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Positive and negative ion mode ESI-MS and MS/MS for studying drug–DNA complexes

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Abstract

We report systematic investigation of duplex DNA complexes with minor groove binders (Hoechsts 33258 and 33342, netropsin and DAPI) and intercalators (daunomycin, doxorubicin, actinomycin D, ethidium, cryptolepine, neocryptolepine, *m*-Amsacrine, proflavine, ellipticine and mitoxantrone) by ESI-MS and ESI-MS/MS in the negative ion mode and in the positive ion mode. The apparent solution phase equilibrium binding constants can be determined by measuring relative intensities in the ESI-MS spectrum. While negative ion mode gives reliable results, positive ion mode gives a systematic underestimation of the binding constants and even a complete suppression of the complexes for intercalators lacking functional groups capable of interacting in the grooves. In the second part of the paper we systematically compare MS/MS fragmentation channels and breakdown curves in the positive and the negative modes, and discuss the possible uses and caveats of MS/MS in drug–DNA complexes. In the negative mode, the drugs can be separated in three groups: (1) those that leave the complex with no net charge; (2) those that leave the complex with a negative charge; and (3) those that remain attached on the strands upon dissociation of the duplex due to their positive charge. In the positive ion mode, all complexes fragment via the loss of protonated drug. Information on the stabilization of the complex by drug–DNA noncovalent interactions can be obtained straightforwardly only in the case of neutral drug loss. In all other cases, proton affinity (in the positive ion mode), gas-phase basicity (in the negative ion mode) and coulombic repulsion are the major factors influencing the fragmentation channel and the dissociation kinetics.

Keywords: Drug–DNA complexes; ESI-MS; MS/MS; Negative ion mode; Gas-phase basicity

1. Introduction

Since intact noncovalent complexes have been observed for the first time by electrospray mass spectrometry [1], the interest in the field has constantly been growing, and ESI-MS has now analytical applications for the study of noncovalent complexes, notably for the determination of the complex stoichiometries, and for the evaluation of equilibrium binding constants. Still, even 15 years later, the investigation of a new family of complexes requires preliminary tests to assess whether the apparent binding constants are reliable [2,3]. An even more exploratory subject concerns the information that can be obtained on the complexes using all the possible MS/MS techniques [4]. Gas-phase dissociation approaches are extremely attractive because it is the only experimental approach that allows probing the purely intermolecular interactions in the absence of solvent [5].

Our group is particularly interested in the research field of drug–DNA interactions. Parsing the binding free energies in those complexes has also been attempted [6–10]. For drug–DNA interactions, Chaires has proposed a conceptual model that assumes that the observed binding free energy ΔG°_{obs} results from the additive contributions of five terms [8]: ΔG°_{conf} (contribution from conformational changes upon complex formation), ΔG°_{t+r} (losses in translational and rotational degrees of freedom), ΔG°_{hyd} (hydrophobic transfer of drug from the solution into its DNA binding site), ΔG°_{pe} (polyelectrolyte contribution), and ΔG°_{mol} (contribution from the formation of noncovalent molecular interactions between the drug and the DNA). These molecular interactions include hydrogen bond formation, van der Waals interactions and electrostatic interactions. Those different terms can all be quantified or determined experimentally except ΔG°_{mol}. It is only possible to estimate the ΔG°_{mol} contribution due to modification of functional

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groups by performing accurate binding studies and structural studies but this is extremely labor intensive. We therefore try to assess whether MS/MS could help getting experimental data on ΔG°_{mol}.

In order to assess the validity of mass spectrometric techniques for DNA–ligand complexes, we used 12-mer duplex DNA, and a panel of ligands having well-known binding modes. Small molecule interaction with duplex DNA can proceed basically via two mechanisms: intercalation and minor groove binding. The two grooves of DNA differ in size and in topology: the major groove is wider and is generally the recognition region for proteins. The minor groove is narrower and surrounded by the sugar-phosphate-sugar chains. Minor groove binders are synthetic molecules that interact with B-DNA in the minor groove. They have a crescent shape and fit in the minor groove without distorting the double helix. Hydrogen bond donor groups at the inner edge make H-bonds with the base pairs of each strand. The drugs we studied here all have a preference for AT-rich regions of DNA for three reasons: (i) the $-NH_2$ group on guanine causes steric hindrance in the groove; (ii) the minor groove is narrower in AT-rich regions, which is favorable to van der Waals contacts with the sugars; and (iii) the AT regions have a more negative electrostatic potential, which is favorable for the binding of positively charged molecules [11,12]. Minor groove binders can form either 1:1 or 2:1 complexes in a given AT rich site. Intercalators bind to DNA by insertion of their planar aromatic chromophore between DNA base pairs [13,14]. Simple intercalators have few substituents, but others like actinomycin D and doxorubicin are more complex, and substituents can interact with the minor or the major groove and participate to a wide variety of molecular interactions. A conceptual model for intercalation has been proposed [8]. First, DNA must undergo conformational transition (the double helix is unwound) to form the intercalation site. Second, the transfer of the intercalator from the solution to the intercalation site is hydrophobic. Finally, a variety of noncovalent molecular interactions can be formed (stacking of the aromatic rings, hydrogen bonding, van der Waals contacts, ...). These intermolecular interactions are those we would like to probe using MS/MS approaches.

Most ESI-MS investigations of DNA and drug–DNA complexes have been carried out using negative ion mode. This follows logically from the knowledge that the phosphodiester backbone of the DNA has a $pK_a < 1$, and is therefore fully deprotonated under most experimental conditions. Several papers demonstrated that this approach gives reliable apparent binding constants in the case of duplex DNA [15,16], quadruplex DNA [17], and different RNA structures [18,19]. Some papers were recently published on ESI mass spectra of DNA acquired using positive ion mode [20,21]. We therefore wanted to assess whether the positive ion spectra are likely to provide an accurate picture of the drug–DNA complexes present in solution. Comparisons between positive and negative ion modes are reported here. The second part of the paper is devoted to a systematic investigation of MS/MS on DNA–drug complexes, in positive and negative ion modes, in order to address the whys and wherefores of tandem mass spectrometry in these systems. Only a few papers on MS/MS of drug–DNA complexes have been published to date [22–26], our goal was to rationalize these observations in the light of a systematic study complexes of well-known structures.

2. Experimental

2.1. Materials

The duplexes Dk33 (d(CGTAAATTTACG)$_2$), Dk66 (d(CGCGAATTCGCG)$_2$) and Dk100 (d(CGCGGGCCCGCG)$_2$) were prepared by heating 100 μM single strand in 100 mM aqueous NH_4OAc to yield a 50 μM stock solution at neutral pH. The single strands were purchased from Eurogentec (Angleur, Belgium) and used without further purification. The drug stock solutions were 100 or 200 μM in water or methanol for the non-water soluble ones. For ESI-MS, drug–duplex equimolar mixtures (2, 4 and 10 μM) were prepared in 100 mM NH_4OAc and 20% methanol. The concentrations of the stock solutions were remeasured no more than 3 days before the ESI-MS experiments. For ESI-MS/MS, relative concentrations are not critical. Usually 10 μM equimolar solutions were used, but a two-fold excess of drug was used for the weak binders to have more signal for the parent ion. All ligands were purchased from Sigma–Aldrich (www.sigma-aldrich.com), except cryptolepine and neocryptolepine [27] which were donated by Luc Pieters (University of Antwerp, Belgium). Cryptolepine, neocryptolepine, ellipticine and *m*-Amsacrine were solubilized in methanol, and all other ligands are solubilized in bi-distilled water.

2.2. Mass spectrometry

Two papers describing the influence of the collision regime on the dissociation of duplex DNA alone have been published [28,29]. Briefly, collision-induced dissociation (CID) of duplex DNA in the quadrupole collision cell of a QTOF2 instrument produces the single strands which share the available charges of the duplex. Upon CID in the QTOF2, the main fragmentation pathway remains the noncovalent dissociation into single strands at high collision energies, but the loss of neutral base (G, A, C) becomes more and more abundant as the hexapole collision voltage is lowered. It was therefore concluded that high collision energies had to be preferred in order to observe predominantly the noncovalent dissociation channels. In order to probe the drug–DNA interaction energy via the MS/MS, we should observe ideally the noncovalent dissociation of the drug from the duplex. Therefore, we used the QTOF instrument for all MS/MS experiments on drug–DNA complexes presented in this study.

All experiments were performed on a Q-TOF Ultima Global (Micromass, now Waters, Manchester, UK) with the normal ESI source. In negative ion mode the capillary voltage was set to −2.2 kV and the cone voltage to 35 V. The RF lens 1 voltage was set to 25 V for Dk33 and complexes, and to 35 V for Dk66 and Dk100 and complexes. In positive ion mode ESI-MS, the capillary voltage was set to 3.0 kV, the cone voltage to 100 V, and the RF lens 1 voltage to 100 V. In both modes, a hexapole collision voltage of 10 V was used for full scan MS. This collision voltage is proportional to the internal energy or the center