Bot. Bull. Academia Sinica (1988) 29: 81-86

Dose-rate effect of ultraviolet light and 4-nitroquinoline 1-oxide treatment on *Saccharomyces cerevisiae*

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(*Received March 3, 1987*; *Accepted December 10, 1987*)

Abstract. The fractionate treatment of Ultraviolet (UV) and 4-nitroquinoline 1-oxide (4NQO) increased the cell survival and decreased the mutation frequency of excision proficient strain of *Saccharomyces cerevisiae* when compared to a single high dose exposure followed by the same period of liquid holding on same strain. The effect of low dose rate is caused by excision repair in UV and 4NQO treated cell. The reason that fractionate effect is much pronounced than liquid holding repair may be due to the higher efficiency in excision of UV and 4NQO damages caused by low dose rate treatment. The effect of fractionate treatment is not observed in excision deficient strain in UV and 4NQO studies.

Key words: Dose fractionate; 4NQO; *Saccharomyces cerevisiae*; Ultraviolet light.

Introduction

The UV-irradiated excision-proficent strains of *Escherichia coli* and *S. cerevisiae* showed "liquid holding recovery" in nonnutrient liquid medium before plating (Harm, 1968; Tang and Patrick, 1977; Ferguson and Cox, 1980). Harm (1968) and Tang and Patrick (1977) showed that the survival of a wild type *E. coli* was increased drastically when cell was treated with a low dose rate of UV instead of an acute treatment with same dose. Their results suggested that more efficient dark repair was carried out under low dose rate exposure condition.

We have found the low dose-rate of UV and 4NQO in *E. coli* by means of cell survival and mutation induction (Dai *et al.*, 1983). A similar effect after UV irradiation was also reported with a repair-proficient strain of *S. cerevisiae* (Ferguson and Cox, 1980).

In this report, we study the low dose-rate effect of UV and 4NQO in excision-proficient strain and excision-deficient strain of *S. cerevisiae* according to the cell survival and reversion frequency of mutation induction.

Since in nature environment, we are exposed under a very low dose rate exposure condition of various environmental mutagen, low dose-rate effect is an important factor for assaying environmental potential hazards to man.

Materials and Methods

Strains

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

Their genotypes were as follows:

Excision-proficient strain xs2167:

a/α RAD/RAD leu 1-1/leu 1-1 his 1-1/his 1-1 lys 1-1/lys 1-1

Excision-dificient strain xs2162:

a/α rad 1/rad 1 leul-1/leu 1-1 his 1-1/his 1-1 lys 1-1/lys 1-1

Media

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.

Cell Culture

A loopful of *S. cerevisiae* was transferred from slant to 50 ml 2X YEPD and incubated at 30°C with shaking for 88 h. The stationary-phase cells were harvested and washed twice with "treatment medium" (0.68% KH_2PO_4, pH 5.4). The cells were resuspended in the same treatment medium and ready for mutagen exposure. The percentage of budding cells in the culture was below 3% for excision-proficient strain and below 4% for excision-deficient strain.

Mutagen Treatment

Cell suspension at a concentration of 10^7 cells/ml was used for either 4NQO or UV treatment. The cells for UV irradiation were carried out in 9 mm glass petri dish at room temperature. All irradiations were performed under dark. UV irradiation was done with two low-pressure mercury Toshiba gericidal lamps, emitting primarily 2537 Å. 4NQO treatment was done at 30°C with shaking in test tube.

Dose-Rate Effect of 4NQO

A typical experiment of dose-rate study was designed as following:

1. Acute Early Treatment: Treatment carried out immediately after cell washing with an acute dose and followed by 24 h's holding at 30°C with shaking. The doses applied on excision proficient strain were 0.25 μM to 15 μM and on excision deficient strain were 0.1 μM and 0.3 μM.
2. Fractionate Treatment: Treatment started right after cell washing. The full dose of 4NQO as Acute Early Treatment described above was splitted into 1/10 and added to the cells once every hour for 10 times. Followed the last treatment, the cells were hold for additional 15 h holding.
3. Acute Late Treatment: Acute dose treatment was performed after the washed cells had been starved 23 h at 30°C. The treatment time for 4NQO was one hour.

At the end of exposure, the cells treated in the above three ways were washed twice with treatment medium by filtration and then the cells were plated for assaying the survival and mutation induction.

Dose-Rate Effect of UV

The method followed the standard procedures as described for 4NQO study except the treated cells were keep in petri dish in the dark without shaking. After treatment, the cells were diluted and plated directly or concentrated by filtration. The UV doses applied on excision-proficient strain were 210 $J.m^{-2}$ and 320 $J.m^{-2}$ and on excision-deficient strain were 5.6 $J.m^{-2}$ and 11.2 $J.m^{-2}$.

For detecting the survival and reverse mutation on xs 2167 and xs 2162, the appropriate cell number was chosen and the cells were plated on SC for survival study and the omission medium lacking leucine was used for reverse mutation study. The plates were incubated for 7 days at 30°C in the

dark and then count for colony formation.

Results

Effect of Fractionate Treatment of UV and 4NQO on the Survival of Excision-Proficient (xs 2167) and Excision-Deficient (xs 2162) strains

Figure 1 indicates that fractionate exposure of UV results in increase survival compared to single exposure at high dose rate on excision-proficient strain. The acute treatment following same period of liquid holding (Acute early treatment) showed lower cell survival compared to fractionate treatment. But liquid holding effect was shown when compared to the cell exposed as acute late treatment at higher UV dose (320 J.m^{-2}). More prounced low dose-rate effect was also observed in this study.

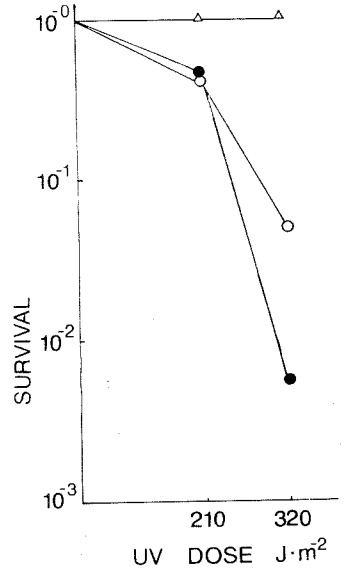

Fig. 1. The effect of UV fractionate treatment on the survival of diploid yeast xs 2167. ○, acute early treatment; △, fractionate treatment; ●, acute late treatment.

Fractionate exposure of UV did not cause the increasing of survival compared to the acute treatment in excision deficient strain (Fig. 2). It suggests that the increase of survival is caused by more efficient of excision repair taking place during UV fractionate treatment on excision-proficient strain.

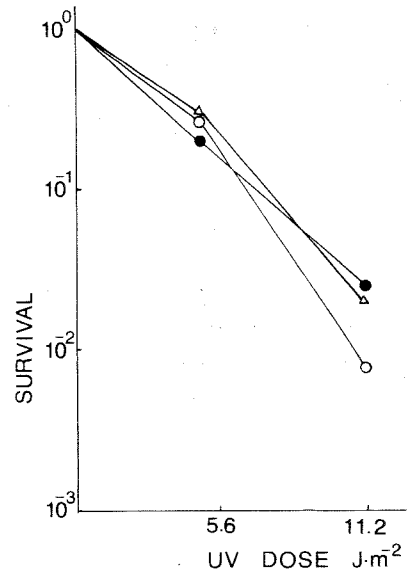

Fig. 2. The effect of UV fractionate treatment on the survival of diploid yeast. xs 2162. ○, acute early treatment; △, fractionate treatment; ●, acute late treatment.

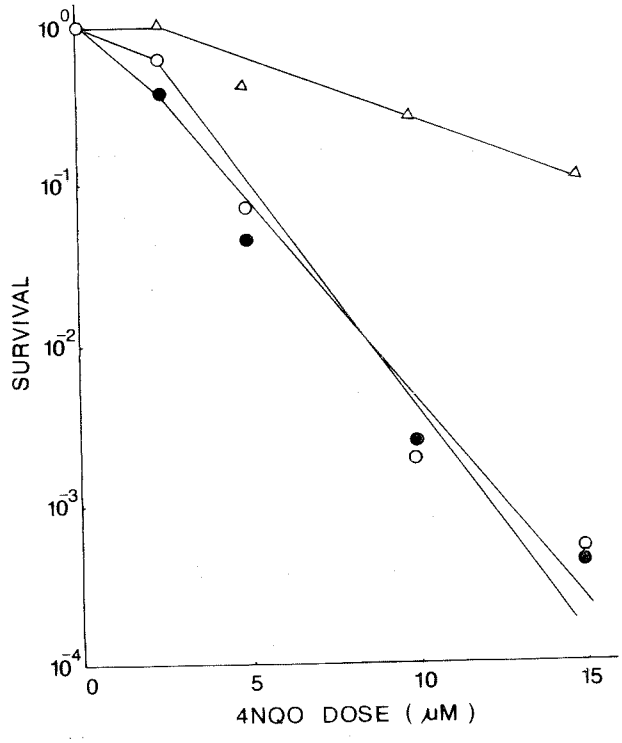

Fig. 3. The effect of 4NQO fractionate treatment on the survival of diploid yeast xs 2167. ○, acute early treatment; △, fractionate treatment; ●, acute late treatment

Figure 3 shows the fractionate treatment of 4NQO on excision-proficient strain results in drastically higher survival. Figure 4 indicates that the fractionate treatment of 4NQO did not increase the colony forming ability on excision-deficient strain. The liquid holding repair was not significant in 4NQO treated wild type cell.

Effect of Fractionate Treatment of UV and 4NQO on the Mutation Induction of Excision-Proficient (xs2167) and Excision-Deficient (xs 2162) strains

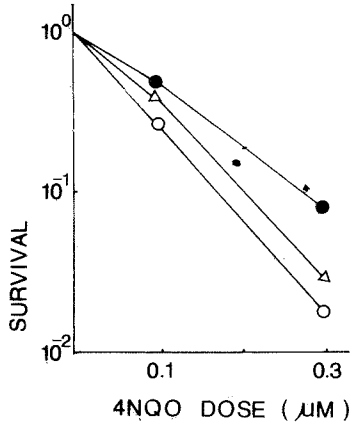

Fig. 4. The effect of 4NQO fractionate treatment on the survival of diploid yeast xs 2162. ○, acute early treatment; △, fractionate treatment; ●, acute late treatment.

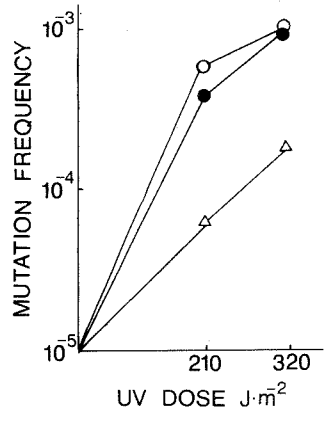

Fig. 5. The effect of UV fractionate treatment on the mutation induction of diploid yeast xs 2167. ○, acute early treament; △, fractionate treatment; ●, acute late treatment.

Figure 5 shows that around 10 folds decreasing of mutation induction was attributed by fractionate treatment in xs 2167. Similar effect was not shown in xs 2162 (Fig. 6).

The fractionate treatment of 4NQO on excision-proficient strains result in lowing mutation frequency (Fig. 7). Our unpublished result in-

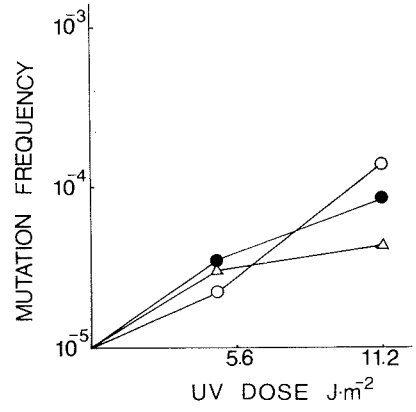

Fig. 6. The effect of UV fractionate treatment on the mutation induction of diploid yeasts xs 2162. ○, acute early treatment; △, fractionate treatment; ●, acute late treatment.

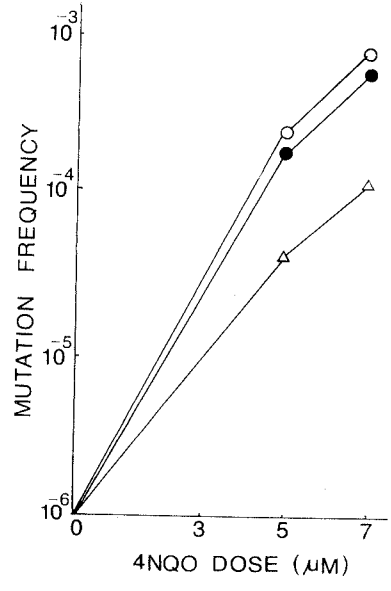

Fig. 7. The effect of 4NQO fractionate treatment on the mutation induction of diploid yeast xs 2167. ○, acute early treatment; △, fractionate treatment; ●, acute late treatment.

dicated that 4NQO was not able to induce mutution in xs 2162. It implies that the excision repair may be involved in the mechanism of mutation induction in diploid yeast xs 2162 by 4NQO treatment.

Discussion

Four pathways or systems which are associated with repair of lesions induced by UV radiation have been identified in the yeast *S. cerevisiae.* The mutant we used in this study, radl, belongs to the *RAD3* epistastis group. It is known that this *RAD3* pathway is responsible for excision of thymine dimers. We use these mutants for study the relationship between excision repair and the fractionation effect of UV and it's mimetic mutagen, 4NQO, on diploid yeast cells.

The total dose of UV on acute and fractionate treatments is the same. From the results of Figs. 2 and 6, we know that there is no increase in survival and decrease of mutation induction caused by fractionate treatment in excision deficient strain. We believe that the fractionate effect of UV in survival and mutation induction (Figs. 1 and 5) is caused by excision repair existed in excision-proficient strain. Same results and conclusion were made by us in our previous study (Dai *et al.*, 1983).

Harm (1968) reported that at a very low dose rate irradiation of UV on *E. coli* the survival increased extensively compared to the single exposure at high dose rate. He concluded that the increasing of survival at low dose rate is attributed to repair, since the effect of low dose rate is small in repair deficient strains of *E. coli.* He proposed that at low dose rate irradiation, the number of lesions present at any time remains relatively low compared to acute treatment. During single dose irradiation, the interference in repair possibly caused overlapping of repair regions in complementary DNA strands. Tang and Patrick (1977) concluded that liquid holding recovery and fluence rate-depend recover took place in excision resynthesis repair proficient but not excision resynthesis repair deficient strains of *E. coli.* They thought that the excision of cyclobutyl dipyrimidine occurred more extensively in low fluence rate condition. Tang *et al.* (1977) found that continuous DNA degradation and resynthesis were taken place in buffer-held, non-irradiated *E. coli* B/r. They suggested that cell survival depends on the delicated balance between DNA turn over and repair of UV-damage. At low dose-rate irradiation, dimers are formed randomly at a low dose rate, more DNA turn over synthesis than after higher fluence-rate irradiation followed by an equivalent liquid holding time. Parry and Parry (1976) found that yeast cells which have been irradiated and held in non-growth conditions were much more resistant to further UV-irradiation. In the split dose treatment of *S. cerevisiae,* Ferguson and Cox (1980) believed that the resistance caused by split dose was due to the relief of dimer interference and the increased efficiency of excision. In our case, we notice that the low dose-rate effect is also much higher than the liquid holding effect. Since the fractionate effect is absent in excision-defective mutant, we may conclude that the enhanced resistant of wild type cells subject to the split dose treatment is dependent on the more efficient excision repair under the nongrowth condition. The excision deficient strain is unable to relief dimer interference along fractionate treatment, so no increase of cell survival when compared to acute dose treated cell.

Our results of Figs. 3 and 7 indicate that fractionate treatment of 4NQO on excision-proficient strain of diploid yeast results in increase of survival and decrease of mutation frequency. The time course study of 4NQO on the same strain (unpublished data) exhibited that the activation of 4NQO on diploid yeast cells was pursued in a quite short period, the reaction was stopped within 1 h by 4NQO being used up or being degraded or by the maximum of the reaction between 4NQO and cells being reached. The

possibility of 4NQO degradation after 1 h treatment could be excluded. The acute early treatment and acute late treatment of 4NQO show similar survival (Fig. 3) and mutation induction (Fig. 7). We believe that the exact reactive dose of 4NQO on acute and fractionate treatment is the same on this wild type yeast cells.

Xeroderma pigmentosum cells which were defective in excision repair showed a reduced level of DNA repair synthesis following a single 4NQO dose treatment and revealed an increased sensitivity to the lethal (Stich and San, 1970). This result suggestes that the excision repair plays an important role in 4NQO damaged cells. Our previous results on *E. coli* showed that the fractionate effect of 4NQO treatment was due to the excision repair of the bacteria (Dai *et al.*, 1983).

Figures 3 and 7 in this paper exhibit that the fractionate treatment of 4NQO on excision-proficient yeast attributes the higher survival and lower mutation frequency. Same results can not be observed in excision-deficient strain (Figs. 2 and 6). We may conclude that the excision repair is responsible for the fractionate effect of survival and mutation induction in 4NQO treated excision-proficient diploid yeast cells. Unfortunately, we could not detect the mutation induction in excision-minus strain after 4NQO treatment to make our conclusion more concrete. Even 4NQO is UV mimetic mutagen, it could not induce mutation on xs 2162 as UV did. Since mutant radl is a weak mutator, it is very interesting if we may study some other excision-minus mutants by 4NQO treatment.

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紫外線和 4NQO 之低劑量率處理對酵母菌之影響

戴　　華

中央研究院植物研究所

以低線量率之 紫外線和 4NQO (4-nitroquinoline 1-oxide) 來處理野生型酵母菌株和以單次高線量率來處理相同菌株作比較，發覺前者可增加細胞之存活率並減少其突變頻率。此影響是因在低線量率處理情況下，細胞行使較高效率之剪除修補 (excision repair)。是以在剪除修補欠損株中無相同低線量率影響發生。