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Inactivation of *Saccharomyces* cells by 8-methoxypsoralen plus pulsed laser irradiation in the wavelength range 308 nm–380 nm

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Summary. Two different strains of *Saccharomyces cerevisiae*, one diploid wild type and one haploid mutant deficient in excision repair were irradiated with laser pulses in the range 308 nm to 380 nm after 8-MOP treatment. Both the shoulder (Dq) and the final slope (Do) of the inactivation curves were dependent on wavelength which showed a broad minimum around 355 nm. No differences in inactivation were recorded after pulsed irradiations between the repetition rates of 5 Hz and 35 Hz. Irradiations with pulses of the energy density from 0.1 mJ/cm^2 up to 26 mJ/cm^2 resulted in a final slope increasing with pulse energy density. This was in contrast to the effects of irradiation alone.

Introduction

Psoralens are known to cause strong photosensitizing effects in the presence of 365 nm light on a variety of organisms (Song and Tapley 1979; Parsons 1980). Furthermore, furocoumarins are used in combination with near UV-light (PUVA) as a very effective treatment for some skin diseases (Parrish et al. 1974; Wolff et al. 1975, 1976; Rodighiero and Dall'Acqua 1976). Psoralens intercalate in a dark reaction between pyrimidine base pairs in duplex DNA. After light absorption covalent bonds can be formed between pyrimidine pairs and 3,4- or 4′,5′-double bonds of the psoralen molecule (monoadducts) after which a sequential conversion of the 4′,5′-monoadducts into interstrand crosslinks is possible (Parsons 1980; Song and Tapley 1979; Dall'Acqua et al. 1978). Dose rate effects with psoralens, especially with 8-MOP, have been reported frequently during the last few years (Averbeck and Averbeck 1978; Amagasa 1981; Grekin and Epstein 1981). Irradiations were usually performed with standard conventional lamps (high pressure mercury lamp, xenon arc lamp, black light lamp) with a maximum emission

Abbreviations: 8-MOP: 8-methoxypsoralen; UV: ultraviolet; PUVA: therapy with *P*soralen plus *UV-A*

around 365 nm. There have only been a limited number of experiments reported where the effects of psoralen action plus laser light have been examined. Laser irradiation is found to be more effective in reducing viability of *Potorous* cells than non-laser light (Peterson and Berns 1978), but no differences are reported for inactivating T2 and RS2 phages (Fenyö et al. 1981) or for erythemic and tanning responses of human skin (Anderson and Parrish 1980). The linking and crosslinking of 8-MOP molecules to DNA with single laser pulses has been demonstrated by Johnston et al. (1977). A slight dependence of the minimum phototoxicity dose after PUVA-treatment of human skin with the repetition rate of laser pulses was detected (Schalla et al. 1980). Little information is known on laser experiments on yeast cells, although much of the recent work on biological activity of psoralens was done with yeasts (Averbeck and Moustacchi 1975; Jain et al. 1976; Averbeck and Averbeck 1978, 1979; Averbeck and Moustacchi 1979; Averbeck et al. 1979).

This paper presents results on the survival of *Saccharomyces* cells after treatment with 8-MOP and pulsed laser irradiation. The influence of wavelength, repetition rate and energy density of the laser pulses was studied in detail.

Materials and methods

Organism

Two different strains of *Saccharomyces cerevisiae* were used, a diploid wild type 211 (Laskowski 1960) and a haploid mutant S 24-12 c (rad 2–20) which is deficient in excision repair (Averbeck et al. 1970). Cells were grown to stationary phase in liquid complete medium containing 0.5% peptone, 1% yeast extract (Difco) and 2% glucose. After 48 h the cell suspension was centrifuged and washed twice in 0.05 *M* KH_2PO_4-buffer. Single cell populations were obtained after fractionated centrifugations. The cell concentration during irradiation was always $5 \cdot 10^3$ cells/ml.

Treatment with 8-MOP

The 8-methoxypsoralen (8-MOP) was kindly provided by Dr. W. Schalla, Berlin. A 10 m*M* stock solution of 8-MOP was prepared in a 50% water-ethanol mixture. Before irradiation the cells were incubated at 30° C for 30 min in a $5 \cdot 10^{-5}$ *M* buffer solution of 8-MOP. All treatments were done under yellow light at room temperature.

Irradiation procedure

The irradiations were performed by an almost parallel laser beam and during the exposure time the cell suspensions were stirred in glass dishes (inner diameter 1.2 cm) containing 0.8 ml of suspension. For 308 nm and 351 nm the irradiation source was an excimer laser, type EMG 101 from Lambda Physik in Göttingen, emitting laser pulses with a length of 10 ns and a

pulse frequency of maximal 40 Hz. Even for the highest doses the irradiation times were shorter than 1 min. The average laser power was measured before and after irradiation by a calorimeter, type 36-0001 from Scientech in Boulder. The power constancy was better than 5%. For the 327, 341, 365 and 380 nm irradiations an additional dye laser, type FL 2000 from Lambda Physik in Göttingen, was used. For these wavelengths the excimer laser, emitting at 308 nm or 351 nm, was used to pump the dye laser, so that pulse length and repetition frequency were the same as above. The dyes included were BM-Terphenyl, p-Terphenyl and BiBuQ, delivered by Lambda Physik. The energy density of the laser pulses were 0.6 mJ/cm^2 for the 327 nm and 1.5 mJ/cm^2 for the other lines. Furthermore for 308 nm and 351 nm the energy density could be extended up to 26 mJ/cm^2 per pulse.

Presentation of results

Immediately after irradiation 0.1 ml of the cell suspension or 1 ml samples for the two longest irradiations were plated fivefold on agar growth medium. After three days of incubation at 30° C the colonies were counted and the results are presented as survival curves reflecting the colony forming ability. Survival data were obtained from at least three independent experiments. In all cases shouldered curves were obtained, the characteristics of which are given by the parameters Dq (extend of shoulder) and Do (final slope) which are calculated within a limit of $\pm 15\%$ of reading.

Results

Control experiments

In all experiments controls were run with suspensions treated with 8-MOP but without irradiation and vice versa. Cells treated only with 8-MOP do not show any inactivation. Cells treated only with irradiation show an inhibiting effect of less than 1%, except for strain S 24-12 c at the shortest wavelength used, i.e. 308 nm. In this case a distinct decrease occured in the dose range normally applied in 8-MOP experiments (Fig. 1 a). As a consequence an exact determination of Do- and Dq-values is rather difficult with this strain, under these conditions.

An inactivation of strain 211 was observed only with 308 nm irradiation, fluences far beyond those used in experiments with 8-MOP. Moreover, inactivation was dependent on the energy density of the laser pulses, i.e. it increases with increasing pulse energy density (Fig. 1 b). In contrast, the effect of pulse energy density on the inactivation of the S 24-12 c strain was only very small (Fig. 1 a).

Variation of repetition frequency

After 8-MOP treatment both strains were irradiated with 351 nm light. The pulses had an energy density of 1.5 mJ/cm^2, but were applied with a repetition rate of 5 Hz, 10 Hz, 15 Hz, 20 Hz, 25 Hz or 35 Hz. As shown in Fig. 2

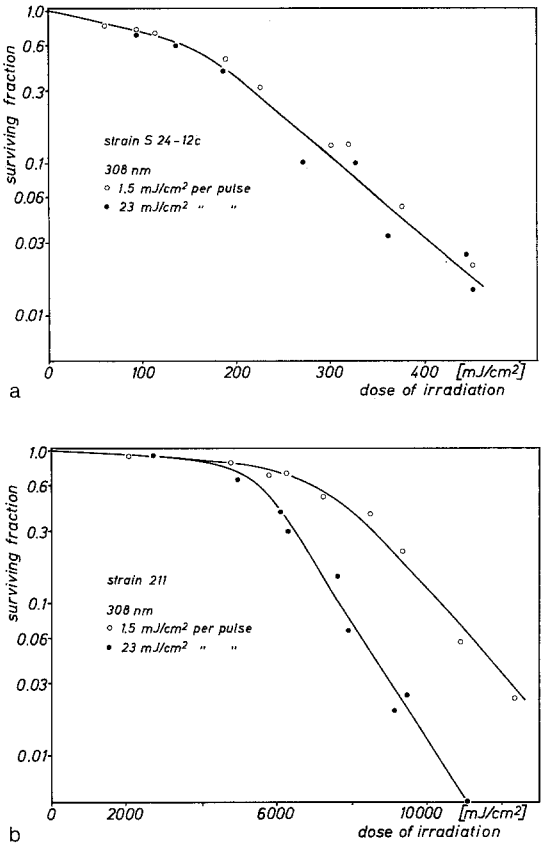

Fig. 1 a and b. Survival of yeast cells for 308 nm irradiation,
a the haploid strain S 24-12 c
b the diploid strain 211

no evidence of any dependence of cell inactivation on the repetition rate could be observed within the range of experimental error.

Variation of pulse energy density

These experiments were performed at two different wavelengths. At 308 nm the 211 strain was irradiated after 8-MOP treatment with four different pulse energy densities varying from 0.13 mJ/cm^2 until 26 mJ/cm^2. At 351 nm, both strains were also examined at the four different energy densities per pulse mentioned above.

Typical inactivation curves are shown in Fig. 3a and 3b. As seen in Table 1 which summarizes the data with increasing density of the laser pulses there is an enhancement in Do whereas the Dq values remain constant.

Variation of wavelength

Six different wavelengths were used to determine the wavelength dependency of the photoinactivation with 8-MOP. The pulse energy density was 1.5 mJ/

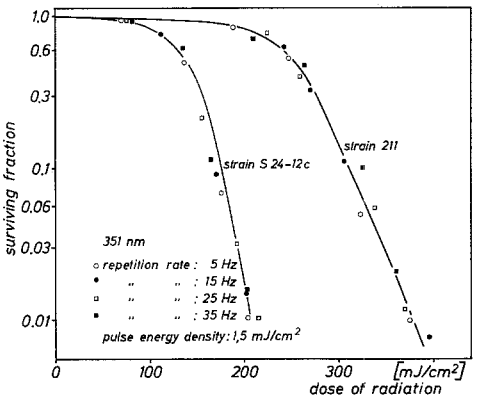

Fig. 2. Survival after 8-MOP treatment and 351 nm irradiation in dependence of the repetition rate

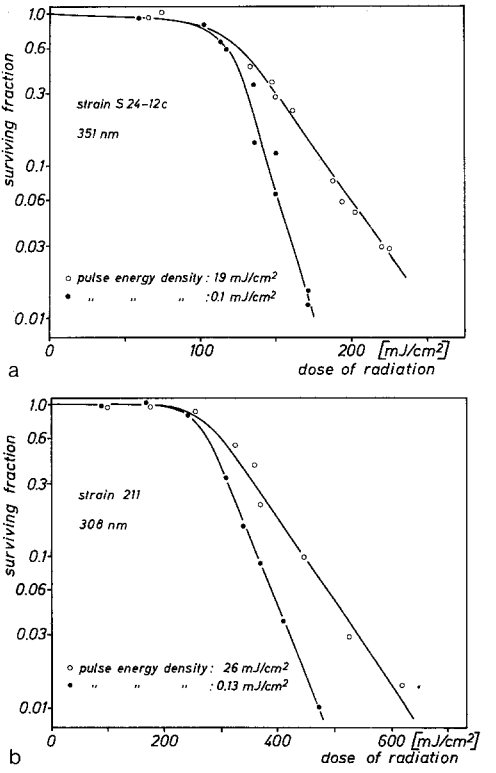

Fig. 3a and b. Survival after 8-MOP treatment and irradiation in dependence of the pulse energy density,
a strain S 24-12 c at 351 nm
b strain 211 at 308 nm

cm^2 except for 327 nm which was run only with it's maximum energy density of 0.6 mJ/cm^2. In spite of the different energy density the data obtained at this wavelength were included. A typical result is represented in Fig. 4 while a summary of all data can be found in Table 2. Do and Dq, as functions of the wavelength, have a broad maximum centered around 335 nm.

Table 1. Survival parameters obtained by variation of pulse energy density

Pulse energy density mJ/cm²	351 nm				Pulse energy density mJ/cm²	308 nm	
	Strain 211		Strain S 24-12 c			Strain 211	
	D_q mJ/cm²	D_o mJ/cm²	D_q mJ/cm²	D_o mJ/cm²		D_q mJ/cm²	D_o mJ/cm²
19	240	60	125	29	26	260	71
6.0	240	41	125	23	7.2	260	64
1.5	240	34	125	19	1.5	260	56
0.1	–	–	125	15	0.13	260	45

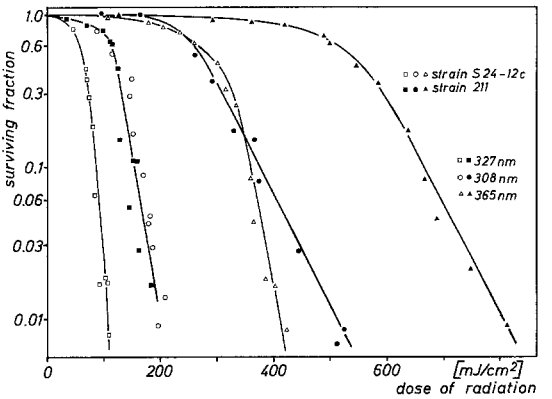

Fig. 4. Survival after 8-MOP treatment and irradiation in dependence of wavelength

Table 2. Survival parameters obtaind by variation of wavelength

Wave-length nm	Strain 211		Strain S 24-12 c	
	D_q mJ/cm²	D_o mJ/cm²	D_q mJ/cm²	D_o mJ/cm²
308	260	56	(~20)	(~60)
327	105	20	56	11
341	105	34	62	15
351	240	34	125	19
365	525	60	300	23
380	3300	450	550	100

Discussion

Data on the inactivation of a *Saccharomyces* cells by 8-MOP were obtained after applying the pulsed UV-radiation of an excimer laser or of a combined system of excimer and dye laser.

The minima found in the Do- and Dq-spectra around 335 nm agree well with those determined for *Escherichia coli* (Bridges et al. 1979; Suzuki et al. 1977) and bacteriophages (Fujita and Suzuki 1978). Thus the minima can be regarded to be independent of the actual biological system. Moreover

their appearance at this wavelength range is in some way connected with the interaction between 8-MOP and DNA. It is important to note that these minima do not correspond to a peak of either 8-MOP or a complex of 8-MOP with DNA (Bridges et al. 1979). One could conclude that the photoreaction pathway goes over the triplet excitation (Poppe and Grossweiner 1975), but another explanation for this behaviour might be an intercalated or linked 8-MOP molecule absorbing at longer wavelengths than the free molecule. However, this absorption spectrum could not be measured directly for yeast cells and the emission data does not support this postulate (Goyal and Grossweiner 1979; Beaumont et al. 1980).

Suzuki et al. (1977) suggested more than one type of lesions being produced by the photoinactivation with 8-MOP, especially since there is a higher mutation frequency at shorter wavelengths. Our present results do not support this suggestion. Nevertheless preliminary experiments in which pulses of two different wavelengths are employed successively do seem to suggest that this is case. The determination of the mutation frequency is in progress.

It is rather difficult to compare our results with those of Averbeck et al. (1978, 1979). The main reason is that their irradiations were done with conventional lamps emitting a broad band centered at 365 nm, whereas our results were produced by laser irradiations which are in principle not only much more monochromatic but also composed of very short and intense pulses. The power density of these pulses varied in our experiments between 10 kW/cm^2 and a few MW/cm^2. Thus, at 308 nm, a photon density was applied which extended to almost $5 \cdot 10^{16}$ photons/cm^2 within 10 ns, which is a time comparable to singlet lifetimes of molecules. Unfortunately the actual quantity of absorbing 8-MOP molecules within the $4 \cdot 10^3$ cells per irradiation procedure is not known, but there is certainly much less than the total number of $2.4 \cdot 10^{16}$ 8-MOP molecules present in the whole suspension volume.

Therefore saturation effects amplified by stimulated emission have to occur and it is not surprising that we needed a higher dose than Averbeck (Averbeck and Averbeck 1978; Averbeck and Moustacchi 1979) for the same inactivation at 365 nm. In addition, these saturation effects are well demonstrated by the dependence of the inactivation on pulse energy density. The reason why the dependency is not manifested in the Dq- but only in the Do-values is not easy to understand. The extent of the shoulder in the inactivation curves is more affected by the repair of lesions and only with higher doses does the differene caused by saturation effects become more evident. The change in spectral behaviour of the intercalated, linked and cross-linked 8-MOP molecules may contribute to this fact.

The lack of dependency of inactivation on the pulse repetition rate shows that there is no time constant within the examined millisecond range. This result contrasts with that found by Schalla et al. (1980) and may be due to the differences in biological endpoints examined.

In conclusion, new experimental data on the photoinactivation with 8-MOP can be obtained with laser systems. The wavelength dependence

which were determined were in accord with those found in the literature. Our spectra however clearly demonstrate that 365 nm is not the most effective wavelength. Moreover, it is possible to obtain fundamental data with pulsed radiation concerning the repetition rate and pulse energy density.

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