Probing the Interaction of Hoechst 33258 with an A–T Rich Oligonucleotide Duplex using ^{1}H NMR Spectroscopy

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Changes in the ^{1}H chemical shifts values for resonances of the decanucleotide duplex d(GGTAATTACC)$_2$ provide an NMR 'footprint' of the interaction of the synthetic dye Hoechst 33258 with the DNA minor groove.

Hoechst 33258 (see Fig. 1) is a fluorescent DNA stain[1] that binds specifically to the minor groove of A–T rich DNA sequences.[2,3] Specific recognition features, identified in several X-ray crystal structures[4–6] and NMR studies[7,8] of complexes of the ligand with DNA, are a deep narrow minor groove with the adenine N3 and thymine O2 on the floor of the groove forming specific hydrogen bonds with NHs on the planar aromatic benzimidazole rings of the drug. The structures and binding orientations presented in Fig. 1(*B*) have been described to date[4–8] and, together with chemical footprinting methods[2,3] employing natural DNA, have identified a binding site size of four base pairs with a minimum requirement for three consecutive A–Ts. While several of these earlier structural studies[4,6] proposed a degree of G–C binding selectivity, associated with accommodating the bulky *N*-methylpiperazine ring of the dye in a wider region of the minor groove, others[5,7,8] have identified binding exclusively within a tract of four A–T base pairs. Some ambiguity regarding the sequence selectivity of this compound still remains despite this body of structural data.

Presented with the decamer duplex d(GGTAATTACC)$_2$, containing twice the minimum A–T requirement, we envisage the possibility of five distinct binding sites in solution, as presented in Fig. 1(*C*), based upon the structural data so far reported. In this study we describe the use of ^{1}H NMR spectroscopy to 'footprint' Hoechst 33258 with the decamer duplex and probe the sequence selectivity of the ligand for A–T rich DNA using this model DNA fragment.

One-dimensional ^{1}H NMR titration studies with drug : duplex ratios between 0 : 1 and 1 : 1 (see Fig. 2) indicate that the asymmetric ligand binds with a dissociation rate constant which places the complex in slow exchange with its components, on the NMR chemical shift time scale. The consequence of this is that the dyad symmetry of the duplex that leads to an NMR equivalence of the two strands for the ligand-free duplex is lifted in the complex. NMR signals are observed for the two non-equivalent strands. Moreover, it is apparent that the ligand binds with high affinity to a single site on the duplex d(GGTAATTACC)$_2$. We see no evidence for multiple, slowly exchanging conformations of the complex that might suggest ligand binding to more than one of the five postulated sites. This is well illustrated by the effects of ligand binding on the thymine methyl resonances. During the course of the titration the three resonances clearly observed between δ 1.0 and 1.4 for the ligand-free duplex are replaced by six resonances characteristic of the asymmetric 1 : 1 complex.

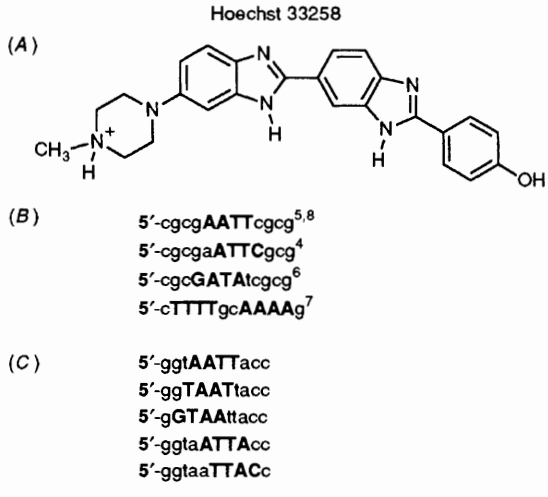

Fig. 1 (*A*) Structure of Hoechst 33258. (*B*) Drug–DNA complexes described to date with bold capitals representing the ligand binding sites. (*C*) Possible binding sites for Hoechst 33258 on the decamer duplex d(GGTAATTACC)$_2$.

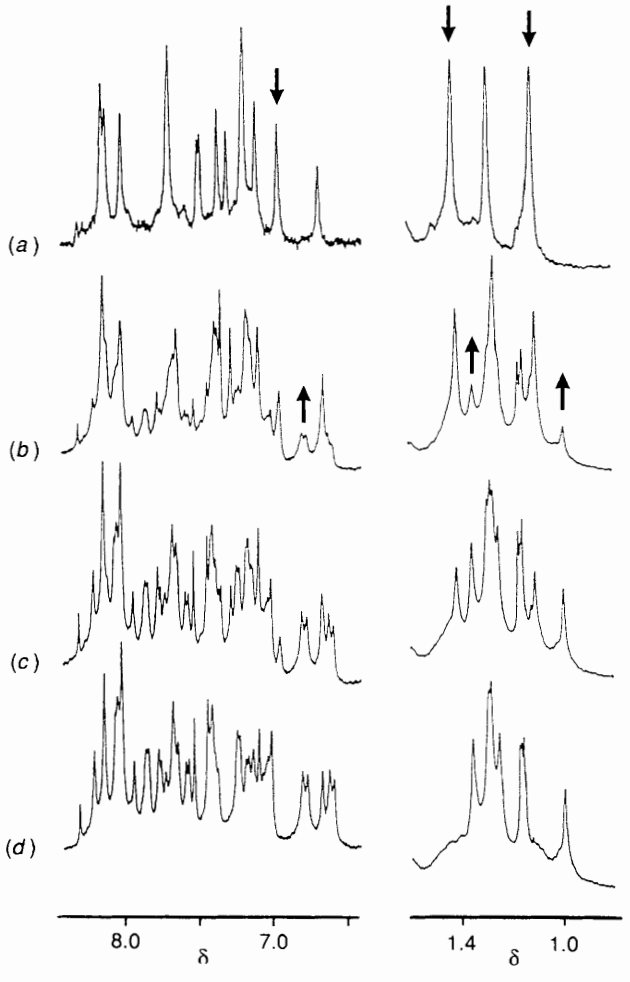

Fig. 2 Aromatic (δ 6.0–8.0) and methyl (δ 1.0–1.4) portions of the 400 MHz ^{1}H NMR spectrum of d(GGTAATTACC)$_2$ recorded at 20 °C. Spectra are labelled: (*a*) ligand-free duplex; arrows identify a few of the resonances that disappear during the titration, (*b*) ligand : duplex ratio of 0.4 : 1; arrows identify resonances from the 1 : 1 complex that increase in magnitude during the titration, (*c*) ratio 0.6 : 1, (*d*) ratio 1 : 1. Solutions of oligonucleotide were buffered with 10 mmol dm^{-3} phospahte, 50 mmol dm^{-3} sodium chloride at pH 7.0.

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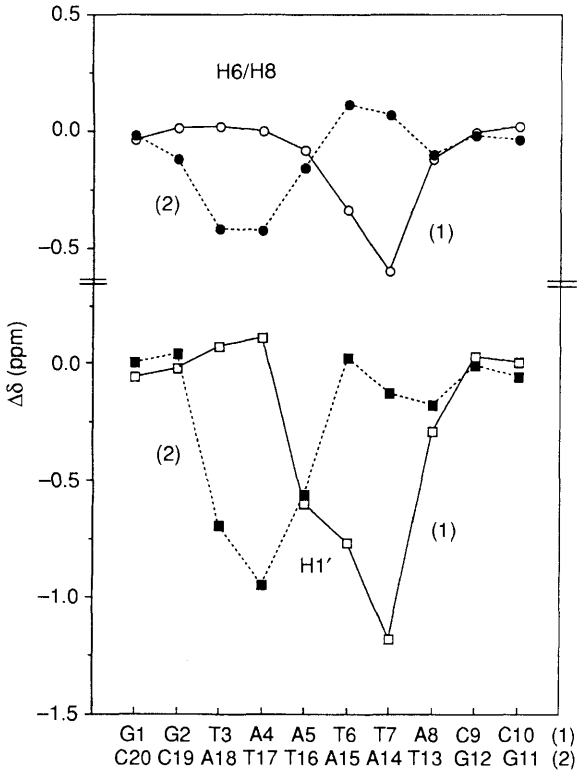

Fig. 3 Changes in ^{1}H chemical shifts values ($\Delta\delta$) at 20 °C for the H6/H8 and H1′ resonances of d(GGTAATTACC)$_2$ upon complexation with Hoechst 33258. Positive shift differences correspond to resonances that move downfield in the presence of the ligand.

Indeed, the aromatic region of the COSY spectrum of the 1 : 1 complex (data not shown) reveals one set of cross-peaks belonging to the bound ligand in a single complex. We are readily able to account for all resonances in the spectrum of the 1 : 1 complex in terms of a single asymmetric species and can discount the possibility of there being equal proportions of a symmetrical 2 : 1 complex and the ligand-free duplex on the basis that signals from the later are monitored during the titration and have disappeared when an equimolar ratio is reached (see Fig. 2).

Although the data are consistent with a slow rate of dissociation of the complex this does not preclude the possibility of fast 'shuffling' between a number of sites by the bound ligand. To examine more closely this latter possibility we present a ^{1}H NMR 'footprint' of Hoechst 33258 binding to d(GGTAATTACC)$_2$. The pattern of perturbations to the ^{1}H chemical shifts of the DNA resonances induced by the Hoechst dye are presented in Fig. 3 for the base H6/H8 and deoxyribose H1′. Resonance assignments are based upon a detailed two-dimensional NMR study to be presented elsewhere. The largest chemical shift perturbations are found within the -AATT- core of the duplex. The H1′ resonances, which are positioned within the minor groove, experience large ring-current shifts from the bound ligand of >1 ppm, in some cases. The graphs for the two strands illustrated in Fig. 3 intersect at the centre of the duplex, highlighting an element of symmetry about the dyad axis. The resonances of one of the T–A base pairs flanking the -AATT- core of the duplex are perturbed more than the others, which is consistent with the asymmetric structure of the drug molecule. The data support the notion that the Hoechst dye is located within the four base pair -AATT- segment of the sequence and correlate well with the pattern of complexation shifts reported for the binding of Hoechst 33258 to the -AATT- segment of d(CGCGAATTCGCG)$_2$. Thus, from our own work and that of Parkinson *et al.*[8] it is clear that the -AATT- sequence carries specific recognition features that are relatively unperturbed by the nature of the flanking base pairs. Despite the fact that all six A–T base pairs within the -TAATTA- tract present the opportunity for similar intermolecular hydrogen bonding networks, the -AATT- core appears to possess the necessary properties for high affinity binding. In all likelihood this remains the region with the narrowest minor groove. No evidence is found for the degree of G–C selectivity previously reported in the crystalline studies of Hoechst–DNA complexes, nor are the data consistent with a 'shuffling' of the ligand between a number of significantly populated sites.

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