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^{1}H NMR determination of the self-association of an acridine homodimer and its complexation with ethidium bromide in aqueous solution

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Abstract

^{1}H NMR spectroscopy (500 MHz) has been used to investigate the self-association in aqueous buffered solution of a bis-intercalator, acridine homodimer (AcrH), and its hetero-association with the aromatic dye, ethidium bromide (EB). The equilibrium constants and thermodynamical parameters (enthalpy and entropy) of self-association have been determined from the observed concentration and temperature dependences of chemical shifts of AcrH protons and the results are consistent with a model consisting of at least four distinct forms of AcrH molecules in solution: unfolded (U), folded (F), a dimer formed from two folded molecules (F_2) and a trimer formed from three folded molecules (F_3). It has also been shown that ethidium bromide complexes strongly to the dimer form (F_2) of the bis-acridine molecule, AcrH. Comparison of the calculated association parameters of AcrH with the previously studied ethidium homodimer (EBH) revealed a correlation between the effectiveness of complexation and the length of chain connecting the chromophores of a bis-intercalator.

Keywords: Acridine homodimer; Ethidium bromide; Self-association; Hetero-association; NMR spectroscopy

1. Introduction

It has been demonstrated that chemical linking of DNA intercalating compounds can lead to composite molecules able to bind to DNA with a very high affinity constant and be very specific in their base pair recognition [1–7]. Some natural and synthetic bis-intercalators have substantial anti-cancer activity and in certain cases are able to overcome multi-drug resistance in cultured cells [6–8]. In addition, they can provide probes for large-amplitude DNA dynamics and may also serve as models for some types of protein–DNA interactions [9].

Over the years a range of bis-intercalators, based on acridine, phenanthridine and anthracycline units connected with linkers of different structure and length, has been synthesized [6,7,10,11] and their complexation with DNA studied, because DNA is considered to be the primary target in their biological action [1–7]. Although a number of models of drug-DNA interaction have been suggested, they are all based on the assumption that a bis-intercalator may exist in an open or unfolded (U) conformation and a closed or folded (F) conformation [3,12]. A few studies on the self-association of acridine and phenanthridine dimeric dyes in solution have provided evidence of the existence of the U- and F-forms in solution [10,11,13,14]. In addition, studies on the interaction of bis-intercalators with mono- and dinucleotides have also been performed in order to gain insight to the specificity of drug-DNA complexation [13,15]. It has been shown that the DNA monomeric units, as well as some other monomeric aromatic ligands, are able to intercalate into the F-form of a bis-intercalator [13,15]. Bearing in mind that monomeric acridines and phenanthridines effectively self-associate in solution via π–π stacking [16], the self-association of bis-intercalators may also be anticipated.

Early NMR studies on the self-association of ethidium (EBH) and acridine (AcrH) homodimers indicated very little probability of the formation of higher order aggregates than U- and F-forms in solution [11,13]. On the other hand, a spectrophotometric titration of AcrH revealed specific spectral changes attributed by the authors to higher order aggregation involving the complexation of the monomeric forms of AcrH molecules in solution [12,14]. We have recently shown by high field NMR that EBH molecules associate in solution in a complicated fashion resulting in mutually intercalated dimers

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and trimers formed mainly of the folded form of the molecules [17]. It was also shown that the folded structure of EBH formed strong hetero-association complexes with propidium iodide, and appeared to be an effective trapper of the aromatic dye [17]. Based on these findings the same mode of the association in solution may also be anticipated for another bis-intercalating compound, acridine homodimer, which is structurally close to ethidium homodimer. In the present work we have studied by 500 MHz ^{1}H NMR spectroscopic methods the self-association of a acridine homodimer and its hetero-association with the phenanthridine dye, ethidium bromide.

2. Experimental

Acridine homodimer ('molecular probes') and ethidium bromide ('sigma') were lyophilized from D_2O and re-dissolved in 0.1 M solution of HEPES buffer in 99.95% D_2O, pD 7.5, containing 10^{-4} M EDTA. The structures of AcrH and EB are shown in Fig. 1.

500 MHz ^{1}H-NMR spectra were recorded on a Bruker DRX spectrometer. Signal assignments of the non-exchangeable protons of AcrH and EB were obtained using two-dimensional homonuclear TOCSY and ROESY experiments. Chemical shift measurements of the non-exchangeable protons of aromatic molecules were made as a function of concentration at 298 and 308 K in the experiments on the self-association of AcrH (Fig. 2(a)) and at 298 K for AcrH-EB hetero-association (Fig. 3(a)); measurements as a function of temperature were made at constant concentration of AcrH and EB (Figs. 2(b) and 3(b)) in the temperature range 278–348 K. Proton chemical shifts were measured relative to an internal reference TMA (tetramethylammonium bromide) and recalculated with respect to DSS (sodium 2.2 dimethyl 2-silapentane-5-sulphonate). All NMR experiments were made in the fast-exchange condition on the NMR time-scale.

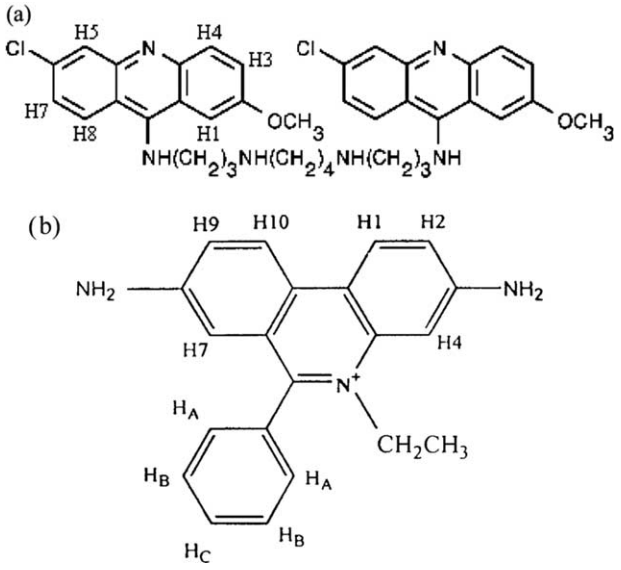

Fig. 1. Structure of (a) acridine homodimer and (b) ethidium bromide.

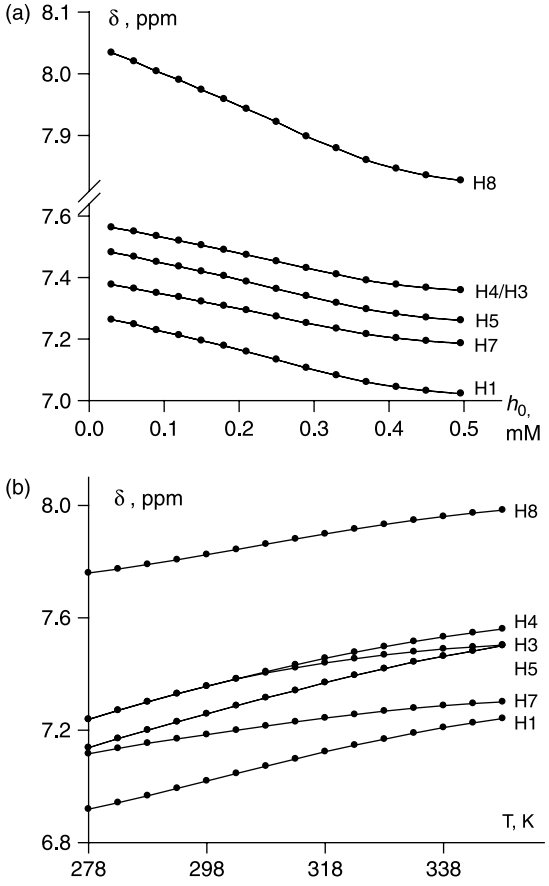

Fig. 2. Dependence of proton chemical shifts of AcrH on: (a) concentration (T=298 K); (b) temperature (h_0=0.496 mM) in aqueous solution, pD 7.5.

3. Results

3.1. Self-association of acridine homodimer

Signal assignments of all the non-exchangeable protons of the ^{1}H NMR spectrum of AcrH in aqueous solution were obtained using both two-dimensional homonuclear TOCSY and ROESY experiments and are in good agreement with the results published previously [13]. The concentration and temperature dependence of proton chemical shifts of AcrH presented in Fig. 2(a) and (b), respectively, exhibit typical behaviour for the association process, i.e. low frequency shift of the concentration curves on increasing the concentration and high frequency shift on increasing the temperature. The behaviour is markedly different from that reported previously for self-association of the bis-intercalator, EBH, in which the experimental concentration and temperature curves for EBH protons were characterized by a highly non-monotonic profile and explained by a competitive contribution of two processes, the formation of dimers (F_2) and trimers (F_3) of EBH molecules in solution [17]. The competitiveness originated from a pronounced shielding of aromatic protons in the F_2 form and concomitant deshielding in the F_3 form due to the electric charges located on the linker chain of EBH. In contrast, the bis-acridine molecule studied in the present work has a pK_a significantly lower (<7) than its monomeric acridine unit