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SURVIVAL OF UV IRRADIATED HAPLOID  
YEAST CELLS MODIFIED BY CADMIUM

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Haploid yeast cells *Saccharomyces cerevisiae*, a,  $\alpha$ , his<sup>-</sup>, which had been grown 24 hours in the presence of cadmium (CdCl<sub>2</sub>, 40  $\mu$ mol/L and 80  $\mu$ mol/L) were irradiated with UV light (254 nm, 50 to 200 J m<sup>-2</sup>s<sup>-1</sup>). Using the colony forming ability as a biological parameter we found that the survival of the cadmium-treated cells after irradiation was higher than that of the irradiated cells without pretreatment in cadmium. A biochemical analysis shows that cadmium-treated cells possess a doubled total amount of deoxyribonucleic acid suggesting this as one of the explanations for the tolerance of cadmium-treated cells to the UV radiation. The observed protective effect of cadmium is not a result of the genetic induction of UV resistance.

## Introduction

The sensitivity of cells ultraviolet radiation can be modified by physical and chemical agents (Peak and Peak 1973, Young and Barth 1982). Among the latter the effects of heavy metals have not been investigated in spite of their presence in the environment and their well-know toxicity to living organisms in general.

Among the major hazardous environmental pollutants, cadmium occupies an important place (Friberg et al. 1974 Sabbioni et al. 1978). In an earlier study (Eger and Perić 1979) we have shown that cadmium (CdCl<sub>2</sub> 40  $\mu$ mol/L and 80  $\mu$ mol/L) exerts a toxic effect on the haploid strain of the yeast cells *Saccharomyces cerevisiae* by inhibiting cell division. In the same yeast strain we examined the photobiological response to ultraviolet radiation and found that exposure doses between 100 J m<sup>-2</sup>s<sup>-1</sup> and 300 J m<sup>-2</sup>s<sup>-1</sup> induced a fall in both total DNA content

and cell survival (Eger and Škreb 1969). The purpose of the present study was to test the response of cadmium treated cells to ultraviolet radiation to see whether cadmium is capable of modifying the effect of UV radiation.

### Material and Methods

Haploid yeast cells (*Saccharomyces cerevisiae*, a,  $\alpha$ , his<sup>-</sup>, Ogur, 1954) from the stationary phase of the fresh pre-grown culture with 2—5% of buds were used for inoculation. The cells were grown in the growth medium containing: 5 g yeast extract (Difco), 30 g glucose (Kemika, Zagreb), 10 g bacto pepton (Difco) and 9 g NaCl (Kemika, Zagreb) in 1 L distilled water at pH 6.5—7.0. The cultures were incubated in the water bath at 30°C and aerated. Cadmium was administered as cadmium chloride (Kemika, Zagreb, analytical grade, 40  $\mu\text{mol/L}$  and 80  $\mu\text{mol/L}$ ). Control cultures were grown at the same time but without cadmium. After 24 hours of growth the number of cells was determined by haemocytometer in each culture. Before radiation the cells were removed from the growth medium, rinsed three times and resuspended in saline.

The source of radiation was a »Philips« germicidal lamp emitting predominantly at 254 nm. The aliquots were irradiated with doses of 50  $\text{J m}^{-2}\text{s}^{-1}$ , 100  $\text{J m}^{-2}\text{s}^{-1}$  and 200  $\text{J m}^{-2}\text{s}^{-1}$  as determined at the surface of solution by a Lатарjet's dosimeter. The distance between the solution and the UV source was about 40 cm. Radiation was performed at room temperature by shaking the cell suspension. The depth of the irradiated suspension was about 4 mm. Immediately after radiation cells were diluted and plated on nutritive agar to assay the colony forming ability.

The cells which grew in cadmium were fractionated successively in 2% cold and 5% hot TCA to obtain the total amount of DNA. DNA was determined by diphenylamine reagent and the intensity of the colour reaction was determined at 600 nm by a UNICAM SP 600 spectrophotometer. The DNA quantity was calculated using deoxyadenosine as standard (Tewari et al. 1966).

### Results

The results showed (Table 1) that cadmium alone significantly decreased the cell survival; with 40  $\mu\text{mol/L}$  of cadmium only about 7% of the cells survived and with 80  $\mu\text{mol/L}$  the survival was about 1%. Radiation with UV, but without cadmium, caused a decrease in the cell survival to 50% at the highest dose applied. Cells pretreated with cadmium before UV radiation showed that radiation caused a smaller effect than it did in control cells. With 40  $\mu\text{mol/L}$  cadmium-radiation decreased the cell survival to 70% while with 80  $\mu\text{mol/L}$  of cadmium the cell survival even increased (10—30%) in comparison to the non-irradiated the population. This effect suggests that through cadmium treatment cells established a new pattern or patterns which made them tolerant to the radiation doses applied. The increase in cell survival after cadmium and UV treatment is due to the increase in plating efficiency, because a large fraction of cells were capable of forming visible colonies, as shown in Table 2.

Table 1. Number of colonies (per millilitre) from UV irradiated cells pretreated with CdCl<sub>2</sub>

RADIATION Jm <sup>-2</sup> s <sup>-1</sup>	CONTROL			PRETREATMENT WITH CdCl <sub>2</sub>		
	40 μmol/L			80 μmol/L		
	X ± S.E.M.	%		X ± S.E.M.	%	
UNIRRADIATED CONTROL						
50	*(1.105 ± 0.053)10 <sup>8</sup>	100		(7.39 ± 0.18)10 <sup>6</sup>	100	(1.34 ± 0.09)10 <sup>5</sup>
100	(0.882 ± 0.046)10 <sup>8</sup>	80		(6.56 ± 0.24)10 <sup>6</sup>	90	(1.52 ± 0.12)10 <sup>5</sup>
200	(0.629 ± 0.018)10 <sup>8</sup>	60		(6.47 ± 0.12)10 <sup>6</sup>	85	(1.62 ± 0.07)10 <sup>5</sup>
	(0.528 ± 0.023)10 <sup>8</sup>	50		(5.63 ± 0.08)10 <sup>6</sup>	70	(1.68 ± 0.06)10 <sup>5</sup>

\* Mean values of three independent experiments each done in triplicate.

Table 2. Plating efficiency of UV irradiated cells pretreated with CdCl<sub>2</sub>. The number of plated cells (per millilitre) is taken as 100%.

RADIATION Jm <sup>-2</sup> s <sup>-1</sup>	PRETREATMENT WITH CdCl <sub>2</sub>		
	CONTROL	40 µmol/L	80 µmol/L
	NUMBER OF PLATED CELLS /ml ± S.E.M.		
	*(1.57 ± 0.09)10 <sup>8</sup>	(2.27 ± 0.18)10 <sup>7</sup>	(2.10 ± 0.10)10 <sup>6</sup>
PLATING EFFICIENCY (%)			
UNIRRADIATED CONTROL	*70 (60 — 80)**	30 (30 — 36)	6 (4 — 8)
50	60 (50 — 70)	30 (25 — 30)	7 (5 — 8)
100	40 (40 — 46)	29 (26 — 30)	8 (6 — 9)
200	30 (30 — 36)	25 (20 — 28)	8 (6 — 9)

\* mean values of three independent experiments

\*\* range given in brackets.

Table 3. The total amount of DNA in cadmium treated and control cells expressed as µg/10<sup>8</sup> cells ± S. E. M.

CONTROL	CdCl <sub>2</sub>	
	40 µmol/L	80 µmol/L
*2.508 ± 0.026	4.900 ± 0.040	5.300 ± 0.046

\* mean values of three independent experiments each done in triplicate.

The role of cadmium in cell survival and cell divisions after UV radiation calls for the investigation of DNA metabolism in cadmium treated cells. Preliminary results in Table 3 show that the cells grown in both cadmium concentration contain double total amounts of DNA.

To detect a possible genetic effect of the cadmium — UV treatment we picked up randomly grown colonies resuspended in saline and irradiated them under the same conditions and with the same exposure doses as previously. These cells behaved as untreated control cells. Hence we conclude that the observed protective effect of cadmium is not a result of the induction of UV resistance.

## Discussion

The results presented in this work suggest a protective effect of cadmium against UV radiation. This effect can be expressed as a biological reaction of the cells to the combined actions of two external agents. It is a question of two heterogeneous agents, one physical and the other chemical, which if applied separately, are capable of inducing specific changes in the cell structures. Despite their heterogeneity these agents have the same target — deoxyribonucleic acid.

The wavelength of the UV spectrum and the radiation doses applied are known to induce the formation of photoproducts in the DNA molecule such as dimers, DNA — DNA cross links, DNA — protein cross links and others which lead to changes of the physico-chemical properties of the molecule (McGrath et al. 1966, Patrick et al. 1976).

Cadmium binds to DNA, to phosphate groups or nucleic bases causing the single strand breakages to decrease the stability of the helix (Eichorn et al. 1979, Mitra et al. 1978).

It is, however, known that  $Cr^{2+}$ ,  $Cu^{2+}$  and  $Ni^{2+}$  produce a protective effect against gamma radiation (Friedberg et al. 1975, Komina mi et al. 1975), but how cadmium modifies the effects of UV radiation is not known.

Although we consider the information that cadmium treated cells have a double amount of DNA to be only preliminary, it is an important indication that DNA metabolism is in progress during the cell incubation with cadmium. We assume that the doubled DNA in the cells treated with cadmium can reduce the effect of UV radiation by reducing the formation of photoproducts or diminishing the lesions that have already been induced. Besides the importance of DNA for the survival of cells after they have been irradiated, the role of the reparatory processes must not be underestimated. It is possible that these processes are intensified by the presence of cadmium in the cell.

Most data about cadmium binding to DNA and cadmium effect on gamma radiation come from in vitro studies. Our investigations, both of the effect of cadmium alone and of the combined cadmium — UV radiation effect, were conducted in vivo. This circumstance leads us to regard the latter effect as a complex expression of the overall cell metabolism because cadmium binds not only to DNA but also to various structures among which the enzymic ones are very important.

To summarize, the cells pre-grown in cadmium and containing a double total amount of DNA are more resistant to UV radiation than the cells which were only irradiated. Consequently, it is concluded that cadmium is capable of modifying the effect of UV radiation.

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## SAŽETAK

## MODIFIKACIJA PREŽIVLJENJA UV ZRAČENIH STANICA HAPLOIDNOG KVASCA S KADMIJEM

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Stanice haploidnog kvasca *Saccharomyces cerevisiae*, a,  $\alpha$ , his<sup>-</sup>, koje su rasle 24 sata u hranljivom mediju s kadmijem (CdCl<sub>2</sub>, 40  $\mu$ mol/L i 80  $\mu$ mol/L) zračene su UV-zračenjem (254 nm, do 200 J m<sup>-2</sup>s<sup>-1</sup>). Koristeći se sposobnošću formiranja kolonija kao biološkim parametrom pokazalo se da stanice pretretirane u kadmiju preživljuju zračenje u višem stupnju od stanica koje nisu rasle prethodno u kadmiju. Biokemijskom kvantitativnom analizom utvrđeno je da stanice koje su rasle u kadmiju imaju dvostruku ukupnu količinu DNK. Smatramo da ta okolnost može biti jedan od uzroka povećane tolerancije kadmijem tretiranih stanica prema UV-zračenju. Genetska analiza je pokazala da protektivni efekt kadmija, koji smo prikazali u ovom radu, nije rezultat genetske indukcije UV-rezistencije.

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