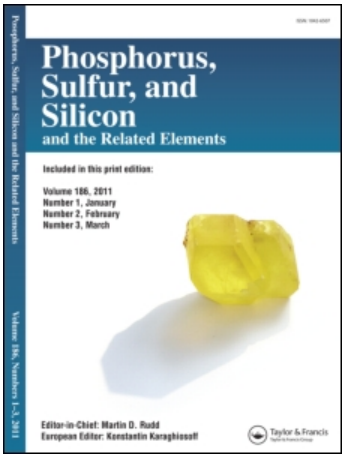

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^{31}P-Nmr as a Probe for Drug-Nucleic Acid Interactions

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The structural impact of covalent and noncovalent interactions of drugs with DNA is an important component for understanding the biochemical and biological consequences of DNA damage. Work in this laboratory has focused on a number of potentially therapeutically important drugs that distort DNA by unwinding, bending DNA into the major or minor groove. These lead to enhanced recognition of DNA by proteins involved in transcription and replication. In this paper, we will present the structures of one of these complexes and show how ^{31}P-NMR can be used to monitor these distortive effects.

Keywords: ^{31}P-NMR; drug-DNA; ecteinascidin; bizelesin; quinolone

SELF-ASSEMBLY COMPLEX OF A 2:2 QUINOBENZOXAZINE–MG^{2+} COMPLEX ON DNA

Introduction

The quinobenzoxazine compounds (typified by A-62176, Figure 1) were initially developed from antibacterial fluoroquinolones (typified by norfloxacin, Figure 1) by scientists at Abbott Laboratories.[1-3] These compounds are active against a number of human and murine cancer cell lines including the multidrug resistant P388/ADR line *in vitro* and several murine and human tumors *in vivo*.[1] An initial study has revealed that the quinobenzoxazines are potent inhibitors of mammalian topo II, both *in vitro* and *in vivo*.[4] According to this study, the quinobenzoxazines appear to belong to the class of topo II suppressors that interfere with the catalytic activity of the topo II at a step prior to the formation of the cleaved complex.

The quinobenzoxazines, which are structurally related to fluoroquinolones, possess a planar tetracyclic ring in place of the fused bicyclic ring of fluoroquinolones (Figure 1). The extended flat aromatic ring structure of quinobenzoxazines enables them to

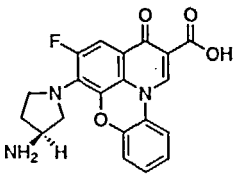

FIGURE 1

form a stable intercalation complex with the DNA helix,[4] while the more limited aromaticity of the fluoroquinolone norfloxacin prevents the formation of a stable intercalation complex with duplex DNA.[5,6] Preliminary data from Abbott Laboratories showed that DNA binding of quinobenzoxazine A-62176, like norfloxacin, is Mg^{2+} dependent.[7] Further biophysical and electrophoretic studies on the ternary complex between the quinobenzoxazine–Mg^{2+} complex and DNA have provided evidence for a 2:2 quinobenzoxazine:Mg^{2+} self-assembly complex, in which one quinobenzoxazine molecule is intercalated into the DNA helix and the second drug molecule is externally bound, held together by two Mg^{2+} bridges.[8]

Results

Mg^{2+} bridges the quinobenzoxazine molecule to the phosphate backbone on DNA

Palumbo and co-workers[7,9] have suggested that the quinolone antibiotics may interact with the phosphate of single-stranded DNA via a bridging Mg^{2+} ion. To explore whether the quinobenzoxazine antineoplastics are bridged via an Mg^{2+} ion to the DNA phosphate backbone, the ^{31}P-NMR spectral changes of $[d(G–C)_5]_2$ in the presence of A-85226 and Mg^{2+} were studied.

Figure 2 shows the ^{31}P-NMR spectra of $[d(G–C)_5]_2$ in the presence and absence of A-85226 in NMR buffer (10 mM NaH_2PO_4 and 100 mM NaCl) containing 100 mM $MgCl_2$. A single resonance signal at –0.88 ppm for all the phosphate groups is observed for $[d(G–C)_5]_2$ (Figure 2A). However, upon addition of A-85226 to the $[d(G–C)_5]_2$ solution, two new resonance signals in the downfield region between –0.72 and –0.10 ppm appear (Figure 2C). For comparison, the intercalator ethidium bromide was also used in this study. Upon addition of ethidium bromide to the $[d(G–C)_5]_2$ oligomer, just one new resonance signal at –0.68 ppm is observed, presumably due to conformational changes on the DNA phosphate backbone induced by intercalation of ethidium bromide into DNA (Figure 2B). This appearance of a new signal slightly downfield from the DNA backbone ^{31}P resonance has been observed in previous studies of

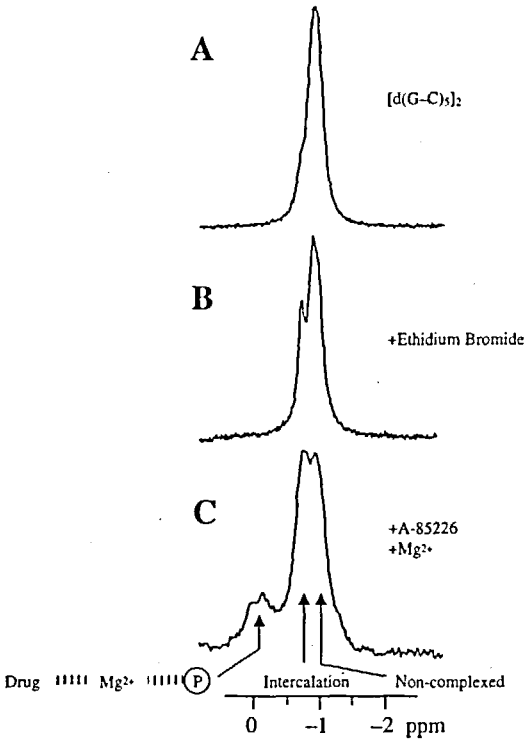

FIGURE 2

DNA–intercalator complexes[10-12] and reflects the local distortion of the backbone that occurs due to formation of the intercalating complex. Significantly, for both ethidium bromide and A-85226, distinct signals are seen for both unperturbed and unwound phosphate backbone ^{31}P resonances, indicating that the exchange between intercalated and free DNA sites is slow on the ^{31}P-NMR time scale for these ligands. Since in the present study the quinobenzoxazine–Mg^{2+} complexes have been confirmed as intercalators, the new downfield signal at –0.72 ppm in Figure 1B can be attributed to intercalation. However, the additional ^{31}P-NMR resonance signal at –0.10 ppm, which is unique to the A-85226–Mg^{2+}–$[d(G–C)_5]_2$ complex, cannot be explained by either purely intercalation or groove binding. The resonance signal at –0.10 ppm must be due to a different effect and is consistent with a distortion of the deoxyribose phosphate

backbone due to formation of an A-62176–Mg^{2+}–(oxygen)phosphate coordination complex.

Acknowledgments

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References

[1] D. T. W. Chu, R. Hallas, J. J. Clement, J. Alder, E. McDonald, and J. J. Plattner, *Drugs Exptl. Clin. Res.*, **18**, 275 (1992).
[2] D. T. W. Chu, R. Hallas, S. K. Tanaka, J. Alder, D. Balli, D., and J. J. Plattner, *Drugs Exptl. Clin. Res.*, **20**, 177 (1994).
[3] J. J. Clement, N. Burres, K. Jarvis, D. T. W. Chu, J. Swiniarski, and J. Alder, *Cancer Res.*, **55**, 830 (1995).
[4] P. A. Permana, R. M. Snapka, L. L. Shen, D. T. W. Chu, J. J. Clement, and J. J. Plattner, *Biochemistry*, **33**, 11333 (1994).
[5] L. L. Shen, J. Baranowski, and A. G. Pernet, *Biochemistry*, **28**, 3879 (1989).
[6] L. L. Shen, L. A. Mitscher, P. N. Sharma, T. J. O'Donnell, D. W. T. Chu, C. S. Cooper, T. Rosen , and A. G. Pernet, A. G., *Biochemistry*, **28**, 3886 (1989).
[7] G. Palù, S. Valisena, G. Ciarrocchi, B. Gatto, and M. Palumbo, (1992) *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 9671 (1992).
[8] J.-Y. Fan, D. Sun, H. Yu, S. M. Kerwin, and L. H. Hurley, *J. Med. Chem.* **38**, 408 (1995).
[9] M. Palumbo, B. Gatto, G. Zagotto, and G. Palù, *Trends Microbiol.*, **1**, 232 (1993).
[10] R. L. Jones and W. D. Wilson, *J. Am. Chem. Soc.*, **102**, 7776 (1980).
[11] W. D. Wilson, R. A. Keel, and Y. H. Mariam, *J. Am. Chem. Soc.*, **103**, 6267 (1981).
[12] W. D. Wilson and R. L. Jones, *Nucleic Acids Res.*, **10**, 1399 (1982).