Bot. Bull. Academia Sinica (1988) 29: 87-91

Synergistic effects of sodium arsenite on UV-irradiated *Saccharomyces cerevisiae*

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(*Received March 6, 1987; Accepted December 18, 1987*)

Abstract. UV-treated wild type yeast cell showed a decreased survival in the presence of sodium arsenite. The magnitude of synergistic effect increased with UV dosage. There was no synergistic effect on the survival of UV-irradiated excision-deficient cells. No increase of UV mutation induction was observed by treatment of sodium arsenite in wild type cell.

Key word: Sodium arsenite; *Saccharomyces cerevisiae*; Ultraviolet light.

Introduction

We are constantly exposed to various chemical compounds in the environment. It is important to study their effect on organisms in order to estimate the risk involved.

The synergistic effect of sodium arsenite to ultraviolt (UV) treated *Escherichia coli* was studied by Rossman *et al.* (1975) and Rossman (1981). Comutagenicity of UV and sodium arsenite was reported by Leonar add Lauwerys (1980) and Lee *et al.* (1985) in mammalian cells. It is interesting to know if sodium arsenite may interfere with excision repair system of UV-damaged *Saccharomyces cerevisiae*. We therefore decide to investigate the synergistic effect of sodium arsenite to UV-irradiated excision proficient and excision-deficient strains of *S. cerevisiae*.

Materials and Methods

Strains

Two strains of diploid *Saccharmyces cerevisiae* were kindly supplied by Dr. T. Saeki (Chiba, Japan).

Their genotypes are as follow:

Excision-proficient strain xs 2167:

a/α RAD/RAD leu 1-1/leu 1-1 his 1-1/his 1-1
l ys 1-1/l ys 1-1

Excision-deficient strain xs 2162:

a/α rad 1-rad 1 leu 1/leu 1-1 his 1-1/his 1-1
1 ys/1 ys

Meida

Growth medium (YEPD) contained 1% Difco yeast extract, 2% Difco bactopeptone, 2% glucose and 2% agar. For liquid incubation 2X YEPD medium was used but omitted agar.

Synthetic Complete Medium (SC) composed of 0.67% Difco yeast nitrogen base without amino acids, 2% glucose and 2% agar supplemented with lysine, histidine, leucine at 20 mg/liter each. Omission medium was SC minus leucine, since only reversion of Leu^+ was reported in this study.

Effect of sodium arsenite on the survival and mutation induction of UV-irradiated ***S. cerevisiae***

Overnight stationary phase cell culture was obtained by growing cells in YEPD at 28°C with shaking. The cells were washed twice with 0.067M potassium phosphate buffer, pH 7.0 and then resupended with phosphate buffer at the concentration of 1×10^7 cells/ml. UV irradiation was carried out with a two 15 W low-pressure mercury Toshiha germicidal lamps which primarily emitted UV at 254 nm. Following UV exposure, appropriate dilution was made and the cell was plated on YEPD for cell survival study and on SC for mutation frequency detection. Different concentrations of sodium arsenite was supplied in both medium in order to investigate the synergistic effect of sodium arsenite on UV-irradiated yeast cells.

Results

The standard survival curves of UV-treated excision proficient strain and excision-deficient strain are shown in Figs. 1a and 1b, respectively. The effect of various concentrations of sodium arsenite on yeast survival is shown in Fig. 2. These results indicated that excision deficient strain was more sensitive to both UV and sodium arsenite than excision proficient strain by means of cell killing.

The effect of various concentrations of sodium arsenite on the survival of UV-irradiated wild type yeast is shown in Fig. 3. In order to compare the synergististic effect of different concentrations of sodium arsenite on UV-treated cell, we normalized the survival of different concentrations of sodium arsenite-treated but no UV-treated cells to one

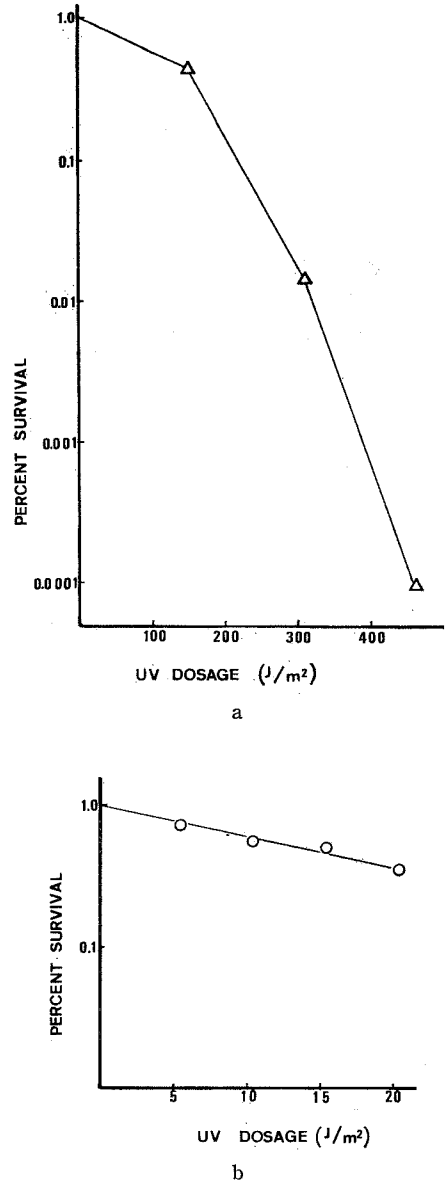

Fig. 1. UV survival curves of strain: (a) xs 2167 (b) xs 2162.

(See Fig. legend) and substracted survivors with UV treatment to survivors without UV treatment. Figure 3 indicates that higher level of sodium arsenite (1.5 mM) treatment exhibited more synergistic effect on cell survival of UV-treated cells. One mM sodium arsenite treatment cause less synergistic effect on UV-treated cells. Figure 4 shows that the survival of same sodium arsenite

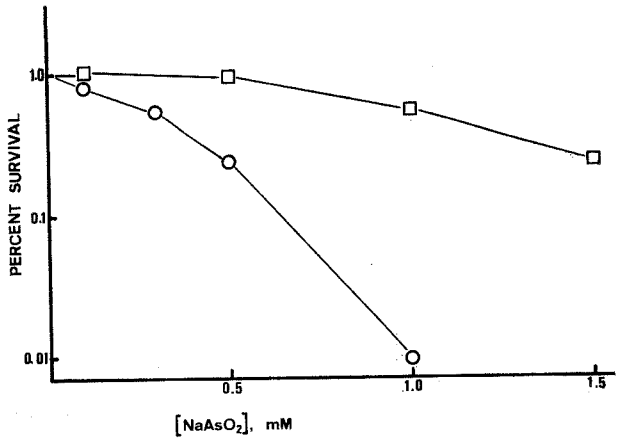

Fig. 2. Sodium arsenite survival curves of strains: xs 2167 (□) and xs 2162 (○).

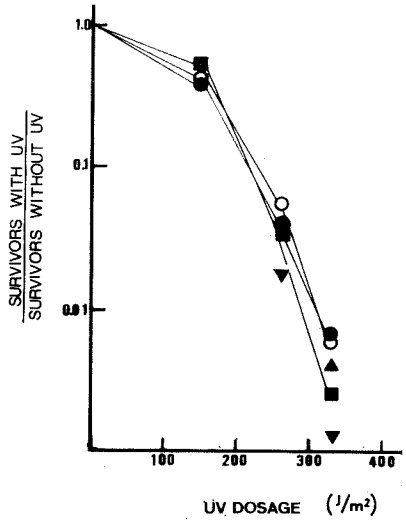

Fig. 3. The effect of different concentrations of sodium arsenite on the survival of UV irradiate xs 2167. The survivals of various levels of sodium arsenite treated cells at unirradiated condition are normalized to 1. Sodium arsenite concentrations: 0mM (○); 0.1mM (●); 0.5 mM (▲); 1.0 mM (■); 1.5 mM (▼).

treated wild type cells decreased with increasing UV irradiation.

Figures 5 and 6 exhibit the result as described in Figs. 3 and 4, respectively, except the cell used is excision-deficient strain xs 2162. In the presence of 1.0 mM sodium arsenite, the chemical seems to enhance the survival of UV-damaged excision-deficient cell (Fig. 5). The synergistic effect shown in UV-treated wild type cell was not observed. Figure 6 indicates that different UV doses exposed excision-deficient cells exhibited no significantly different response to additional sodium arsenits treatment.

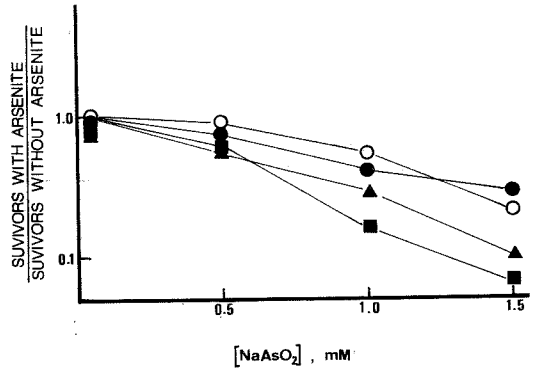

Fig. 4. The effect of sodium arsenite on the survival of different UV dose treated xs 2167. The survivals of various dosages of UV treated cells without sodium arsenite treatment are normalized to 1. UV dosage: 0 J/m² (○); 147 J/m² (●) with 42% survival; 330 J/m² (■) with 0.64% survival.

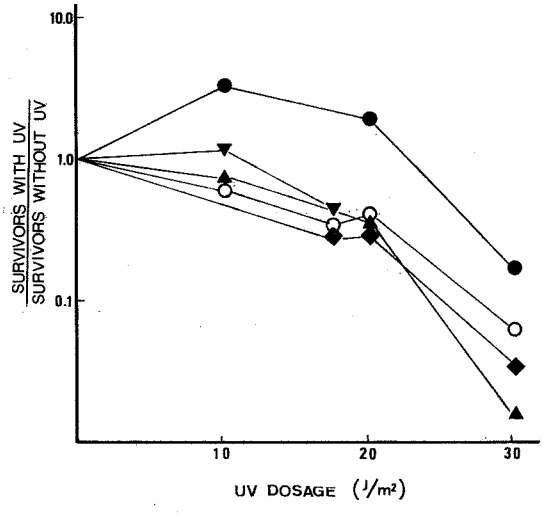

Fig. 5. The effect of different concentrations of sodium arsnite on the survival of UV irradiated xs 2162. The survivals of various levels of sodium arsenite treated cells at unirradiated condition are normalized to 1. Sodium arsenite concentrations: 0 mM (○); 0.1 mM (■); 0.3 mM (▲); 0.5 mM (▼); 1.0 mM (●).

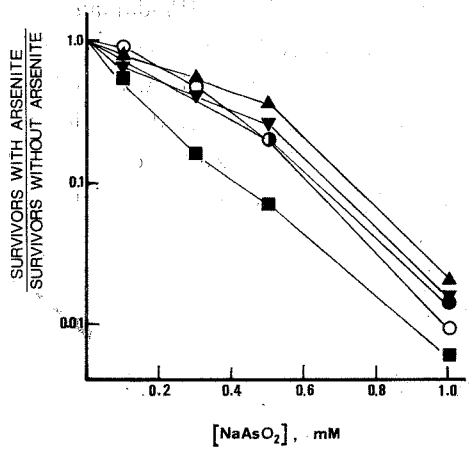

Fig. 6. The effect of sodium arsenite on the survival of different UV doses treated xs 2162. The survivals of various dosages of UV treated cells without sodium arsenite treatment are normalized to 1. UV dosages: 0 J/m² (○); 10.5 J/m² (▲) with 58% survival; 17.5 J/m² (◆) with 36.3% survival; 20.5 J/m² (●) with 41.6% survival; 30.5 J/m² (■) with 6.5% survival.

In order to find out if mutation induction of UV-treated will type cell was synergisticlly effected by sodium arsenite, the auxotrophy reversion frequency is shown in Table 1. No enhancement of UV-mutagenesis by various concentrations of sodium arsenite was found in excision proficient strain.

Discussion

Rossman *et al.* (9975) reported that both UV-irradiated wild type and excision-deficient cells showed decreasing survival in the presence of sodium arsenite. In our study, same phenomenon is found in wild type cell but not in excision-deficient cell (Figs. 3 and 5). The more significant synergistie effect shows when higher dose of sodium arsenite was applied. The magnitude of the effect also increase with increasing UV dosage in wild type cell (Fig. 4). Higher level arsenite treatment may increase the survival of UV-damaged excision-minus cells (Fig. 5) The mechanism of increasing the survival of UV-treated excision deficient cells in the presence of sodium arsenite is not clear. But it is obviously that sodium arsenite may interfere with excision repair system after UV treatment. So the survival of UV treated repair proficient cells decreased when sodium arsenite was applied and same phenomenon was not observed in repair deficient strain.

Rossman (1981) reported the increasing of UV-mutagenesis by treatment of sodium arsenite in *E. coli*. Kharab and Singh (1985) also concluded that sodium arsenite gave a positive result for reversion in yeast cell. Our results shows that sodium arsenite may interfere with excision repair and cause the synergistic effect on cell killing but not mutation induction. Since excision repair is error-free, it is no surprise that sodium arsenite does not increase the mutation induction in UV-exposed wild type cell even it can increase cell killing by interfere with excision repair.

Table 1. *Absence of comutagenic effect by sodium arsenite in xs2167*

$NaAsO_2$ (mM)	Mutation frequency ($\times 10^{-5}$)				
	UV dosage (J/m²)				
	0	36.25	61.25	122.5	210
0	0.04	1.2	3.6	8.7	24
0.05	< 0.01	1.1	2.2	7.0	24
0.10	< 0.01	1.5	3.3	9.2	23
0.30	< 0.01	1.4	2.8	7.0	28
0.05	< 0.01	1.2	3.2	8.1	23
1.00	< 0.01	1.0	2.0	5.0	20

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亞砷化鈉對紫外線處理之酵母菌之影響

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紫外線處理之野生型酵母菌株可因亞砷化鈉之存在而增加其致死百分率，但不增加其突變頻率，此亞砷化鈉對紫外線之相加致死效果可因紫外線劑量之增加而更顯著。但此亞砷化鈉對紫外線之相加致死效果並不存在於修復欠損株中。