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Interaction of DNA with Bis(diiminosuccinonitrilo)platinum(II)

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Interaction of DNA with bis(diiminosuccinonitrilo)platinum(II) has been studied by UV-visible absorbance spectra, fluorescence spectra and viscosity measurements. The UV-visible absorption spectra of the metal complex exhibit hypochromism with a small blue shift on interaction with DNA. Scatchard plot analyses indicate that the binding sites of the metal complex on DNA are different from those of ethidium bromide. Viscosity experiments reveal that the binding of the metal complex decreases the relative viscosity of DNA. These results suggest that the platinum diimine complex interact with DNA by surface binding. These studies are helpful for us to understand the action mechanism of bis(diiminosuccinonitrilo)platinum(II) as a potential photodynamic therapeutic agent, and further to develop it.

Keywords Platinum(II) diimine complex, bis(diiminosuccinonitrilo)platinum(II), DNA binding

Introduction

As a fascinating method for cancer diagnosis and treatment of various types of tumours by the combined action of oxygen, light and sensitizers, photodynamic therapy (PDT) has received extensive attention.[1] Nowadays, the most investigated photosensitizers are various porphyrins, chlorins, bacteriochlorins, phthalocyanines, naphthalocyanines, texaphyrins and their metalloderivatives.[2] For example, photofrin, which is a mixture of hematoporphyrin monomers, dimers, and oligomers, has been approved for clinical use.[2] The synthetic tetrakis(*m*-hydroxyphenyl)chlorin (*m*-THPC, Forscan) and the luthetium texaphyrin complexes have also been accepted for clinical applications.[2] However, there are many other metal complexes capable of photosensitised singlet oxygen generation.[3] Platinum diimine complexes are attractive due to their high efficiency as dye sensitizers. For example, some platinum diimine dithiolate complexes, such as Pt(bpy)(tdt), Pt(bpy)(bdt) (bpy = 2,2'-bipyridyl, bdt = 1,2-benzenedithiolate, tdt=3,4-tolulenedithiolate), have been shown to produce singlet oxygen with moderate efficiency.[4-7] Weinstein *et al.*[8-10] have synthesised a series of red-luminescent diimine or cyclometalated platinum(II) complexes, some of which can generate singlet oxygen efficiently. Barton *et al.*[11] found that photoexcited $[(dppz)Pt(mes)_2]Cl_2$, upon intercalation into the DNA π stack, promoted reductive and oxidative damage within the DNA duplex. These findings encourage us to develop new platinum(II) diimine complexes as potential photodynamic therapeutic agents.

Bis(diiminosuccinonitrilo)platinum(II) ($Pt(disn)_2$, Figure 1), structurally similar to its analogue bis(diiminosuccinonitrilo)nickel(II),[12-14] is a planar molecule, packed in a similar fashion as in metalloporphyrins.[15] The compound has a strong absorption band in red light region (λ_{max} = 633 nm, $\varepsilon = 10^4$ mol^{-1}•L•cm^{-1} in dimethoxyethane),[16] and its electrochemical behaviour and electron spin resonance spectra have been studied.[16,17]

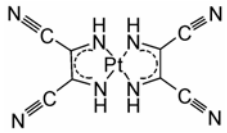

Figure 1 Structure of $Pt(disn)_2$.

In this paper, we report the interaction between $Pt(disn)_2$ and DNA by UV-visible absorption spectra, fluorescence spectra, and viscosity measurements.

Experimental

Calf thymus DNA was purchased from Sino-American Biotechnology Co. in China. $Pt(disn)_2$ was prepared according to literature procedures.[18] All the solvents used were analytical reagents.

The solution of calf thymus DNA was made by dissolving the DNA in 50 mmol•L^{-1} aq. NaCl. Its purity was checked by UV spectroscopy to ensure that the ratio of A_{260} to A_{280} was 1.8—2.0, indicating that the DNA was sufficiently free of protein.[19] DNA concentration per nucleotide was determined by ε_{260} = 6600 mol^{-1}•L•cm^{-1}.[20]

All the experiments involving calf thymus DNA

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were carried out at pH 7.2 employing buffer (4.5 mmol•L^{-1} Tris, pH 7.2, 45 mmol•L^{-1} NaCl, 10% Me_2SO).

UV-visible absorption spectra were recorded on a Hewlett Packard HP-8453 spectrophotometer. Fluorescence spectra were recorded on a Perkin-Elmer fluorescence spectrophotometer. The sample was excited at 520 nm and its emission spectra were scanned in the range 540—700 nm. Viscosity experiments were performed at (20±0.1) ℃ using an Ubbelodhe-type viscometer.

UV-visible absorption spectral studies

$Pt(disn)_2$ (1.00×10^{-5} mol•L^{-1}) and calf thymus DNA were mixed together in different molar ratios (c_{DNA} : $c_{complex}$=0, 1, 5, 15), then stood in darkness for 30 min at room temperature. Afterwards, their UV-visible absorption spectra were recorded respectively.

Inhibition effect of $Pt(disn)_2$ on DNA-associated EB fluorescence enhancement

$Pt(disn)_2$ ($c_{complex}$: c_{DNA}=0, 1.0, 1.5, 2.0, 2.5) solutions at different concentrations were added into DNA-ethidium bromide (EB) mixtures ($c_{DNA}=1.00\times10^{-4}$ mol•L^{-1}, $c_{EB}=1.02\times10^{-5}$ mol•L^{-1}), respectively. After the reaction mixtures stood in darkness for 30 min at 25 ℃, their fluorescence spectra were recorded at 25 ℃, respectively.

Measurement of fluorescence Scatchard plots

Calf thymus DNA (1.27×10^{-5} mol•L^{-1}) was mixed with $Pt(disn)_2$ in different molar ratios ($c_{complex}$: c_{DNA}=0, 0.25, 0.5). After the reaction mixtures stood in darkness for 30 min, spectrophotometric titrations were performed by stepwise addition of EB (2.54×10^{-4} mol•L^{-1}) to the above reaction mixtures, and the fluorescence intensities were measured at 25 ℃.

Viscosity measurements

Calf thymus DNA (1.00×10^{-3} mol•L^{-1}) was mixed with $Pt(disn)_2$ of different concentrations. After the reaction mixtures stood in darkness for 30 min, the flow time of the reaction mixtures was recorded at (20±0.1) ℃, respectively. Relative viscosities for DNA in the presence and absence of $Pt(disn)_2$ were calculated accordingly.

Results and discussion

UV-visible absorption spectra studies

As shown in Figure 2, $Pt(disn)_2$ has a strong absorption band at around 627 nm. With the increase of DNA amounts, the band shows hypochromism ($\Delta\varepsilon$=26%, when c_{DNA} : $c_{complex}$=15) accompanied by a small blue shift of 6 nm.

The intrinsic binding constant of $Pt(disn)_2$ with DNA was determined by using the equation,[21,22] $D/(\varepsilon_a-\varepsilon_F)=D/(\varepsilon_B-\varepsilon_F)+1/K(\varepsilon_B-\varepsilon_F)$, where D is the concentration of DNA in base pairs, K is the intrinsic binding constant, ε_a corresponds to the apparent extinction coefficient ($A_{obsd}/c_{complex}$), ε_B and ε_F correspond to the extinction coefficient of the bound form of $Pt(disn)_2$ and the extinction coefficient of free $Pt(disn)_2$, respectively. The ratio of slope to intercept in the plot of $D/(\varepsilon_a-\varepsilon_F)$ vs. D gives the value of K as $(1.65\pm0.65)\times10^5$ mol•L^{-1}, suggesting weaker affinity of $Pt(disn)_2$ to DNA than those well-known DNA binders such as ethidium and daunomycin.[23]

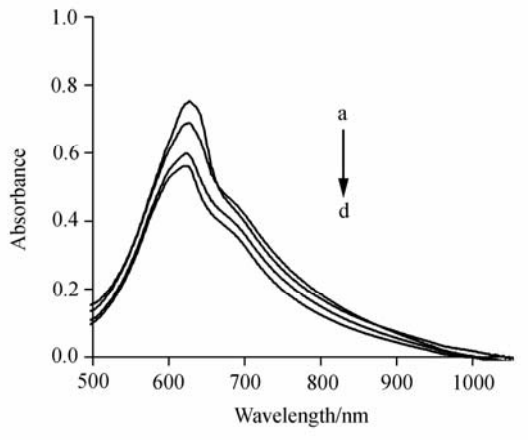

Figure 2 Absorption spectra of $Pt(disn)_2$ at pH 7.2 in buffer (4.5 mmol•L^{-1} Tris, pH 7.2, 45 mmol•L^{-1} NaCl, 10% Me_2SO) in the presence of calf thymus DNA at different concentrations. The ratio of DNA to $Pt(disn)_2$ increases in the order of 0, 1, 5, 15 for curves a—d, respectively.

Interaction between an intercalator and DNA bases commonly results in hypochromism and a red shift of the transition of the intercalated chromophore.[24] Interaction between $Pt(disn)_2$ and DNA also results in hypochromism, but in a blue shift of 6 nm as well. Thus, the platinum(II) diimine complex does not bind to DNA by an intercalation mechanism. Combined with other evidences (see below), we thought that $Pt(disn)_2$ might interact with DNA by surface binding.

Interestingly, interaction of a cobalt(III) complex with DNA also results in moderate hypochromism and a small blue shift of 3 nm of absorption band of the metal complex, and the binding mode between the complex and DNA was proved to be surface binding.[25]

Inhibition effect of $Pt(disn)_2$ on DNA-associated EB fluorescence enhancement

As shown in Figure 3, the fluorescence intensity of DNA-EB adduct decreases with the increasing concentrations of the metal complex, suggesting that the metal complex inhibit EB binding. We noticed that a trinuclear copper(II) complex, which interacts with DNA by surface binding, also induced a slight fluorescence intensity decrease when the complex was added to the DNA-EB system.[26]

Scatchard plots of fluorescence data

The fluorescence data were processed by Scatchard plots as described in the literature.[27] The binding of EB

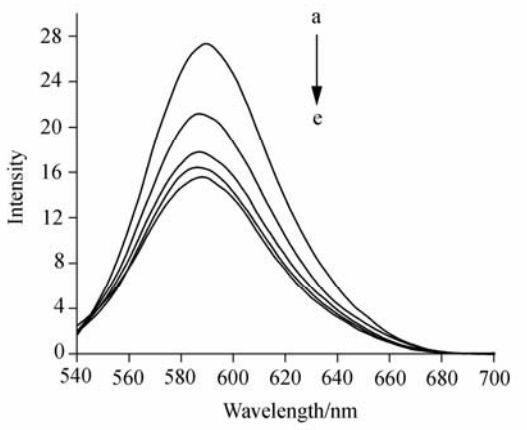

Figure 3 Inhibition of $Pt(disn)_2$ on DNA-associated EB fluorescence enhancement. The ratio of $Pt(disn)_2$ to DNA increases in the order of 0, 1.0, 1.5, 2.0, 2.5 for curves a—e, respectively.

to DNA is expressed using the equation, $r/c=K(n-r)$, where r is the number of mole of EB bound to one mole of DNA phosphate, n the number of binding sites per DNA phosphate, K the intrinsic association constant to a site, and C the free EB concentration. Using data of fluorescence intensity to determine r, binding isotherms were obtained and the corresponding Scatchard plots were constructed.

As shown in Figure 4, inhibition of $Pt(disn)_2$ on DNA-EB binding produces a Scatchard plot in which the intercept on the abscissa (n) decreases while the slope (K) keeps almost constant in the presence of increasing amounts of the metal complex. The result suggests that $Pt(disn)_2$ and EB have different binding sites on DNA,[27] and $Pt(disn)_2$ might bind to DNA by surface binding, which is in good agreement with the studies by UV-visible absorption spectra.

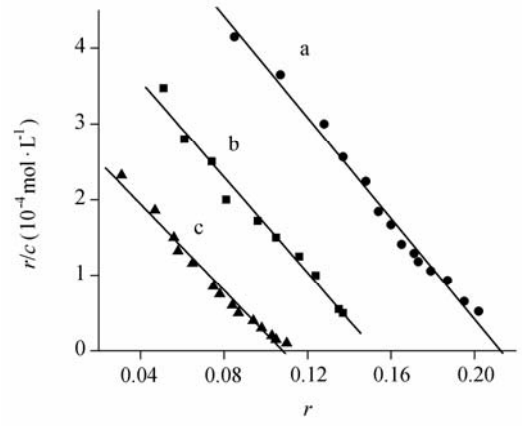

Figure 4 Fluorescence Scatchard plots of the binding of EB to DNA in the absence and the presence of $Pt(disn)_2$. The ratio of $Pt(disn)_2$ to DNA increases in the order of 0, 0.25, 0.50 for lines a—c, respectively.

Viscosity measurements

In order to compare with other data previously reported,[23] viscosity is presented as $(\eta/\eta_0)^{1/3}$(Figure 5).

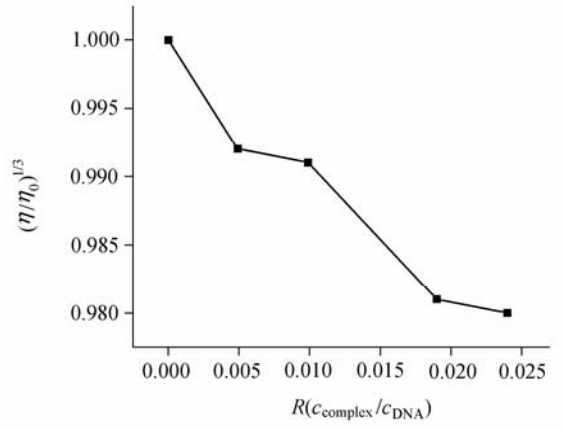

Figure 5 Effect of increasing amounts of $Pt(disn)_2$ on the relative specific viscosity of calf thymus DNA.

Viscosity changes are sensitive to length increases, and hydrodynamic measurements are regarded as an essential method to support an intercalation model in the absence of crystallographically structural data or NMR data.[23] The intercalator ethidium was found to increase the relative specific viscosity of DNA due to the lengthening of DNA double helix resultant from intercalation; the groove-binding antibiotic Hoechst 33258 did not appreciably alter DNA viscosity; whereas Δ-tris(phenanthroline)ruthenium(II) decreased the relative specific viscosity of DNA, and the behavior was explained by a binding mode that produces bends or kinks in DNA helix.[23]

Figure 5 shows that $Pt(disn)_2$ decreases the relative specific viscosity of DNA. We thought that the interaction of $Pt(disn)_2$ with DNA by surface binding caused bends or kinks in DNA helix, hence leading to the decrease of the relative specific viscosity of DNA. The conclusion is in agreement with above-mentioned other studies.

Conclusion

$Pt(disn)_2$ has an extensive electrochemistry with stable oxidized or reduced species obtained in various cases.[13,16] Cyclic voltammetry experiments indicate that $Pt(disn)_2$ can be oxidized by a one-electron, reversible process to give an unstable cation.[17] Our preliminary investigation also shows that $Pt(disn)_2$ is sensitive to red light ($\lambda>600$ nm) (unpublished data). Thus, it is possible that $Pt(disn)_2$ is photooxidized to a cation form which can be stabilized by DNA via electrostatic interaction. However, the exact mechanism is still unknown, and the investigation on photophysical properties of $Pt(disn)_2$ is still underway. $Pt(disn)_2$ has a strong absorption band within the phototherapeutic window (620—850 nm). Our studies reveal that $Pt(disn)_2$ can interact with DNA by surface binding. Thus, $Pt(disn)_2$ might be a promising candidate for potential photodynamic therapeutic use. Further studies need to be carried out.

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