

# Inhibition of the Mitochondrial $F_1$ -ATPase by Rose Bengal Mediated Photooxidation.† Interaction of the $Fe^{2+}$ Chelate of Bathophenanthroline with the Sensitizer

Elzbieta Glaser,<sup>a</sup> Enrique Cadenas,<sup>b</sup> Siv Andell<sup>a</sup> and Lars Ernster<sup>a</sup>

<sup>a</sup>Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-10691 Stockholm and

<sup>b</sup>Department of Pathology II, Linköping University, S-581 85, Linköping, Sweden

Glaser, E., Cadenas, E., Andell, S. and Ernster, L., 1988. Inhibition of the Mitochondrial  $F_1$ -ATPase by Rose Bengal Mediated Photooxidation. Interaction of the  $Fe^{2+}$  Chelate of Bathophenanthroline with the Sensitizer. – Acta Chem. Scand., Ser. B 42: 175–182.

Rose Bengal mediated photooxidation of mitochondrial  $F_1$ -ATPase and its  $\beta$ -subunit resulted in inactivation and loss of about 50 and 60 % of their histidine residues, respectively. The  $\beta$ -subunit was not cleaved upon photooxidation. Photooxidation of histidine probably results in changes in the conformational stability of  $F_1$ -ATPase leading to its inactivation. The participation of singlet molecular oxygen during the photooxidation process is suggested by the selective loss of histidine residues, while other amino acids, also sensitive to singlet oxygen attack, were not affected.

Photochemical damage of  $F_1$ -ATPase was prevented by various phenanthroline compounds, the order of efficiency being bathophenanthroline-Fe chelate > bathophenanthroline > orthophenanthroline-Fe chelate > bathophenanthroline-sulfonate-Fe chelate. The prevention by bathophenanthroline-Fe chelate of photochemical damage is interpreted on the basis of its interaction with the photosensitizer, Rose Bengal, probably implying a chemical reaction which decreases the actual concentration of the sensitizer and, thereby, the extent of photoinactivation.

It is well established that the  $F_1$ -ATPases\* of mitochondria, chloroplasts and bacteria consist of 5 subunits ( $\alpha$ - $\epsilon$ ), with a stoichiometry of  $\alpha_3\beta_3\gamma\delta\epsilon$ .<sup>1</sup> The  $\beta$ -subunit, with a molecular weight of approximately 50 kD, is generally considered to contain the catalytic center of the enzyme. There is evidence that the 3 $\beta$  subunits function in an alternating fashion with respect to the binding and release of ATP and of ADP +  $P_i$  in the course of the catalytic cycle of the enzyme,<sup>2,3</sup> but the details of this “alternate-site” or “binding-change” mechanism are not yet fully understood. Likewise, although the idea that  $F_1$  functions as a

proton-motive ATPase and ATP-synthase, in accordance with the chemiosmotic hypothesis, is widely accepted,<sup>4</sup> the precise mode of interaction of  $F_1$  with the proton-translocating  $F_0$  moiety of the ATPase (or ATP-synthase) system is still unclear.<sup>5</sup>

Various inhibitors have proved useful in recent years in an attempt to settle these questions.<sup>1</sup> In our laboratory we have been particularly interested in the effects of *tris*bathophenanthroline) $Fe^{2+}$  ( $BPh_3Fe^{2+}$ ) and related octahedral BPh-metal chelates,<sup>6,7</sup> which we have found to act as powerful inhibitors of soluble mitochondrial  $F_1$ -ATPase as well as of  $F_1$ -ATPases from chloroplasts and bacteria and other enzymes catalyzing the hydrolysis or synthesis of pyrophosphate bonds.<sup>8</sup> A striking feature of the inhibition of mitochondrial  $F_1$ -ATPase by  $BPh_3Fe^{2+}$  is its reversibility by various uncouplers of oxidative phosphorylation. These compounds, which are

† Communication at the Meeting of the Swedish Biochemical Society in Uppsala, August 21–22, 1987.

\* Abbreviations used: BSA, bovine serum albumin; Tris, tris(hydroxymethylamino)methane;  $F_1$ , coupling factor 1 of adenosine triphosphatase; BPh, bathophenanthroline = 2,7-diphenyl-1,10-phenanthroline.

believed to act as "proton conductors" across energy-transducing membranes, have been shown to relieve the inhibition of soluble  $F_1$  by  $BPh_3Fe^{2+}$  by forming an adduct to the chelate.<sup>6</sup> Evidence was presented that  $BPh_3Fe^{2+}$  binds to  $F_1$  with a 3:1 molar stoichiometry, probably to the  $\beta$ -subunit of the enzyme, and that this binding is not altered by uncouplers although the inhibition of the ATPase activity is abolished.<sup>6</sup> These findings were interpreted as indicating that  $BPh_3Fe^{2+}$  has two types of binding sites on the  $\beta$ -subunit of  $F_1$ ,<sup>9</sup> one binding to the three bathophenanthroline moieties and not involved in the inhibition, and another binding through  $Fe^{2+}$ , probably causing the inhibition of the ATPase activity by binding to tyrosyl residues; this latter binding is prevented by the addition of negatively charged uncouplers.

Another effect of  $BPh_3Fe^{2+}$  on mitochondrial  $F_1$  that we noticed was that it prevented the inactivation of the ATPase activity occurring upon photooxidation of the enzyme by exposure to light in the presence of Rose Bengal.<sup>7</sup> This effect was also abolished by uncouplers. The inactivation by photooxidation, which had been described by Godinot *et al.*,<sup>10</sup> is usually due to destruction of essential histidine residues. How-

ever, from the pH profile of the inactivation these authors concluded that the inactivation of mitochondrial  $F_1$ -ATPase by photooxidation probably was not related to attack of essential histidine residues. The purpose of the present study was to identify by direct amino acid analysis the amino acid residue(s) involved in the inactivation of mitochondrial  $F_1$ -ATPase by photooxidation and destruction of these residues in order to characterize the mechanism by which  $BPh_3Fe^{2+}$  prevents this effect.

### Materials and methods

$F_1$ -ATPase was purified from beef heart mitochondria according to the method of Horstman and Racker.<sup>11</sup> The  $\beta$ -subunit of  $F_1$  was isolated according to the method of Yoshida *et al.*,<sup>12</sup> except that beef heart mitochondrial  $F_1$ , and not  $F_1$  from the thermophilic bacterium PS3 was used as starting material. Sephadex G-25 was used to displace guanidine hydrochloride with urea.

Photooxidation of  $F_1$  was performed at room temperature in a reaction mixture of volume 1 ml containing 0.03 mg  $F_1$ , 25 mM Tris/acetate (pH 7.5), 0.2  $\mu$ M Rose Bengal and 0.08–8  $\mu$ M  $Fe^{2+}$ -phenanthroline chelate. The samples were

**Table 1.** Amino acid composition of  $F_1$  (mol %) and its  $\beta$ -subunit (number of residues) derived from photooxidized  $F_1$ . Assay conditions as described in the Materials and methods section.

Amino acid	$F_1$		$\beta$ -subunit of $F_1$			
	Native	Photooxidized		Native	Photooxidized	
		- $BPh_3Fe$	+ $BPh_3Fe$		- $BPh_3Fe$	+ $BPh_3Fe$
Lys	8.50	6.83	8.08	23	24	23.5
His	2.38	1.20	2.16	9	3.5	7.5
Arg	5.75	4.91	5.64	21	18	18
Asp	7.49	8.07	7.63	37	38	36
Thr	6.24	6.00	5.69	30	30	30
Ser	5.20	5.76	5.58	25	25	23
Glu	10.76	11.5	10.76	56	58	58
Pro	4.76	4.63	4.59	28.5	28	29
Gly	7.32	8.53	8.11	41	44	43
Ala	7.79	7.98	7.31	37	39	38
Val	9.36	9.60	10.54	52	52	53
Met	2.23	2.24	2.03	9	8	11
Ile	6.51	6.85	6.75	32	34.5	32
Leu	9.19	9.84	9.34	44.5	43	46
Tyr	3.12	2.75	2.77	15	14	14
Phe	3.36	3.29	3.02	19	18	17

irradiated at 23°C for 1 min with a 1000 W photolamp from a distance of 12 cm, corresponding to a light intensity at the sample position of 0.25 W cm<sup>-2</sup>. Under these conditions, photooxidation in the absence of the Fe<sup>2+</sup>-bathophenanthroline chelate caused an inhibition of F<sub>1</sub>-ATPase activity of 75–85%. Photooxidation of histidine was carried out under similar conditions, directly in the O<sub>2</sub>-electrode chamber in a volume of 0.5 ml, at 30°C. The concentrations of histidine and Rose Bengal were 1 mM and 1 μM, respectively.

Fluorescence of Rose Bengal was measured at an excitation wavelength of 525 nm and emission wavelength of 566 nm at concentrations of 1 mM histidine and 1 μM Rose Bengal. Absorption spectra of Rose Bengal in the absence and presence of Fe<sup>2+</sup>-bathophenanthroline chelate were measured in water using reagent concentrations of 5 μM. Protein was determined by the method of Lowry *et al.*<sup>13</sup> using BSA as standard. Amino acid analysis was performed after hydrolyzing the proteins in 6 M HCl in vacuum at 110°C for 24 h; the hydrolysed samples were then applied to a Beckman 121 M amino acid analyzer.

## Results

**1. Photosensitization of F<sub>1</sub>-ATPase: inactivation and sites of damage.** Rose Bengal mediated photosensitization damage to F<sub>1</sub>-ATPase and its β-subunit probably proceeds by a type II mechanism leading to singlet oxygen (<sup>1</sup>O<sub>2</sub>) generation in a sequence of steps involving excitation of the sensitizer (S) to the first singlet excited state [eqn. (1)], its conversion to the triplet state by intersystem crossing [isc; eqn. (2)], and energy-transfer quenching of the triplet sensitizer by ground state O<sub>2</sub> to produce an electronically excited state of O<sub>2</sub>, singlet oxygen (<sup>1</sup>O<sub>2</sub>) [eqn. (3)]:



Reaction 3 is usually very fast, accounting for almost all the quenching of triplet sensitizer by O<sub>2</sub>. Several acceptors (A), among them amino acids, react at relatively high rates with <sup>1</sup>O<sub>2</sub>, thus accounting for the O<sub>2</sub> consumption accompany-

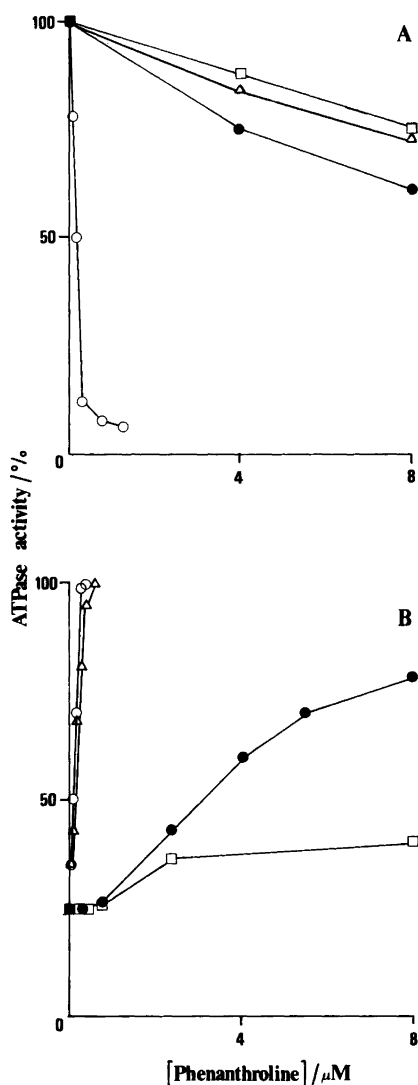


Fig. 1. Inhibition (A) and protection (B) of F<sub>1</sub>-ATPase activity by different Fe<sup>2+</sup> chelates. Assay conditions as described in the Materials and methods section. (○), bathophenanthroline Fe<sup>2+</sup> chelate; (●), orthophenanthroline Fe<sup>2+</sup> chelate; (□), bathophenanthrolinesulfonate Fe<sup>2+</sup> chelate; (△), bathophenanthroline. In Fig. 1B, the control in the absence of phenanthroline was irradiated in the presence of Rose Bengal, causing an inhibition of about 75% of the ATPase activity.

ing the photooxidation process [eqn. (4)]. Though individual amino acids vary in their susceptibility to sensitized photooxidation, histidine,

tryptophan, methionine, tyrosine and cysteine are readily affected by photooxidation.<sup>14,15</sup>



The role of  ${}^1\text{O}_2$  in photosensitized inactivation of  $F_1$ -ATPase by Rose Bengal was investigated. The amino acid compositions of  $F_1$  and its  $\beta$ -subunit before and after photooxidation are shown in Table 1. The  $\beta$ -subunit of  $F_1$  was isolated from  $F_1$  after photooxidation. Photooxidation resulted in a marked difference in the content of histidine but no significant differences in the content of any other amino acids. The slight decrease in the lysine content of  $F_1$  (but not the isolated  $\beta$ -subunit) was not statistically significant. Histidine is probably oxidized via a  ${}^1\text{O}_2$  mechanism, its reaction rate with  ${}^1\text{O}_2$  being about  $5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ .<sup>16</sup> Approximately 50% of the histidine residues of  $F_1$  (24 residues out of 50 per  $F_1$ ) were destroyed by irradiation of  $F_1$  in the presence of Rose Bengal. In the  $\beta$ -subunit of  $F_1$  about 60% of the histidine residues (i.e. 5 or 6 out of 9 per  $\beta$ -subunit) were destroyed upon photooxidation. The  $\beta$ -subunit as a whole was not fragmented by the photooxidation process, as revealed by gel electrophoresis (not shown). Since there are three  $\beta$ -subunits per  $F_1$ , 15 of 18 histidine residues out of the 24 residues that are destroyed in the whole  $F_1$  are located in the  $\beta$ -subunits. The localization of the 6 or 9 remaining histidine residues which were not destroyed upon photooxidation

was not determined. Untreated  $\gamma$ -,  $\delta$ -, and  $\epsilon$ -subunits contain 40% of the total histidine residues of  $F_1$ , and the remaining histidine residues in the photooxidized  $F_1$  might be located in these subunits. No histidine residues appeared to be destroyed in the  $\alpha$ -subunit of  $F_1$  (not shown).

II. *Effect of phenanthroline compounds on  $F_1$ -ATPase activity and on photooxidation of  $F_1$ -ATPase.* Fig. 1A shows the inhibition of the  $F_1$ -ATPase activity by bathophenanthroline and  $\text{Fe}^{2+}$  chelates with bathophenanthroline, orthophenanthroline and water-soluble bathophenanthrolinesulfonate. As shown earlier,<sup>6</sup> bathophenanthroline and the  $\text{Fe}^{2+}$  chelates with orthophenanthroline and bathophenanthrolinesulfonate have much weaker inhibitory effects on  $F_1$ -ATPase than the  $\text{Fe}^{2+}$  chelate with bathophenanthroline.

Fig. 1B shows that the photosensitization damage to  $F_1$ -ATPase is prevented by the various phenanthroline compounds. There is a correlation between the capacity of different  $\text{Fe}^{2+}$  chelates to inhibit the ATPase activity, and their capacity to prevent photooxidation damage to  $F_1$ -ATPase. However, bathophenanthroline, which does not inhibit the ATPase activity, prevents the inactivation of the enzyme during photooxidation almost as efficiently as its  $\text{Fe}^{2+}$  chelate. The  $\text{Fe}^{2+}$  chelate with bathophenanthroline prevents almost completely the destruction of histidine residues during irradiation of  $F_1$  in the presence of Rose Bengal (Table 1).

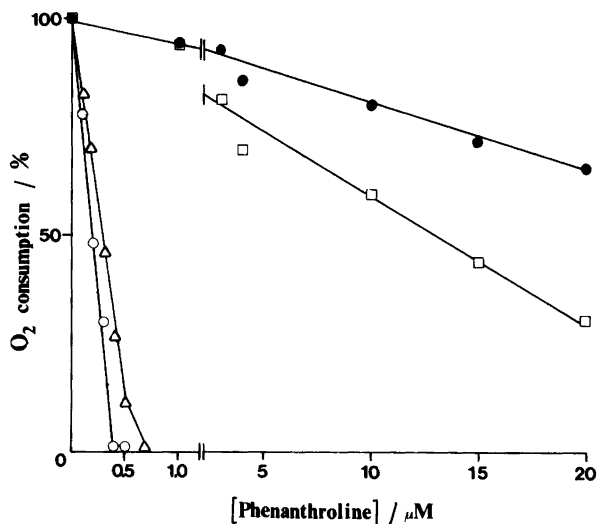


Fig. 2. Inhibition of oxygen consumption elicited by Rose Bengal mediated photooxidation of histidine by different analogues of phenanthroline. Experimental conditions as described in the Materials and methods section. (○), bathophenanthroline  $\text{Fe}^{2+}$  chelate; (●), orthophenanthroline  $\text{Fe}^{2+}$  chelate; (□), bathophenanthrolinesulfonate  $\text{Fe}^{2+}$  chelate; (Δ), bathophenanthroline.

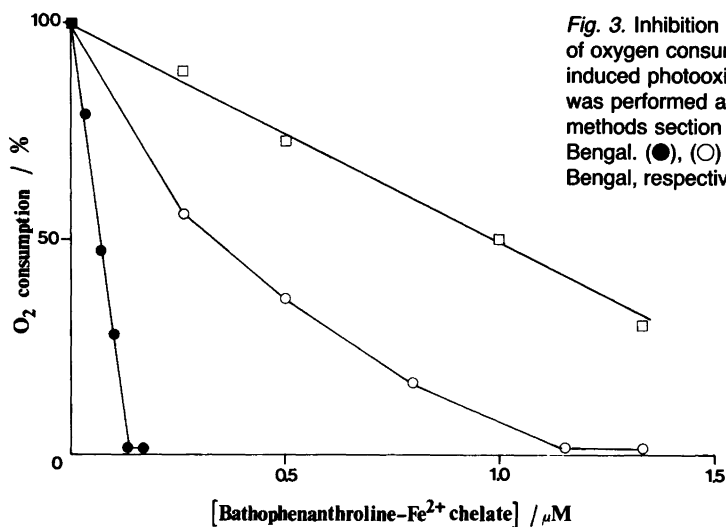


Fig. 3. Inhibition by bathophenanthroline Fe<sup>2+</sup> chelate of oxygen consumption elicited by Rose Bengal induced photooxidation of histidine. Photooxidation was performed as described in the Materials and methods section with varying concentrations of Rose Bengal. (●), (○) and (□): 1, 2 and 3 μM Rose Bengal, respectively.

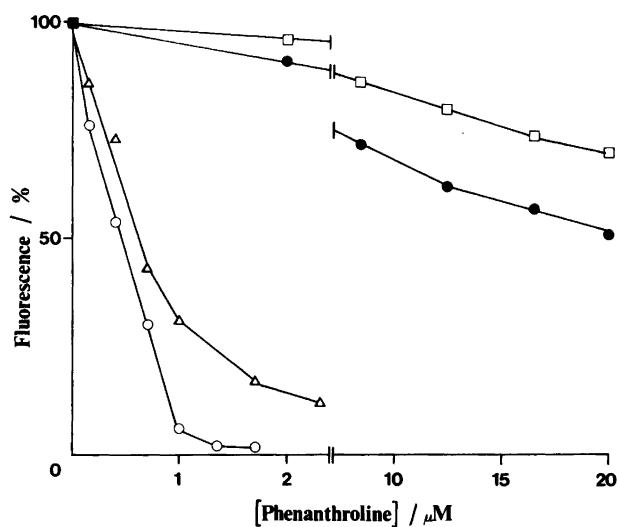


Fig. 4. Quenching of Rose Bengal fluorescence by different phenanthroline compounds. Assay conditions as in the Materials and methods section. (○), bathophenanthroline Fe<sup>2+</sup> chelate; (●), orthophenanthroline Fe<sup>2+</sup> chelate; (□), bathophenanthrolinesulfonate Fe<sup>2+</sup> chelate; (Δ), bathophenanthroline.

The inhibition of photosensitized inactivation of F<sub>1</sub>-ATPase by bathophenanthroline and its chelate mentioned above could be interpreted in terms of: (a) interaction of bathophenanthroline and its Fe<sup>2+</sup> chelate with F<sub>1</sub>, thus shielding F<sub>1</sub> against <sup>1</sup>O<sub>2</sub> attack; (b) reaction with or quenching of <sup>1</sup>O<sub>2</sub> by bathophenanthroline, thus establishing competition for <sup>1</sup>O<sub>2</sub> between bathophenanthroline and the photooxidation-sensitive amino acid residues of F<sub>1</sub>; (c) reaction with or quenching of

the sensitizer by bathophenanthroline, thus decreasing the actual concentration of the sensitizer and, thereby, the extent of photooxidation of F<sub>1</sub>.

O<sub>2</sub>-consumption originating from the photooxidation of histidine (eqn. 4) in the presence of Rose Bengal was used to evaluate the possibilities listed above. Bathophenanthroline, its Fe<sup>2+</sup> chelate, and the Fe<sup>2+</sup> chelates with orthophenanthroline and bathophenanthrolinesulfonate inhibit the O<sub>2</sub>-consumption accompanying pho-

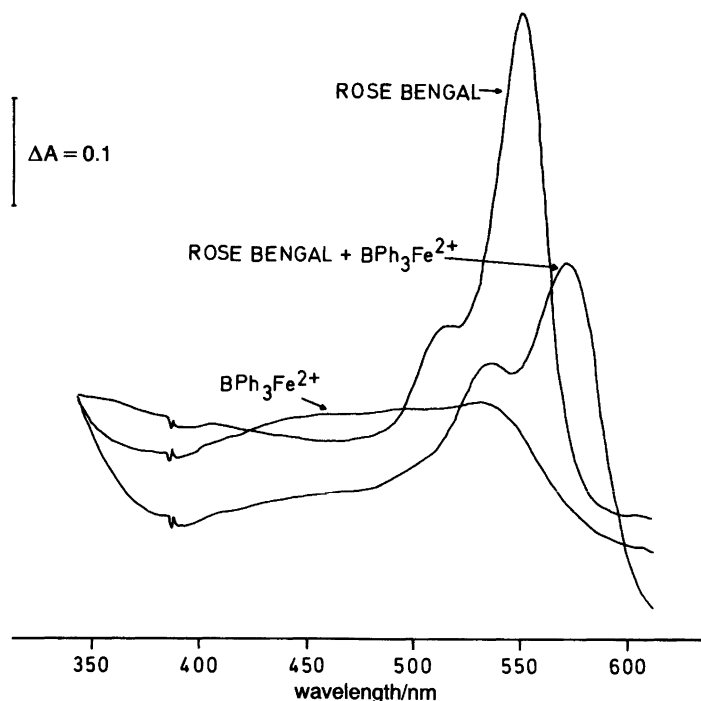


Fig. 5. Absorption spectra of bathophenanthroline  $\text{Fe}^{2+}$  chelate and of Rose Bengal in the absence and presence of bathophenanthroline  $\text{Fe}^{2+}$  chelate. Spectra were recorded at equimolar concentrations ( $5 \mu\text{M}$ ) of Rose Bengal and bathophenanthroline  $\text{Fe}^{2+}$  chelate.

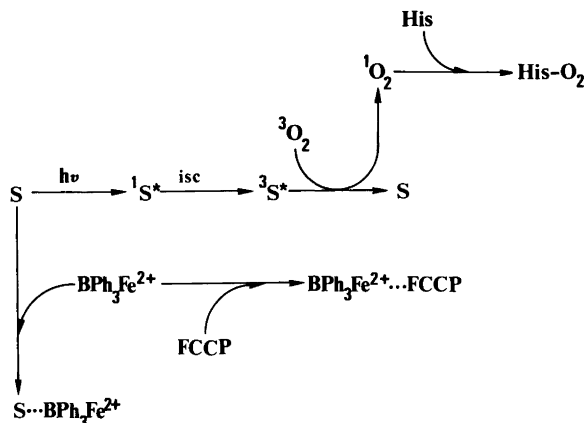
tooxidation of histidine by Rose Bengal (Fig. 2). This implies that there is not competition for  $^1\text{O}_2$  between the acceptor (histidine) in the present model and the phenanthroline compound. The inhibition exerted by the  $\text{Fe}^{2+}$  chelate with bathophenanthroline on the  $\text{O}_2$ -consumption coupled to histidine photooxidation is somewhat more efficient than the inhibition by bathophenanthroline in the absence of  $\text{Fe}^{2+}$ . Maximal inhibition is obtained at a concentration of  $0.4 \mu\text{M}$  in the presence of  $\text{Fe}^{2+}$  and  $0.7 \mu\text{M}$  in the absence of  $\text{Fe}^{2+}$ . The inhibitory effect of other chelates is much weaker.

Fig. 3 shows the inhibition of  $\text{O}_2$ -consumption by the  $\text{Fe}^{2+}$  chelate with bathophenanthroline at three concentrations of Rose Bengal, viz. 1, 2 and  $3 \mu\text{M}$ . Increasing concentrations of the chelate are required to inhibit  $\text{O}_2$ -consumption when the concentration of Rose Bengal is raised: i.e., a 10-fold increase in the concentration of  $\text{Fe}^{2+}$  bathophenanthroline chelate is necessary when the concentration of Rose Bengal is doubled. These results indicate a possible interaction be-

tween the photosensitizer and the  $\text{Fe}^{2+}$  chelate with bathophenanthroline. This view is supported by the efficient quenching of Rose Bengal fluorescence by both bathophenanthroline (70% at  $1 \mu\text{M}$ ) and its  $\text{Fe}^{2+}$  chelate (95% at  $1 \mu\text{M}$ ) (Fig. 4). 20–30-fold higher concentrations of the  $\text{Fe}^{2+}$  chelates with orthophenanthroline and bathophenanthrolinesulfonate are required for half-maximal quenching of Rose Bengal fluorescence, the latter chelate being slightly more efficient than the former.

Quenching of fluorescence could be interpreted as arising from a chemical reaction between Rose Bengal and bathophenanthroline or physical quenching of the singlet (fluorescent) state of the sensitizer. Quenching of the triplet state of Rose Bengal ( $^3\text{S}^*$ ) is not likely, since bathophenanthroline, at  $1 \mu\text{M}$ , would have to compete with  $220 \mu\text{M}$   $\text{O}_2$  (eqn. 3) (cf. Fig. 2), the latter reaction proceeding almost at the diffusion-controlled rate.

Additional evidence for an interaction between  $\text{Fe}^{2+}$ -bathophenanthroline chelate and Rose Ben-



Scheme 1.

gal is obtained from the absorption spectral shift that occurs when the chelate is mixed with the sensitizer (Fig. 5): the absorbance maximum of Rose Bengal at 550 nm is shifted to about 566 nm in the presence of Fe<sup>2+</sup> bathophenanthroline-chelate. No such shift could be observed with either bathophenanthroline in the absence of Fe<sup>2+</sup> or the Fe<sup>2+</sup> orthophenanthroline-chelate (not shown).

These results are compatible with a chemical interaction between Rose Bengal and the Fe<sup>2+</sup> bathophenanthroline-chelate. In the absence of Fe<sup>2+</sup>, bathophenanthroline probably acts by quenching the first singlet state of Rose Bengal.

## Discussion

Photooxidation of F<sub>1</sub>-ATPase in the presence of Rose Bengal results in inhibition of the catalytic activity of the enzyme. The results presented here indicate that the photooxidation is associated with photochemical damage to about 50% of all histidine residues of F<sub>1</sub> (24 residues out of 50). It is also shown that about 60% of the histidine residues (5 or 6 out of 9) of the β-subunit of F<sub>1</sub> isolated after photooxidation are destroyed. This implies that 15 or 18 of the 24 histidine residues destroyed upon photooxidation are located on the β-subunit of the enzyme.

It remains to be established how many of the damaged histidine residues are critical for the loss of the ATPase activity. Photooxidation of succinate dehydrogenase in the presence of Rose Bengal is associated with the modification of an essential histidine residue at or near the active site

of the enzyme.<sup>17</sup> Studies on the effect of modification of the β-subunit of F<sub>1</sub>-ATPase by histidine reagents on the binding of P<sub>i</sub> and ATP<sup>18</sup> reveal that histidine residues participate in the binding of both P<sub>i</sub> and of the γ-phosphate group of ATP.

The effect of protection against photooxidation can be interpreted in terms of direct shielding of F<sub>1</sub> against <sup>1</sup>O<sub>2</sub> attack or by the reaction of bathophenanthroline or its Fe<sup>2+</sup> chelate with the photooxidizing system, i.e. <sup>1</sup>O<sub>2</sub> or the sensitizer. Although we cannot draw any definitive conclusions at present, our results suggest an interaction between bathophenanthroline and its Fe<sup>2+</sup> chelate with the sensitizer, resulting in inhibition of <sup>1</sup>O<sub>2</sub> formation. This conclusion is based on the following facts: (1) the protection against photooxidation is obtained in a model system in which histidine is photooxidized in the presence of Rose Bengal and O<sub>2</sub>-consumption is monitored polarographically (cf. Fig. 2); (2) increasing concentrations of the chelate are required to inhibit O<sub>2</sub>-consumption when the concentration of Rose Bengal is raised (cf. Fig. 3); (3) the shift in the absorption spectrum occurs when BPh<sub>3</sub>Fe<sup>2+</sup> is mixed with the sensitizer (cf. Fig. 5).

A possible interpretation of the results presented above is summarized in Scheme 1. The upper part of this scheme shows the photooxidation and indicates the site of damage of F<sub>1</sub>-ATPase, and the lower part shows the prevention of photooxidative damage to F<sub>1</sub>-ATPase by phenanthroline compounds and its reversal by uncouplers. It remains an open question whether these two effects are related to each other, since our

results indicating a direct interaction between phenanthroline compounds and the sensitizer do not exclude a shielding effect of  $\text{BPh}_3\text{Fe}^{2+}$  on  $\text{F}_1$ .

**Acknowledgements.** We are grateful to Professor Hans Jörnvall, Karolinska Institute, for carrying out the amino acid analysis. This research was supported by grant No. 2162-123 from the Swedish Natural Science Research Council, and grants Nos. 7697 and 4481 from the Swedish Medical Research Council.

## References

1. Kagawa, Y. In: Ernster, L., Ed., *Bioenergetics*, Elsevier, Amsterdam 1984, pp. 149-186.
2. Cross, R. L. *Annu. Rev. Biochem.* 50 (1981) 681.
3. Kandpal, R. P. and Boyer, P. *Biochim. Biophys. Acta* 890 (1987) 97.
4. Boyer, P. D., Chance, B., Ernster, L., Mitchell, P., Racker, E. and Slater, E. C. *Annu. Rev. Biochem.* 46 (1977) 955.
5. Slater, E. C. *Eur. J. Biochem.* 166 (1987) 489.
6. Carlsson, C. and Ernster, L. *Biochim. Biophys. Acta* 638 (1981) 345.
7. Carlsson, C. and Ernster, L. *Biochim. Biophys. Acta* 638 (1981) 358.
8. Carlsson, C. and Ernster, L. In: Anachenko, S. N., Ed., *IUPAC Symposium on Frontiers of Bioorganic Chemistry and Molecular Biology*, Pergamon Press, Oxford 1980, pp. 1-9.
9. Ernster, L. *Curr. Topics Cell* 24 (1984) 313.
10. Godinot, C., Penin, F. and Gautheron, D. C. *Arch. Biochem. Biophys.* 192 (1979) 225.
11. Horstman, L. L. and Racker, E. *J. Biol. Chem.* 245 (1970) 1336.
12. Yoshida, M., Sone, N., Hirata, H. and Kagawa, Y. *J. Biol. Chem.* 252 (1977) 3480.
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. *J. Biol. Chem.* 193 (1951) 265.
14. Nilsson, R., Merkel, P. B. and Kearns, D. R. *Photochem. Photobiol.* 16 (1972) 117.
15. Matheson, I. B. C. and Lee, J. *Photochem. Photobiol.* 29 (1979) 879.
16. Foote, C. S. In: Pryor, W. A., Ed., *Free Radicals in Biology*, Academic Press, New York 1976, Vol. II, pp. 85-133.
17. Hederstedt, L. and Hatefi, Y. *Arch. Biochem. Biophys.* 247 (1986) 346.
18. Khananshvilii, D. and Gromet-Elhanan, Z. *Proc. Natl. Acad. Sci. U.S.A.* 82 (1985) 1886.

Received September 29, 1987.