Sequence Specificity of (Cyanomorpholino)adriamycin Adducts in Human Cells†

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ABSTRACT: The highly reiterated α DNA tandem repeat was extracted from HeLa cells incubated with (cyanomorpholino)adriamycin (CMA) using mild techniques and subsequently probed for drug adducts by exonuclease III. The sequence specificity of the CMA-induced blockages was compared with that for blockages induced on the same DNA fragment when reacted *in vitro*. The sequence specificity of the drug-induced blockages was the same on both the isolated and the intact cell α DNA templates, with blockages predominantly associated with GpG sequences on either strand of the DNA.

The anthracycline antibiotic, adriamycin, has been used successfully in the treatment of a wide spectrum of neoplasia, particularly against leukemias and advanced cancers, for over 2 decades (Jones, 1982). Its use, however, is limited by several severe side effects, including cardiotoxicity, myelosuppression, and acquired resistance (Jones, 1982). Much research has been directed toward the synthesis of adriamycin derivatives with increased antitumor activity and fewer limiting side effects. Of the hundreds of compounds synthesized to date, 3′-(3-cyano-4-morpholinyl)-3′-deaminoadriamycin ((cyanomorpholino)adriamycin, CMA[1]) (Figure 1) is one of the derivatives with the greatest potential for fulfilling these requirements (Acton *et al.*, 1988). CMA is over 1000-fold more potent than adriamycin both *in vitro* (Begleiter & Johnston, 1985) and *in vivo* (Acton *et al.*, 1984). Several factors may contribute to the enhanced activity of this drug. Firstly, the increased lipophilicity of CMA results in rapid uptake through the cell membrane, resulting in a high intracellular concentration, especially in the nucleus (Wassermann *et al.*, 1986). Secondly, CMA rapidly induces DNA cross-links, the formation of which correlates closely with the cytotoxicity of this drug (Scudder *et al.*, 1988). We have recently determined the sequence specificity of CMA-induced DNA adducts *in vitro* as the 2-bp sequences GpG and GpC, apparently reflecting intrastrand and interstrand cross-links through G residues, respectively (Cullinane & Phillips, 1992). The N^2 of guanine has now also been implicated as a DNA binding site of the drug (Cullinane & Phillips, 1993).

Several methods have been utilized previously in the determination of the DNA sequence specificity of covalent binding agents and coordination complexes in cells. Linear PCR has been used to detect platinum compounds (Murray *et al.*, 1992), conversion of N^7 alkylation sites to single-strand breaks has been exploited for the detection of nitrogen mustard adducts (Grunberg & Haseltine, 1980; Hartley *et al.*, 1992), and a combination of both techniques has been used to detect dimethyl sulfate adducts (Saluz & Jost, 1989). While the aim of the present work was to investigate the sequence specificity of CMA in cells, the use of any of these methods was precluded by our recent findings that all DNA cross-links

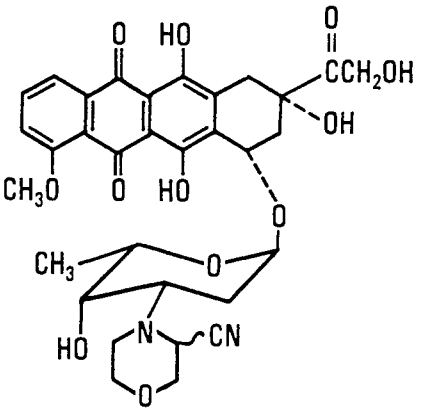

FIGURE 1: Structure of CMA.

induced by CMA *in vitro* displayed a heat lability with a melting temperature of 70 °C (10-min exposure) (Cullinane & Phillips, 1993).

A suitable DNA template for the detection of CMA in cells was α DNA. This chromatin subfraction is a 340-bp tandem repeat, representing approximately 1% of the total human genome, and is of sufficient homogeneity to define a consensus DNA sequence. As such, it has previously proven an appropriate template for the analysis of DNA damage induced by a range of different drugs (Grunberg & Haseltine, 1980; Murray & Martin, 1985; Hartley *et al.*, 1992; Murray *et al.*, 1992).

Following extraction of the total cellular DNA, damage induced by CMA was assayed on the α DNA template by the use of exonuclease III. This enzyme catalyzes the removal of mononucleotides from the 3′-terminus of double-stranded DNA templates (Sambrook *et al.*, 1989). The presence of a drug covalently bound to a labeled DNA template has previously been shown to impair the progression of the enzyme, yielding digestion products of lengths that indicate the drug binding site on the DNA (Tullius & Lippard, 1981; Royer-Pokora *et al.*, 1981). This feature of the enzyme has been exploited in the present work for the detection of CMA binding to α DNA. We show that the sequence specificity of adducts induced by CMA on isolated DNA *in vitro* is maintained in intact cells and demonstrate the enhanced efficiency of formation of CMA-induced adducts in the cellular environment.

MATERIALS AND METHODS

Materials. CMA was a gift from Dr. E. M. Acton (NCI, Bethesda, MD). The drug was dissolved in DMF and stored

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[1] Abbreviations: CMA, (cyanomorpholino)adriamycin; TE, Tris–EDTA; DMF, dimethylformamide; *cis*-DDP, *cis*-diamminedichloroplatinum(II); DME, Dulbecco's Modified Eagle medium; bp, base pair.

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in the dark at −20 °C. Bis(acrylamide), polynucleotide kinase, exonuclease III, and urea were purchased from IBI. Acrylamide was obtained from Bio-Rad, while calf intestinal phosphatase was from Promega. RNase T1 and restriction enzymes *Eco*RI (50 units/μL) and *Hae*III were from Boehringer Mannheim, and [γ-^{32}P]ATP (5000 Ci/mmol) was obtained from Amersham. RNase A was purchased from Sigma. X-ray film was from Kodak International, and NA45 paper was purchased from Schleicher and Schuell.

Cell Culture. Approximately 10^7 HeLa cells were incubated at 37 °C for 2 h with 0–2 μM CMA in DME cell culture medium containing 10% fetal calf serum. The cells were then harvested by scraping and pelleted before being washed twice in phosphate-buffered saline.

DNA Isolation Procedures. The extraction of the genomic DNA from HeLa cells was based on that of Murray and Martin (1985), except that all incubations were performed at 37 °C. Briefly, the washed cell pellets were resuspended in 300 μL of buffer comprising 50 mM Tris (pH 7.5) and 20 mM EDTA. The cells were lysed by the addition of SDS (0.2%) and proteinase K (0.1 mg/mL) and passed through a 23-gauge syringe three times prior to incubation at 37 °C for 60 min. A second aliquot of proteinase K was added, and the incubation was extended for an additional 60 min at 37 °C. The lysate was extracted twice with phenol and once with chloroform and then precipitated twice with ethanol. The nucleic acid pellet was resuspended in 100 μL of TE, to which was added RNase A (60 μg/mL) and RNase T1 (40 μg/mL), and then incubated at 37 °C for 30 min.

Isolation and Labeling of α DNA. The procedures for the preparation of the α DNA fragment were analogous to those used by Hartley *et al.* (1992). Genomic DNA was digested with *Eco*RI at 37 °C for 3 h. The digest was electrophoresed through a 1.5% agarose gel containing 0.5 μg/mL ethidium bromide, the position of the 340-bp α DNA was determined by UV visualization, and the fragment was run onto NA45 anion-exchange paper. The DNA fragment was eluted from the paper by incubation at 45 °C for 2 h in 20 mM Tris (pH 8.0), 1.5 M NaCl, and 2 mM EDTA. A phenol/chloroform extraction was performed, and the DNA was concentrated by ethanol precipitation. The fragment was dephosphorylated with calf intestinal phosphatase and 5′-labeled in the presence of polynucleotide kinase and [γ-^{32}P]ATP using routine methods (Sambrook *et al.*, 1989). Digestion of the dual-labeled fragment with *Hae*III produced two singly labeled fragments of 296 and 44 bp, which were resolved on a 6% (29:1) native polyacrylamide gel. The position of the 296-bp fragment was determined by autoradiography, the band was excised, and the DNA eluted from the polyacrylamide slice overnight at 37 °C in 10 mM Tris (pH 7.5), 1 mM EDTA, and 150 mM NaCl. An additional phenol/chloroform extraction was performed, and the DNA was precipitated, resuspended in TE, and stored at −20 °C prior to assay by exonuclease III digestion.

Drug Treatment of Isolated DNA. The 5′-labeled 296-bp α DNA fragment, isolated from untreated HeLa cells, was reacted *in vitro* with 0.75 or 1.5 μM CMA in 40 mM Tris (pH 8.0), 100 mM KCl, 3 mM $MgCl_2$, and 0.1 mM EDTA for 60 min at 37 °C. The DNA was precipitated with ethanol prior to exposure to exonuclease III.

Exonuclease III Assay. The 296-bp templates reacted *in vitro* and within intact cells were digested with 16 units of exonuclease III for 60 min at 37 °C in 6.6 mM Tris (pH 8.0) and 0.66 mM $MgCl_2$. The reactions were terminated by the addition of formamide loading buffer and heat denatured at 90 °C for 5 min prior to electrophoresis through a 10% (19:1) denaturing polyacrylamide gel. The gel was fixed and dried as described previously (Skorobogaty *et al.*, 1988) and autoradiographed using Kodak X-Omat AR film. Band quantitation was performed using a Molecular Dynamics Model 400B PhosphorImager.

RESULTS

The thermal lability of CMA adducts seen previously *in vitro* (Cullinane & Phillips, 1993) imposed constraints upon the methodology employed to detect the DNA binding sites of CMA adducts in intact cells. As a consequence, elevated temperature incubations (>45 °C) were avoided in the isolation and 5′-labeling of the 296-bp α DNA template from cells incubated with CMA. In addition, exonuclease III was used as the probe for drug adducts on the template due to its activity at physiological conditions (i.e., its ability to digest a double-stranded DNA template at neutral pH at 37 °C).

Total genomic DNA was isolated from HeLa cells and digested with *Eco*RI to release the 340-bp α DNA fragment. A singly labeled 296-bp α DNA fragment was prepared as described by Hartley *et al.* (1992) and was utilized as a substrate for the exonuclease III detection of CMA-induced adducts *in vitro*. The DNA was reacted with 0–1.5 μM CMA for 1 h prior to digestion of the template with exonuclease III. The digestion products were resolved by electrophoresis and are shown in Figure 2. The undigested control lanes show that the DNA template essentially consists of a full-length band of 296 bp. Exonuclease III digestion of DNA incubated in the absence of drug resulted in almost total digestion of the full-length band, as indicated by the low level of radioactivity in the lane. Digestion of the CMA-treated templates, however, resulted in specific digestion products not evident in the control lanes, indicating the sensitivity of the nuclease to the presence of CMA-induced adducts on the DNA template.

The labeled 296-bp α DNA fragment was prepared from DNA extracted from HeLa cells exposed to 0–2 μM CMA and subsequently assayed for the presence of bound drug by the use of exonuclease III. The results of the gel are shown in Figure 3. The undigested DNA fragments again almost totally consisted of the 296-bp full-length band. Exonuclease treatment of the control DNA fragment resulted in a background of inefficient enzyme digestion products, with a digestion pattern similar to that of the purified DNA fragment (Figure 2). Enzyme digestion of the drug-treated DNA again revealed the presence of blockages not evident in the control lane, indicating the presence of CMA-induced adducts on the DNA template.

The amount of DNA exposed in intact cells to CMA was ~85 μg (one HeLa cell contains 8.5 pg of DNA; Adams *et al.*, 1986), which is vastly in excess of the amount exposed to isolated DNA *in vitro* (~0.5 μg). Despite this difference, comparable levels of CMA-induced damage were evident on the α DNA template from each source upon exposure to similar drug concentrations.

Sequence Specificity. A slight offset is apparent between the electrophoretic mobility of the Maxam–Gilbert G-sequencing lane and exonuclease III digestion products (Figures 2 and 3). As the enzyme-degraded products contain a 3′-hydroxyl group, they are of slightly lower mobility than a corresponding G-sequencing product, which contains a terminal 3′-phosphoryl group (Royer-Pokora *et al.*, 1981).

The sequence specificity of CMA-induced adducts on α DNA, following exposure of the drug to both purified DNA

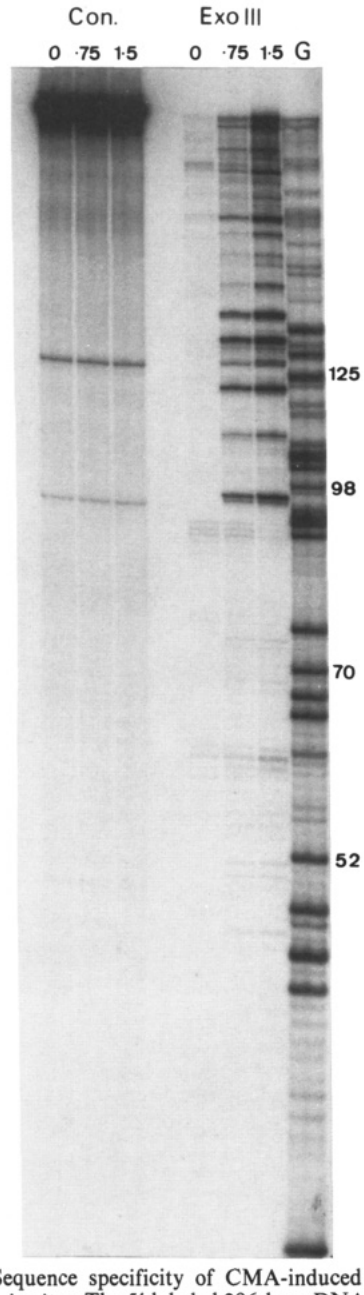

FIGURE 2: Sequence specificity of CMA-induced adducts with isolated DNA *in vitro*. The 5′-labeled 296-bp α DNA fragment was reacted with 0–1.5 μM CMA (as shown) for 60 min at 37 °C, and the DNA subsequently was digested with exonuclease III at 37 °C for 60 min. The digested products (denoted as Exo III), together with undigested reacted DNA (denoted as Con.), were denatured and electrophoresed through an 8% denaturing polyacrylamide gel. Lane G represents a Maxam–Gilbert sequencing lane. The lengths of several of these sequencing products are shown on the right-hand side of the autoradiogram.

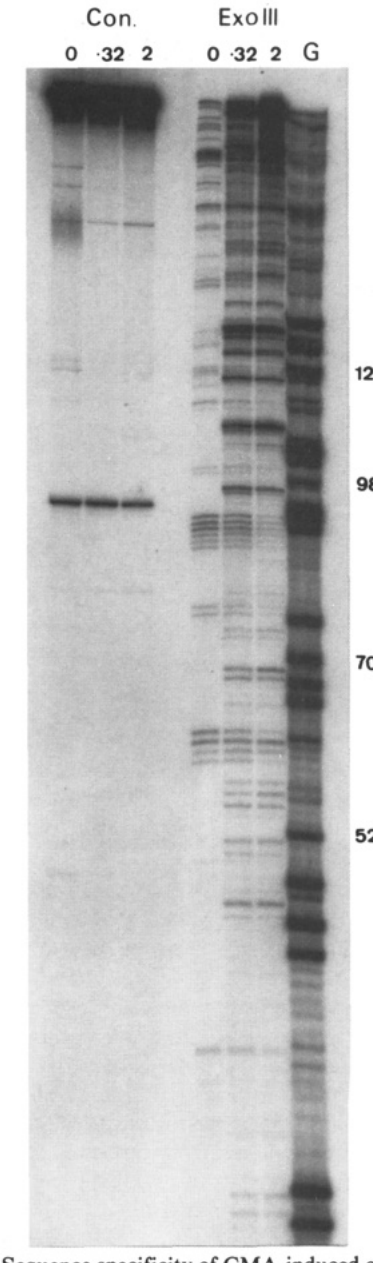

FIGURE 3: Sequence specificity of CMA-induced adducts in HeLa cells. The 5′-labeled 296-bp α DNA fragment was isolated from HeLa cells following incubation with 0–2 μM CMA (as shown) for 60 min. The DNA was digested with exonuclease III, and the digestion products were resolved on an 8% denaturing polyacrylamide gel.

and cells, is summarized in Figure 4 in terms of the relative intensity of blockages to exonuclease III. Of the 160 bases resolved, 12 well-defined blockage regions were evident on the purified template treated *in vitro* (Figure 4A). Five of these regions correspond to the inhibition of enzyme digestion 2 or 3 bases prior to a GpG sequence on the degraded strand (blockage sites 1, 2, and 4–6), while three regions consisted of enzyme blockages 2–5 bases prior to CpC sequences on the degraded strand (blockage sites 3, 8, and 11). The combination of these two sets of blockages reflect eight of the nine such GpG and CpC sequences in the DNA region probed and, as suggested previously, appear to reflect the presence of intrastrand cross-links on the degraded and non-degraded strands, respectively (Cullinane & Phillips, 1992). A single CpC site at position 55 does not appear to impair enzyme digestion. Due to the proximity of the sequence to the 5′-end of the fragment, the absence of any drug-induced enzyme blockage associated with this site may reflect the failure of any enzyme to reach and probe this region of the template, as a result of inhibition at prior drug binding sites. The remaining four drug-induced enzyme blockage sites were of much reduced intensity compared with those at GG and CC sequences. Three of these sites occurred 2 bases prior to the G residue in an AGA sequence. Other such sequences were present on the template, but did not result in blockage of the enzyme digestion.

CMA-induced enzyme blockages at all GpG sites were similar, consisting of an intense block 3 bases prior and a lower intensity block 2 bases prior to the GpG sequence. In contrast, the highest intensity CMA-induced blockage at each of the CpC sequences was at least 4 bases prior to the apparent drug binding site.

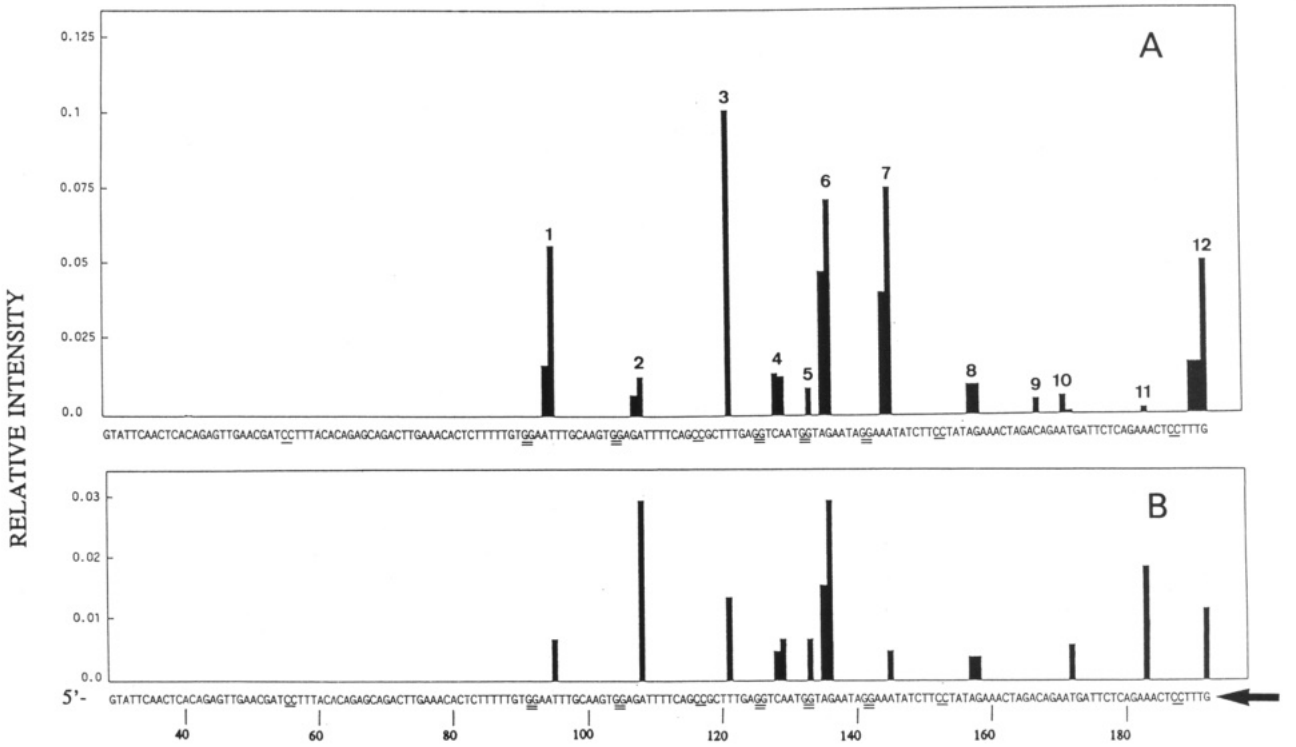

FIGURE 4: Quantitation of exonuclease III blockages. The relative intensity of CMA-induced exonuclease III blockages is shown with respect to the length of each DNA digestion product detected in the 0.75 μM lane of CMA reacted with isolated DNA *in vitro* (A) and in the 0.32 μM lane of CMA reacted with HeLa cells (B). The numbering represents the length of the DNA fragment beginning at the 5'-labeled end. The arrow shows the direction of exonuclease III digestion. All GpG sequences are highlighted with double underlining and CpC sequences with single underlining.

The *in vitro* sequence specificity of CMA-induced adducts was maintained on the DNA template extracted from cells treated with CMA (Figure 4B). The additional background bands present on the cellular template result in an underestimation of the number of drug-induced blockages at sites probed after these regions and, therefore, restrict the comparison between the *in vitro* and the cellularly reacted templates to a qualitative analysis. Eleven drug-induced sites were evident on the DNA template reacted with CMA in intact cells, all of which were detected on the purified DNA template, indicating the retention of *in vitro* drug specificity in the cellular environment. Most blockage sites were characterized by enzyme inhibition at a single base corresponding to the 3'-most blockage at a site detected on the purified DNA template.

DNA Interstrand Cross-Linking. CMA has been widely reported to cross-link cellular DNA (Begleiter & Johnston, 1985; Scudder *et al.*, 1988). The presence of CMA-induced DNA interstrand cross-links, previously suggested to occur through GpC sequences (Cullinane & Phillips, 1992), was not detected by the exonuclease assay on either the *in vitro* or the intact cell templates. In order to directly measure the formation of such cross-links in cells, a highly sensitive electrophoresis assay was employed. The DNA template probed was the 680-bp α DNA fragment, which consists of two tandemly repeated 340-bp *Eco*RI fragments linked as a result of a point mutation in the internal *Eco*RI site, rendering the site inaccessible to the endonuclease (Wu & Manuelidis, 1980). The 680-bp fragment was isolated in preference to the 340-bp fragment as its higher molecular weight facilitates the electrophoretic resolution of the single- and double-stranded DNA bands. The DNA fragment was isolated from cells reacted with 3 μM CMA and subsequently 5'-end-labeled prior to denaturation in 45% formamide and electrophoresis through a 2.5% agarose gel at 50 V overnight. DNA

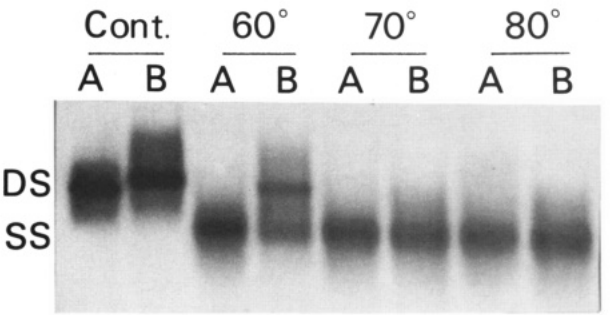

FIGURE 5: DNA interstrand cross-linking by CMA in intact HeLa cells. The 680-bp α DNA imperfect tandem repeat was isolated from cells incubated for 60 min in the absence (A) and presence (B) of 3 μM CMA. The fragment was 5'-end-labeled and subsequently denatured in 45% formamide for 5 min at the temperatures shown. Unheated double-stranded control samples are shown as Cont. Aliquots were loaded onto a 0.8% agarose gel and electrophoresed through TAE overnight at 50 V. Double-stranded DNA is denoted as DS and single-stranded DNA as SS.

interstrand cross-links were detected as a resistance of drug-treated DNA to separate into single strands under DNA-denaturing conditions.

The results of the cross-linking gel are shown in Figure 5. Analysis of the electrophoretic mobility of the 680-bp fragment from cells exposed to CMA reveals a migration retardation of this non-denatured DNA fragment in comparison to that of the control. A similar drug-induced retardation was observed previously on an *in vitro* reacted DNA fragment (Cullinane & Phillips, 1993). Heating of the control DNA at 60 °C for 5 min resulted in the complete denaturation of the DNA, made evident by a single band migrating more rapidly than the double-strand band due to its lower molecular weight. In contrast, the CMA-treated fragment was resistant to strand separation at 60 °C with 65% remaining as a double strand, indicating the presence of drug-induced DNA inter-

strand cross-links on the template. Denaturation of both the control and CMA-treated templates at 70 °C resulted in strand separation on both fragments, consistent with the presence of heat-labile DNA interstrand cross-links in cells analogous to those observed *in vitro* (Cullinane & Phillips, 1993).

DISCUSSION

The formation of DNA cross-links by CMA has long been implied in the mechanism of action of this drug (Westendorf *et al.*, 1985; Scudder *et al.*, 1988; Lau *et al.*, 1992). Previously, we have investigated cross-link and adduct formation by CMA and determined the DNA sequence specificity of drug binding *in vitro* (Cullinane & Phillips, 1991, 1992, 1993). In the present study, we report the first evidence of the sequence specificity of CMA-induced adducts in intact cells.

The results of the exonuclease assay clearly demonstrate that the *in vitro* DNA sequence specificity of CMA is maintained in HeLa cells, with 11 of 12 sites seen *in vitro* also detected on the cellular template. The ability to detect such CMA-induced adducts in cells clearly demonstrates that the methods used in the extraction, isolation, and probing of the α DNA fragment template were sufficiently mild to preserve the DNA-bound drug component.

The major sites of drug damage occurred at GpG and CpC sequences, reflecting the preferential formation of DNA intrastrand cross-links through adjacent guanine residues on the degraded and non-degraded strands, respectively. Similar blockages have been detected previously *in vitro* using *Escherichia coli* RNA polymerase and λ-exonuclease as probes of drug binding (Cullinane & Phillips, 1992, 1993).

Exonuclease III digestion was blocked consistently at a defined distance prior to a CMA adduct site. As observed previously, this distance is dependent on the type of DNA damage present. Exonuclease III is able to digest to within 1 base of DNA photoproducts (Royer-Pokora *et al.*, 1981) and within 4–6 bases of saframycin A adducts (Rao & Lown, 1992). CMA-induced intrastrand cross-links present on the degraded strand, together with *cis*-DDP lesions (Tullius & Lippard, 1981), block the enzyme 3 bases prior to the drug binding site. It has been suggested that this variable distance of drug-induced enzyme inhibition may be a consequence of local DNA denaturation about the drug binding site (Tullius & Lippard, 1981). This explanation, however, is unlikely to account for the differential ability of the enzyme to approach GpG and CpC sequences on a CMA-reacted template, representing an intrastrand cross-link on the degraded and non-degraded strands, respectively, as any DNA denaturation associated with drug binding on either strand would yield a denatured region of essentially identical size. As a consequence, CMA-induced enzyme blockage associated with both of these sequences would be expected to be inhibited the same distance prior to the drug binding site, and this has not been observed. An alternate explanation of the differential inhibition of the enzyme by CMA adducts on the degraded and non-degraded strands assumes that the active site of the enzyme is positioned back from the DNA substrate recognition function. As exonuclease III requires duplex DNA as its substrate, the leading substrate recognition function of the enzyme may interact strongly with the non-degraded strand, thus detecting damage on this strand prior to the detection of damage on the degraded DNA strand.

CMA-induced DNA interstrand cross-links, thought to form at GpC sequences (Cullinane & Phillips, 1992), were not detected by the exonuclease probe. The low reactivity of these sites to CMA (relative to intrastrand cross-links) has been observed previously (Cullinane & Phillips, 1992), and this necessitated the use of a more sensitive assay to detect these lesions in cells. The interstrand cross-linking assay directly confirmed the presence of low levels of DNA interstrand cross-links induced by CMA in HeLa cells. DNA interstrand cross-links were heat-labile, consistent with the inherent instability of the aminal bond (Petrusek *et al.*, 1981; Lown *et al.*, 1982) thought to link the drug to the N^2 of guanine (Cullinane & Phillips, 1993).

No drug-induced DNA interstrand cross-links were evident on α DNA following denaturation at 70 °C. In contrast, CMA-induced cross-links formed on plasmid DNA *in vitro* display a T_m of 70 °C (Cullinane & Phillips, 1993). The difference in the melting temperatures of α DNA (38% GC content) and bacterial DNA (50% GC content) suggests that the stability of the cross-link may be related to the stability of the DNA duplex. The comparatively low melting temperature of α DNA may therefore result in the decreased stability of cross-links seen on this template, as compared to those cross-links formed on the plasmid DNA template. Anthramycin, a pyrrolo[1,4]benzodiazepine antibiotic, which also binds DNA *via* an aminal link to the N^2 of guanine (forming monofunctional adducts), also demonstrates instability at temperatures greater than the DNA melting temperature (Hurley *et al.*, 1979).

Given the complex environment of the DNA within a cell, it is striking to observe the conservation of the *in vitro* sequence specificity of CMA-induced damage when reacted with isolated DNA *in vitro*, compared to the same template in intact cells. Although α DNA is neither transcribed nor translated and its role in the cell remains unclear (Wu & Manuelidis, 1980), it is widely used as a representative of cellular DNA. As such, it would be expected that some DNA sequences might be protected from DNA damage, but this clearly was not the case with CMA, with each of the drug sites detected *in vitro* maintained in the HeLa cells. This complete maintenance of the *in vitro* sequence selectivity of a drug has been demonstrated previously for several other DNA binding agents in intact cells, including the nitrogen mustards (Grunberg & Haseltine, 1980; Hartley *et al.*, 1992), bleomycin (Murray & Martin, 1985), Hoechst 33258 (Murray & Martin, 1988), and a series of platinum compounds (Murray *et al.*, 1992). The sequence selectivity of CMA in HeLa cells therefore further supports the view that simple *in vitro* assays provide a reliable prediction of the sequence specificity of drug binding in intact cells (Hartley *et al.*, 1992).

The efficiency of DNA binding by CMA in intact cells is surprising. While several studies have investigated the binding of various drugs to DNA both *in vitro* and in intact cells, all have noted the significantly higher drug concentrations required with intact cells to obtain a level of drug-induced damage comparable to that detected on the purified template (Grunberg & Haseltine, 1980; Murray & Martin, 1985; Hartley *et al.*, 1992; Murray *et al.*, 1992). In comparison, the level of DNA damage induced by CMA on α DNA in cells was similar to that observed at similar concentrations and incubation conditions with isolated DNA, and this may reflect the rapid uptake and localization of the drug to the nuclear DNA in intact cells (Wassermann *et al.*, 1986).

The exceptional potency of CMA in cells appears to be conferred by structural properties rarely combined in a single compound. The presence of the morpholinyl group increases the lipophilicity of this drug over that of the parent compound, adriamycin, facilitating the rapid uptake of the drug into the cell (Wassermann *et al.*, 1986). Once inside the cell, the

chromophore has been suggested to act as a targeting function, directing the drug through the cell to localize on the DNA (Denny, 1989). At the DNA level, the presence of the cyano moiety confers the DNA cross-linking activity of the drug (Acton *et al.*, 1988). While many alkylating agents apparently are nonselective toward DNA (e.g., mustards), reacting with various cellular nucleophiles, CMA shows strong DNA specificity, exhibiting minimal reactivity toward proteins (Westendorf *et al.*, 1985).

Recent studies investigating the efficiency of repair of *cis*-DDP lesions have found that DNA intrastrand cross-links, which represent more than 85% of total platinum lesions, are poorly repaired, while interstrand cross-links, forming less than 1% of total lesions, are removed more efficiently (Calsou *et al.*, 1992). This evidence, together with the observation that *cis*-DDP resistance in one cell line was associated with increased intrastrand cross-link repair (Eastman *et al.*, 1988), has led to the proposal that the efficiency of *cis*-DDP as an anticancer drug is closely related to its ability to induce intrastrand DNA cross-links (Calsou *et al.*, 1992). CMA forms predominantly intrastrand cross-links with a 10-fold preference compared to interstrand cross-links (Cullinane & Phillips, 1992). By analogy with *cis*-DDP, the formation of such lesions might be expected to be poorly repaired in human cells, and this may well contribute to the unusually high potency of this drug.

CONCLUSIONS

The sequence specificity of CMA binding on the 296-bp α DNA template *in vitro* was maintained in intact HeLa cells. The cellular environment greatly facilitated the formation of CMA-induced adducts. The potency displayed by this drug in cells demonstrates its potential as an improved anthracycline, with considerable possibilities as an active anticancer drug. Further investigation of the pharmacological properties of CMA are therefore warranted, despite the known additional toxicities of this compound.

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