Acta Biochimica Polonica

Vol. 40 No. 4/1993

QUARTERLY

Short Communication

Cis-DDP induced alteration of DNA structure studied by scanning tunneling microscopy*

Tomasz H. Zastawny[a], Wielisław Olejniczak[b] and Ryszard Oliński[a]

[a]*Department of Clinical Biochemistry, Medical School, M. Skłodowskiej-Curie 9, 85–094 Bydgoszcz, Poland and* [b]*Department of Solid State Physics, Institute of Physics, University of Łódź, S.Banacha 12/16, 90–237 Łódź, Poland*

Received 20 May, 1993

Scanning tunneling microscopy (STM) was used to study the structural changes of DNA induced by the antitumor drug *cis*-diamminedichloroplatinum. The STM image showed a dramatic structural perturbation of the DNA by complexed Pt with the characteristic bend of the double helix.

Cis-diamminedichloroplatinum (cis-DDP) is one of the drugs most commonly used in the treatment of human neoplasia. DNA is generally accepted as the critical intracellular target for the drug, with the major damage being intrastrand crosslinks [1].

However, the biologically significant platinum-DNA lesions induced in DNA by DNA-Pt complexes may not simply be due to adducts *per se*, but to a combination of adducts formation and concomitant perturbation of three dimensional structure of DNA, as has been suggested by Kozelka & Lippard [1 - 3]. Therefore investigation of conformational changes induced in the secondary and tertiary structure in DNA molecules by the binding of cis-DDP[1] is of prime importance.

Scanning tunneling microscope offers attractive prospects for the detailed analysis of DNA structure [4 - 7]. The present results confirm the feasibility of application of this method for determination of the structural changes induced in DNA by the binding of cis-DDP.

MATERIALS AND METHODS

The fully computerized scanning tunneling microscope was used. The microscope was constructed at the Department of Solid State Physics, Institute of Physics, University of Łódź, and was supplemented with the CAMAC controlling unit. For the imaging head, the bimorph type coarse approach system was used. The scanning system was "Tripol" and the computer was connected to the microscope in the IBM PC/AT clone equipment with an SVGA (800 × 600) graphics card and Multisymec color monitor (Mitsubishi).

The STM system was operated in air, in the constant height mode. A voltage of 1.2 - 1.5 V was used and a tunneling current of the medium value of 1.5 nA. Samples (deposited on a vacuum sublimated gold film) were negative with respect to the tip. All images were obtained using electrochemically etched tungsten tips. DNA was dissolved in deionized water at

*This work was supported in part by grant No. 20972 91.01. from the State Committee for Scientific Research.

[1]Abbreviations: cis-DDP, *cis*-diamminedichloroplatinum; NMR, nuclear magnetic resonance; STM, scanning tunneling microscopy.

a concentration of 1 mg/ml. The DNA sample was not sonicated, cis-DDP was dissolved in deionized water and the reaction between DNA and DDP was run at 37°C for 24 h. The nucleotide: Pt ratio (rb) determined by the AAS method was 0.001. For the STM observation a droplet of the solution was allowed to dry in air for 2 h on a gold-plated glass.

RESULTS AND DISCUSSION

Figure 1 shows a fragment of a DNA molecule visualized with a high resolution scanning tunneling microscope. The right handedness of the molecule is clearly visible. The periodicity of the helix ranged from 28 to 32 Å and the width of the DNA was approximately 20 Å. Thus the image shown in Fig. 1 could fit either the A or B model of DNA. Under dehydration, DNA is known to adopt the A conformation [8 - 10]. Since the DNA molecules were dried in air, one would expect them to be in the A form, characterized by a pitch of 28.2 Å and a width of 23 Å. However, local variations in humidity of the environment and the presence of a DNA fragment in B conformation cannot be ex-

Fig. 1. Scanning tunneling microscopy image of DNA.

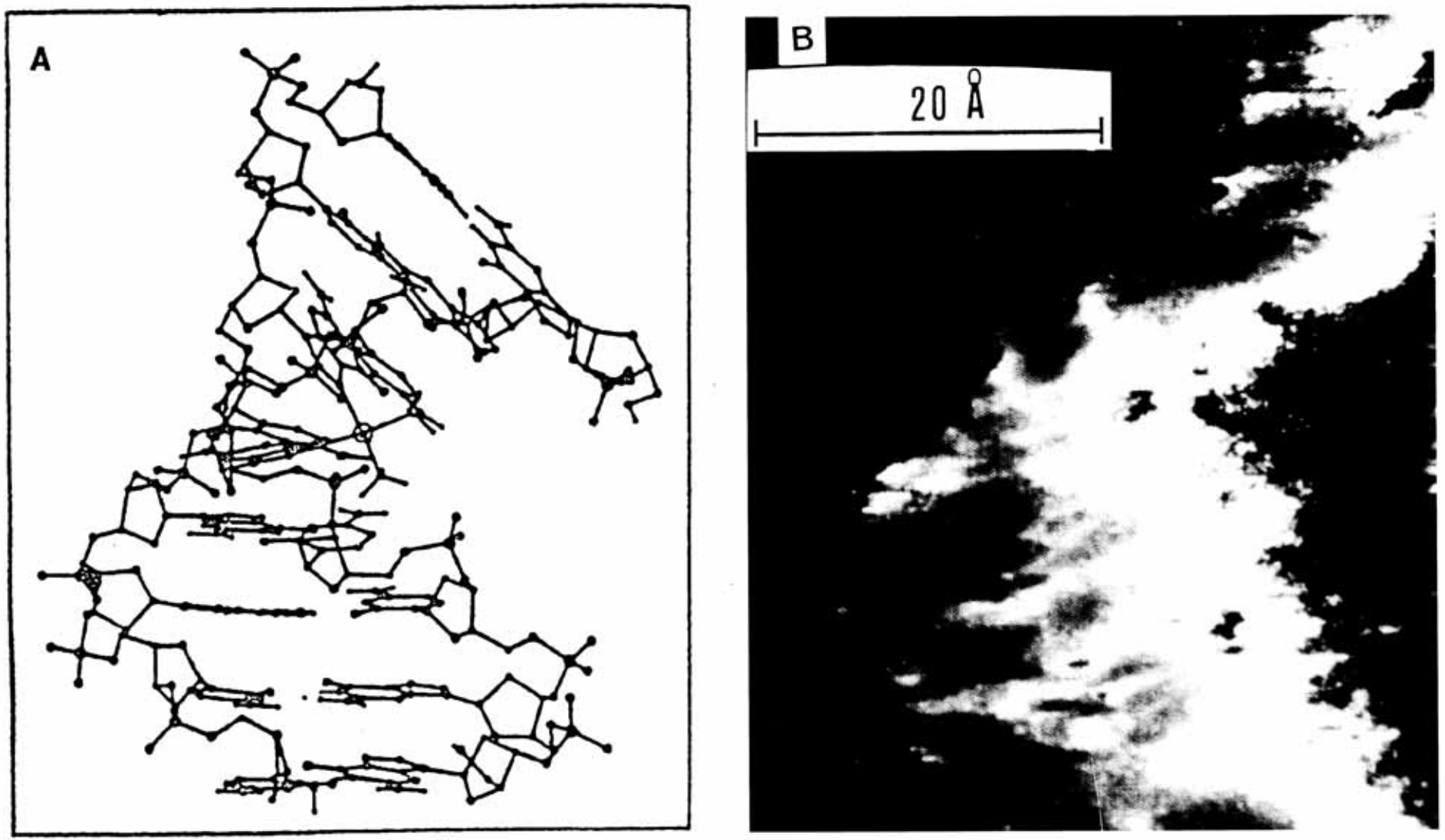

Fig. 2. A. Molecular model of structural perturbations in DNA caused by cis-DDP, proposed by Sanquist & Lippard [1]. B. Scanning tunneling microscopy image of the DNA-Pt complex

cluded. STM images of DNA molecules prepared in a similar way were interpreted by others either as being in B or A conformation [4, 5].

The results presented and discussed above, as well as other studies [4 - 7] proved that STM offers an unique advantages by allowing a close insight into DNA conformation. Consequently, we applied the method to investigate the structural changes of DNA induced by cis-DDP. The image presented in Fig. 2 B shows a dramatic structural perturbation of the DNA structure on formation of the DNA-Pt complex, with the characteristic bend of the double helix. It is interesting that the observed image fits well the theoretical model proposed by Lippard and co-workers [1 - 3] for DNA-Pt complexes (compare A and B in Fig. 2). The disappearance of the features characteristic for the double stranded DNA helix (seen in Fig. 1) may be due to the kink of the helix (see Fig. 2) as well as to Pt induced unwinding and base destacking [1 - 3].

After reaction with DNA cis-DDP can form various kinds of adducts. The principal lesion is the intrastrand link between two adjacent guanines or adjacent adenine-guanine bases [11]. These kinds of adducts account for greater than 90% of the reaction products [11]. They are believed to be responsible for the observed DNA helix distortion [11].

Up to now structural investigations of cis-DDP adducts have been performed using a variety of techniques including NMR spectroscopy and X-ray crystallography. These techniques made possible exact measurements of sugar conformation and hydrogen-bonding interactions, but structural features of duplex DNA containing cis-DDP adducts mostly proved elusive [1 - 3]. Using STM we were able for the first time to visualize structural changes induced in native DNA molecules by the binding of the antitumor drug cis-DDP.

The experiments reported here demonstrate the great potential of the STM technique for precise characterization of DNA and possible investigation of the alteration in DNA conformation induced by cis-DDP. Further studies with defined polynucleotides are in progress to determine the influence of particular adducts on DNA structure.

REFERENCES

1. Sundquist, W.I. & Lippard, S.J. (1990) *Coord. Chem. Rev.* **100**, 293 - 296.
2. Kozelka, J., Petsko, G.A. & Lippard, S.J. (1985) *J. Am. Chem. Soc.* **107**, 4079 - 4081.
3. Kozelka, J. & Chottard, J.-C. (1990) *Biophys. Chem.* **35**, 165 - 178.
4. Beebe, T.P., Wilson, T.E., Ogletree, D.F., Katz, J.E., Balhorn, R., Salmeron, M.B. & Siekhaus, W.J. (1989) *Science* **243**, 370 - 372.
5. Crecenti, A., Selci, A.C., Felici, A.C., Generosi, R., Gori, E., Djaczenko, W. & Chiarotti, G. (1989) *Science* **245**, 1226 - 1227.
6. Arscott, P.G., Lee, G., Bloomfield, V.A. & Evans, D.F. (1989) *Nature (London)* **339**, 484 - 486.
7. Oliński, R., Zastawny, T.H., Olejniczak, W., Wojczuk, S. & Chwiałkowski, M. (1992) *Acta Biochim. Polon.* **39**, 15 - 19.
8. Depew, R.E. & Wang, J.C. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **348**, 147 - 159.
9. Levitt, M. & Greer, J. (1977) *J. Mol. Biol.* **114**, 181 - 293.
10. Ivanov, V., Michenkova, L.E., Minyat, E.E. & Schyolkina, A.K. (1983) *Cold Spring Harb. Symp. Quant. Biol.* **47**, 243 - 250.
11. Bruhn, S.L., Toney, J.H. & Lippard, S. (1990) *Progress in Inorganic Chemistry: Bioinorganic Chemistry* (Lippard, S.J., ed.) **38**, 477 - 517.