Free Rad. Res., Vol. 26, pp. 201–208
Reprints available directly from the publisher
Photocopying permitted by license only

Effects of Photofrin® Photodynamic Action on Mitochondrial Respiration and Superoxide Radical Generation

CHRISTIAN SALET[a,*], GIULIANA MORENO[a] and FERNANDA RICCHELLI[b]

[a]*Laboratoires de Biophysique et de Photobiologie, INSERM U 201 et CNRS URA 481, Muséum National d'Histoire Naturelle, 43 rue Cuvier, 75231 Paris Cédex 05 France and* [b]*Centro C.N.R. Metalloproteine, Dipartimento di Biologica, Università di Padova, Via Trieste 75, 35121 Padova Italy*

(Received 9 May 1996; In final form 22 August 1996)

Cyanide-resistant respiration increases after irradiation of isolated mitochondria in the presence of Photofrin®. This suggests an enhancement of electron leakage which has been evaluated by measuring superoxide radical formation in submitochondrial particles incubated with 6 μg/ml Photofrin® in the medium and irradiated with increasing doses of light at 365 nm. After a dose of 4.5 kJ/m^2 has been delivered, superoxide generation increases by a factor of ~2.5 at the level of NADH dehydrogenase and by a factor ~1.5 in the cyt bc_1 region. These effects have been compared with changes observed in NADH-, succinate- and ascorbate-driven respiration and their implications discussed.

Keywords: Photofrin®, photosensitization, mitochondria, electron leakage, electron transfer

INTRODUCTION

In recent years, there has been growing interest in the photodynamic therapy of cancer using light and Photofrin (PF).[1] Numerous studies have attempted to delineate the mechanisms responsible for cell death in these experimental conditions, but these mechanisms might be different according to the cell type and are, in fact, not fully understood.[2] However, mitochondria have been considered repeatedly as primary targets, at least in some cases, because one of the first effects of photodynamic action is to reduce the intracellular ATP level in normal and tumoral cells.[3–8] Moreover, we have demonstrated that laser micro-irradiation of areas rich in mitochondria can induce cell death whereas micro-irradiation of the nucleus or hyaloplasm does not do so.[9] While impairment of oxidative phosphorylation can be associated with that of many mitochondrial enzymes, a detailed study on isolated mitochondria has shown that photoalteration of the ADP/ATP translocator plays a crucial role in the decline of mitochondrial ATP synthesis (for a general review see[10]).

Less attention has been focused on functional damage to the electron transport chain itself. In mitochondrial respiration, electron transport from NADH or $FADH_2$ to terminal oxygen takes place in the inner mitochondrial membrane and

*Corresponding author.

is coupled with the activity of proton pumps able to polarize this membrane. According to Mitchell's hypothesis, the electro-chemical gradient formed in this manner provides the potential energy necessary for ATP synthesis. The majority of oxygen consumed by mitochondria is reduced to water in a four-electron process by cyt oxidase. Even under normal conditions, a minor pathway (1–2%) occurs in a one-electron process with formation of the superoxide radical (Fig. 1). Recently, we have shown that photodynamic action can increase electron leakage along the respiratory chain.[11] The present investigation examines whether such an effect can occur when mitochondria are photosensitized with PF. Our results show that cyanide-resistant respiration increases in isolated mitochondria at the same time as electron leakage, as indicated by the measure of superoxide radical generation in submitochondrial particles (SMP). The contribution of the different respiratory chain segments to the changes in electron leakage, as well as electron transport, has been evaluated. The consequences of an excess of radical formation after photodynamic action are discussed.

MATERIALS AND METHODS

Chemicals

All the chemicals were of the purest available grade and were used without further purification. Epinephrine, catalase (EC 1.11.1.6), rotenone and antimycin were purchased from Sigma Chemical Co. (St. Louis, MO), NADH and superoxide dismutase (EC 1. 15. 11) (SOD) from Boehringer Mannheim France S.A. PF, generously given by Laboratories Lederle, Oullins, France, contains 2.5 mg/ml of dihematoporphyrin ethers.

Preparation of Mitochondria and Submitochondrial Particles

Mitochondria were isolated from the livers of male Wistar rats (250–300g) which had fasted overnight as previously described[12] and submitochondrial particles (SMP) were prepared by sonication according to Boveris.[13] Briefly, 15 mg mitochondrial protein/ml, measured according to the method of Waddel and Hill,[14] were sus-

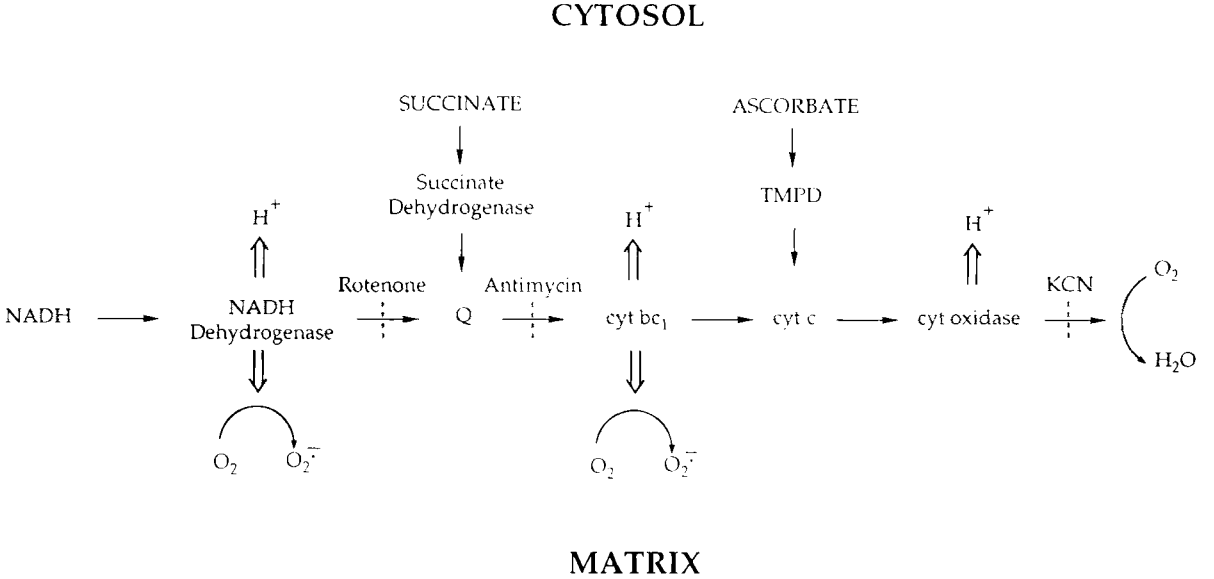

FIGURE 1 Scheme of the mitochondrial respiratory chain showing sites of substrate entry, sites of inhibition by rotenone, antimycin and KCN, as well as sites of H^+ production and potential sites of $O_2^{-\cdot}$ generation.

pended in a medium (MSM) containing 0.23 M mannitol, 0.07 M sucrose, and 50 mM Tris-HCl, at pH 7.4. The suspension was sonicated at ice-cold temperature with the probe of a Cell Disruptor B15 (Branson Sonifier, Danbury, CT) at 30% output. A twelve-second sonication period was repeated 7 times at 15 s intervals. After centrifugation of the suspension at 15,000g × 10 min, the supernatant was centrifuged at 100,000g × 50 min. The pellet was suspended in the same medium and recentrifuged at 100,000 g × 50 min. The final submitochondrial pellet was suspended in MSM at a concentration of ~40 mg/ml. All operations were performed at 4°C.

Measurement of O_2 Consumption

Oxygen consumption rates of mitochondria or SMP (0.5 mg protein/ml) in MSM were measured polarographically under magnetic stirring in a thermostated (25°C) water-jacketed vessel, fitted with a Clark electrode (Yellow Springs Instruments, Yellow Springs, OH) as described previously.[12] Respiration was activated with 300 μM NADH, 7 mM succinate or 5 mM ascorbate + 0.2 mM TMPD. Cyanide-resistant respiration was studied in the same medium containing 2 mM KCN and 7 mM succinate + 2 μM rotenone as substrate.

$O_2^{-}\cdot$ Production

The production of $O_2^{-}\cdot$ was measured by the superoxide dismutase-sensitive oxidation of epinephrine to adrenochrome.[15] SMP (0.5 mg protein/ml) was suspended in MSM containing 1 mM epinephrine, and 150 U/ml catalase.[13] The reaction was started by the addition of the different substrates and inhibitors (*i.e.*, 100 μM NADH + 1 μM rotenone, 7 mM succinate + 2 μM antimycin or 5 mM ascorbate + 0.2 mM TMPD + 2 mM KCN) and followed spectrophotometrically at 485–575 nm ($\varepsilon = 2.96\ mM^{-1}\ cm^{-1}$) with a dual-wavelength spectrophotometer (SLM Aminco, DW 2000, SLM Instruments, Inc., Urbana, IL) at room temperature. The formation of $O_2^{-}\cdot$ was ascertained from the inhibition of adrenochrome formation by adding 150 U/ml of SOD to the reaction medium (data not shown).

Conditions of Irradiation

Mitochondria or SMP (0.5 mg protein/ml), suspended in MSM in the presence of ~6 μg/ml PF giving an absorbance of 0.8/cm at the irradiation wavelength of 365 nm, were irradiated with a Philips HPW 125 W lamp (Philips, Eindhoven, The Netherlands) after 2 min incubation in the dark. Previous experiments[16] indicated that the uptake of PF in isolated mitochondria reached a plateau within 2 min. The fluence rate incident to the preparation was 25 W/m^2 measured with a Black Ray UV meter (J 221, Ultraviolet Products, Inc., San Gabriel, CA). All irradiations were performed at 25°C under magnetic stirring. It was confirmed that neither photosensitizer addition in the dark at the concentrations indicated above nor illumination alone were able to produce any change in oxygen consumption and $O_2^{-}\cdot$ production.

The results are presented as the mean of at least two experiments in duplicate and were analyzed using the Student t-test.

RESULTS

Effect of Irradiation on Cyanide-Resistant Respiration in Isolated Mitochondria

Cyanide is a powerful inhibitor of cyt oxidase and the so-called cyanide-resistant respiration has been used as an indirect measure of $O_2^{-}\cdot$ formation in mitochondria.[17,18] In a preliminary experiment, we have studied cyanide-insensitive respiration before and after irradiation of isolated mitochondria incubated with 6 μg/ml PF in MSM and energized with 7 mM succinate + 2 μM rotenone in the presence of 2 mM KCN: a dose of 1.5 kJ/m^2 at 365 nm increases its value from 2.0 ±

0.26 to 2.56 ± 0.19 nmol O_2 min^{-1} mg $protein^{-1}$ (with a risk of error, $p = 0.05$).

Effects of Irradiation on Superoxide Production and Respiration in SMP

Changes in cyanide-resistant respiration are technically difficult to measure. Nevertheless, the ~25% increase reported above suggests that $O_2^{-}\cdot$ formation is enhanced by photosensitization. In order to determine the sites of increased electron leakage, SMP were prepared and carefully washed to remove Mn-superoxide dismutase, then the formation of $O_2^{-}\cdot$ was measured according to the classical technique of Boveris[13] before and after irradiation in the presence of PF. Moreover, the effects on $O_2^{-}\cdot$ formation have been compared with those on respiration when electrons are introduced at various sites along the electron transport chain. Respiration rate in mitochondria is essentially controlled by phosphorylation rate,[19] but coupling in SMP no longer exists. Consequently, respiration is not limited and reaches an upper values as in mitochondria treated with protonophores. As respiration is two orders of magnitude greater than oxygen consumption because of leakage, it is worthwhile noting that the total oxygen consumption, measured polarographically, can be considered as a measure of respiration.

Effects at the Level of NADH Dehydrogenase

This site contributes to about one-third of total $O_2^{-}\cdot$ production along the electron transport chain.[13] The rate of adrenochrome min^{-1} mg $protein^{-1}$ increases from 0.10 ± 0.013 to 0.27 ± 0.03 nmol ($p \leq 0.05$) when rotenone-blocked SMP energized with NADH are irradiated with a dose of 4.5 kJ/m^2 in the presence of 6 µg/ml PF. As shown in Figure 2, this enhancement is an increasing function of the dose of radiation. The effects on $O_2^{-}\cdot$ generation appear to be significant, whereas NADH-driven respiration is more slightly impedded.

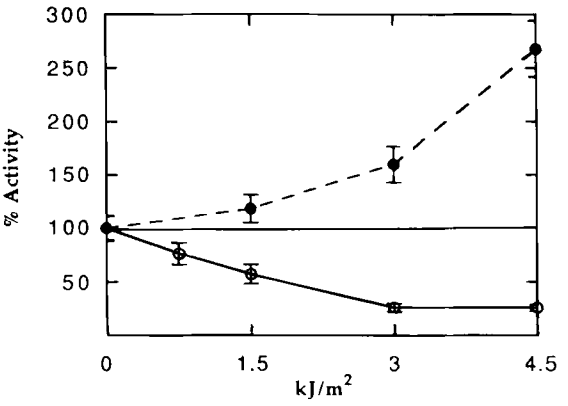

FIGURE 2 Relative oxygen consumption and $O_2^{-}\cdot$ production rate in SMP *versus* the incident dose of irradiation (kJ/m^2) at the level of NADH-dehydrogenase. SMP (0.5 mg/ml) were incubated in the dark in MSM containing PF (~ 6 µg/ml) for 2 min and irradiated with various doses of light at 365 nm. Oxygen consumption was measured polarographically after addition of 300 µM NADH. $O_2^{-}\cdot$ production was monitored spectrophotometrically at 485–575 nm. One mM epinephrine, 1 µM rotenone and 150 U/ml catalase were added to the SMP suspension. The reaction was started by addition of 100 µM NADH. Error bars, SD. O —— O, oxygen consumption; ● --- ●, $O_2^{-}\cdot$ production.

Effects at the Level of Ubiquinol-cyt C Oxido-Reductase (cyt bc1)

This site is the main source of $O_2^{-}\cdot$ and is studied in the presence of antimycin which favours the reaction of Q^{-} with molecular oxygen to produce superoxide radical after inhibition of e^{-} transfer from cyt b_{562} in the Q cycle. When SMP are energized with succinate, the production of adrenochrome is 0.28 ± 0.038 nmol min^{-1} mg protein.$^{-1}$ After irradiation under the same conditions as above, this value increases to 0.45 ± 0.011 ($p \leq 0.05$), a variation in percentage smaller at this site than at the NADH dehydrogenase site. Figure 3 shows that respiration decreases and $O_2^{-}\cdot$ production increases at equivalent rates.

Effects at the Level of Cyt Oxidase

Cyt oxidase complex is known to be a proton pump,[20] but electron leakage has never been reported at its level. In our case, no $O_2^{-}\cdot$ generation in SMP blocked with 2 mM KCN and acti-

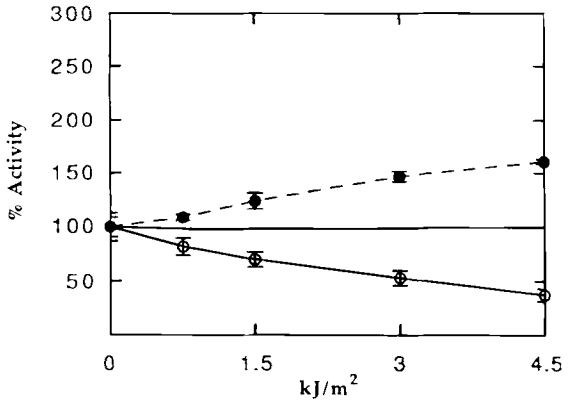

FIGURE 3 Relative oxygen consumption and $O_2^{-}\cdot$ production rate in SMP *versus* the incident dose of irradiation (kJ/m^2) at the level of ubiquinol-cyt c oxido-reductase (cyt bc_1). SMP (0.5 mg/ml) were incubated in the dark in MSM containing PF (~ 6 µg/ml) for 2 min and irradiated with various doses of light at 365 nm. Oxygen consumption and $O_2\cdot$ production were measured in the presence of 2 µM antimycin as described in Fig. 1 after addition of 7 mM succinate. Error bars, SD. O —— O, oxygen consumption; ● --- ●, $O_2\cdot$ production.

vated with 5 mM ascorbate + 0.2 mM TMPD was detected, even after irradiation. Moreover, the effects on ascorbate-driven respiration are small: for a dose of irradiation of 4.5 kJ/m^2, the decline in respiration is less than 10% (data not shown), a value to compare with the strong decline of NADH- or succinate-driven respiration (Fig. 2 and 3).

DISCUSSION

Few attempts have been made to study the effects of photodynamic action on the mitochondrial electron transport chain. It has been shown that irradiation of isolated mitochondria in the presence of porphyrins is correlated to an immediate decline of respiratory control ratio.[21,22] Moreover, irradiation of SMP in the presence of rose Bengal, a photosensitizer which generates $^1O_2$ with a high quantum yield as porphyrins,[23,24] decreases the activities of the electron carries.[25] It has also been shown that protein alteration plays a major role in the loss of enzyme activities, while fatty acid oxidation has only a secondary role.[25] Recently, we have been able to show that photodynamic action can increase the leakage of the electron transport chain in SMP sensitized with rose Bengal.[11] The results here show that cyanide-resistant respiration increases following irradiation of isolated mitochondria in the presence of PF. In addition, after such photosensitization, the electron leakage responsible for this effect has been studied using the formation of the superoxide radical at the various sites of coupling along the respiratory chain.

The first site is the NADH-dehydrogenase, a large enzyme consisting of some twelve subunit proteins. This complex, the composition of which is not yet known in detail, accepts electrons from NADH and passes them on to ubiquinone with a small leakage leading to $O_2^{-}\cdot$ generation. After PF photosensitization, the formation of this radical increases strikingly. Respiration at this site is also affected. The structural modifications of the complex after singlet oxygen attack are unknown, and clearly need further study.

Electron leakage also increases with the duration of illumination at the cyt bc_1 level. The enhancement is less pronounced than in the NADH-dehydrogenase region, but it cannot be considered as negligible because this second site is the main source of superoxide radicals. Furthermore, the electron transport rate from succinate to cyt oxidase + oxygen decreases. Although this finding does not specify which electron carrier is most affected, the work of Miki *et al.*[26] suggests that cyt bc_1 is partly responsible for this effect. According to these authors, cyt bc_1 is impeded when the purified complex is irradiated under aerobic conditions in the presence of hematoporphyrin; the photoinactivation site is the iron-sulfur cluster of Rieske's protein with the destruction of two histidine residues serving as ligands. Interestingly, the same authors have reported that photosensitization causes the formation of proton leaking channels in the complex embedded in phospholipid vesicles, which

seems to contradict results concerning the stability of the mitochondrial inner membrane potential after moderate photodynamic action.[27] It now seems that photodamage of the inner mitochondrial membrane leads to an augmentation of electron leakage at this site.

The loss of activity of the third complex, the cyt oxidase, has been reported previously by many investigators[28,29] who have studied cyt c oxidation rate in the presence of an homogenate of cells photosensitized with PF. Surprisingly, we have found that PF photosensitization has only limited effects on SMP respiration with ascorbate as substrate, in contrast to previous results,[28,29] including those of Musser and Oseroff.[30] Musser and Oseroff used the changes in reduction rates of tetrazolium salts as criteria for measuring damage at various points of the respiratory chain in intact cells treated with PF + light. These authors emphasize that cyt oxidase is extremely vulnerable because triphenyltetrazolium chloride reduction was found to be inhibited almost immediately. According to Slater,[31] this compound has a high specific affinity for cyt oxidase, but the effects of irradiation are studied on a shunt to the functional electron transport chain; the high sensitiveness of the so-constructed redox couple is not theoretically incompatible with small changes in electron transfer rate in the sequence cyt c-cyt oxidase-O_2 without appearance of electron leakage to free oxygen. Even if the consequences of oxidation depend on the oxidizing species,[32] our results concerning the action of singlet oxygen are similar to those of Davies and co-workers[33] demonstrating that the internal activity of the cyt oxidase complex is highly resistant to the action of hydroxyl or superoxide radicals. In any case, a comparison between the slopes of respiration decline (Fig. 2 and 3) suggests that damage to cyt oxidase is certainly not the main rate-limiting factor in the inhibition of the respiratory chain after photodynamic action. It is clear that $O_2^{-}\cdot$ production increases as a function of the duration of irradiation along the electron transport chain, especially at the level of NADH dehydrogenase. Superoxide anion can undergo a variety of reactions, some of which ultimately yield to hydroxyl radical, a very potent oxidant. As a rule, SOD, catalase and glutathione peroxidase maintain low levels of activated oxygen species. However, it has been demonstrated that the functional characteristics of liver mitochondria are damaged in rats with congenitally enhanced capacity to generate free radicals.[34] Moreover, the formation of 8-hydroxydesoxyguanosine in mtDNA parallel hydroxyl radical generation due to mitochondrial electron transfer[35] and multiple deletions appear in mt DNA of skin cells after long exposure in the sun.[36] As such, it is plausible that primary damage due to $^1O_2$ formation leads, in the course of time, to further mitochondrial damage resulting from an excess of free oxidizing radicals, especially in tumor cells where diminished amounts of MnSOD have been found.[37]

Acknowledgements

This research was partially supported by C. N. R. grant no. 102006.04.9305026/27 (Italy-France cooperative scientific program) and by a grant from the E.U. through the HCM programme, "PDT Euronet" no. ERBCHRXCT 930178. We are very grateful to F. Vinzens for her excellent technical assistance.

References

[1] T. J. Dougherty (1993). Photodynamic therapy, *Photochemistry and Photobiology*, **58**, 895–900.

[2] L. C. Penning, K. Tijssen, J. P. Boegheim, J. Van Steveninck and T. M. A. R. Dubbelman (1994). Relationship between photodynamically induced damage to various cellular parameters and loss of clonogenicity in different cell types with hematoporphyrin derivative as sensitizer, *Biochimica et Biophysica Acta*, **1221**, 250–258.

[3] D. Kessel (1986). Sites of photosensitization by derivatives of hematoporphyrin, *Photochemistry and Photobiology*, **44**, 489–493.

[4] R. Hilf, R. S. Murant, U. Narayanan and S. L. Gibson (1986). Relationship of mitochondrial function and cellular adenosine triphosphate levels of hematoporphyrin

derivative-induced photosensitization in R3230AC mammary tumors, *Cancer Research*, **46**, 211–217.
[5] T. L. Ceckler, R. G. Bryant, D. P. Penney, S. L. Gibson and R. Hilf (1986). ^{31}P-NMR spectroscopy demonstrates decreased ATP levels *in vivo* as an early response to photodynamic therapy, *Biochemical and Biophysical Research Communication*, **140**, 273–279.
[6] J. P. J. Boegheim, H. Scholte, T. M. A. R. Dubbelman, E. Beems, A. K. Raap and J. Van Steveninck (1987). Photodynamic effects of hematoporphyrin-derivative on enzyme activities of murine L929 fibroblasts, *Journal of Photochemistry and Photobiology, B: Biology*, **1** 61–73.
[7] L. C. Penning, K. Tijssen, J. van Stevenick and T. M. A. R. Dubbelman (1994). Hematoporphyrin derivative-induced photodynamic inhibition of Na^+/K^+-ATPase in L929 fibroblasts, Chinese hamster ovary cells and T24 human bladder transitional carcinoma cells, *Photochemistry and Photobiology*, **59**, 336–341.
[8] M. Dellinger, F. Ricchelli, G. Moreno and C. Salet (1994). Hematoporphyrin derivative (Photofrin®) photodynamic action on Ca^{2+} transport in monkey kidney cells (CV-1), *Photochemistry and Photobiology*, **60** 368–372.
[9] G. Moreno and C. Salet (1985). Cytotoxic effects following micro-irradiation of cultured cells sensitized with haematoporphyrin derivative, *International Journal of Radiation Biology*, **47**, 383–386.
[10] C. Salet and G. Moreno (1990). Photosensitization of mitochondria. Molecular and cellular aspects, *Journal of Photochemistry and Photobiology B: Biology*, **5**, 133–150.
[11] C. Salet and G. Moreno (1995). Photodynamic action increases leakage of the mitochondrial electron transport chain, *International Journal of Radiation Biology*, **67**, 477–480.
[12] C. Salet, G. Moreno and F. Vinzens (1982). Photodynamic effects induced by furocoumarins on a membrane system. Comparison with hematoporphyrin, *Photochemistry and Photobiology*, **36**, 291–296.
[13] A. Boveris (1984). Determination of the production of superoxide radicals and hydrogen peroxide in mitochondria, *Methods in Enzymology*, **105**, 429–432.
[14] W. J. Waddel and C. Hill (1956). A simple ultraviolet spectrophotometric method for the determination of protein, *Journal of Laboratory and Clinical Medicine*, **48**, 311–314.
[15] H. P. Misra and I. Fridovich (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase, *Journal of Biological Chemistry*, **247**, 3170–3175.
[16] M. Dellinger, C. Vever-Bizet, D. Brault, G. Moreno and C. Salet (1990). Uptake and photodynamic efficiency of hematoporphyrin, hydroxyethylvinyldeuteroporphyrin and hematoporphyrin derivative (Photofrin II®): a study with isolated mitochondria. *Photochemistry and Photobiology*, **51**, 185–189.
[17] H. M. Hassan and I. Fridovich (1977) Regulation of the synthesis of superoxide dismutase in *Escherichia coli*. Induction by methyl viologen. *Journal of Biological Chemistry*, **252**, 7667–7672.
[18] H. M. Hassan and I. Fridovich (1979) Intracellular production of superoxide radical and of hydrogen peroxide by redox active compounds. *Archives of Biochemistry and Biophysics*, **196**, 385–395.
[19] B. Chance and G. Williams (1956) The respiratory chain and oxidative phosphorylation. *Advances in Enzymology*, **17**, 65–134.
[20] M. K. Wikström (1977) Proton pump coupled to cytochrome c oxidase in mitochondria. *Nature*, **266**, 271–273.
[21] S. Sandberg and I. Romslo (1980) Porphyrin-photosensitized photodamage of isolated rat liver mitochondria. *Biochimica et Biophysica Acta*, **593**, 187–195.
[22] C. Salet and G. Moreno (1981) Photodynamic effects of hematoporphyrin on respiration and calcium uptake in isolated mitochondria. *International Journal of Radiation Biology*, **39**, 227–230.
[23] T. Ito (1978) Cellular and subcellular mechanisms of photodynamic action: the $^1O_2$ hypothesis as driving force in recent research. *Photochemistry and Photobiology*, **28**, 493–508.
[24] K. R. Weishaupt, D. J. Gomer and T. J. Dougherty (1976) Identification of singlet oxygen as the cytotoxic agent in photoinactivation of a murine tumor. *Cancer Research*, **36**, 2326–2329.
[25] C. Giulivi, M. Sarcansky, E. Rosenfeld and A. Boveris (1990) The photodynamic effect of Rose Bengal on proteins of the mitochondrial inner membrane. *Photochemistry and Photobiology*, **52**, 745–751.
[26] T. Miki, L. Yu and C.-A. Yu (1991) Hematoporphyrin-promoted photoinactivation of mitochondrial ubiquinol-cytochrome c reductase: selective destruction of the histidine ligands of the iron-sulfur cluster and protective effect of ubiquinone. *Biochemistry*, **30**, 230–238.
[27] C. Salet, G. Moreno, A. Atlante and S. Passarella (1991) Photosensitization of isolated mitochondria by hematoporphyrin derivative (Photofrin®): effects on bioenergetics. *Photochemistry and Photobiology*, **53**, 391–393.
[28] S. L. Gibson and R. H. Hilf (1983) Photosensitization of mitochondrial cytochrome c oxidase by hematoporphyrin derivative and related porphyrins *in vitro* and *in vivo*. *Cancer Research*, **43**, 4191–4197.
[29] F. Khanum and V. Jain (1989) Effects of hematoporphyrin derivative on the cellular energy metabolism in the absence and presence of light. *Photochemistry and Photobiology*, **50**, 647–651.
[30] D. A. Musser and A. R. Oseroff (1995) The use of tetrazolium salts to determine sites of damage to the mitochondrial electron transport chain in intact cells folloving *in vitro* photodynamic therapy with Photofrin II. *Photochemistry and Photobiology*, **59**, 621–626.
[31] T. F. Slater, B. Sawyer and U. Strüli (1963) Studies on succinate-tetrazolium reductase systems. III. Points of coupling of four different tetrazolium salts. *Biochimica et Biophysica Acta*, **77**, 283–293.
[32] C. Prinsze, T. M. A. R. Dubbelman and J. Van Steveninck (1990) Protein damage, induced by small amounts of photodynamically generated singlet oxygen or hydroxyl radicals, *Biochimica et Biophysica Acta*, **1038**, 152–157.
[33] Y. Zhang, O. Marcillat, C. Giulivi, L. Ernster and K. J. A. Davies (1990). The oxidative inactivation of mitochondrial electron transport chain components and ATPase, *Journal of Biological Chemistry*, **265**, 16330–16336.
[34] R. I. Salganik, I. G. Shabalina, N. A. Solovyova, N. G. Kolosova, V. N. Solovyov and A. R. Kolpakov (1994). Impairment of respiratory functions in mitochondria of rats with an inherited hyperproduction of free radicals, *Biochemical and Biophysical Research Communications*, **205**, 180–185.
[35] C. Giulivi, A. Boveris and E. Cadenas (1995). Hydroxyl radical generation during mitochondrial electron trans-

fer and the formation of 8-hydroxydesoxyguanosine in mitochondrial DNA, *Archives of Biochemistry and Biophysics*, **316**, 909–916.
[36] C.-Y. Pang, H.-C. Lee, J.-H. Yang and Y.-H. Wei (1994). Human skin mitochondrial DNA deletions associated with light exposure, *Archives of Biochemistry and Biophysics*, **312**, 534–538.
[37] L. W. Oberley and G. R. Buettner (1979). Role of superoxide dismutase in cancer: a review, *Cancer Research*, **39**, 1141–1149.