions of lipid during preparation of liposomes by conducting experiments under anaerobic conditions in red safety light. Peroxidation of liposomal phospholipid was measured by spectrophotometrically monitoring the production of thiobarbituric acid-reactive substance (TBARS) in hot acid-digested liposomes, as described by Buege and Aust.[14]

Determination lysozyme activity. Lysozyme solutions, subjected to various treatments, were assayed for the enzyme activity as in Sellak *et al.*[15] For this, the rate of decrease in light scattering at 450 nm by *Micrococcus lysodeiklicus* suspensions due to the bacteriolytic activity of lysozyme was taken as an index of enzyme activity.

Measurement of $^1O_2$ production. The imidazole derivative plus N,N-dimethyl-4-nitrosoaniline (imidazole-RNO) method was employed to assay $^1O_2$ production in samples by irradiation. The reaction mixtures contained RNO (5 μ*M*), histidine (10 m*M*), and either RB (6.5 μ*M*), Fd (20 μ*M*) or PM-Fd (20 μ*M*); hereafter we will call these mixtures RB/histidine/RNO, Fd/histidine/RNO, and PM-Fd/histidine/RNO systems, respectively.

Chemicals and instruments. Chemicals were purchased from Sigma Chemical Co. (St. Louis, MO), Fluka Chemie AG (Buchs, Switzerland) and Merck (Frankfurter, Germany). These were of the highest grade available and used as supplied. Throughout the work spectrometric measurements were done with a Cary 118-C spectrophotometer (Varian Assoc., Palo Alto, CA). The light fluence rates of visible light (λ > 400 nm) were determined by using an IL 1700 Research radiometer/photometer (International Light Inc., NewBuryport, MA), while those of UV-A (320 - 400 nm) were by a DRC-1000 X digital radiometer with a DIX 365 detector (Spectroline, Westbury, New York). Photolysis was performed in a thermostated quartz cuvette at 20°C. UV-A was obtained from an Osram Xe-Arc lamp (3 kW), using a combination of UV band pass filter and a glass plate of 5 mm thickness. A f/3.4 grating monochromator (Applied photophysics, London, UK) was used to obtain monochromatic light from the same Xe-Arc source.

RESULTS AND DISCUSSION

Photodynamic reactions by [2Fe-2S] via $^1O_2$ mechanism

Apoprotein of Fd has a number of amino acid residues, that are sensitive to $^1O_2$ and thus act as effective chemical quenchers of $^1O_2$, such as 1 histidine, 4 methionine, 1 tyrosine, 5 cystein and 1 tryptophan residues. It would therefore be reasonably assumed that $^1O_2$ generated from the iron-sulfur group is scavenged to a significant extent by these residues before it reaches to and reacts with trap compounds generally used for measuring $^1O_2$ production.

In Fd, four cystein residues are directly associated with iron in [2Fe-2S] and some $^1O_2$-sensitive residues may also be involved in maintaining the structural integrity of the chromophore: all these residues can be regarded as the essential $^1O_2$-sensitive residues. It seems, however, that other $^1O_2$-sensitive, but not essential, residues may simply act as $^1O_2$ scavengers, competing with the trap compounds for reaction with $^1O_2$. In this context, it would be desirable to deprive, at least, the nonessential residues of the $^1O_2$-quenching capacity.

RB is a well-known photosensitizer *via* type II mechanism. Because the quantum yield of $^1O_2$ production by RB, [Φ^1O_2 (RB)], is very large, one can readily supply biological samples with high concentrations of $^1O_2$ by irradiating them in the presence of RB even with relatively weak light. By exposing Fd solution containing RB to 550 nm monochromatic light and carrying out anion exchange chromatography (Fig. 1-A), we were able to prepare PM-Fd. The absorption spectrum (Fig. 1-B) and the

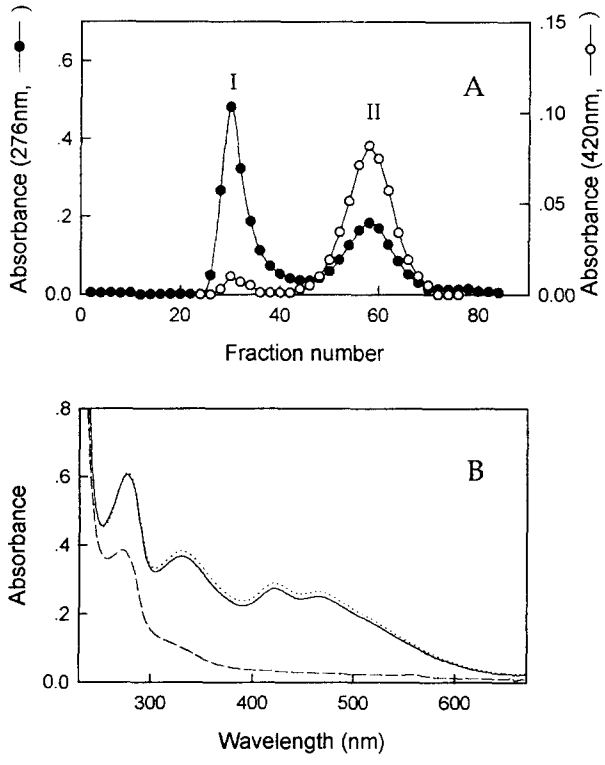

Figure 1. Panel A, anion exchange (DEAE-Sepharose CL 6B) chromatogram of Fd subjected to the RB/light treatment, showing two distinctive protein fractions I and II. Panel B, absorption spectra of fraction I (broken trace) and fraction II (solid trace), as compared with the spectrum of intact Fd (dotted trace). For the RB/light treatment, the mixture of Fd (20 μ*M*) and RB (6.5 μ*M*) in 10 m*M* phosphate buffer (pH 7.3) was irradiated with 550 nm monochromatic light (350 W/m^2) for 20 min at 10°C.

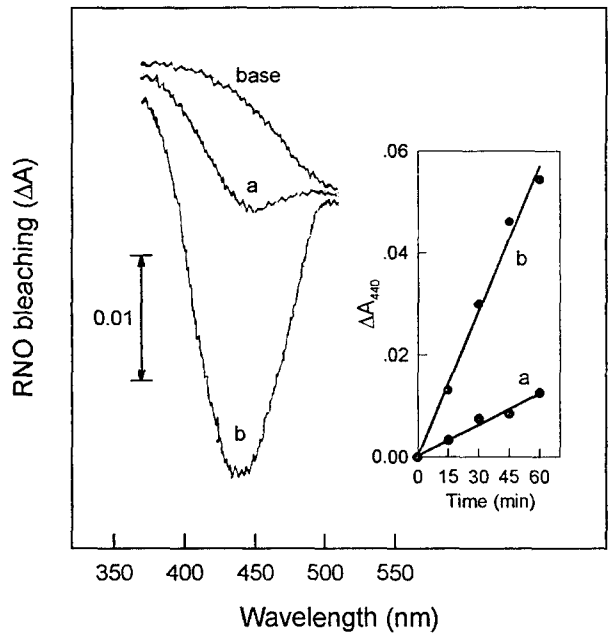

Figure 2. RNO bleaching in the Fd/histidine/RNO (a) and PM-Fd/histidine/RNO (b) systems, subjected to irradiation with 350 nm monochromatic light (180 W/m^2) for 30 min at 10°C. The zero order kinetics of the bleaching of the respective systems was shown in inset. As for the composition of the photolysis samples, see Materials and Methods. PM-Fd corresponds to the fraction II in Fig. 1-A.

labile sulfur content (data not shown) of PM-Fd were found practically identical with those of the untreated Fd, indicating that the iron-sulfur chromophore remained intact during the RB/light treatment.

The relative efficiencies of $^1O_2$ photoproduction by intact Fd and PM-Fd were evaluated by the degrees of RNO bleaching in the Fd/histidine/RNO and PM-Fd/histidine/RNO systems. As it turned out (Fig. 2), PM-Fd was for more effective in photosensitization than intact Fd: this observation appears pertinent with the notion that the $^1O_2$-reactive amino acid residues of Fd scavenge $^1O_2$ generated from the visible absorbing chromophore.

Since some $^1O_2$-sensitive residues were assumed to be involved in maintaining the chromophore structure of Fd, it seems very unlikely that the RB/light treatment brought about a complete deprivation of the $^1O_2$ quenching activity of Fd, while keeping the structural intactness of [2Fe-2S]. The prolonged treatment, in fact, resulted in total loss of the labile sulfur from Fd as well as a drastic change in light absorption property, as can be seen in the spectrum of fraction I of the treated Fd (see Fig. 1-B).

Simple and convenient as it may be, chemical trap method for $^1O_2$ measurement, in general, suffers lack of specificity; for many trap compounds can also be oxidized to the same products by free radical processes. Therefore, other techniques together with this are usually employed to provide the supporting data for the involvement of $^1O_2$ in photosensitization reactions, which are based on checking kinetic effects of the addition of $^1O_2$ quencher and deuterium oxide (D_2O) to samples prior to irradiation.

We used histidine-RNO, phospholipid vesicles and lysozyme as chemical traps. The imidazole-RNO method, developed by Kraljić and Mohsni,[16] is probably one of the most sensitive and simple techniques; its usefulness for the detection of $^1O_2$ has been demonstrated with such biological systems as mitochondria and thylakoids under high irradiance conditions.[2,3] Lysozyme has also been used as a $^1O_2$ trap because it is readily susceptible to $^1O_2$-mediated inactivation: note that there exist three tryptophan residues at the active site of this bacteriolytic enzyme.[17,18] Liposome made of unsaturated phospholipid also traps $^1O_2$ particularly produced in membranes,

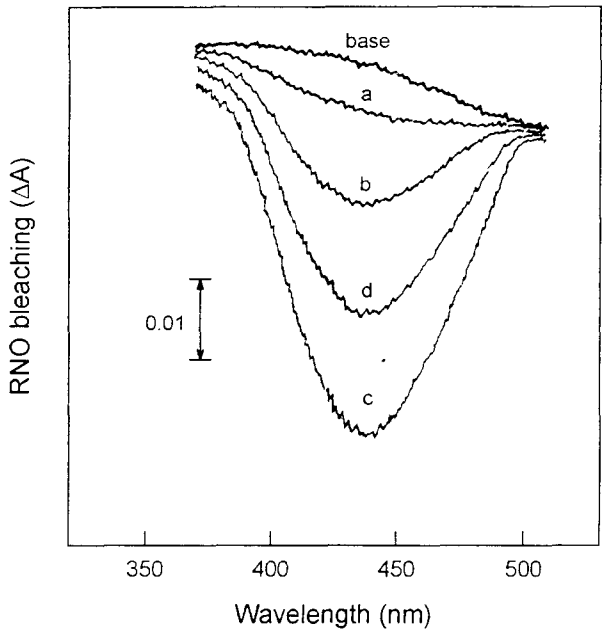

Figure 3. Effects of (a) deaeration (N_2-purging) of, (b) azide (30 m*M*) addition to, and (c) 50% mediume-deuteration of the PM-Fd/histidine/RNO system on RNO bleaching. Trace (d) shows the bleaching of the control. Photolysis was performed with UV-A (200 W/m^2) from a Xe-Arc lamp for 30 min at 10 °C. The compostion of the PM-Fd histidine RNO system was the same as in Fig. 2.

resulting in lipid peroxidation, and thus has been used to check whether $^1O_2$ produces in photolysis systems under investigation.[7]

RNO bleaching in the PM-Fd/histidine/RNO system by irradiation was substantially enhanced by medium deuteration and suppressed by an efficient $^1O_2$ quencher, azide (Fig. 3). As expected, the patterns of both lysozyme inactivation and liposomal membrane lipid peroxidation (Fig. 4), induced by the combined action of light plus PM-Fd, were strikingly similar to that of the bleaching of RNO. All these photochemical reactions were O_2-dependent: see that the relative degrees of RNO bleaching, the lysozyme inactivation and the lipid peroxidation were small enough to be negligible when the respective samples were irradiated under anaerobic conditions. Considering that the lifetime of $^1O_2$ ($\tau_{^1O_2}$) is much longer in D_2O than in H_2O (2-4 μs in H_2O *vs* about 40-50 μs in D_2O)[20] and shortened by the presence of azide (< 0.5 μs in 10 m*M* azide, for instance[21]). The above results, as shown in Figs. 3 and 4, clearly indicate that the binuclear Fe-S center can act as an endogenous photosensitizer in biological systems *via* type II mechanism.

In the case of the effect of medium deuteration on those O_2-dependent photochemical reactions, an increase in the quantum yield of $^1O_2$ production ($\Phi_{^1O_2}$)

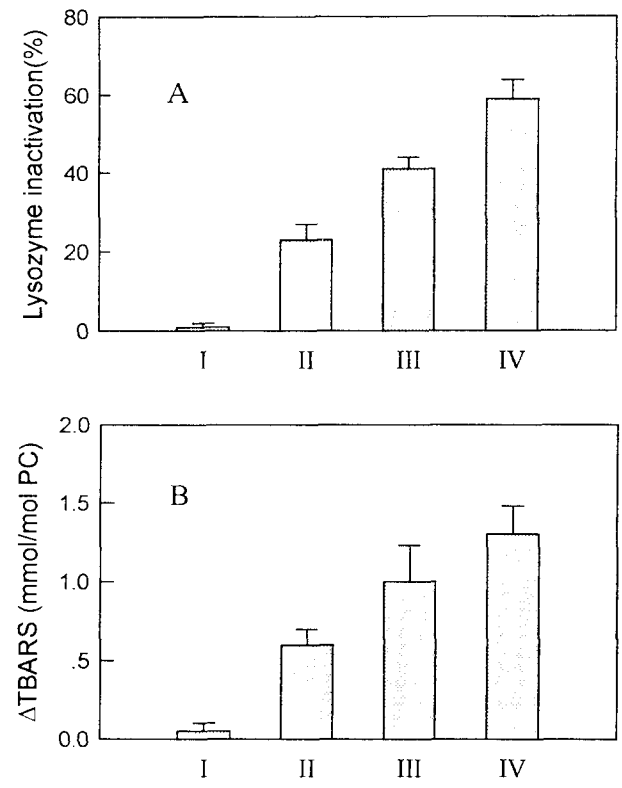

Figure 4. Inactivation of lysozyme (panel A) and lipid peroxidation of liposomes (panel B), induced by PM-Fd dependent photosensitization. The samples containing PM-Fd (20 μ*M*) and either lysozyme (2500 units) or liposomes (0.7 m*M*) were either (I) deaerated by N_2-purging, (II) admixed with 30 m*M* azide, (III) aerated with air bubbling or (IV) medium-deuterated by 50%. Photolysis conditions were the same as in Fig. 3.

in D_2O relative to H_2O might also be considered together with the extension of $\tau_{^1O_2}$; for a small but significant increase in $\Phi_{^1O_2}$ in D_2O has been observed with a photosensitizer, tetrakis-(4-sulfonatophenyl) porphyrin, which arises from the enhanced triplet quantum yield of the porphyrin derivative, presumably due to a decrease in the rate of internal conversion.[22] Although it is very unlikely that the phenomenon is a common feature of type II photosensitizers, this point is certainly interesting and worthy of further extensive experimental scrutiny.

Quantum yield of $^1O_2$ production by [2Fe-2S]

It would be appropriate to briefly describe the kinetic treatment for the measurement of $^1O_2$ by chemical trap method. The rate of $^1O_2$ formation by energy transfer from a photosensitizer to molecular oxygen obeys Eq.(1)

$$\frac{d[^1O_2]}{dt} = I_{abs}\,\Phi_{^1O_2} \qquad (1)$$

where I_{abs} is the intensity of light absorbed by the

sensitizer and Φ^1O_2 is the quantum yield of $^1O_2$ production. According to Gandin *et al.*,[23] when a $^1O_2$ trap compound (A), which reacts specifically with $^1O_2$ and does not interact with the triplet state of photosensitizer, is present in sample, stationary state approximation leads to an expression for the rate of disappearance of A, as given by Eq.(2)

$$-\frac{d[A]}{dt} = k_c[^1O_2][A] = I_{abs}\,\Phi^1O_2\,\frac{k_c[A]}{k_c[A] + k_q[A] + k_d} \qquad (2)$$

where k_d is the rate constant of deactivation of $^1O_2$ *via* the solvent ($^1O_2 \rightarrow {}^3O_2$), and k_c and k_q are the rate constants of chemical scavenging ($^1O_2 + A \rightarrow AO_2$) and physical quenching ($^1O_2 + {}^3A \rightarrow {}^3O_2 + A$), respectively. When $k_d \gg k_c[A] + k_q[A]$, Eq.(2) reduces to the following form

$$-\frac{d[A]}{dt} = I_{abs}\,\Phi^1O_2\,\frac{k_c}{k_d}\,[A] \qquad (3)$$

The slope of first order plot for disappearance of A will give $I_{abs}\,\Phi^1O_2\,k_c/k_d$. If k_c and k_d are known for the corresponding photosensitizer, Φ^1O_2 is calculated from the slope, corrected for the 'real' absorption by the sensitizer from total absorbance of sample at the illuminating wavelength. Even in the case that the constants are not available, Φ^1O_2 can readily be determined if the rate of disappearance of A for a reference photosensitizer, whose Φ^1O_2 is known, is measured under the same experimental conditions; for the ratio of the corrected slopes give a relative scale of Φ^1O_2 by different photosensitizers.

The imidazole-RNO method is based upon the secondary bleaching of RNO that is induced by the reaction of $^1O_2$ with imidazole or its derivatives; the intermediate, a transannular peroxide, causes RNO bleaching. Since RNO *per se* is not a $^1O_2$ scavenging compound, Eq.(3) is not directly applicable for determining Φ^1O_2. However, a linear relationship has been observed between the rate of RNO bleaching and the rate of disappearance of imidazole when such conditions are met as the imidazole ($^1O_2$ scavenger) concentration being in a large excess (*ca.* 10 m*M*) and the change in RNO being kept under 10% of its initial concentration.[23] Thus, an expression is given that shows the bleaching of RNO following a zero order kinetics with the slope proportional to Φ^1O_2.

$$-\frac{d[RNO]}{dt} = C\,I_{abs}\,\Phi^1O_2 \qquad (4)$$

Table 1. The production of singlet oxygen by photosensitizers at 350 nm

buffer	sensitizer	R/f_s*	Φ^1O_2
0.01M K-PO_4	RB	3.5×10^{-1}	0.75
(pH 7.3)	Fd	6.0×10^{-4}	0.0013
	PM-Fd	2.2×10^{-3}	0.0047
0.01M Tris-HCl	RB	8.5×10^{-2}	0.19
(pH 7.3)	Fd	5.0×10^{-4}	0.0011
	PM-Fd	1.3×10^{-4}	0.0028

*R is the rate of RNO bleaching (ΔA_{440}/min) and the fraction of light absorbed by sensitizer was measured with $f_s = A_s(1\text{-}10^{-At})/A_t$. Data are averages of 6 to 9 measurements, done by using two different concentrations for each sensitizer; the relative value of spread was not greater than 25%.

where the apparent constant C would involve the concentration of imidazole and the rate constant for the reaction of RNO with the transannular peroxide, in addition to k_c and k_d.

The slopes of zero order plots for RNO bleaching (A_{440}) were measured with samples containing RB, Fd and PM-Fd, respectively, and corrected for the absorption by the corresponding sensitizers, by dividing the bleaching rate (R) by the fraction of light absorbed by sensitizer (f_s), as described by Blum and Grossweiner:[24] f_s was calculated with $F_s = A_s/A_t \cdot (1\text{-}10^{-At})$, where A_s is the initial absorbance of sensitizer in buffer and A_t is the absorbance of sensitizer plus RNO in solution. From the ratio of the corrected slopes of RNO bleaching in phosphate buffer, that was found to be 100 : 0.17 : 0.64 for RB : Fd : PM-Fd, the quantum yields of $^1O_2$ production by Fd and PM-Fd, denoted as Φ^1O_2 (Fd) and Φ^1O_2 (PM-Fd), were determined to be 0.0013 and 0.0047, repectively, scaled to Φ^1O_2 (RB) = 0.75 in the same buffer (Table 1).

Φ^1O_2 (Fd) is about a quarter of Φ^1O_2 (PM-Fd), indicating that at least three quarters of total $^1O_2$ photogenerated from [2Fe-2S] of intact Fd are quenched by the $^1O_2$-reactive amino acid residues of the protein. However, the possibility cannot be ruled out that PM-Fd still contained some of the $^1O_2$-sensitive residues, keeping the $^1O_2$-quenching activity to a certain degree; for photomodification of Fd was carried out only to the extent that the structural property of the chromophore remained virtually unaffected. In this respect, the value 0.0047 for PM-Fd should be regarded as the minimum, rather than the true, quantum yield of $^1O_2$ production by the binuclear Fe-S center.

Also noteworthy from Table 1 is the dependence of Φ^1O_2 on buffer composition, as manifested by the

values obtained with Tris buffer that are substantially lower than those obtained with phosphate buffer for all sensitizers examined: this tendency was particularly prominent in RB. It may be conjectured from this observation that certain buffer components are somehow involved in the deactivation and/or quenching processes of $^1O_2$. The plausibilty of this explanation seems very low, however, because the relative degrees of change in $\Phi_{^1O_2}$ with Tris buffer *vs.* phosphate buffer are markedly different between the sensitizers. The other possible explanation is that buffer components may affect such photophysical processes as intersystem crossing ($^1Sen^* \rightarrow {}^3Sen^*$) and triplet decay ($^3Sen^* \rightarrow Sen$), and thereby cause a change in $\Phi_{^1O_2}$. Whatever the underlying mechanisms are, it is clear that buffer composition is an important parameter, which undoubtedly influences photosensitization efficiency and should be taken into account for a photobiological work with either endogenous or exogenous photosensitizing agents in aqueous media.

CONCLUSION

The results presented herein have confirmed our previous suggestion that the blue light-absorbing prosthetic groups of iron-sulfur proteins are the major endogenous sensitizers in ordinary cells, capable of inducing photodynamic reactions *via* type II mechanism. Compared with such effective photosensitizer as RB which uses up to 75% of total absorbed photons for producing $^1O_2$ in aerated phosphate buffer, photosensitization efficiency of spinach Fd appears small. Because of this, one may make little account of physiological significance for the photobiological role of Fe-S centers. However, in certain biological systems which are inevitably exposed to bright light for a long period (take chloroplasts and mitochondria in plant leaves, for instance), the low efficiency does not necessarily implicate an insignificant degree of photodynamic damage incurred by Fe-S centers. Furthermore, if the $^1O_2$-generating chromophores are located in the apolar interior of cellular membranes (note that many nonheme iron proteins are the integral membrane proteins), $\tau_{^1O_2}$ is presumed to be remarkably prolonged, as evinced by the fact that $\tau_{^1O_2}$ is *ca.* 20 μs in hexane while it is only 2-4 μs in water. Such a sizable extention of $\tau_{^1O_2}$ can result in a substantial enhancement of photodynamic effects particularly on the structures and functions of biomembranes, notwithstanding the low efficiency of endogenous photosensitizers.

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REFERENCES

1. Evans, M. C. W. (1982) Iron-sulfur centers in photosynthetic electron transport. In *Iron-Sulfur Proteins* (Edited by T. G. Spiro), pp. 249-284. Wiley-Interscience press, New York.
2. Jung, J., H. Kim and M. Cho (1990) Action spectra for the generation of singlet oxygen from mitochondrial membranes from soybean (Glycine max) hypocotyls. *Photochem. Photobiol.* **52**, 561-566.
3. Jung, J. and H. S. Kim (1990) The chromophores as endogenous sensitizers involved in the photogeneration of singlet oxygen in spinach thylakoids. *Photochem. Photobiol.* **52**, 1003-1009.
4. Jung, J. and Y.-J. Kim (1990) Inactivation of citric acid cycle enzymes as a result of photodynamic sensitization by mitochondrial inner membrane. *Photochem. Photobiol.* **52**, 1011-1015.
5. Jung, J. and Y. W. Kim (1991) Involvement of thylakoid membrane-dependent photosensitization in photoinhibition of Calvin cycle activity in spinach chloroplasts. *Photochem. Photobiol.* **54**, 833-839.
6. Kim, C. S. and J. Jung (1992) Iron-sulfur centers as endogenous blue light sensitizers in cells: a study with an artificial nonheme iron protein. *Photochem. Photobiol.* **56**, 63-68.
7. Kim, C. S. and J. Jung (1993) The susceptibility of mung bean chloroplasts to photoinhibition is increased by an excess supply of iron to plants: a photobiological aspect of iron toxicity in plant leaves. *Photochem. Phtotobiol.* **58**, 120-126.
8. Kim, C. S. and J. Jung (1994) Possible involvement of Fe-S centers as major endogenous photosensitizers in high light-caused loss of membrane structure and function of mitochondria. *J. Photoscience.* **1**, 9-14.
9. Chung, S. K. and J. Jung (1995) Inactivation of the acceptor side and degradation of the D1 protein of photosystem II by singlet oxygen photogenerated from the outside. *Photochem. Photobiol.* **61**, 383-389.
10. Kim, C. S. amd J. Jung (1995) Inactivation of the respiratory chain in plant mitochondria by visible light: the primary target for photodamage and endogenous photosensitizing chromophores. *J. Photochem. Photobiol. B: Biol.* **29**, 135-139.
11. Buchanan B. B. and D. I. Arnon (1971) Ferredoxin from photosynthetic bacteria, algae, and higher plants. *Methods Enzymol.* **23**, 413-440.
12. Petering, D., A. F. James, and D. Palmer (1971) The oxygen sensitivity of spinach ferredoxin and other iron-sulfur proteins. *J. Biol. Chem.* **246**, 643-653.
13. New, R. R. C. (1990) Preparation of liposomes. In *Liposome: a practical approach* (Edited by R. R. C. New), pp. 33-104, IRL press, Oxford.
14. Buege, J. A. and S. D. Aust (1978) Microsomal lipid peroxidation. *Methods Enzymol.* **52**, 302-310.
15. Sellak, H., E. Franzini, J. Hakim, and C. Pasquier (1992)

Mechanism of lysozyme inactivation and degradation by iron. *Arch. Biochem. Biophys.* **299**, 172-178.
16. Kraljic, I. and S. El. Mohsni (1978) A new method for the detection of singlet oxygen in aqueous solutions. *Photochem. Photobiol.* **28**, 557-581.
17. Senthil, V., L. R. Jones, K. Senthil, and L. I. Grossweiner (1994) Hypercin photosensitization in aqueous model systems. *Photochem. Photobiol.* **59**, 40-47.
18. Jones, L. R. and L. I. Grossweiner (1994) Singlet oxygen generation by photofrin in homogenous and light-scattering media. *J. Photochem. Photobiol. B: Biol.* **26**, 249-256.
19. Girotti, A. W. (1990) Photodynamic lipid peroxidation in biological systems. *Photochem. Photobiol.* **51**, 497-509.
20. Gorman, A. A. and M. A. J. Rodgers (1989) Singlet oxygen. In *Handbook of Organic Photochemistry* (Edited by J. C. Scaiano) Vol. II, pp. 229-247. CRC press, Boca Raton.
21. Nilsson, R. and D. R. Kearns (1973) A remarkable deuterium effect on the rate of photosensitized oxidation of alcohol dehydrogenase and trypsin. *photochem. photobiol.* **17**, 65-68
22. Tanielian, C., C. Wolff and M. Esch (1996) Singlet oxygen production in water: aggregation and charge-transfer effects. *J. Phys. Chem.* **100**, 6555-6560.
23. Gandin, E., Y. Lion and A. Vande Vorst (1983) Quantum yield of singlet oxygen production by xanthene derivatives. *Photochem. Photobiol.* **37**, 270-278.
24. Blum, A. and L. I. Grossweiner (1985) Singlet oxygen generation by hematoporphyrin IX. Uroporphyrin I and hematoporphyrin derivative at 546 nm in phosphate buffer and in the presence of egg phosphatidylcholine liposomes. *Photochem. Photobiol.* **41**, 27- 32.