Degradation of DNA and Structure-Activity Relationship between Bleomycins A_2 and B_2 in the Absence of DNA Repair†

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ABSTRACT: The contribution of DNA repair to the net number of DNA breaks produced during chemical degradation of DNA was determined by using temperature-sensitive mutant cells deficient in ATP-dependent DNA ligase [poly(deoxyribonucleotide):poly(deoxyribonucleotide) ligase, EC 6.5.1.1]. In a very sensitive assay for determining lesions introduced into *Saccharomyces cerevisiae* DNAs, 2-^{14}C- and 6-^{3}H-prelabeled DNAs from ligase-proficient and ligase-deficient cells were sedimented together through precalibrated, isokinetic alkaline sucrose gradients. DNA ligation was slower after chemical degradation of DNA by bleomycin than after γ irradiation. DNA breaks increased approximately linearly with drug concentrations, and were approximately equivalent for ligase-proficient and ligase-deficient cells. These results were unexpected because ligase-deficient, but not ligase-proficient, cells lacked the capacity to eliminate DNA breaks produced by bleomycin. The results indicated that DNA repair did not occur during the chemical degradation of DNA under the experimental conditions. Bleomycin B_2 produced considerably more DNA breaks than bleomycin A_2 over a range of concentrations in ligase-proficient cells, which tolerated higher numbers of DNA breaks in general than ligase-deficient cells. The chemical analogues are structurally identical except for their cationic C-terminal amine. The actual number of DNA breaks produced by bleomycin A_2 or bleomycin B_2, and not the concentration of bleomycin A_2 or bleomycin B_2 per se, determined the amount of cell killing. DNA repair is critical in quantitating DNA breaks produced by chemicals, but was ruled out as a factor in the higher DNA breakage by bleomycin B_2 than bleomycin A_2.

The unique chemical action of the low molecular weight family of glycopeptidic bleomycins (M_r ~1500) (Suzuki et al., 1969; Haidle et al., 1972; Kuo & Haidle, 1973; Kuo et al., 1973; Takeshita & Grollman, 1979; Burger et al., 1980; Giloni et al., 1981; Murugesan et al., 1985; Sugiyama, 1985; Mirabelli et al., 1982a,b; Wu et al., 1983) produces both single- and double-strand breaks [e.g., see Suzuki et al. (1969), Carter et al. (1978), Hecht (1979), Moore and Vossler (1980), Mirabelli et al. (1985), Keller and Oppenheimer (1987), Berry et al. (1985), Moore and Little (1985), Moore (1988a,b), Iqbal et al. (1976), Kohn and Ewig (1976), Yamamoto and Hutchison (1981), Murray and Martin (1985), and Grimwade and Beerman (1986)] and 5′-phosphate and 3′-phosphoglycolate termini in DNA molecules (D'Andrea & Haseltine, 1978; Takeshita et al., 1978; Grollman & Takeshita, 1980; Giloni et al., 1981; Murugesan et al., 1985; Sugiyama, 1985). Bleomycins recognize 5′-phosphoguanylyl(3′-5′)thymidine or 5′-phosphoguanylyl(3′-5′)cytosine sequences most frequently, releasing the pyrimidines when they are located to the 3′ side of guanosine (D'Andrea & Haseltine, 1978; Takeshita et al., 1978; Takeshita & Grollman, 1979; Grollman & Takeshita, 1980; Mirabelli et al., 1982a,b) and leaving DNA alkali-labile (Wu et al., 1983; Sugiyama et al., 1985). Deoxyribose degradation also produces 3-(pyrimidin-1-yl)-2-propenal and 3-(purin-9-yl)-2-propenal (Burger et al., 1980; Giloni et al., 1981; Murugesan et al., 1985) and 3′-(phosphoro-2-*O*-glycolic acid)-oligonucleotide derivatives (Giloni et al., 1981; Uesugi et al., 1984; Murugesan et al., 1985). DNA strand breaks are stoichiometric with the production of base propenals (Burger et al., 1982). After enzymatic removal of the unusual phosphoglycolate and extension of the remaining DNA strand by one nucleotide, ATP-dependent DNA ligase [poly(deoxyribonucleotide):poly(deoxyribonucleotide) ligase, EC 6.5.1.1] is required for resynthesis of a phosphodiester bond between adjacent 3′-hydroxyl and 5′-phosphoryl termini and chemical reestablishment of full-sized DNA molecules (Moore, 1988a).

In previous studies designed to determine factors critical in the chemical action of the bleomycin–phleomycin group of glycopeptides on cells, phleomycins were dramatically more active than structurally related bleomycins in producing breaks in DNA molecules in *Saccharomyces cerevisiae* (Moore, 1982a, 1988b). Clinical preparations of bleomycin and phleomycin were chosen for the previous studies because of their clinical relevance. A major structural difference between anticancer bleomycins and phleomycins is that bleomycins contain a coplanar bithiazole (Figure 1) in place of thiazoline and thiazole in phleomycins. In addition, the C-terminal amines of bleomycins differ both chemically and quantitatively from phleomycins. A strict structure–activity relationship could not be established in the previous studies without conducting a comparative analysis of phleomycin and bleomycin with identical C-terminal amines.

In the current study, we have investigated activities of purified bleomycin A_2 and purified bleomycin B_2 on DNA in *S. cerevisiae*. These chemical analogues are structurally identical except for their cationic C-terminal amine (Figure 1). The

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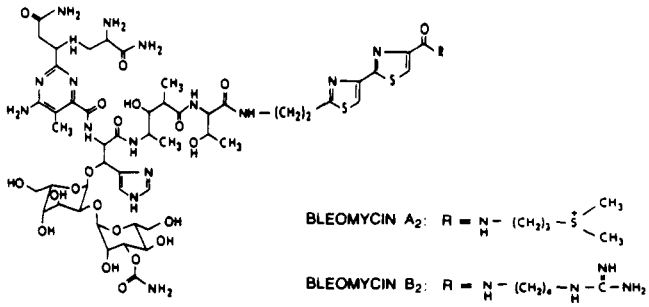

FIGURE 1: Chemical structures of bleomycin A_2 and bleomycin B_2 (Takita et al., 1978; Umezawa, 1976; Naganawa et al., 1977).

C-terminal amine moieties of both congeners bear one positive charge and are similar in length. We first sought to determine the role of the chemical difference in DNA cleavage and if the structural difference in the C-terminal amine might cause a difference in the number of DNA breaks produced. The average molecular weight of yeast chromosomes is approximately 3×10^8, and this DNA size is ideal for velocity sedimentation through sucrose, particularly at low centrifugal speeds. This assay, moreover, is quite sensitive and accurate, particularly under limited reaction conditions. In conducting this investigation, we have considered some of the important factors affecting quantitation of DNA breaks by chemicals, and how such factors could influence structure–activity relationships of chemicals. For example, DNA degradation and repair take place simultaneously as a dynamic process in cells proficient in mechanisms to rapair damaged DNA, and it is usually impossible to determine the fraction of DNA breaks which are actually lost during repair of DNA. DNA repair in this context implies that the continuity of DNA molecules becomes reestablished, but does not reflect the fidelity of the mechanism. The current investigation measures the contribution of DNA repair to the net number of DNA breaks produced during a finite period of chemical degradation of DNA by employing temperature-sensitive mutant cells deficient in ATP-dependent DNA ligase (Hartwell, 1967, 1976; Hartwell et al., 1973; Barker & Johnston, 1983; Barker et al., 1985).

Experimental Procedures

Strains and Culturing Conditions. Strain CM-1293 [ligase-proficient; constructed in this laboratory, as previously described (Moore & Schmick, 1979; Moore, 1988b)] and strains A364A (ligase-proficient, *CDC9*) and H288 (ligase-deficient, *cdc9-9*) (Hartwell, 1967, 1976; Hartwell et al., 1973; Johnston & Nasmyth, 1978; Moore, 1982b,d; Barker & Johnston, 1983) were routinely grown with aeration in nonsynthetic complete medium at 23 °C (YPAD; Moore, 1982a,b) and harvested by centrifugation at 4 °C in a Dupont Sorvall RC-5B SS34 rotor at 2500*g*.

Purification of Bleomycin A_2 and Bleomycin B_2. Bleomycin A_2 and bleomycin B_2 were purified by adaption of the fractionation method previously described by Sakai and Riordan (1979). Briefly, Blenoxane [approximately 55–70% (usually 68–69%) bleomycin A_2 and approximately 25–32% bleomycin B_2 (Crooke & Bradner, 1976; Dr. William T. Bradner, personal communication)] was eluted on a column (30 × 2.5 cm; flow rate was approximately 2 mL/min) of CM-Sephadex C-25 (Pharmacia) using a linear gradient (0.02–0.80 M, 1 L total) of ammonium formate (pH 6.5). Elution was monitored at 292 nm using a variable-wavelength detector (Varian Instruments).

Fractions were collected and lyophilized until residual ammonium formate and H_2O were removed. Fractions were then resolubilized in a minimal amount of distilled H_2O (1–3 mL) and desalted on a Sephadex G-10 (Pharmacia) column (88.5 × 1.2 cm). Elution was at approximately 15 mL/h.

2-^{14}C- and 6-^{3}H-Prelabeled DNAs. Cells were grown from starting inocula of fresh cells (5×10^6 cells/mL) in supplemented synthetic minimal medium (Petes & Fangman, 1972; Forte & Fangman, 1976; Moore & Vossler, 1980; Moore, 1982a,b, 1988a,b) to which [6-^{3}H]uracil (specific activity, 20–30 Ci/mmol; New England Nuclear Corp., Boston, MA) was added to 7 μCi/mL or [2-^{14}C]uracil (specific activity, 40–60 Ci/mmol; New England Nuclear Corp.) was added to 5 μCi/mL. Cells were harvested by centrifugation at 4 °C in an RC-5B Sorvall SS34 rotor at 3000*g*, washed once in deionized water, chased in supplemented synthetic minimal medium (containing 0.0017% uracil) without radiochemical for 60–90 min, and washed twice with deionized water. RNA was hydrolyzed completely as described below.

Equivalent numbers of ligase-proficient and ligase-deficient cells were mixed and suspended prior to incubation with bleomycin, and all subsequent experimental procedures were carried out on the mixed suspensions. The two radioisotopes were alternated in replicated experiments between ligase-proficient and ligase-deficient cells, with no detectable effect on sedimentation profiles or molecular weights of DNAs. DNA sedimentation profiles and molecular weights for strains CM-1293, A364A, and H288 are indistinguishable (Moore, 1982b, 1988a,b). In addition, no differences were observed throughout this study between profiles or molecular weights of 2-^{14}C- or 6-^{3}H-prelabeled DNAs from cells incubated with identical concentrations of bleomycin A_2 or between profiles or molecular weights of 2-^{14}C- or 6-^{3}H-prelabeled DNAs from cells incubated with identical concentrations of bleomycin B_2.

Reaction Conditions. Rigorous consistency was followed in culturing conditions and drug lots, as well as pH, cell density, and temperatures during reactions. Bleomycins were dissolved and diluted in deionized water (pH 5) just prior to use. Absorbance was monitored at 292 nM; 1×10^7 washed cells/mL of deionized water (pH 5) was incubated with bleomycin at 23 °C for 20 min with aeration. Neither metal ions nor reducing agents were added to reaction mixtures. Throughout this incubation, the pH of the reaction was 5. EDTA was added to 0.05 or 0.5 M at the end of 20 min when indicated. At the end of reactions, cells were immediately pelleted by centrifugation at 4 °C in an RC-5B Sorvall SS34 rotor at 3000*g*, washed twice at 4 °C with deionized water or 0.05 M EDTA, and converted to spheroplasts or plated as described previously (Moore, 1982b).

Sedimentation of DNA and Molecular Weight Determinations. The 2-^{14}C- and 6-^{3}H-prelabeled DNAs from ligase-proficient and ligase-deficient cells were sedimented together through precalibrated, isokinetic alkaline sucrose gradients (Moore, 1982b; Carrier & Setlow, 1971; Ehmann & Lett, 1973; Reynolds & Friedberg, 1980, 1981; Moore, 1988a,b). DNA from control cells (cells incubated without bleomycin) was included in each rotor. Additional sedimentation procedures were as described previously (Moore, 1982b).

The gradient system was precalibrated with phage markers to establish the relationship of sedimentation distance, time, and force to molecular weight. This relationship was used to compute weight-average molecular weights from the amount of radioactivity sedimenting in different positions throughout the gradient. With the use of a computer program, weight-average molecular weights (M_r) were calculated from distributions of radioactivity in gradients and related to number-average molecular weights (Ehmann & Lett, 1973; Res-

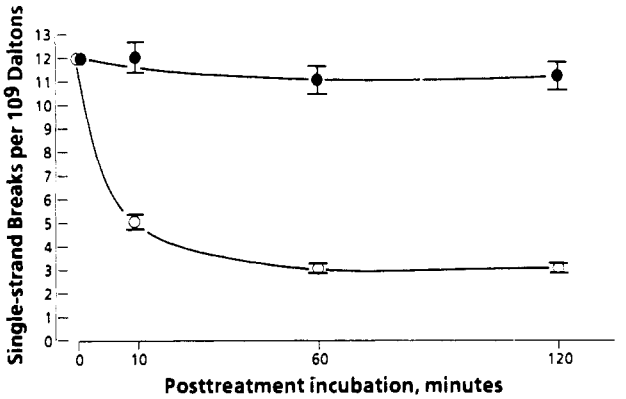

FIGURE 2: Comparisons of numbers of single-strand breaks in DNA from ligase-proficient (open circles) and ligase-deficient (closed circles) cells after posttreatment incubation in nonsynthetic complete medium containing 0.05 M EDTA. Cells were grown at 23 °C with aeration to early stationary phase, "chased" 90 min at 23 °C, and incubated (1×10^7 cells/mL) 20 min with bleomycin (6.7×10^{-7} M, 23 °C). The reaction was terminated by the addition of EDTA to 0.05 M. Posttreatment incubation was carried out at 23 °C. Error bars for each time point are standard errors of the means determined for three independent experiments.

nick & Martin, 1976; Reynolds & Friedberg, 1980, 1981; Moore, 1982b, 1988a,b). From the distributions of radiolabeled DNA under the established sedimentation conditions, the numbers of single-strand breaks relative to the untreated control were calculated as

$$10^8 \frac{M_r(\text{untreated})/M_r(\text{treated}) - 1}{M_r(\text{untreated})/2}$$

Alkali-labile lesions left in DNA by the release of free bases by bleomycins are converted to DNA breaks, and thus included in the DNA breaks measured in this assay system. In comparing ligase-proficient and ligase-deficient cells or bleomycin A_2 and bleomycin B_2, certain assumptions were made and are inherent in using this method to approximate numbers of DNA breaks. However, any error associated with the assumptions should be equivalent for ligase-proficient and ligase-deficient cells or for bleomycin A_2 and bleomycin B_2.

Results

Structure–activity relationships of chemicals on DNAs in living cells are very difficult to establish unless DNA repair is inhibited. Thus, we designed experimental conditions in which DNA repair should be minimized and then used mutant cells deficient in DNA ligase to test the extent to which DNA repair was eliminated under these experimental conditions.

Production of an Equivalent Number of DNA Breaks in Ligase-Proficient and Ligase-Deficient Cells and Removal of DNA Breaks during Posttreatment Incubation. Ligase-deficient (*cdc9*) *S. cerevisiae* was previously shown to lack the capacity to remove bleomycin-induced DNA breaks during posttreatment incubation in deionized water (37 °C), 0.05 M EDTA (37 °C), or medium containing 0.05 M EDTA (30 °C) (Moore, 1988a). For the current investigation, we wished to avoid elevated temperatures because low molecular weight "Okazaki fragments" accumulate in DNA synthesized at elevated temperatures (Johnston & Nasmyth, 1978) and because colony-forming ability rapidly decreases at restrictive temperatures [unpublished experiments; also see Johnston (1983)]. Thus, ligase-deicient cells were tested for their capacity to join DNA breaks at the permissive temperature of 23 °C by incubating the cells at 23 °C before, during, and after incubation with bleomycin.

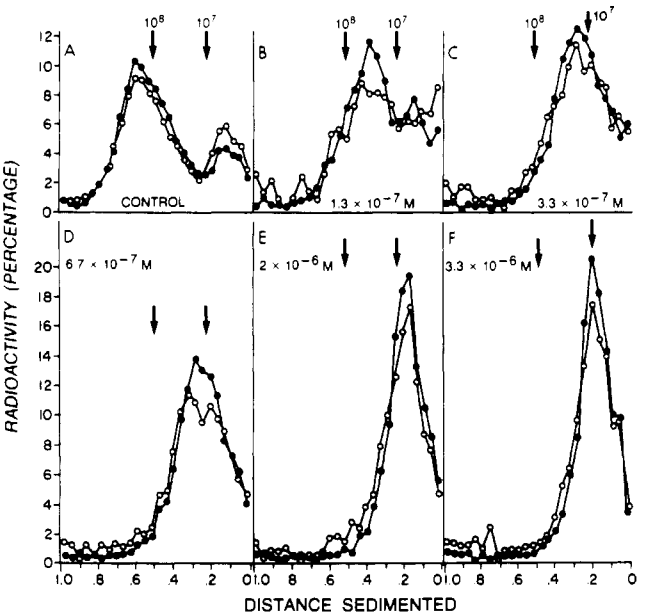

FIGURE 3: Typical sedimentation profiles of DNA from untreated (panel A) and treated (panels B–F) cells. Cells (1×10^7 cells/mL) were pelleted at the end of 20-min exposures and washed with cold deionized water without the addition of EDTA. Distributions of radioactivity were indistinguishable whether DNA from ligase-proficient *CDC9* (closed circles) and ligase-deficient *cdc9* (open circles) yeast was sedimented separately or together.

An equivalent number of DNA breaks were introduced into ligase-proficient and ligase-deficient cells in order to determine the capacities of the cells to eliminate a low number of DNA breaks. Cells were subsequently washed and incubated without bleomycin to allow for repair of DNA breaks. Figure 2 contrasts the removal of DNA breaks in ligase-proficient and ligase-deficient cells during the second incubation period. As Figure 2 illustrates, approximately 43% of the DNA breaks remained in ligase-proficient cells after 10-min posttreatment incubation, in contrast to 100% of the DNA breaks in the ligase-deficient cells. Approximately 26% of the original low number of DNA breaks remained in ligase-proficient cells after 1 or 2 h, in contrast to approximately 93% in ligase-deficient cells. Since the number of breaks in ligase-deficient cells did not differ significantly over the 2-h incubation (Figure 2), it can be concluded that the ligase-deficient cells completely or nearly completely lacked the capacity to eliminate DNA breaks even at the permissive temperature of 23 °C.

Net Numbers of DNA Breaks Produced in Ligase-Proficient and Ligase-Deficient Cells. We next determined the numbers of DNA breaks produced in ligase-proficient and ligase-deficient cells over a range of chemical concentrations under experimental conditions designed to eliminate DNA repair in ligase-proficient cells. The reaction period was limited to 20 min, and was not followed by posttreatment incubation. We expected that ligase-proficient cells would exhibit fewer DNA breaks than ligase-deficient cells if DNA molecules were repaired. On the other hand, if DNA molecules were not repaired concomitantly with chemical degradation by bleomycin, equivalent numbers of DNA breaks would be measured in ligase-proficient and ligase-deficient cells.

The distributions of radioactivity in sedimentation profiles indicated the sedimentation rates of DNAs from ligase-proficient and ligase-deficient cells were equivalent prior to DNA hydrolysis by the chemical action of bleomycin (Figure 3, panel A). For untreated *S. cerevisiae*, the 80–90% of DNA which is nuclear in origin sediments approximately 70–75% of the gradients in a major peak [ca. M_r (2–2.4) $\times 10^8$], while mi-

Table I: Mean Numbers of DNA Breaks (per 10^8 Daltons) Assayed in Replicate Experiments When an Equivalent Fraction of Logarithmic-Phase Cells Survived and Approximate Molarity (M) of Bleomycin A_2 or Bleomycin B_2 Estimated To Reduce Survival to This Fraction[a]

	surviving fraction							
	0.75		0.50		0.37		0.10	
	DNA breaks[b]	M	DNA breaks[b]	M	DNA breaks[b]	M	DNA breaks[b]	M
bleomycin A_2	1	1.2×10^{-6}	1.5	3.9×10^{-6}	2.2	5.3×10^{-6}	5.2	$12^c \times 10^{-6}$
bleomycin B_2	1	0.5×10^{-6}	1.5	1.7×10^{-6}	2.2	2.3×10^{-6}	5.2	4.7×10^{-6}

[a] DNA breaks and survival were measured simultaneously on the same cell populations in each of six independent experiments. [b] Per 10^8 daltons. [c] Estimated by extrapolation.

tochondrial DNA from untreated cells sediments in the top 25% in the minor peak under the sedimentation conditions routinely used (8000 rpm for 19.5 h).

As shown in Figure 3, bleomycin treatments [(1.3–33.3) × 10^{-7} M] resulted in dose-dependent reductions in sedimentation rates (panels B–F). Moreover, the distributions of radioactivity in sedimentation profiles indicated the sedimentation rates of DNAs from ligase-proficient cells were indistinguishable from sedimentation rates of DNAs from ligase-deficient cells. The numbers of single-strand breaks routinely calculated from distributions of radioactivity similar to those shown in profiles A–F (Figure 3) were equivalent for ligase-proficient and ligase-deficient cells and increased linearly with drug concentration.

DNA Breaks by Bleomycin A_2 and Bleomycin B_2. Distributions of radioactivity in sedimentation profiles of 2-^{14}C- and 6-^{3}H-prelabeled DNAs from ligase-proficient (CM-1293) cells incubated with low concentrations of purified bleomycin A_2 or purified bleomycin B_2 were used to determine if similar numbers of DNA breaks were produced by bleomycin A_2 and bleomycin B_2 over a range of concentrations. For each concentration, DNAs from cells incubated with bleomycin A_2 sedimented faster than DNAs from cells incubated with bleomycin B_2, indicating the higher molecular weight of DNAs from bleomycin A_2 treated cells than from bleomycin B_2 treated cells. EDTA was added to 0.5 M to terminate reactions and reduce posttreatment activities of bleomycins (Fugiwara & Kondo, 1973; Moore, 1982c; unpublished results), including the activity of a cytotoxic diffusible factor which is likely to be bleomycin A_2 or bleomycin B_2 or metabolic product(s) of the chemicals (Moore, 1982c). The addition of EDTA at this molarity significantly reduced numbers of DNA breaks produced by bleomycin A_2 and bleomycin B_2.

Numbers of DNA breaks produced by bleomycin A_2 and bleomycin B_2 in cells in the logarithmic phase of growth are presented in Figure 4. DNA breaks increased approximately linearly with increasing concentrations of bleomycin A_2 or bleomycin B_2, even at very low concentrations. Bleomycin B_2 produced more DNA breaks than bleomycin A_2. Bleomycin B_2 produced approximately 1 DNA break per 1×10^{-6} M, while bleomycin A_2 produced approximately 0.5 DNA break per 1×10^{-6} M.

DNA Breaks as a Function of Survival. We investigated how DNA breaks caused by bleomycin A_2 and bleomycin B_2 relate to lethal properties of these analogues. A major concern in the literature at the present time is whether DNA breaks relate to killing by structural congeners of bleomycin. We reasoned that if DNA breaks relate to killing by bleomycin A_2 and bleomycin B_2 in a quantitative way, then equivalent numbers of DNA breaks should cause approximately equivalent killing, irrespective of the congener. In Figure 5, DNA breaks produced by bleomycin A_2 and bleomycin B_2 are plotted against the fraction of cells which were not killed by bleomycin A_2 and bleomycin B_2. DNA breaks and survival were measured simultaneously on the same population of cells in each experiment. Numbers of DNA breaks were directly proportional to fractions of killed cells (Figure 5).

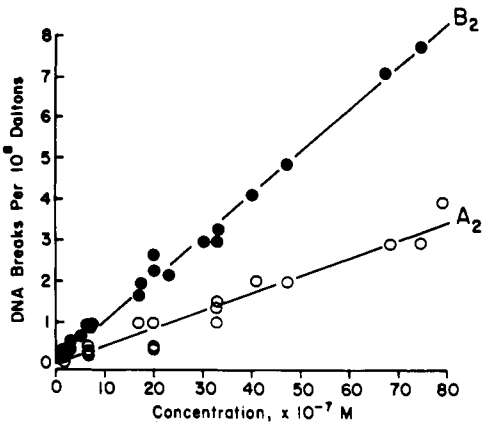

FIGURE 4: Numbers of DNA breaks produced by bleomycin A_2 (open circles) or bleomycin B_2 (closed circles) in logarithmic-phase cells in individual experiments. Cells were grown, harvested, washed, and incubated (20 min) as described for Figure 3, except that EDTA was added to 0.5 M at the end of treatment periods.

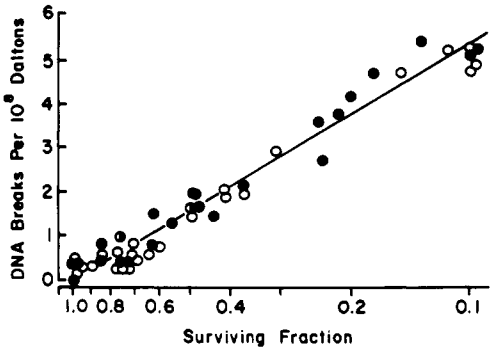

FIGURE 5: DNA breaks produced by bleomycin A_2 (open circles) and bleomycin B_2 (closed circles) in cells during the logarithmic phase of growth plotted as a function of the fraction of cells which survived.

Thus, approximately equivalent numbers of DNA breaks, whether produced by bleomycin A_2 or bleomycin B_2, killed approximately equivalent fractions of the cell population. Stated another way, more molecules of bleomycin A_2 than bleomycin B_2 should be required to produce equivalent numbers of DNA breaks and equivalent killing in cells. Table I summarizes the mean number of DNA breaks measured in cells in the logarithmic phase of growth when equivalent fractions of the cells were killed, along with the approximate, higher molarities of bleomycin A_2 than bleomycin B_2 which were required to produce the DNA breakage and killing.

Discussion

It has been possible to determine that DNA largely remains unrepaired during a finite period of chemical degradation of DNA in *S. cerevisiae*. In order to determine if the structural

difference in the C-terminal amine might cause a difference in the number of DNA breaks produced by bleomycin A_2 and bleomycin B_2, it was important to measure DNA breaks under experimental conditions where DNA breaks were not repaired because rates of DNA repair or amounts of reparable DNA could differ.

The final catalysis of covalent joining of single-strand breaks in double-helical DNA involves synthesizing a phosphodiester bond between adjacent 3′-hydroxyl and 5′-phosphoryl termini (Lehman, 1974; Olivera & Lehman, 1967). This esterification was inhibited in ligase-deficient cells (Figure 2). The similar numbers of DNA breaks produced in ligase-proficient and ligase-deficient cells (Figures 2 and 3) over a range of chemical concentrations indicated that DNA repair does not measurably reduce the number of DNA breaks produced during the chemical degradation of intracellular DNA under these experimental conditions. Thus, DNA repair cannot be the basis for the larger amounts of DNA breakage by bleomycin B_2 than bleomycin A_2. It cannot be ruled out that steps prior to the final step of esterification contribute to the total number of DNA breaks caused by chemical degradation of DNA, but this contribution should be, and in fact appears to be, equivalent in ligase-proficient and ligase-deficient cells.

In the experimental conditions used for the current study, DNA repair was inhibited in ligase-proficient cells because the cells were never exposed to growth medium or nutrient conditions during or after incubation with bleomycin A_2 or bleomycin B_2. Instead, direct effects of bleomycin A_2 and bleomycin B_2 in deionized water were studied, independent of growth and nutrient conditions. Yeast can be incubated for long periods in water without acquiring DNA breaks and without any detectable effects on survival of the cells because of the cell wall. These experimental conditions are important ones which distinguish the studies of bleomycins with yeast from those with procaryotic or cultured mammalian cells, and which avoid some of the serious problems affecting quantitation of DNA breaks. In addition, we avoided long chemical exposures because of the modulation of DNA breakage (Moore & Vossler, 1980; Moore, 1982c; Berry et al., 1985; Moore & Little, 1985) and cytotoxicity (Moore, 1982c) caused by bleomycin or its metabolites during prolonged exposures. We also assayed DNA breaks at low, physiologically meaningful chemical concentrations, enabling DNA breaks to be quantitated at physiologically meaningful survival. In previous studies of *S. cerevisiae*, bleomycin was dramatically less reactive with intracellular DNA than equimolar concentrations of structurally related phleomycin (Moore, 1988b). After similar numbers of DNA breaks were introduced into cells by low concentrations of bleomycin or phleomycin, repair of DNA molecules was comparable during posttreatment incubation periods and required the function of the same nuclear genes in *S. cerevisiae* (Moore, 1980, 1982a, 1988b). This is additional strong evidence that DNA repair is not the basis for the widely differing activities of the chemical congeners in the bleomycin–phleomycin group.

The finding that bleomycin B_2 produced higher DNA breakage than bleomycin A_2 prompted us to consider three possibilities. One, we considered the possibility that DNA breaks produced by bleomycin A_2 and bleomycin B_2 could be directly proportional to killing. Second, if DNA breaks were related to killing in this manner, then a definitive, limited number of DNA breaks should produce predictable killing. Third, if DNA breaks are a principal cause of killing by bleomycin A_2 and bleomycin B_2, then the number of DNA breaks produced by bleomycin A_2 or bleomycin B_2, and not the concentration per se of bleomycin A_2 or bleomycin B_2, should determine the amount of cell killing. Each of these possibilities was borne out in the current study. Thus, the C-terminal polyamine in bleomycin B_2 is more chemically reactive than the C-terminal dimethylsulfonium group in bleomycin A_2 in producing DNA breaks and killing under the experimental conditions employed.

Berry et al. (1985) concluded that growth inhibition and net DNA damage by bleomycin congeners in cultured human (KB) cells were not directly correlated with each other. In the case of bleomycin A_2 and bleomycin B_2, however, growth was inhibited to a greater extent by bleomycin B_2 than bleomycin A_2 in 24-h treatments of cultured human cells, and more DNA breaks were produced by bleomycin B_2 than bleomycin A_2 after 24 h of growth in the presence of the congeners. Berry et al. (1985) determined that DNA repair occurs during long exposure periods to bleomycin A_2 since fewer DNA breaks were assayed after 24-h treatments than after 1-h treatments.

Finally, the involvement of DNA ligase in the overall process of restoring full-sized DNA molecules from the DNA fragments produced by bleomycin appears to differ from the involvement of DNA ligase in the overall process of restoring full-sized DNA molecules from DNA fragments produced by ionizing radiation. This difference is apparent even though bleomycin is often considered "radiomimetic". A particular concentration of bleomycin (Moore, 1982d, 1988a, this report) or a given dose of ionizing radiation (Moore, 1982a) kills significantly more ligase-deficient cells than ligase-proficient cells. The number of breaks assayed for each dose was very similar, however, for ligase-proficient and ligase-deficient cells after either γ irradiation (Moore, 1982a) or incubation with bleomycin (Figure 5). It is thus concluded that ligase-proficient cells tolerate higher numbers of DNA breaks than ligase-deficient cells, even at temperatures permissive for ligase-deficient cells.

The slower ligation after chemical degradation of DNA by bleomycin (Moore & Little, 1985; Moore, 1988a; this report) than after γ irradiation (Moore & Little, 1985; Moore, 1988a) could be due to posttreatment enzymic reactions required to prepare the DNA product of the bleomycin reaction to be a substrate for DNA ligase. This difference is particularly striking when one considers the significantly fewer numbers of breaks purposefully produced in the experiments in the current study than in previous studies. The possibility cannot be ruled out, however, that persistent chemical reactivity of bleomycin or their metabolites (Moore & Vossler, 1980; Moore, 1982c) contributes to this difference.

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