

OPTIMISATION OF EXPERIMENTAL CONDITIONS FOR OBSERVATION OF DOUBLE-STRANDED DNA USING ELECTROSPRAY IONISATION MASS SPECTROMETRY

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Deoxyribonucleic acid (DNA) is a target of a number of anticancer drugs including cisplatin and daunomycin. These anticancer drugs bind and modify DNA in different ways. The study of the precise nature of DNA-drug interactions is important in understanding the structural basis of antitumour activity and thus will help in the design of new and improved antitumour drugs.

Electrospray ionisation mass spectrometry (ESI-MS) has been successfully employed to study a wide range of covalent and noncovalent interactions of different drugs and proteins with DNA. Most ESI-MS studies concerning the interactions of drugs with DNA have focussed on complexes with single-stranded (ss)DNA. This is in part because ESI-MS conditions are not ideal for preserving stable, double-stranded (ds)DNA. In the cell, however, interactions with drugs involve dsDNA. Determination of ESI-MS conditions where maximum amounts of dsDNA can be observed will aid in evaluation of the utility of ESI-MS for studying drug-DNA interactions. Recently we studied intercalating complexes of daunomycin and nogalamycin with oligodeoxynucleotides as models for cellular DNA.¹ The amount of dsDNA relative to ssDNA was maximised by: (i) annealing DNA in the presence of intercalator in relatively high concentrations of ammonium acetate (0.1 M), (ii) avoiding the use of organic solvents, and (iii) using gentle ionisation conditions.

Although significant amounts of dsDNA-intercalator complexes were observed under these conditions, ssDNA persisted. Intercalators are known to enhance the stability of dsDNA. In the case of drugs using other binding modes (e.g. cisplatin), however, duplex stability may be decreased making observation of complexes with dsDNA in the gas phase challenging.

We have recently defined conditions where dsDNA and platinated dsDNA are more readily observed. Spectra were acquired using a Qtof-2 ESI-mass spectrometer (Micromass, UK). DNA or drug-ssDNA complexes were annealed with their complementary DNA strands in 0.1 M ammonium acetate. Prior to analysis, dsDNA was diluted to a concentration of 1-5 pmol/L using 0.1 M ammonium acetate, pH 8.5. Spectra were acquired in positive ion mode, using a low desolvation temperature (≤ 80 °C), capillary voltage of 2.3 kV and cone voltage in the range (40-60 V).

Results will be presented for various self-complementary and non-self complementary oligodeoxynucleotides with and without bound cisplatin.

1. A. Kapur, J.L. Beck, M.M. Sheil (1999) *Rapid Commun. Mass Spectrom.*, 13, 2489.
