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^{1}H-NMR study of the interaction of distamycin A and netropsin with the parallel stranded tetraplex [d(TGGGGT)]$_4$†

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The first ^{1}H-NMR investigation of the reversible interaction of two small minor groove binding molecules with a synthetic tetraplex DNA structure is reported.

Nucleic acids reversibly interact with a broad range of small organic molecules, such as intercalators or minor groove binders, which represent one of the most important lines of drug development and of current chemotherapy against cancer, viral, and some parasitic diseases. Even though these compounds are known to bind to double stranded DNA, several investigations involving the interaction of small ligands with unusual structures of nucleic acids have been reported,[1–3] including multistranded tetraplex forms.[1,4–7] A multitude of proteins have been shown to interact with these structures which, particularly, are implicated in the molecular biology of telomeres.[8–10] A high level of telomerase activity has been associated with cancer cells and may be essential for their immortality. G-quartet DNA[11] inhibits telomerase activity and, therefore, compounds that bind to this arrangement can negatively interfere with telomerase activity. This is the strongest argument in favour of the potential role of G-tetraplexes as a potential target for anticancer drug design. In this frame, we wish to report here the preliminary results from a NMR investigation of the reversible interaction of the minor groove binding agent distamycin A (Dist-A) and the related drug netropsin (Net) with the synthetic tetraplex [d(TGGGGT)]$_4$.

A wide array of G-tetraplex topologies, groove widths, loop conformations, and alternative DNA base associations have been observed to date.[4–7] The structure of the four stranded hexamer [d(TGGGGT)]$_4$ used for the present study has been already characterized by NMR and X-ray crystallography.[12] This tetraplex possesses a four-fold symmetry with all strands parallel to each other, which afford four grooves of identical medium width, and all nucleosides in an anti conformation. The 1D NMR proton spectra (T = 300 K; 500 MHz), recorded at several points during the titration of the tetraplex with Dist-A, are shown in Fig. 1. The first addition of 0.5 mol equiv. of Dist-A to the oligonucleotide caused several notable variations in the spectrum. Particularly, a general change in chemical shift of DNA proton resonances was observed, whereas a new set of broad signals appeared between 6 and 7 ppm which were assigned to the drug protons. An increase of Dist-A concentration up to 2 mol equiv. caused drug resonances to gradually grow in intensity and a progressive drift of DNA signals. Anyhow, during the early stage of the titration, the four strands were magnetically equivalent upon interaction with the drug. Surprisingly, at nearly 2:1 ligand–tetraplex stoichiometry, a further addition of drug caused a complication in the spectrum due to the presence in solution of different species in slow exchange on the chemical shift time scale. In fact, a new set of proton signals could be observed, whose intensities rose with increasing amounts of drug with a concomitant falling off of the original signals, which completely disappeared at a ratio of 4:1 drug–DNA. At this point, in spite of a general broadening of all signals, a single, well-defined species, was plainly observable in solution and any further addition of ligand did not lead to substantial changes. The final NMR spectrum showed that the binding of the ligand to the tetraplex caused the loss of the original four-fold symmetry of the free tetraplex. Particularly, 8 imino proton, 4 methyl and 12 aromatic proton resonances were clearly discernible, thus pointing to a two-fold symmetry for the 4:1 complex.

In order to clarify the binding mode of Dist-A, several NOESY spectra at 300 K (500 MHz) of the final complex were acquired at different mixing times (250, 180, 100, and 50 ms). The whole of the data provided by these experiments allowed us a preliminary conclusion that the tetraplex retained the original

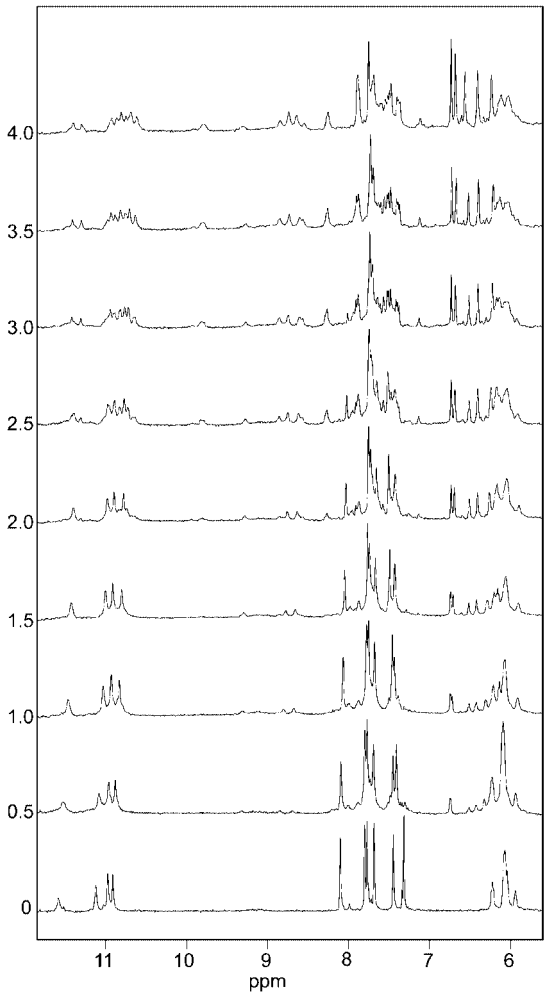

Fig. 1 Titration with Dist-A of 1.4 mM solution of [d(TGGGGT)]$_4$ containing 2 mM phosphate buffer, 0.2 mM EDTA and 70 mM KCl, pH 7.3, in 9:1 H_2O–D_2O at 300 K (500 MHz). ^{1}H NMR spectra were recorded using pulsed-field gradient WATERGATE[15] for H_2O suppression. A time domain deconvolution was used to further reduce the H_2O signal. The drug–DNA mole ratios are shown along the side of the spectra.

† Electronic supplementary information (ESI) available: 2D NOESY and ^{1}H NMR spectra for netropsin/tetraplex and distamycin/tetraplex complexes. See http://www.rsc.org/suppdata/cc/b1/b100460n/

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conformation upon binding of the drug, since all intranucleotide and internucleotide connectivities of the free tetraplex were still visible. However, the evaluation of possible perturbation at the binding sites, such as small changes of the glycosidic bond angle, would require a comprehensive analysis of the NOE buildup rate and a quantitative analysis of internucleotide base to sugar NOE intensities, which have not been carried out at the present. Furthermore, the presence of ligand–ligand contacts between formyl hydrogen (FH) at 7.68 ppm and amidinium protons at 9.75 ppm and among NH1 amide proton at 8.25 ppm, C20 hydrogen at 1.49 ppm and the afore cited hydrogen at 9.75 ppm, indicated that the drug molecules simultaneously bound to the tetraplex two by two, with each term of the dimeric pairs with an antiparallel orientation and in close contact to its partner (as observed with duplex DNA).[13] An alternative explanation, exchange between two orientations of one molecule, so that the ligand–ligand NOEs arise from a combination of transferred NOE and exchange effects, could be ruled out since the NOE contacts were still visible in the NOESY spectra acquired at short mixing time (*i.e.* 50 ms), where spin diffusion phenomenon is dramatically reduced.

NOESY spectra also showed DNA–ligand contacts between imino proton at 11.28 and C20 hydrogen at 1.49 ppm and imino proton at 11.38 and both NH1 amide proton at 8.25 ppm and amidinium protons at 9.75 ppm. Other cross peaks in correspondence with DNA and ligand resonances were present in the NOESY spectra but line broadening and signal overlapping prevented us from unequivocally ascertaining which of them were actually due to ligand–DNA contacts.

It is noteworthy that, throughout the whole titration, a single set of signals was present for Dist-A protons, which only grew in intensity and did not show any significant change in chemical shift values with increasing drug concentration. This observation suggests that, (i), even at low ligand–DNA stoichiometries (*e.g.* 0.5 : 1), simultaneous binding of two Dist-A side by side, in a highly cooperative mode, is dominant; (ii), both in the 2 : 1 and in the 4 : 1 complexes, the bound pair of Dist-A, reorientates itself in a fast process on the NMR time scale, similar to that observed for the binding of Dist-A to duplex DNA structures.[14]

In conclusion, the above preliminary results can be interpreted as follows. Below 2 : 1 ligand–tetraplex stoichiometry, Dist-A, in a dimeric form, binds each groove of the tetraplex to form short-lived complexes on the NMR time scale. Therefore, only one set of signals for the four strands is observed. The fast exchange behavior of the lower complex (2 : 1 Dist-A–tetraplex) could be changed only to an intermediate regime by decreasing the temperature of the system. As the temperature was reduced from 300 to 280 K, there was only a general broadening of the resonances, with peaks belonging to Dist-A broadening more than the other peaks, probably due to the slower reorientation of the Dist-A dimer. At higher drug–DNA ratios, a second Dist-A dimer tightly and specifically binds the tetraplex, to give a 4 : 1 complex, in slow exchange with the 2 : 1 complex, as indicated by the presence of separate proton resonances for the two species. This behavior can be explained assuming that binding of the second drug pair is more favorable than binding of the first one. Finally, the dyad symmetry of the 4 : 1 complex is consistent with a model comprising two Dist-A dimers simultaneously spanning, in fast reorientation, two opposite grooves of the tetraplex, as illustrated in Fig. 2. Notably, a fully saturated complex with all grooves occupied by drug molecules was not observed. Binding of dimer Dist-A may, most likely, expand the binding groove (as observed with duplex DNA),[13] and simultaneously reduce the size of the two adjacent grooves, thus preventing a further interaction with other Dist-A molecules.

Addition of Net to the oligonucleotide (data not shown) caused gradual changes in the chemical shift and a broadening of DNA proton resonances. At a ligand–DNA ratio of 4 : 1 the titration was virtually completed. The four strands were found to be magnetically equivalent throughout the titration, since no splitting of resonances was observed at any stage. This behavior

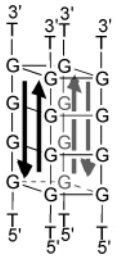

Fig. 2 Pictorial illustration of the proposed binding mode of Dist-A (arrows) to parallel-stranded tetraplex $[d(TGGGGT)]_4$.

could be explained assuming that the ligand is in fast exchange on the NMR time scale with its binding sites on the tetraplex. This fast exchange behavior could not be changed to a slow or intermediate regime by altering the temperature of the system. NOESY experiments at 300 K (500 MHz) performed at different mixing times did not show any long-range ligand–ligand interactions, whereas only a single ligand–DNA contact between H3 proton at 6.43 ppm and aromatic DNA proton at 7.72 could be unambiguously identified. These data can be tentatively explained assuming that electrostatic repulsion between the ends of the doubly charged Net molecules prevents their side-by-side arrangements into the grooves. Thus, in contrast to Dist-A, netropsin complexes the tetraplex with one drug molecule bound per groove. As for Dist-A, a comprehensive analysis of NOESY spectra showed that the structure of the tetraplex remained similar to the original conformation upon binding of netropsin, as indicated by the presence of all the intranucleotide and internucleotide connectivities of the free tetraplex.

The present communication represents the first ^{1}H-NMR investigation of the reversible interaction of groove binding agents with a tetraplex structure of DNA. Detailed structural analysis of the above 4 : 1 distamycin A–DNA complex as well as studies involving other DNA sequences and/or ligands are currently in progress.

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