Effects of 2-Deoxy-D-glucose on DNA Repair and Mutagenesis in UV-irradiated Yeast

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We have studied the effects of 2-deoxy-D-Glucose (2-DG) on the recovery of potentially lethal damage (PLDR), repair of chromosomal DNA, cyclobutane pyrimidine dimers (CPDs), reverse mutation and gene-conversion in UVC (254 nm) irradiated yeast. As studied by pulsed-field gel electrophoresis, post-irradiation chromosomal DNA repair kinetics in a phosphate buffer (PB) with 10 mM glucose (G) was biphasic, where the first phase exhibited a decrease and the second phase showed an increase in the band intensities. A post-irradiation treatment in PB+G (10 mM) with 2-DG (10, 20, 50 mM) reduced the decrease in the DNA band intensities in the first phase of DNA repair. As compared to a post-irradiation (125 J/m²) treatment in PB+G (10 mM), a treatment in PB+G (10 mM) + 2-DG (10 mM) showed a decreased PLDR, but increased revertants and gene-convertants.

INTRODUCTION

Woodward in 1952 and subsequent studies1) reported that 2-deoxy-D-glucose (2-DG) is a glucose antimetabolite which inhibits the glycolytic pathway and decreases the intracellular ATP, GTP and UTP pool2). 2-DG has also been shown to reduce DNA repair and the recovery of potentially lethal damage (PLDR), induced by ionizing as well as non-ionizing radiation in respiratory deficient mutants of yeast3) and Ehrlich ascites tumor cells4). A decreased energy supply in the presence of 2-DG is considered to be the major reason for reduced DNA repair because ATP is essential for the activity of several DNA repair enzymes5). Currently, clinical trials are in progress for expediting the use of 2-DG as an adjuvant to tumor-radiotherapy6). Such trials are based on the hypothesis that 2-DG reduces post-irradiation repair in tumor cells to a greater extent than that of normal cells, because tumor cells are more dependent on the

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glycolytic pathway.

2-DG has been shown to reduce PLDR in respiratory proficient yeast cells\(^7-9\)), which also generate ATP through TCA, similar to normal mammalian cells. Further, because 2-DG is a non-specific metabolic energy inhibitor, both error-free and error-prone repairs, being energy dependent, are affected. Our studies showed that a post-irradiation treatment with 2-DG increased the reverse mutation and gene conversion in UVC\(^7\), X-irradiated\(^8\) and UVA+Psoralen treated\(^9\) yeast. The proposed use of 2-DG in clinics calls for further investigations on mutagenic and recombinogenic effects of 2-DG. Since cyclobutane pyrimidine dimers (CPDs) are among the major DNA lesions produced by UVC (254 nm), and CPDs also play an important role in cytotoxicity and mutagenecity\(^10,11\)), it was considered worth while to examine the effects of 2-DG on CPDs’ repair. Respiratory proficient yeast, \textit{Saccharomyces cerevisiae}, was used as a model system. \textit{Saccharomyces cerevisiae} is an eukaryote, and a number of its DNA repair enzymes, genes and transcripts are involved in the dark repair of UV-induced damage, and also participate in ionizing radiation-induced DNA repair\(^12\). Since homologies exist between the DNA repair genes of yeast and humans\(^13\), this study may have some implications towards evaluating the clinical use of 2-DG.

**MATERIALS AND METHODS**

**Organism and growth media**

Stationary-phase diploid strain D7 of \textit{Saccharomyces cerevisiae}\(^14\)} (genotype: a/α trp5–12/trp5-27, ilv1-92/ilv1-92), grown on a yeast extract peptone dextrose (YPD) agar medium (Himedia), was used. Yeast extract nitrogen base without amino acids, dextrose and bacto-agar were obtained from Difco; all amino acids of biochemical grade (99% purity) were from Sisco Res. Labs, India; β-Mercaptoethanol and Proteinase K were from E. Merck; RNase was from Boehringer Mannheim; GELase and T4 endonucleaseV were from Epicentre Technologies, U.S.A. All other chemicals and enzymes were obtained from Sigma Chemicals U.S.A.

**Irradiation and post-irradiation treatment**

A cell suspension (10\(^8\) cells/ml) in a phosphate buffer (PB) (pH 6.0, 67 mM, at 4°C) was irradiated under stirring with ultraviolet light 254 nm (UVC) from a germicidal tube (15 W, Philips). The fluence was 2.09 J/m\(^2\)/sec (photometer, model no. IL1400A, International Light inc. U.S.A.) and the dose was varied by altering the exposure time. A post-irradiation treatments were given by incubating the irradiated cells at 30°C for different time intervals in PB containing 10 mM glucose (PBG) with or without 2-DG (10, 20, 50 mM).

**Estimation of PLDR, recombinogenesis, mutagenesis, DNA damage and repair**

Survivors, gene convertants and revertants were scored using a defined synthetic complete medium, a tryptophan omission medium and an isoleucine omission medium respectively\(^14\)). The difference between surviving fractions obtained post-irradiation treatment and that obtained immediately after UVC exposure, was the estimated PLDR.
To assess DNA damage and repair, samples were prepared as described. Briefly, cells were treated with lyticase and immobilized in low-melting agarose plugs. The plugs were treated with LET buffer [0.5 M EDTA pH 8.0, 0.01 M Tris(hydroxymethyl)-aminomethane pH 7.0, 7.5% β-Mercaptoethanol] for 16 h, 37°C followed by NDS buffer [0.01 M Tris(hydroxymethyl)-aminomethane pH 7.0, 0.5 M EDTA pH 8.0, 1% n-lauryl sarcosine, 2 mg/ml ProteinaseK] for 20 h, 50°C. Sufficient washings were given in EDTA (0.5 M, pH 8.0) before electrophoresis. The treated plugs were pulsed-field gel electrophoresed (PFGE) for 20 h (60 sec pulse for first 13 h and 90 sec pulse for next 7 h) at 200 V, using CHEF DRII (BioRad, USA), to resolve genomic DNA into a number of chromosomal bands. To study the effect of a T4 endonucleaseV treatment, DNA was first extracted from LET- and NDS-buffer treated agarose plugs using GELase enzyme. The extracted large molecular mass DNA was divided in two parts; one was treated with T4 endonucleaseV, and the other served as a control (the procedures for GELase and T4 endonucleaseV treatments were as recommended by supplier). Samples were subjected to PFGE (CHEF-DRII, BioRad, USA) for 3 h at 150 V, 1–12 sec ramp, to differentially resolve small-size fragments (<100 kb) (Application Manual, 1990, BioRad) from larger chromosomal-size DNA (smallest chromosome being 250 kb).

Gels were stained with ethidium bromide for 0.5 h at 28°C and destained with RNAse for 3 h at 37°C. Densitometry was performed using Diversity Database software (BioRad, USA). Since DNA plug pieces for gel loading were cut by approximation, a well-to-well variation in the amount of DNA was expected. To normalize this variation, the intensity ratio (ρₙ) of a band was calculated as ρₙ = Iₙ/Iₚ, where Iₙ is the fluorescence intensity of nth band in a lane, Iₚ is sum of the fluorescence intensities of all bands and the well in the lane and n = 1 to 169. The area under an individual peak obtained by densitometry represented the fluorescence intensity (I) of that band. For the sake of clarity, inverted gel images are presented in the text.

All experiments were repeated at least three times, each with three replicates. The data was subjected to the standard methods of statistical analysis.

RESULTS

Effect of 2-DG on PLDR, recombinogenesis and mutagenesis

Table 1 shows that the post-irradiation treatment in PBG for 24 h enhanced PLDR. The presence of 2-DG (10 mM) inhibited PLDR partially at all UVC doses, as compared to the samples treated in PBG alone; the effect was most pronounced at 125 J/m². A post-irradiation (125 J/m²) treatment in PBG with 2-DG (10 mM) caused a 2.8-times increase in the revertants and a 1.6-times increase in the convertants as compared to a post-irradiation treatment in PBG alone.

Effect of 2-DG on chromosomal DNA bands

Figure. 1 shows that chromosomal DNA from untreated cells could be resolved into 16 bands. At 0 h, after UVC exposure (125 J/m²), no observable effect was recorded on the fluo-
Fig. 1a. Chromosomal profile of diploid strain D7 of Saccharomyces cerevisiae. [Unirradiated, lane 1; UVC irradiated and processed at 0 h, lane 2; processed after incubation in PBG for 20 min, lane 3; 30 min, lane 4; 40 min, lane 5; 50 min, lane 6; 60 min, lane 7; 5 h, lane 8; 24 h, lane 9; chromosomal marker, lane 10]. Only well-resolved bands viz. Nos. 5, 10, 11 and 12 were studied in detail.

Table 1. Post-irradiation modulation of surviving fraction, mutagenesis and recombinogenesis by 2-DG in diploid strain D7 of Saccharomyces cerevisiae exposed to UVC; values are mean of three separate experiments ± standard deviation. (Spontaneous frequency per 10^6 survivors for convertants and revertants is 13 ± 1 and 0.6 ± 0.1 respectively)

<table>
<thead>
<tr>
<th>UVC Fluence (J/m²)</th>
<th>Post-irradiation treatment</th>
<th>Surviving fraction (%)</th>
<th>Revertants/10^6 survivors</th>
<th>Convertants/10^6 survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>time (h)</td>
<td>medium, PB + G (mM)</td>
<td>2-DG (mM)</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>0</td>
<td>0</td>
<td>59 ± 3</td>
<td>49 ± 5</td>
</tr>
<tr>
<td>24</td>
<td>10</td>
<td>0</td>
<td>88 ± 4*</td>
<td>35 ± 5*</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>0</td>
<td>43 ± 4</td>
<td>112 ± 10</td>
</tr>
<tr>
<td>24</td>
<td>10</td>
<td>0</td>
<td>83 ± 5</td>
<td>33 ± 6</td>
</tr>
<tr>
<td>24</td>
<td>10</td>
<td>10</td>
<td>69 ± 4*</td>
<td>73 ± 8*</td>
</tr>
<tr>
<td>125</td>
<td>0</td>
<td>0</td>
<td>22 ± 6</td>
<td>242 ± 14</td>
</tr>
<tr>
<td>24</td>
<td>10</td>
<td>0</td>
<td>72 ± 3</td>
<td>50 ± 7</td>
</tr>
<tr>
<td>24</td>
<td>10</td>
<td>10</td>
<td>51 ± 3*</td>
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<td>150</td>
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<td>0</td>
<td>4 ± 1</td>
<td>400 ± 30</td>
</tr>
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<td>10</td>
<td>0</td>
<td>10 ± 2</td>
<td>300 ± 20</td>
</tr>
<tr>
<td>24</td>
<td>10</td>
<td>10</td>
<td>7 ± 1</td>
<td>350 ± 40</td>
</tr>
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</table>

PB: phosphate buffer; 2-DG: 2-deoxy-D-glucose; G: glucose

* p < 0.05, Student’s t-test (significant change as compared to samples treated in PBG alone)
Fig. 1b. The densitograph showing intensity alterations of chromosomal bands in untreated controls (a), in samples exposed to UVC and processed at 0 h (b), processed after incubation in PBG for 40 min (c), 50 min (d), 60 min (e), 24 h (f).

Fluorescence intensity and mobility of the DNA bands, suggesting the absence of strand breaks. A post-irradiation treatment in PBG caused biphasic changes in the fluorescence intensities of the chromosomal bands. In the first phase, the fluorescence intensities of several bands decreased, and the effects were appreciable after 40–50 min. The decrease in the fluorescence intensity differed from band to band. In the second phase, the fluorescence intensities of the chromosomal bands increased, and continued up to 24 h. For the reliability of the results, only
well-resolved bands (5, 10, 11, 12) were selected for detailed studies.

A post-irradiation treatment in PBG+2-DG (Fig. 2) caused a smaller decrease in $\rho_{5,10,11,12}$ during the first phase, as compared to PBG, and the effects were 2-DG concentration dependent. The quantity and time kinetics of $\rho_n$ alterations were different for every chromosomal band.

Fig. 2. Quantitative effects of 2-DG, on the relative fluorescence intensity of selected DNA bands as a function of time. [Unirradiated control □; Irradiated with UVC and treated with 2DG/G = 0 □; 2-DG/G = 1 □; 2-DG/G = 2 □; 2-DG/G = 5 □].

Effects of 2-DG on repair of CPDs

Figure. 3 shows the differences among samples before and after a T4 endonucleaseV
Fig. 3. DNA fragments separated before (lane 1–6) and after a T4 endonucleaseV treatment (lane 7–12). [Untreated control (lane 1, 7); irradiated with UVC and processed at 0 h (Lane 2, 8); processed after 40 min incubation in PBG without 2-DG (lane 3, 9); with 2-DG, 10 mM (lane 4, 12), 20 mM (lane 5, 11); 50 mM (lane 6, 10)].

treatment. Prominent variations were observed in the fluorescence intensity of the last band (henceforth addressed as band \( \alpha \)). This band had a diffused nature (size \( \leq 100 \) kb, as judged by a \( \lambda \) ladder; not shown in the picture). Before T4 endonucleaseV treatment (lane 1–6), the intensity of band a was very faint in untreated control (lane 1), and in samples subjected to a post-irradiation treatment in PBG+2-DG (50 mM) (lane 6). While the intensity of band \( \alpha \) after a T4 endonucleaseV treatment did not change conspicuously in untreated controls (lane 7), it increased the most in samples treated with T4 endonucleaseV immediately after UVC exposure (lane 8). In all samples subjected to a post-irradiation treatment in PBG+2-DG (lane 10–12), the band \( \alpha \) intensity increased after a T4 endonucleaseV treatment; the increase was 2-DG concentration dependent.

DISCUSSION

UVC dose-dependent increases in the cytotoxicity, recombinogenicity and mutagenicity (Table 1) corroborate earlier studies\(^7\).\(^{14}\). An increased PLDR, accompanied by decreased revertants and gene-convertants following a post-irradiation (75–125 J/m\(^2\)) treatment with PBG (Table 1), suggested that the repair was largely error-free. At a higher UVC dose (150 J/ m\(^2\)), very high lethality (96%, Table 1) due to excessive genomic damage could be the reason for small effects on the PLDR, recombinogenicity and mutagenicity. A lower surviving fraction and a higher number of gene-convertants and revertants following a post-irradiation (75–
125 J/m²) treatment in PBG+2-DG, as compared to PBG, indicated decreased PLDR, but increased error-prone repair.

Since alterations in the ethidium bromide fluorescence in UVC-irradiated samples could be observed better in chromosomal bands, as compared to total DNA (personal communication), the genomic DNA was resolved into a number of bands (Fig. 1). Because of a poor resolution of band Nos. 1–4, 6–10 and a considerable overlap of smear on band Nos. 13–16, the intensity ratios (ρ) were only calculated for band Nos. 5, 10–12. The biphasic nature of ρ alterations during a post-irradiation treatment in PBG for 24 h (Fig. 1, 2) indicated the predominance of different processes in each phase. While a decrease in ρ during the first phase (Fig. 1, lane 3–6) suggested decreased DNA contents per chromosome and/or an unwinding of the DNA helix, an increased ρ during the second phase (lane 7–9) could be due to increased DNA and/or a refolding of the repaired stretches. Because initial steps of UVC-induced repair involve unwinding of DNA helix near to the site of a lesion to allow the accessibility of repair enzymes and the removal of CPDs bearing oligonucleotides, it is suggested that a decreased ρ for approx. 1 h after irradiation (Fig. 2) represented the first phase of UVC-induced repair. Decreased DNA contents per chromosome and the appearance of fragments during a post-irradiation treatment in PBG is known. An increase in ρ from 5 to 24 h (Fig. 2) represented the second phase of DNA repair, and suggested the preponderance of some processes i.e. polymerization, ligation, DNA helix refolding. This observation corroborates well with the increased PLDR observed during a 24 h post-irradiation treatment in PBG (Table 1).

A smaller decrease in r following a post-irradiation treatment with 2-DG, as compared to PBG alone (Fig. 2), during the first few hours has also been reported with X-irradiated and UVA+Psoralen-treated yeast. A reduced decrease in ρ following a post-irradiation treatment with 2-DG could be due to either (i) very fast repair or (ii) an inhibition of the first phase of repair. To resolve this, DNA from plugs of all samples was treated with T4 endonucleaseV, an enzyme that specifically creates nicks in regions having CPDs. Since two very closely placed nicks on opposite strands are likely to result in double strand breaks, attempts were made to resolve any fragmented DNA molecules of size <100 kb (band α, Fig 3). A 45 min post-irradiation treatment time was selected because an approx. 45 min treatment in PBG maximally decreased ρ in most bands (Fig. 2). Since a T4 endonucleaseV treatment did not increase the intensity of band α in unirradiated controls (Fig. 3), but enhanced it observably in irradiated samples processed at 0 h, the intensity of band α was treated as an index of the CPDs number. There was no observable increase in the band α intensity after a T4 endonucleaseV treatment in samples treated with PBG (Fig 3, lane 9), which suggested the absence of appreciable numbers of CPDs, which in turn indicated the removal of CPDs during the post-irradiation treatment. Similarly, the increased band α intensities after a T4 endonucleaseV treatment in PBG+2-DG treated samples suggested the presence of an appreciable number of CPDs due to incomplete CPDs removal. These remaining CPDs in the 2-DG treated samples could be the cause of the reduced decrease in ρ following a post-irradiation treatment with PBG+2-DG (Fig. 2), and not the faster repair. This assumption was further supported because both an increase in the band α intensities (Fig 3, lane 10–12) and a
reduction in the decrease in $\rho_{5,10,11,12}$ (Fig. 2) were 2-DG concentration dependent. This indicated that a treatment with 2-DG decreased the first phase of DNA repair. The decreased CPDs’ removal in the presence of 2-DG explains the observed reduction in PLDR and the increase in mutagenesis (Table 1), because CPDs cause cytotoxicity and mutagenicity in murine fibroblasts and normal human cells$^{10}$ and tumorigenicity in hairless mice$^{11}$. Mutagenesis at the ILV locus was expected because genes of ilv1 are known to be reverted by a variety of allele-specific or locus-specific suppressors$^{17}$, and UVC can induce a spectrum of mutations, which may be GC-AT transitions as well as multiple tandem and non-tandem base substitutions$^{10}$.

At the molecular level, the DNA conformational alterations mediated by a set of topoisomerases are some of the initial and essential steps of DNA repair. Because Topoisomerase II is strictly ATP dependent in yeast$^{18}$, it is likely that its activity was adversely affected in the presence of 2-DG. This, however, needs a further confirmation. Other ATP-requiring DNA repair enzymes (topoisomerases I, III, exonucleases$^{19}$) and important gene products (rad1p, rad10p, rad3p, rad25p which participate in DNA repair$^{20}$) could also be adversely affected in the presence of 2-DG. Decreased ATP contents in the respira-

tory-proficient diploid strain D7 of yeast in the presence of 2-DG have been recorded by in

vivo magnetic resonance spectroscopy$^{21}$.

A number of ATP-dependent UV repair enzymes and important gene products of yeast

viz. rad1p, rad10p, rad3p, rad25p, bearing homologies with human DNA repair genes and pro-

teins$^{12,13}$, are also involved in the repair of lesions induced by ionizing radiation$^{22}$. Even

though subtle differences exist between the physiology and proliferation behavior of respira-

tory-proficient yeast and normal human cells, 2-DG has been reported to decrease the energy

phosphates (ATP, GTP and UTP) in both yeast$^{2,21}$ and human cells$^{23}$. In view of proposed

clinical applications of 2-DG, it is suggested that similar studies should be performed in nor-

mal mammalian cells before recommending the use of 2-DG as an adjuvant to radiotherapy.

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