Inhibition of poly(ADP-ribose) polymerase causes increased DNA strand breaks without decreasing strand rejoining in alkylated HeLa cells

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Treatment of alkylated HeLa cells with 3-aminobenzamide, an inhibitor of poly(ADP-ribose) polymerase, increased the number of DNA strand breaks but did not affect the rate of strand rejoining. This suggests that an increase in DNA incision, not a decrease in ligation, results from the inhibition of poly(ADP-ribose) polymerase in cells recovering from DNA damaged by alkylating agents.

Poly(ADP-ribose) *DNA strand break* *DNA repair*

1. INTRODUCTION

Inhibition of poly(ADP-ribose) polymerase (ADPRP) by 3AB in cells recovering from alkylation damage has been reported to increase DNA repair replication, DNA strand break frequency, and cell death [1]. One explanation of these findings is that DNA ligase II is not activated for DNA repair when ADPRP is inhibited [2]. In this model the increased DNA strand break frequency, observed during repair in the presence of 3AB, reflects a decreased rate of repair due to reduced ligation. The increased repair replication is interpreted as longer patches being formed at repair sites that fail to be ligated.

Recently, Walker and co-workers [3] and Cleaver [4] have found that repair patch size is, in fact, not increased when 3AB is present during DNA repair in alkylated cells. Their results suggest that more, not longer, repair patches result from DNA damage when ADPRP is inhibited by 3AB. If increased strand break frequency were due to a failure in ligation, then ligation would be necessarily rate limiting under these conditions. In contrast to this constraint, we found that the rate of resealing of DNA strand breaks in alkylated HeLa cells was unaffected by the presence of 3AB, even though the actual strand break frequency was increased by 3AB. These findings are consistent with the results of Walker et al. [3] and Cleaver [4], and suggest that DNA incision, and not ligation, is affected by ADP-ribosylation in cells recovering from alkylation damage.

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Abbreviations: 3AB, 3-aminobenzamide; araC, cytosine-β-D-arabinofuranoside; DMS, dimethyl sulfate; PBS, phosphate-buffered saline; AP, apurinic/apyrimidinic

2. MATERIALS AND METHODS

2.1. *Cell labelling and alkylation*

HeLa S3 cells were grown in Eagle's MEM medium containing 10% calf serum (Gibco), penicillin (50 U/ml) and streptomycin (50 μg/ml). Cells were labelled for 40 h with 0.015 μCi [^{14}C]thymidine per ml (58.0 mCi/mmol) followed by 5–7 h in fresh medium without label. Treatment of cells with araC (Sigma) began 30 min prior to DMS addition. DMS (gift from Dr I.G. Walker,

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this university) was first dissolved in methanol before dilution with PBS and addition to cell cultures. The final concentrations with DMS-treated and control cells were 0.003% methanol and 0.3% PBS. Labelled cells in 60-mm dishes were treated with DMS for 2.0 or 2.5 min followed by incubation in fresh medium with the indicated drugs for the recovery period. Cells that were exposed to γ-radiation were kept on ice and exposed at 68 rad/min with a ^{137}Cs gamma emitter (Gammacell 20, Atomic Energy of Canada). 3AB was obtained from Pfaltz and Bauer.

2.2. *Alkaline elution*

The procedure used was essentially that of Kohn et al. [5]. Cells were harvested by rinsing with 3.0 ml ice-cold PBS/EDTA (140 mM NaCl, 2.7 mM KCl, 16 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.02% EDTA), followed by scraping into 1.0 ml PBS/EDTA. Cells were diluted with 4.0 ml cold medium and 5.0 ml cold PBS and kept on ice. Approx. 3×10^5 cells were loaded onto 2 μm polycarbonate filters (Nucleopore) followed by 3 washes with 5.0 ml cold PBS. Cells were lysed with 5.0 ml of 2% SDS/0.025 M EDTA, pH 9.7. Alkaline elution with 0.02 M EDTA-tetrapropylammonium hydroxide (Aldrich)/0.1% SDS, pH 12.1, was carried out at 0.035–0.038 ml/min. Polycarbonate filters were prepared for scintillation counting by first dissolving in Protosol (New England Nuclear).

3. RESULTS AND DISCUSSION

Alkaline filter elution was performed with HeLa S3 cells at various times after a brief exposure to a low concentration of DMS. At the concentrations of DMS used in these experiments, there is virtually no cell death (S. Warmels and K. Ebisuzaki, unpublished). The slope of the DNA elution curve which reflects the presence of alkali-labile sites, predominantly strand breaks, was used to compare strand break frequencies [5]. The slope of an elution curve was calculated as the average of the slopes between individual fractions of the curve. The decrease in the slope of the elution curve results from the rejoining of strand breaks by ligation. Therefore, the change in the slope was followed over time to determine the rate of resealing of DNA breaks during DNA repair.

Fig.1 shows a typical elution profile from HeLa S3 cells after treatment with DMS. In the presence of a non-toxic concentration (5 mM) of 3AB (S. Warmels and K. Ebisuzaki, unpublished), the slope, and therefore the number of strand breaks, has increased. AraC (30 μM) also increased the strand break frequency, presumably by inhibiting DNA repair polymerization and thereby preventing subsequent strand rejoining [6]. A combination of 3AB and araC further increased the level of breaks in DMS-treated cells. However, incubation of cells with 3AB and araC alone did not induce strand breakage. Fig.2 shows the repair of strand breaks during the recovery period after DMS treatment. In the absence (fig.2A) or presence (fig.2B) of araC the number of strand breaks present during DNA repair is increased by 3AB. In fig.3, strand breaks are expressed relative to an initial value of 100% at 10 min after DMS treatment. This allows a comparison of the rate of repair of strand breaks between cells containing different initial levels of breaks. A lower dose of DMS (75 μM for 2.5 min) was used in experiments that included araC due to the potentiation of strand breaks by the drug, especially when used in combination with

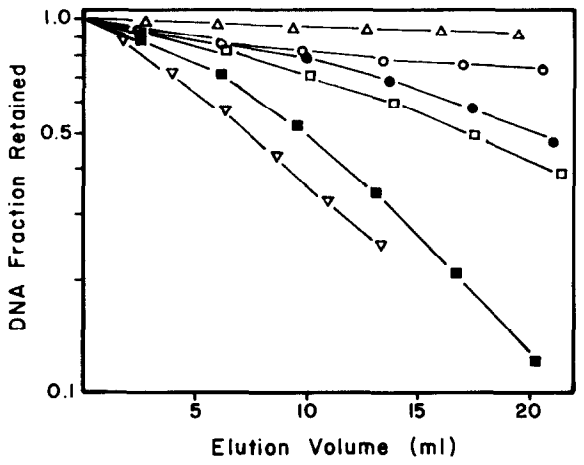

Fig.1. Effect of 3AB and araC on strand break frequency in alkylated HeLa cells. Cells were exposed to 75 μM DMS for 2.5 min and then allowed to recover for 20 min in fresh medium containing: nothing (○), 5 mM 3AB (●), 30 μM araC (□), 30 μM araC and 5 mM 3AB (■). Control cells incubated for 70 min with 30 μM araC and 5 mM 3AB (△). Cells eluted immediately after irradiation with 204 rads of γ-rays (▽). All experiments shown have been repeated.

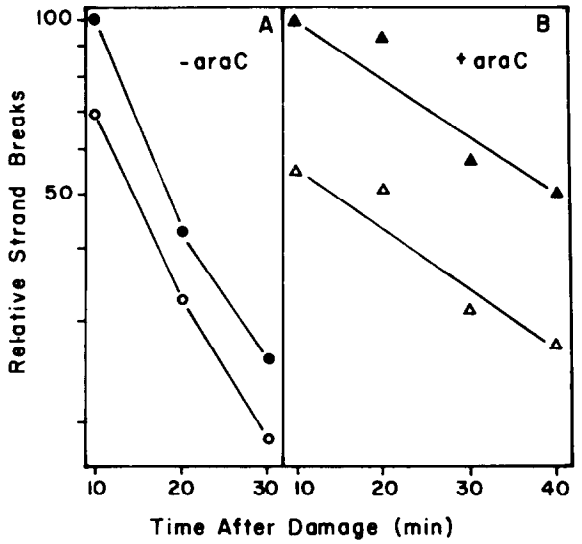

Fig.2. Loss of strand breaks during recovery from DMS damage. Cells exposed to DMS were allowed to recover in the absence (○, △) or presence (●, ▲) of 3AB for the indicated time. The DMS dose was 150 μM for 2.0 min in the absence of araC [A (○, ●)] or 75 μM for 2.5 min in the presence of araC [B (△, ▲)].

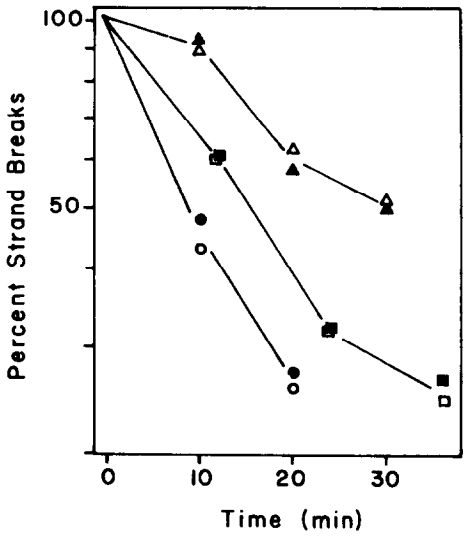

Fig.3. Rate of DNA resealing in the absence and presence of 3AB and araC. Strand breaks are expressed relative to an initial value of 100% taken at 10 min after DMS exposure. Cells were treated with 75 μM DMS for 2.5 min and allowed to recover with 30 μM araC plus (▲) or minus (△) 5 mM 3AB. Cells incubated without araC after treatment with DMS, 50 μM for 2.5 min (□, ■), or 150 μM for 2.0 min (○, ●), in the absence (○, △, □) or presence (●, ▲, ■) of 5 mM 3AB.

3AB (see fig.1). Fig.3 shows that the repair of strand breaks in the absence of araC is more rapid than in the presence of araC at 2 different doses of DMS (50 μM for 2.5 min and 150 μM for 2.0 min). Although more breaks exist in cells recovering from damage in the presence of 3AB (figs 1 and 2), the rate of resealing of these breaks is not affected by inhibition of ADPRP by 3AB (fig.3). In contrast, araC clearly changes the rate of resealing of breaks, as expected from an inhibition of DNA polymerization. AraC serves as a positive control in fig.3 by increasing the strand break frequency by limiting ligation. Addition of 3AB to araC-inhibited cells causes a further increase in strand breaks, while the resealing rate is not further decreased. These results suggest that the 3AB-induced increase in DNA strand breaks in alkylated HeLa S3 cells is due to an increase in an early repair reaction and not a decrease in ligation.

Poly(APR-ribose) polymerase is activated by nicked DNA to modify covalently nuclear proteins, including itself, by ADP-ribosylation [7,8]. The level of ADP-ribosylation of proteins may be a mechanism to control DNA-protein interaction [9]. The production of strand breaks at methylated purines by base excision repair requires the initial formation of AP sites by spontaneous acid hydrolysis or enzymatic cleavage of the *N*-glycosidic bond [10]. Subsequent AP-endonuclease action would generate a nick in the DNA [11], capable of activating ADPRP. The observed increase in damage-induced strand breaks when ADPRP is inhibited suggests that ADPRP may act to modulate further strand break formation either by the ADP-ribosylation of an endo- or AP-endonuclease, or by controlling the accessibility of chromatin to nucleolytic activity.

Koide and co-workers have demonstrated that ADP-ribosylation of Ca^{2+}/Mg^{2+}-dependent endonuclease from rat liver [12] and bull seminal plasma [13] results in enzyme inhibition, probably by preventing its interaction with DNA. The possibility that the rat liver enzyme is involved in DNA repair has been suggested [14]. Cleaver [4] has recently suggested that the increased repair replication in alkylated cells treated with 3AB might result from increased cutting by Ca^{2+}/Mg^{2+}-dependent endonuclease, not neces-

sarily acting at damaged sites. This increased cutting is not observed in undamaged cells treated with 3AB, but might be dependent on altered chromatin structure in damaged cells. The control of DNA incision by ADPRP may explain the observed effects of ADPRP inhibitors on DNA repair.

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