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DNA binding of a proflavine derivative bearing a platinum hanging residue

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ABSTRACT

New platinum(II) complex of 3,6-diamine-9-[6,6-bis(2-aminohethyl)-1,6-diaminohexyl]acridine, AzaPt, has been synthesised and characterised. Behaviour of AzaPt in solution (protonation and possible self-aggregation phenomena) has been investigated by spectral methods (absorbance and fluorescence) at $I = 0.1$ M and 25 °C, and the equilibrium parameters of binding to calf thymus DNA have been established. Two different modes of DNA binding by the complex were detected, which depend on the polymer to dye molar ratio (P/D). At relatively low P/D values the mode was interpreted as binding by the polyamine residue external to the base pairs, while at high P/D values the binding corresponds to intercalation of the proflavine residue. Such interpretation is supported by the observed salt effect on binding and the temperature variation of the binding constants, which allowed estimating the ΔH and ΔS values contributions. Spectrophotometric analysis of the long time range binding revealed that AzaPt is involved in a slow reaction, interpreted as an attack by the platinum ion on the nucleobases. The time constant for such interaction was calculated and found to be the same order of magnitude as for processes responsible for the action of anti-tumour drugs that do covalently bind to polynucleotides.

1. Introduction

Acridines are compounds known for a long time to interact with DNA by intercalation [1,2]. For this reason and due to their interesting spectral properties [3,4], these compounds found wide use in pharmaceutical and dye industries.

Approved by the Food and Drug Administration in 1978, cisplatin, carboplatin, and oxaliplatin are platinum anticancer drugs that are currently administered for the treatment of several forms of cancer [5–7]. While progress has been made in elucidating the mechanism of cisplatin action [8–10], overcoming side effects [5] and drug resistance [11] proved to be difficult. Consequently, research effort into design of new platinum compounds continues, and the aim is to improve significantly their efficiency in cancer treatment [12–14], as well as reduce undesirable side effects.

As intercalation itself was found to underpin the activity of many anticancer agents [15,16] and the binding to nucleic acids constitutes a prelude to a slow attack of Pt(II) on the nitrogen base donors [17,18], it appeared promising to synthesise a bifunctional molecule that bears both an intercalating acridine moiety and a separate Pt(II) centre. The intercalating aromatic residue provides an anchorage for the molecule on a double helix, whereas the metal centre may also enter into the helix or reach out towards the polymeric backbone, exerting a given function there [19]. Some bifunctional acridine-platinum complexes, targeted as potential anticancer drugs, where the platinum bearing pendant residue was linked to the acridine N10 [17] C3 or C6 [18,20] atom, have been successfully tested [21,22].

This paper reports on the synthesis and binding properties of a metallointercalator (AzaPt, Fig. 1), characterized by a proflavine unit and a diethylenetriamine moiety coordinated to a platinum(II) ion to calf thymus DNA. Here, the metal containing fragment is linked to the C9 position of the acridine group. Thermodynamic aspects of complex interaction with DNA, via fast non-covalent linkage and slow covalent attack on the bases of nucleic acid, were investigated.

2. Experimental

2.1. Materials

All chemicals employed for the synthesis of the platinum(II) complex of 3,6-diamino-9-[6,6-bis(2-aminoetyl)-1,6-diaminohexyl]acridine (AzaPt, Fig. 1, for its synthesis see ref. [19]) and all subsequent studies were reagent grade and were used without further purification. $Pt(DMSO)_2Cl_2$ used in the synthesis of AzaPt was prepared according to a reported procedure [23].

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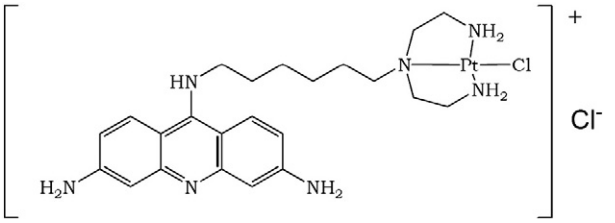

Fig. 1. Chemical structure of the platinum(II) complex of 3,6-diamino-9-[6,6-bis (2-aminoetyl)-1,6-diaminohexyl]acridine (AzaPt).

2.1.1. Synthesis of AzaPt·3DMSO

A solution of 3,6-diamino-9-[6,6-bis(2-aminoetyl)-1,6-diaminohexyl] acridine (52.0 mg, 0.127 mmol) in MeOH (6 mL) was added, over 1 h, to a boiling suspension of $Pt(DMSO)_2Cl_2$ (56.5 mg, 0.134 mmol) in MeOH (12 mL). The suspension was refluxed for 4 h and then left overnight at room temperature. The excess of $Pt(DMSO)_2Cl_2$ was removed by filtration and the resulting solution evaporated in vacuum to dryness. Red residue obtained was dissolved in minimum amount of hot MeOH and left to crystallize. The complex was collected by filtration as a dark orange powder and dried in vacuum at room temperature. Yield 30 mg (26%); ^{1}H NMR (D_2O, pH 2, 400 MHz) $\delta = 7.64$ (doublet, 2 H), 6.63 (d, 2 H), 6.25 (singlet, 2 H), 3.55–3.12 (multiplet, 8 H), 1.62–1.24 (m, 8 H); ^{13}C NMR (D_2O, pH 2, 75.4 MHz) $\delta = 160.0$, 147.7, 143.5, 124.4, 123.1, 110.7, 108.0, 46.0, 45.8, 35.2, 32.1, 27.8, 26.7; electrospray ionization mass spectrometry (ESI-MS): m/z: 639.27 (Calcd. 640.11) ($[C_{23}H_{35}N_7PtCl]^+$), 319.63 (Calcd. 320.06) ($[C_{23}H_{35}N_7PtCl]^{2+}$); elemental analysis calcd (%) for $C_{29}H_{53}N_7S_3O_3PtCl_2$: C 38.27, H 5.87, N 10.77; found: C 38.5, H 5.9, N 10.8.

AzaPt dye solutions were prepared by dissolving the required amount of the solid in doubly distilled water and keeping them in the dark at 4 °C; their concentration is expressed as molarity and labelled as C_D. Calf thymus DNA (CT-DNA) was purchased from Sigma as the lyophilised sodium salt. It was dissolved in water and sonicated as described below. Stock solutions of the polynucleotide were standardised spectrophotometrically, using the value $\varepsilon = 13200\ M^{-1}cm^{-1}$ at 260 nm provided in the sample certificate, and their concentrations are expressed as molarity of the base-pairs. Poly(dA-dT)•poly(dA-dT) was purchased from Sigma, dissolved in 0.1 M NaCl at pH = 7.0 and standardised spectrophotometrically by using the value $\varepsilon = 13200\ M^{-1}cm^{-1}$ at $\lambda = 262$ nm (molarity of base pairs) [24]. The nucleic acid concentration in the working solutions is labelled as C_P. For the study of AzaPt interaction with DNA all solutions were buffered by 0.01 M NaCac (sodium cacodylate, $(CH_3)_2AsO_2Na$), while the ionic strength was adjusted by suitable additions of sodium chloride. Doubly-distilled water from a Millipore Milli-Q water purification system was used as the reaction medium and for the preparation of all solutions.

2.2. Methods

2.2.1. Potentiometric measurements

All pH measurements ($pH = -\log [H^+]$) employed for the determination of the complex protonation constant were carried out in 0.10 M NMe_4Cl ionic medium at 25.0 ± 0.1 °C, using the equipment and procedure described previously [25]. The combination Hamilton electrode (LIQ-GLASS 238000/08), used as the hydrogen ion concentration probe, was calibrated by titrating known amounts of HCl with CO_2-free NaOH solutions and determining the equivalence point by Gran's method [26]. The latter allows determination of the standard electrode potential E° and the ionic product of water (at 25.0 ± 0.1 °C in 0.10 M NMe_4Cl, $pK_w = 13.83(1)$) among other parameters. Two titration runs (about 70 data points each) were performed in the pH range 2.5 to 10.5. In both experiments the complex concentration was 3×10^{-4} M. As shown below, no self-aggregation of the complex occurs at this concentration. HYPERQUAD computer program [27] was used to calculate the equilibrium constant from e.m.f. data. The analysis of the titration curves revealed no complex dissociation in the pH range studied.

2.2.2. DNA sonication

DNA samples (ca. 2×10^{-3}M CT-DNA, 10 mL) were sonicated in a MSE-Sonyprep sonicator by applying seven consecutive cycles of 10 s sonication and 20 s pause with an amplitude of 14 μm. The sonicator tip was introduced directly into the solution, which was kept in an ice bath to minimize thermal effects caused by the sonication. Agarose gel electrophoresis tests confirmed that the polymer length was reduced to ca. 800 base pairs.

2.2.3. Spectral measurements

Absorption spectra were recorded on a Perkin-Elmer Lambda 35 spectrophotometer and fluorescence emission spectra on a Perkin-Elmer LS 55 spectrofluorometer. Both instruments were equipped with jacketed cell holders, providing temperature control to within ± 0.1 °C. DNA titrations were carried out at 25.0 ± 0.1 °C by adding increasing amounts of the polynucleotide directly into the cell that contained dye solution. Experimental data were analysed by means of non-linear least-square fitting procedures performed by a JANDEL (AISN software) program.

As the study of the DNA/dye interaction on a long time scale (days) revealed some dye degradation, UV-visible (UV-Vis) spectra have been collected in a differential mode. Namely, same amount of AzaPt was placed both in the sample (AzaPt + DNA mixture) and in the reference cuvette.

3. Results

Proflavine and proflavine-like dyes are known to undergo self-aggregation processes that may interfere with the equilibrium studies [28,29]. Hence, possible self-aggregation of AzaPt was investigated by recording the dye absorbance spectrum at different concentrations. The absence of spectral shifts or appearance of shoulders in the spectra and the linearity of the absorbance/concentration plots suggest no dye aggregation occurs in the C_D range 0 to 4×10^{-4} M (Fig. 1S of Supporting Information). The extinction coefficient of AzaPt at the wavelength of maximum absorption turned out to be $\varepsilon(390\ nm) = 2.11 \times 10^3\ M^{-1}cm^{-1}$.

According to fluorescence measurements, the maximum intensity of light emission is observed at $\lambda_{em} = 498$ nm for $\lambda_{ex} = 380$ nm. Fluorescence is preferred to absorbance as it allows working with lower concentrations of the dye. High dye concentrations can cause aggregation phenomena on the DNA backbone that overlap with the binding process and make the analysis of the dye-DNA interaction much more difficult.

Potentiometric (pH-metric) titrations, performed at a complex concentration of 3×10^{-4} M to avoid self-aggregation, indicated that, in the pH range 2.5 to 10.5, the complex is involved in a single protonation equilibrium characterized by the constant $\log K = 8.45(3)$. This means that under the experimental conditions employed in the binding studies (pH 7, $[AzaPt] = 4 \times 10^{-4}$ M) the complex exists almost entirely (>96%) in its protonated form, bearing an overall 2+ charge. The variation of absorption and emission spectra of AzaPt with pH (Fig. 2S of Supporting Information) is consistent with the behaviour previously reported for the free ligand [19], indicating that protonation of AzaPt takes place on the heteroaromatic proflavine nitrogen.

The interaction of the AzaPt dye with DNA is evidenced through changes in the fluorescence spectra that occur when increasing amounts of polynucleotide are added to a dye solution. Similar to the behaviour observed for the binding of the metal free dye to DNA [19], the binding isotherm is of biphasic nature. It clearly reveals two different modes of interaction (Fig. 2).