The Electrophoretic Separation of Curved Cisplatin-Modified DNA Fragments on Polyacrylamide Gels Is Dependent on the Voltage Gradient

Julia Yaneva[a,b] and Jordanka Zlatanova[a,c]

[a] Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331–7305
[b] Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
[c] Institute of Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Z. Naturforsch. **53c,** 921–923 (1998);
received April 27/May 12, 1998

Cisplatin, Curved DNA, Electric Field, Electrophoresis, Polyacrylamide Gels

Polyacrylamide gel electrophoresis has been widely used to study DNA fragments containing sequence-dependent curvature. The anomalous electrophoretic behavior of curved DNA fragments on such gels allows their separation from straight fragments of the same length. Here we demonstrate that polyacrylamide gels can be successfully used to resolve DNA fragments modified at a single site by the antitumor drug *cis*-diamminedichloroplatinum(II) (*cis*-DDP, cisplatin) from their unmodified counterparts. However, the resolution strongly depends on the voltage gradient, being completely lost when it drops below a certain threshold level. The parameters of the electric field do not affect separation of 'normal' DNA fragments of comparable length.

Introduction

Electrophoresis in polyacrylamide gels is a potent method allowing separation of DNA fragments in a wide range of sizes (Lane *et al.*, 1992). It is especially useful for DNA fragments smaller than 100 bp which fail to be resolved on agarose gels. The use of polyacrylamide gel matrix also allows the resolution of DNA fragments of the same length but differing in conformation. Thus, DNA fragments that contain a stable curvature are anomalously retarded on such gels, and can be separated from straight fragments of the same length (Marini *et al.*, 1982; Stellwagen, 1983). The anomalous mobility of curved fragments that underlies such a separation is completely eliminated at 37 °C (Stellwagen, 1983) but does not seem to depend on the strength of the electric field.

Our recent experiments have focused on the binding of some chromatin proteins to DNA modified by the antitumor drug *cis*-diamminedichloroplatinum(II) (*cis*-DDP, cisplatin) (Yaneva *et al.*, 1997; Paneva *et al.*, 1998). The cytotoxic effect of cisplatin is believed to result mainly from its interaction with DNA, via the formation of covalent adducts between certain DNA bases and the platinum compound. The structure of *cis*-DDP-modified DNA has been assessed by biochemical, biophysical, and, more recently, by direct structural studies (for a recent review, see Zlatanova *et al.* (1998)). These studies reveal that the covalent cross-links formed by cisplatin binding to DNA result in drastic structural distortions in the modified DNA. The details of these structural alterations in the DNA double helix differ for the different types of adducts formed (Zlatanova *et al.*, 1998). Nonetheless, all adducts seem to unwind and bend DNA. As expected on the basis of the known electrophoretic behavior in polyacrylamide gels of curved versus straight DNA, the *cis*-DDP-modified DNA fragments are retarded compared to the same fragments in their unmodified form. We (Yaneva *et al.*, 1997; Paneva *et al.*, 1998), and others (reviewed in Zlatanova *et al.* (1998)), have previously shown that this is the case for globally modified fragments which contain multiple and usually different kinds of modified sites. Whether or not DNA fragments containing single-site adducts can be successfully separated from their unmodified counterparts seems less clear.

In this report we present data that fragments containing single *cis*-DDP-modified sites at the center of the molecule are very well resolved from the unmodified control fragments when polyacrylamide gels are run at a given voltage gradient. Decreasing the voltage gradient below a certain threshold leads to loss of resolution. This effect is independent of the temperature at which the gels

Abbreviations: cis-DDP, *cis*-diamminedichloroplatinum(II).

Reprint requests to Prof. Jordanka Zlatanova.
Fax: (541) 737 0481.
E-mail: zlatanoj@ucs.orst.edu.

0939–5075/98/0900–0921 $ 06.00

are electrophoresed. Similar changes in the electric field parameters do not affect separation of 'normal' (unmodified) fragments of similar length.

Experimental Procedures

A 34 bp DNA fragment of the sequence:

5'-GTTGATTGATCAATATGCTCATGTCATGATCAAC-3'

was synthesized and modified with *cis*-DDP for 12–16 hr at 37°C following annealing with its complementary strand. *Cis*-DDP was purchased from Sigma (St. Louis, Missouri, USA). The mixture of modified and unmodified fragments was electrophoresed on 15% polyacrylamide slab gels (100 mm x 70 mm x 1.0 mm) [29:1 acrylamide/N,N'-methylenebis(acrylamide)] in a Vertical Mini-Gel System (Idea Scientific) using a Model 500 BioRad power supply. The gel was run in TAE [40 mM Tris(hydroxymethyl)aminomethane-HCl, pH 8.3, 25 mM acetate, 1 mM EDTA] buffer at room temperature at the voltage gradients denoted in the figures.

Plasmid pUC19 was isolated following standard procedures (Sambrook *et al.*, 1989). Restriction endonucleases HinfI and MspI were purchased from New England BioLabs. Digestions were performed according to the manufacturer.

Results and Discussion

Figure 1 presents the electrophoretic gel patterns of mixtures of unmodified and *cis*-DDP-modified fragments 34 bp in length, obtained at different voltage gradients. The DNA fragment (see Experimental Procedures) is designed to contain a single centered target site for modification, d(GC/GC), which upon incubation with *cis*-DDP creates an interstrand covalent cisplatin adduct between the guanines on the opposite strands. The solution structure of such interstrand adducts has been solved by NMR (Huang *et al.*, 1995; Paquet *et al.*, 1996) and reveals a number of structural alterations to the double helix: extrahelicity of the complementary deoxycytidines, unwinding and local reversal of its handedness, and a bend of ~40° induced by the stacking of the two cross-linked guanines with the surrounding bases. Figure 1 shows that while gels run at 30 and 25 V/cm clearly resolved the modified from the control fragment,

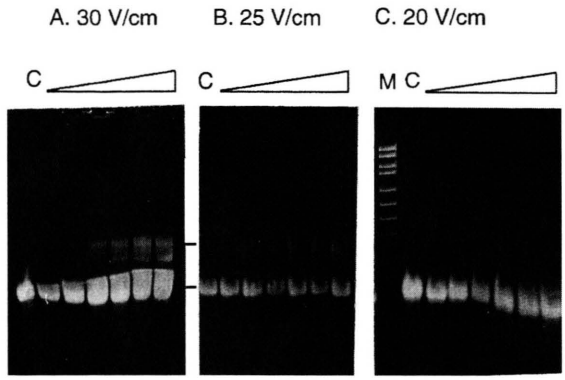

Fig. 1. Polyacrylamide gel electrophoretic pattern of a mixture of unmodified and *cis*-DDP-modified DNA fragments. **C** denotes the unmodified 34 bp fragment; the successive lanes contain DNA modified at increasing Pt/nucleotide molar ratio as follows: 0.0125, 0.0250, 0.0375, 0.0500, 0.075, and 0.1000. Note the increasing amount of retarded modified fragment upon increasing the Pt/nucleotide ratio. Even at the highest ratio used only between 10 and 20% of the DNA was modified, as expected from the low efficiency of formation of the interstrand cisplatin crosslink (Paquet *et al.*, 1996). Lane **M** contains the DNA size marker, *Msp*I/pUC19.

decreasing the voltage gradient to 20 V/cm led to a complete loss of resolution. The effect was evidently not related to the different temperatures of the gels (the heat production is a quadratic function of the field strength, which is proportional to the applied voltage), since the lower voltage gels would produce less heat, and hence would be expected to improve the resolution (Stellwagen, 1983). That the temperature was not a factor in the observed phenomenon follows also from the observation that exactly the same patterns were obtained when the different voltage gels were run in the cold (not shown).

As another control, gels run under the same conditions were used for separation of restriction fragments in a widely used DNA electrophoretic marker. As shown in figure 2, the separation of the marker fragments even in the range of lengths below 100 bp was in no way affected by the parameters of the electric field.

The reason for the observed dependence of the electrophoretic migration of cisplatin-modified DNA fragments on the electric field remains unclear at this point. However, this peculiar phenomenon should be borne in mind in experiments involving polyacrylamide gel electrophoresis as a method to study DNA containing stable bends,

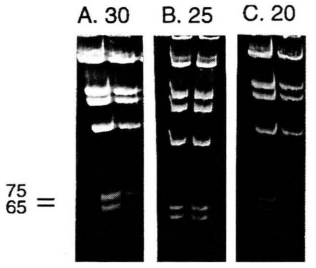

Fig. 2. Polyacrylamide gel electrophoretic pattern of a mixture of several restriction fragments produced by *Hinf*I cleavage of plasmid pUC19. A, B, and C present the patterns produced by running the gels at 30, 25, and 20 V/cm. All other conditions as in figure 1. Note that the separation of fragments 75 and 65 bp in length is good and not affected by the applied voltage gradient.

like the one produced by cisplatin-mediated chemical modifications.

Acknowledgments

This research was funded by NIH grant GM50276 to Kensal van Holde and JZ, by Fogarty International Research Collaboration Award (FIRCA TW00568–02) to KvH and JZ, and a National Foundation for Research grant K529/95, Bulgaria, to JY.

Huang H., Zhu L., Reid B. R., Drobny G. P and Hopkins P. B. (1995), Solution structure of a cisplatin-induced DNA interstrand cross-link. Science **270**, 1842–1845.

Lane D., Prentki P. and Chandler M. (1992), Use of gel retardation to analyze protein-nucleic acid interactions. Microbiol. Rev. **56**, 509–528.

Marini J. C., Levene S. D., Crothers D. M. and Englund P. T. (1982), Bent helical structure in kinetoplast DNA. Proc. Natl. Acad. Sci. USA **79**, 7664–7668.

Paneva E., Spassovska N., Grancharov K., Zlatanova J. and Yaneva J. (1988), Interaction of histone H1 with *cis*-platinum modified DNA. Z. Naturforsch. **53**, 135–138.

Paquet F., Pérez C., Leng M., Lancelot G. and Malinge J.-M. (1996), NMR solution structure of a DNA decamer containing an interstrand cross-link of the antitumor drug *cis*-diamminedichloroplatinum(II). J. Biomol. Str. Dyn. **14**, 67–77.

Sambrook J., Fritsch E. F. and Maniatis T. (1989), Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Stellwagen N. C. (1983), Anomalous electrophoresis of deoxyribonucleic acid restriction fragments on polyacrylamide gels. Biochemistry **22**, 6186–6193.

Yaneva J., Leuba S. H., van Holde K. and Zlatanova J. (1997), The major chromatin protein histone H1 binds preferentially to *cis*-platinum-damaged DNA. Proc. Natl. Acad. Sci. USA **94**, 13448–13451.

Zlatanova J., Yaneva J. and Leuba S. H. (1998), Proteins that specifically recognize cisplatin-damaged DNA: a clue to anticancer activity of cisplatin. FASEB J. **12**, in press.