Anomalous Mobility of Cadmium(II)- and Copper(II)-treated Lambda DNA Restrictions Digests on Agarose Gels

VASANTHA NARASIMHAN* and ANDREW T. WALKER
Skidmore College, Saratoga Springs, NY 12866, U.S.A.
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A considerable wealth of information has been accumulated over the recent years regarding the flexibility and stereodynamics of the conformation of the DNA molecule [1–3]. Several localized conformational possibilities such as bends, kinks, cruciforms and curvatures have been examined in recent research reports in connection with their possible role in the regulation of genetic expression [4–7]. Our past work has indicated that certain metal ions are capable of inducing significant conformational alterations in the DNA molecule even at very low concentrations [8, 9]. For example, carcinogenic ions such as cadmium(II) induced a B to Z transition in the DNA polymer poly d(G–C), whereas non-carcinogenic ions such as copper(II) and the anti-tumor agent cisplatin caused a B to A transition in the same polymer [9]. In our continued effort to understand the biological consequences of such conformational changes, we have undertaken a study of the substrate quality of DNA samples treated with Cd(II), Cu(II) and cisplatin and its *trans*-isomer towards a variety of enzymes. Restriction endonucleases lend themselves as suitable choices for such studies since the separation of restriction fragments in agarose gels is conformation dependent and any alterations in conformation could be visualized by alterations in mobilities of fragments. Since our earlier studies have indicated that the conformational alterations caused by metal ion binding are more prominently felt by alternating GC-rich sequences [9], we have chosen three enzymes, Cfo I, Hha I and Tha I, all of which require a short run of alternating GC sequence at their restriction sites. In addition, two other enzymes, Bam HI and Hind III, were chosen as controls. The restriction site specificities of the five enzymes are listed below:

Cfo I and Hha I: 5′-GCG$^{\downarrow}$C-3′
3′-C$_{\uparrow}$GCG-5′

Tha I: 5′-CG$^{\downarrow}$CG-3′
3′-GC$_{\uparrow}$GC-5′

BAM HI: 5′-G$^{\downarrow}$GATCC-3′
3′-CCTAG$_{\uparrow}$G-5′

Hind III: 5′-A$^{\downarrow}$AGCTT-3′
3′-TTCGA$_{\uparrow}$A-5′

Experimental

Lambda DNA and the restriction enzymes were purchased from Bethesda Research Laboratories and P. L. Biochemicals. Cadmium chloride and cupric chloride were purchased from the Fisher Scientific Company while *cis*-dichlorodiammineplatinum(II) (cisplatin) and *trans*-dichlorodiammineplatinum(II) were products of the Sigma Chemical Company.

The DNA solution (200 μl) containing 16 μg of DNA in Tris-HCl buffer of pH 8.0 was mixed with 10 μl of the appropriate metal salt solution that would yield an '*r*' value or metal-ion-to-DNA-phosphorus ratio of 5.0. The mixture was allowed to incubate in the refrigerator overnight. The samples were then filtered through a microgel filtration device using Sephadex G-25 to remove the excess metal ions. The filtered samples were stored at −20 °C until further use. The restriction digests were prepared by treating an aliquot of the above metal-damaged DNA samples containing 2 μg of DNA with 4 units of the restriction enzyme for 60 min at 37 °C in Tris-HCl buffer of pH 7.8. The reaction was stopped by the addition of a quenching solution containing 5% SDS, 25% sucrose and 0.05% bromophenol blue. The digests were immediately electrophoresed on a 1% agarose gel using Tris-acetate buffer of pH 7.5 for 18–20 h at 4 mA per sample. Gels were stained with an ethidium bromide solution containing 1.0 mg of ethidium bromide per liter of distilled water for 10 min, rinsed with two washings of distilled water, and photographed under ultraviolet light.

Results and Discussion

Figure 1 shows the electrophoretic pattern of the native lambda DNA as well as its metal-ion-treated counterparts, before their reaction with any restriction enzymes. It is seen clearly that the metal-damaged DNA samples have not undergone any fragmentation by themselves which may interfere with the interpretation of the results from the restriction digests.

The fragmentation patterns obtained with the two enzymes Cfo I and Tha I are presented in Figs. 2 and 3, respectively. It is clearly evident that the mobilities of the fragments from cadmium- and copper-treated samples are considerably different

*Author to whom correspondence should be addressed.

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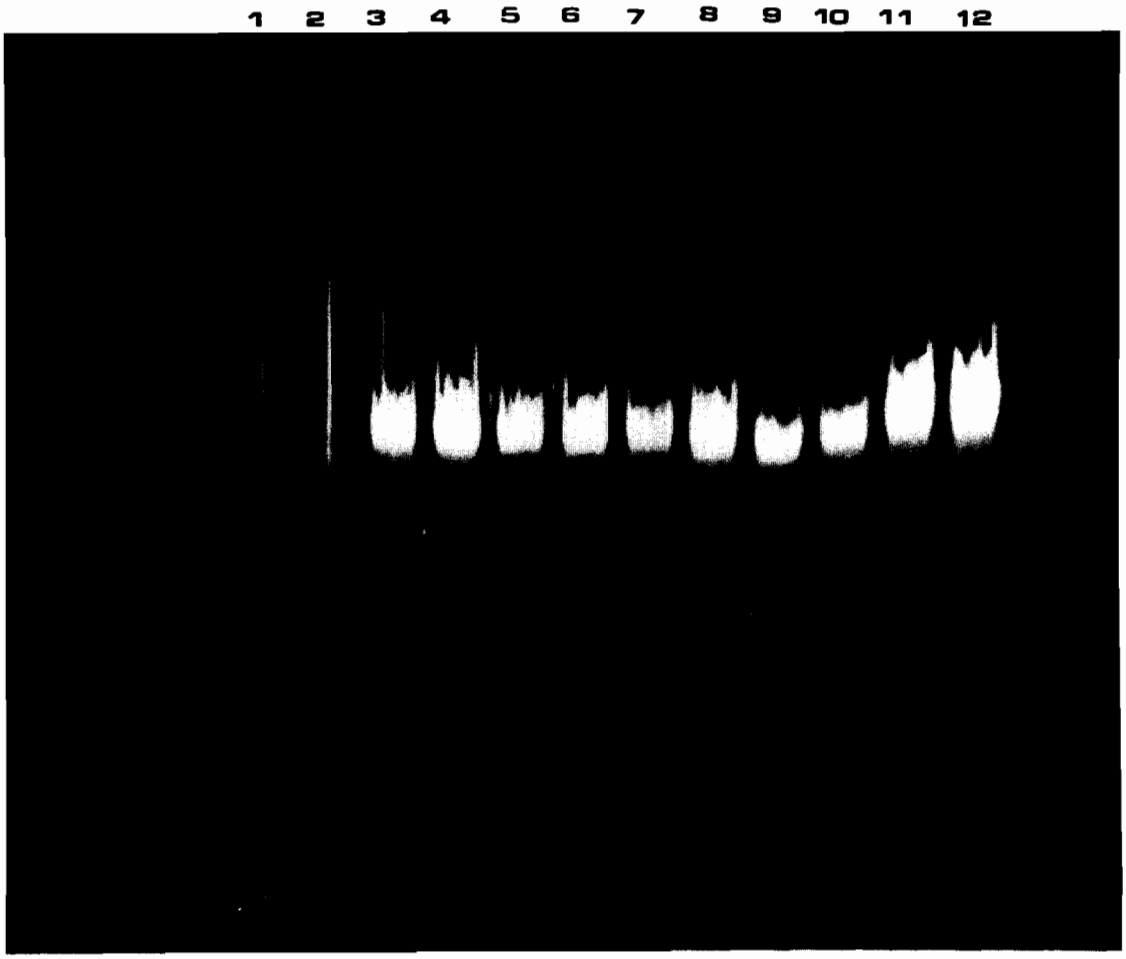

Fig. 1. Electrophoresis of native and metal-treated lambda DNA samples on 1% agarose gel. From left to right: lanes 1, 2 = native lambda DNA (unincubated and unfiltered through the microgel column); lanes 3, 4 = DNA–Mg(II); lanes 5, 6 = DNA–Cd(II); lanes 7, 8 = DNA–Cu(II); lanes 9, 10 = DNA–*trans*-isomer of cisplatin; lanes 11, 12 = DNA–cisplatin.

from those of the native lambda DNA. DNA samples treated with cisplatin or its *trans*-isomer showed complete inhibition of the restriction reaction by these two enzymes. Similarly, no fragmentation was obtained from any of the metal-treated DNA samples on digestion with Bam HI, Hind III or Hha I, indicating that these enzymes were completely inhibited by the metal-treated DNA substrates (unpublished results). Hha I is an isoschizomer of Cfo I and these two enzymes produce identical fragmentation patterns with native lambda DNA. However, in the cadmium- and copper-treated samples, Cfo I produces fragments of altered mobilities, whereas Hha I is completely inhibited. We attribute this anomaly to probable differences in the conformational requirements of these two enzymes at the DNA restriction sites which may have been altered by treatment with cadmium and copper. The results obtained for the cadmium samples from the Cfo I digestion and Tha I digestion are noteworthy in light of the fact that the restriction site symmetries of these two enzymes are just mirror images of each other. The two prominent fragments in the Cfo I digestion of native lambda DNA have been assigned approximate sizes of 3.5 and 2.8 kilobase pairs, respectively, by running a commercial lambda DNA–Hind III digest alongside our lambda DNA–Cfo I digests (unpublished results). In the cadmium- and copper-treated samples, these major fragments exhibit a slower mobility. The group of fast moving fragments in the native lambda DNA have migrated farther away from the two major fragments, whereas these fragments in the cadmium- and copper-treated DNA samples exhibit a much slower mobility, even slower than the two major fragments. Such anomalous slow mobilities of restriction fragments have been observed in DNA samples under different conditions in polyacrylamide gels and these have been attributed to various conformational anomalies due to bends and kinks in the fragments [10–12]. Our present results clearly show that the fragments obtained from the metal-damaged DNA samples have definitely undergone conforma-

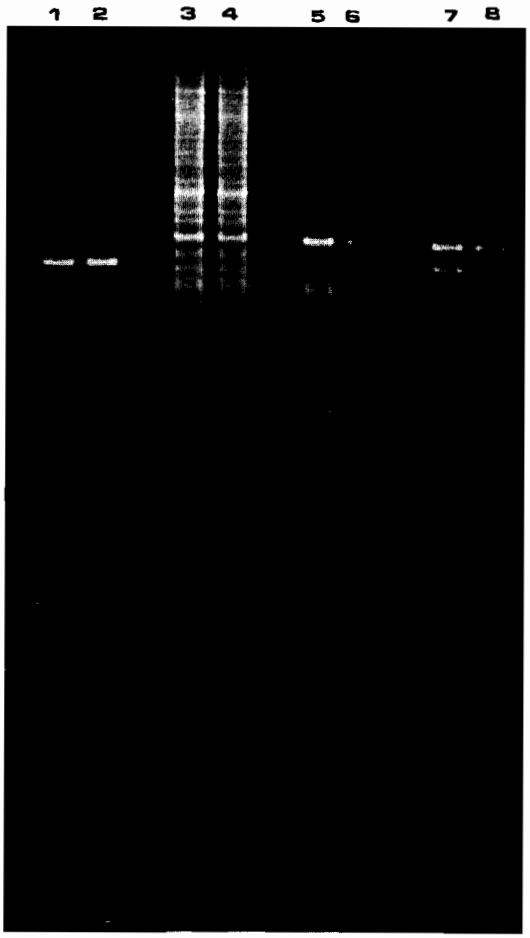

Fig. 2. Electrophoresis of lambda DNA–Cfo I digest on 1% agarose gels. From left to right: lanes 1, 2 and lanes 7, 8 are from native lambda DNA and Mg(II)-treated DNA, respectively, lanes 3, 4 are from cadmium-treated DNA; and lanes 5 and 6 are from copper-treated DNA.

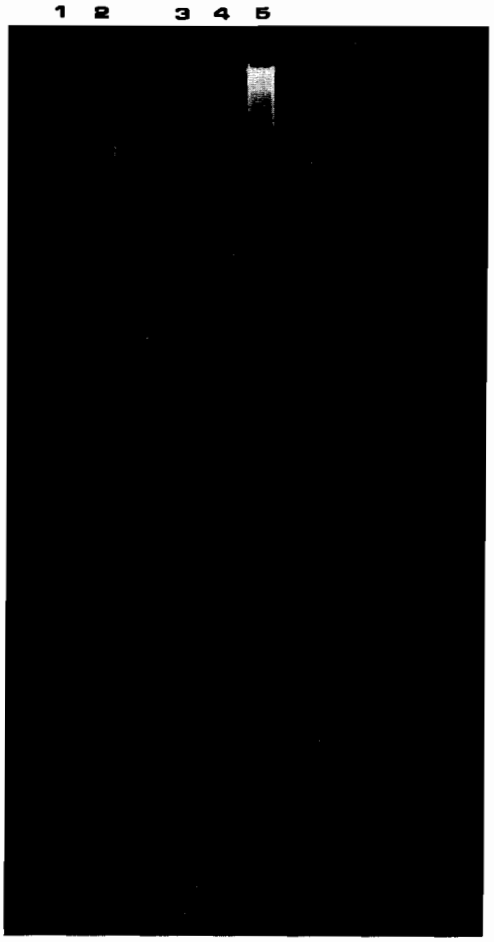

Fig. 3. Electrophoresis of lambda DNA–Tha I digests on 1% agarose gels. From left to right: lanes 1 and 2 are from native lambda DNA and Mg(II)-treated DNA, respectively; lanes 3, 4 are from cadmium-treated DNA; and lane 5 is from copper-treated DNA.

tional changes leading to large variations in their effective lengths. We hypothesize that these conformational changes have been caused by bends and kinks in the fragments due to localized unpairing or mispairing of the bases as a result of the binding and removal of the cadmium(II) and copper(II) ions.

In the case of Tha I digestion, copper completely inhibits the restriction reaction, while the fragments from cadmium-treated DNA show a higher mobility than those of native lambda DNA. Considering the fact that the symmetry site requirements of these two enzymes are exactly opposite to each other (refer to the first section), the polarities of the fragments obtained from Cfo I and Tha I are somewhat opposite to each other. It has been shown both theoretically and experimentally that such reversal of polarities of the fragments can cause a reversal of electrophoretic mobilities [13, 14]. Thus we believe that the curvature or the 'metal-damage scar' caused by the binding and removel of cadmium ions has resulted in slow moving fragments in the case of Cfo I digestion and fast moving fragments in the case of Tha I, reflecting the reverse polarity of these two sets of fragments. Such a reversal of mobilities also leads us to eliminate the possibility that the metal ions might still be bound to the DNA, thus increasing the effective molecular weight of the fragments, since, if that were the case one would expect a reduction in mobility in both cases or complete inhibition of the restriction reactions. As this does not happen in the cadmium-treated samples, it is clear that cadmium binding and removal does indeed cause significant 'scars' in the DNA molecule.

The differences obtained between the behavior of the cadmium- and copper-treated samples with respect to these two enzymes pose yet another puzzle. Both metal ions have obviously caused similar damage to the DNA molecule, as seen from the results of Cfo I digestion. However, in the case of Tha I, the copper-treated sample completely inhibits

the enzyme reaction, which leads us to conclude that the exact geometries of the conformational alterations caused by copper and cadmium are not identical to each other; those produced by copper probably at or near the restriction sites of Tha I obviously prevent the formation of a proper enzyme–substrate complex and hence the restriction reaction is inhibited.

In summary, our unique results presented here clearly point out that indeed some type of permanent conformational damage is caused by the transient binding and removal of cadmium and copper ions. This damage is significant enough to interfere with the normal substrate function of DNA towards its enzymes, of which the restriction endonucleases are only a selected group. The mutually opposing nature of the results obtained from the Cfo I and Tha I digestion of the cadmium-treated DNA samples lends strong support to the hypothesis that the conformational damages are attributable to localized bends and curvatures in the DNA molecule. Experiments are in progress in our laboratory at present to further evaluate the nature of these conformational damages and their effects on other types of substrate and template functions of DNA.

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