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EXPERIMENTAL ARTICLES

Photodynamic Damage to Yeast Subcellular Organelles Induced by Elevated Levels of Endogenous Protoporphyrin IX

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Abstract—The 2,2'-dipyridyl-induced accumulation of protoporphyrin IX in *Saccharomyces cerevisiae* cells was shown to be accompanied by the photoinhibition of cell respiration and the enhancement of the photoinduced permeability of plasma membranes to the fluorescent dye primuline. The visible-light illumination (at 400–600 nm) of the mitochondria and plasma membranes isolated from yeast cells with a high level of endogenous protoporphyrin IX intensified lipid peroxidation in these subcellular organelles. Comparative studies showed that the rad 52 mutant cells, which are deficient in the postreplicative recombinational DNA repair system, are considerably more sensitive to the inactivating action of visible light than are the wild-type cells and the rad 3 mutant cells, which are deficient in the excision DNA repair system. The contribution of photodynamic damage to the yeast subcellular organelles to the lethal photodynamic effect is discussed.

Key words: protoporphyrin IX, photodynamic damage, visible light.

It is known that endogenous protoporphyrin IX is an efficient photosensitizer of photodynamic processes in biological objects exposed to visible light [1–3]. The phototoxicity of endogenous protoporphyrin IX is due to its ability to generate reactive oxygen species (predominantly, singlet oxygen), which readily react with biologically important macromolecules and thereby cause their photooxidation, impairment of their functional activity, and eventually cell death [3, 4].

In the previous work [5], we showed that the growth of *Saccharomyces cerevisiae* cells in the presence of 2,2'-dipyridyl (a chelating agent of ferrous ions) is accompanied by the accumulation of endogenous protoporphyrin IX and considerable enhancement (by about seven times) of the sensitivity of the yeast cells to the inactivating action of visible light.

The aim of this work was to investigate photodynamic damage to the subcellular structures of *S. cerevisiae* cells and the impairment of their functional activity under conditions inducing the accumulation of endogenous protoporphyrin IX in these cells.

MATERIALS AND METHODS

Experiments were carried out with the wild-type haploid *Saccharomyces cerevisiae* strain 711α-ade1 and two mutant strains, rad 3 and rad 52, which are deficient in the excision DNA repair and the postreplicative recombinational DNA repair systems, respectively. The strains were kindly provided by I.P. Arman from the Institute of Molecular Genetics, Russian Academy of Sciences.

The strains were grown under the standard conditions [1]. The accumulation of endogenous protoporphyrin IX in yeast cells was induced by adding the ferrous-ion chelator 2,2'-dipyridyl 4 h before the growth termination. Then yeast cells were harvested and washed twice with distilled water by centrifuging them at 1500 *g* for 5 min.

Mitochondria from *S. cerevisiae* cells were isolated by the method of Mattoon and Balcavage [6]. Plasma membranes were isolated by a modification of the method of Bussey *et al.* [7] using a 1% solution of the lyophilized gastric juice of the *Helix pomatia* snail. The plasma membranes of yeasts isolated by this method do not contain mitochondria, which is evident from the absence of porphyrins (typical components of mitochondria) in the plasma membrane preparation [8].

Protoporphyrin IX was extracted from the preparations of plasma membranes (10 mg protein) and mitochondria (15 mg protein) by shaking them for 30 min at 4°C with 9 ml of a chloroform–methanol (2 : 1) mixture. The concentration of protoporphyrin IX in the chloroform phase was determined by the method described earlier [9]. Protein was quantified by the method of Lowry *et al.* (procedure N 690, Sigma).

The source of visible light within a spectral region of 400–600 nm, illumination conditions, and the measurement of photoinhibited cell respiration were described in the aforementioned work [9].

Photoinduced changes in the permeability of plasma membranes were measured with the fluorescent dye primuline [8] using a Lumam I-3 luminescence microscope. Primuline was added at a concentration of

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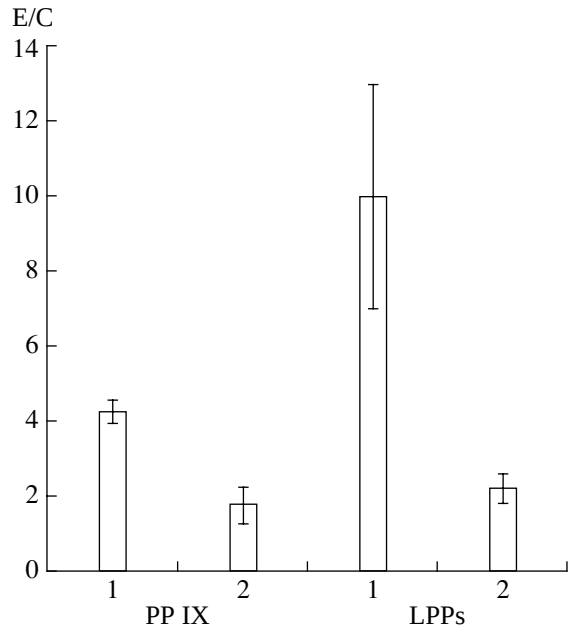

Fig. 1. The accumulation of protoporphyrin IX (PP IX) and the TBA-reactive lipid peroxidation products (LPPs) induced by exposure to visible light (at a dose of 7.2 J/cm^2) in (1) the mitochondria and (2) the plasma membranes of the wild-type *S. cerevisiae* 711α-ade1 cells grown in the presence (E) and absence (C) of 0.2 mM 2,2'-dipyridyl. The concentration of protoporphyrin IX in the mitochondria (1) and the plasma membranes (2) of the control cells was 0.08–0.015 and 0.017 ± 0.003 nmol/mg protein, respectively. The concentration of TBA-reactive products in the same subcellular organelles was 0.5 ± 0.1 and 0.2 ± 0.05 nmol/mg protein, respectively. The data presented in the figure are the means of triplicate experiments. E and C stand for "experiment" and "control," respectively.

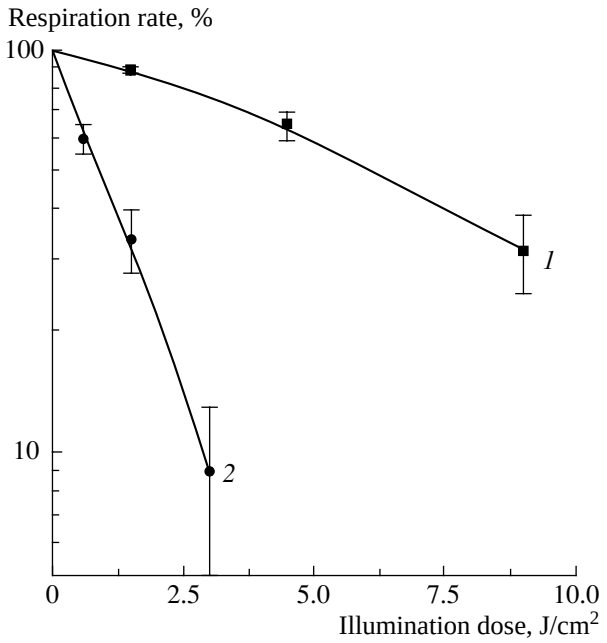

Fig. 2. The dose–response curves of the respiration of the wild-type *S. cerevisiae* 711α-ade1 cells grown (*2*) in the presence and (*1*) absence of 0.2 mM 2,2'-dipyridyl and exposed to visible light (400–600 nm). The data presented in the figure are the means of five replicate experiments.

0.5 mM immediately before the exposure of yeast cells to visible light. The number of primuline-stained cells was determined 10 min after the addition of primuline. In the control, yeast cells stained with primuline were not exposed to visible light.

The effect of visible light on the isolated mitochondria and plasma membranes was studied at concentrations of 1.5 and 0.75 mg protein/ml, respectively. The photoinduced lipid peroxidation products (PLPPs) in the plasma membranes and mitochondria were determined spectrophotometrically with 2-thiobarbituric acid (TBA) [10]. To this end, aliquots (0.15 mg protein) of the irradiated plasma membranes and mitochondria were added to 2 ml of a mixture containing 0.3% TBA, 0.2% sodium dodecyl sulfate, and 50 μM $FeCl_3$. The samples were incubated at 100°C for 10 min, cooled to room temperature, and analyzed for the content of the TBA adducts with PLPPs by measuring absorbance at 532 nm (the TBA–PLPP adducts have ε = 150 mM^{-1} cm^{-1}).

All the reagents used were purchased from Sigma (United States).

RESULTS AND DISCUSSION

It is known that photodynamic processes induced by endogenous protoporphyrin IX occur predominantly at the sites of its accumulation [3, 4]. On the other hand, it is known that the final steps of protoporphyrin IX synthesis take place in the inner mitochondrial membrane [11], due to which the concentration of protoporphyrin IX in yeast mitochondria may increase by more than four times (Fig. 1).

The accumulation of protoporphyrin IX in the yeast mitochondria was accompanied by the enhancement of the photoinduced lipid peroxidation (Fig. 1) and the inhibition of cell respiration by visible light (Fig. 2). This suggests that yeast mitochondria are one of the primary targets for the destructive action of visible light, especially under conditions promoting the accumulation of endogenous protoporphyrin IX. On the other hand, it is not evident that the photoinduced inhibition of mitochondrial respiration is of crucial importance to the yeast *S. cerevisiae*, which is a facultative anaerobe and can derive energy from glycolysis.

For this reason, of interest are experimental data on the redistribution of protoporphyrin IX accumulated in animal mitochondria to other cellular compartments, including the plasma and nuclear membranes [12, 13]. The photodynamic destructive processes occurring in the nuclear membrane may induce damage to the genetic apparatus of cells [4].

The exposure of the isolated plasma membranes to visible light caused a 1.8-fold increase in the content of

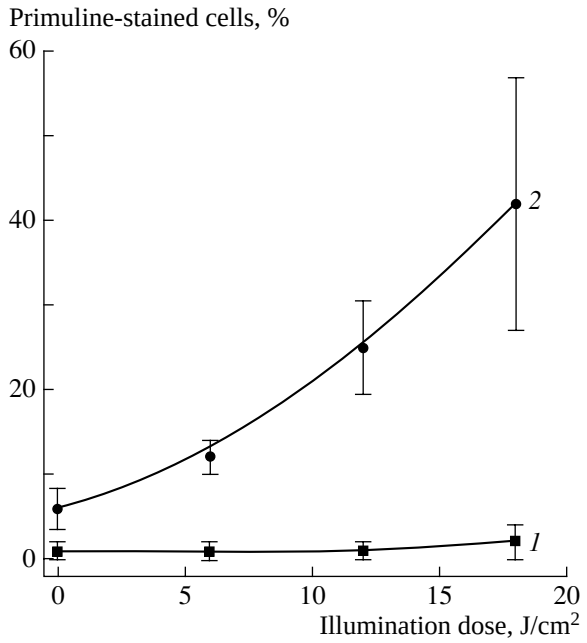

Fig. 3. The effect of visible light (400–600 nm) on the permeability of the plasma membranes of the wild-type *S. cerevisiae* 711α-ade1 cells grown in the (*2*) presence and (*1*) absence of 0.2 mM 2,2'-dipyridyl. The permeability of the plasma membranes was estimated as the percentage of cells stained with the fluorescent dye primuline (0.5 mM). The data presented in the figure are the means of five replicate experiments.

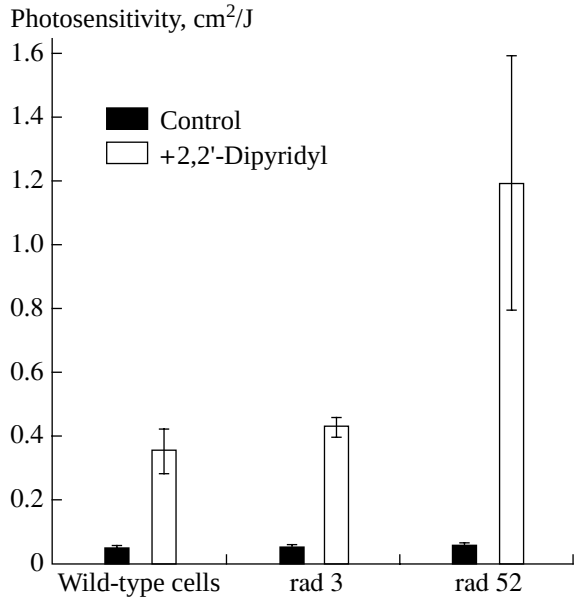

Fig. 4. The photosensitivity of the wild-type *S. cerevisiae* cells and the rad 3 and rad 52 mutant cells deficient in excision DNA repair and postreplicative recombinational DNA repair systems, respectively. Cells were grown in the presence (experiment) or absence (control) of 0.2 mM 2,2'-dipyridyl. Photosensitivity was expressed as the reversal of 37% cell survival ($1/D_{37}$). D_{37} is defined as the dose of visible light at which the cell survival rate is 37%. The data presented in the figure are the means of triplicate experiments.

protoporphyrin IX (Fig. 1). The accumulation of this photosensitizer in the membranes enhanced photoinduced lipid peroxidation in these subcellular structures (Fig. 1) and impaired their barrier functions, as is evident from the increased permeability of the membranes to the fluorescent dye primuline (Fig. 3).

To investigate the role of the postreplicative recombinational DNA repair system in the restoration of the potentially lethal photodynamic damage to the genetic apparatus of *S. cerevisiae*, we used two mutant strains, rad 52 (it is deficient in the postreplicative recombinational DNA repair system and hence is sensitive to the formation of DNA breaks [14]) and rad 3 (it is deficient in the excision DNA repair system [15]). It is known that the DNA lesions caused by reactive oxygen species include the apurine/apyrimidine regions of DNA and its breaks [16, 17], which are induced during the excision repair of oxidized bases [18]. The minor repair pathway is the excision repair of nucleotides catalyzed by the enzymes that efficiently repair the nucleotide lesions (such as pyrimidine dimers and adducts) caused by UV light [16, 18].

As was shown in preliminary experiments, yeast cells that are mutant in the DNA repair system contain the same amounts of protoporphyrin IX as do the wild-type cells. The rad 52 mutant cells, which are deficient in the postreplicative recombinational DNA repair system, turned out to be much more sensitive to the inactivating action of visible light than the wild-type cells (Fig. 4). This suggests that the photoinactivation of the rad 52 cells with the induced accumulation of protoporphyrin IX results from DNA lesions, which are typically repaired by the postreplicative recombinational DNA repair system.

The excision repair–deficient rad 3 mutant cells virtually did not differ in photosensitivity from the wild-type cells (Fig. 4). This suggests that the contribution of the excision repair system to the repair of the protoporphyrin IX–mediated photodynamic DNA damage is not great. On the other hand, there is evidence [15] that the rad 3 mutant cells have an efficient system for the repair of the double-stranded DNA breaks formed in the photoinactivated yeast cells with the induced accumulation of endogenous protoporphyrin IX.

It is difficult to estimate the contribution of DNA lesions to the photoinactivation of the wild-type cells, although there are grounds to believe (taking into account the fact that DNA repair processes are energy-dependent) that this contribution may increase significantly in energy-depleted yeast cells with photoinhibited respiration [19].

To conclude, the photodynamic inactivation of yeast cells with the induced accumulation of endogenous protoporphyrin IX may result from the photosensitized destructive processes occurring in various cellular compartments.

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