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Ruthenium(III)-edta type complexes for DNA-metallation

DEBABRATA CHATTERJEE
Chemistry Section, Central Mechanical Engineering Research Institute, Durgapur 713 209, India
e-mail: root@cscmeri.ren.nic.in

Abstract. Interactions of $[Ru^{III}(edta)(H_2O)]^-$ (edta = ethylenediaminetetraacetate) with DNA related molecules L (L = DNA-bases, nucleosides, nucleotides and single strand calf-thymus DNA) were studied by spectrophotometric, electrochemical and kinetic methods. The spectral features of substituted product $[Ru^{III}(edta)L]^-$ complexes were characterized by a strong ligand-to-metal charge transfer band in the UV region (293–310 nm). However, a blue colour is developed (λ_{max} = 582 nm) at high *p*H (> 7·0) for L = GMP (guanosine-5′-monophosphate) Dissociation of N^1-H proton of purine portion of coordinated GMP is suggested to promote ligand-to-metal charge band at lower energy (visible) region. The $E_{1/2}$ values corresponding to Ru^{III}/Ru^{II} redox couple for $[Ru^{III}(edta)L]^-$ complexes are in the range –0·28 to –0·12 V (*vs* SCE). Formation kinetics of $[Ru^{III}(edta)L]^-$ was studied using stopped-flow methods. Comparing the reactivity of $[Ru^{III}(edta)H_2O)]^-$ with L in terms of spectral, electrochemical and kinetic data, it is proposed that binding of DNA with Ru(III)-edta complex takes place through adenine base unit.

Keywords. Ruthenium(III)-edta; DNA-bases; nucleosides; nucleotides; DNA-metallation; kinetics.

1. Introduction

We have been engaged in exploring the various intriguing reactions of edta type complexes of ruthenium(III) [1–5]. Polyaminopolycarboxylate ligands (edta, hedtra, medtra) are somewhat similar in donor sets to many metalloenzymes which utilize carboxylate, amine, or imidazole donors from Asp, Glu, Lys or His amino acids to bind a metal centre. Our own research has emphasized ligands which provide such donors from simple polyaminopolycarboxylate chelates. Furthermore, the remarkable lability of the aquo molecule in $Ru^{III}(edta)(H_2O)^-$ complex provides an advantage of easy coordination of incoming ligand to ruthenium(III) centre. The interest in the present work is related to the probability of using $Ru^{III}(edta)(H_2O)^-$ complexes as DNA binders for developing a new family of antitumor metallodrugs and reagents for molecular biochemistry. Hence, in order to examine the interaction of $Ru^{III}(edta)(H_2O)^-$ with DNA we have studied the reactions of $Ru^{III}(edta)(H_2O)^-$ with DNA-related molecules and DNA itself (single strand calf-thymus DNA). The present paper reports the results of spectral, electrochemical and kinetic investigations of reactions of $[Ru^{III}(edta)H_2O)]^-$ with DNA-bases (adenine, cytosine, thymidine) nucleosides (adenosine, cytidine), nucleotides (adenosine-5′-monophosphate, cytidine-5′-monophosphate, guanosine-5′-monophosphate, uracyl-5′-monophosphate) and single strand calf-thymus DNA itself in aqueous solution.

2. Experimental

2.1 *Materials*

The salt $K[Ru^{III}(Hedta)Cl]\cdot 2H_2O$ was prepared by following the published procedure[6] and characterized. $K[Ru^{III}(Hedta)Cl]$ complex is rapidly aquated when dissolved in water and exists predominantly in its labile form $Ru^{III}(edta)(H_2O)^-$ in the *p*H range 5–6[7]. Calf-thymus DNA (single strand, four base pair) obtained from Sigma was purified further by exhaustive dialysis against phosphate buffer and water. A solution of DNA (10^{-5} M) in phosphate buffer (*p*H 7·2) gave a ratio of UV absorbances at 260 and 280 nm of 1·8 indicating that the DNA is sufficiently free of protein[8]. The concentration of DNA is expressed here in terms of nucleotide phosphate concentration calculated from UV absorbance at 260 nm, considering the absorption coefficient (ε_{260}) to be 6600 M^{-1} cm^{-1}[9]. All other chemicals used were of AR grade. Double distilled water was used throughout the experiments.

2.2 *Instrumentation and techniques*

Absorption spectra of the experimental solutions were recorded on a Shimadzu UV-VIS 160 spectrophotometer equipped with a TCC 240A temperature controller. Electrochemical studies were performed on a Princeton Applied Research (PAR 174) electrochemical instrument operating on cyclic voltammetry (CV) and differential pulse voltammetry (DPV) mode. A glassy working electrode and a saturated calomel electrode (SCE) as reference were employed for this purpose. KCl was used as supporting electrolyte.

2.3 *Kinetic studies*

Kinetic measurements were carried out with a Hi-Tech stopped-flow spectrophotometer (SF-51) attached to an on-line data analyser (Apple II-e) with which kinetic traces could be evaluated. All the reactions were monitored in the wave length region 330–350 nm where appreciable spectral difference between $Ru^{III}(edta)(H_2O)^-$ and $[Ru^{III}(edta)L]^-$ (product) exists. The instrument was thermostated at ± 0·1°C. Rate constant data were measured under pseudo-first order conditions of excess (10–100 fold) of L. Acetic acid-acetate, phosphate and borate buffers were used to maintain the *p*H of the kinetic solutions, whereas KCl was used to control the ionic strength. The *p*H measurements were carried out with a Digisun *p*H meter.

3. Results and discussion

The reaction of $[Ru^{III}(edta)(H_2O)]^-$ with L (L = DNA = base, nucleosides, nucleotides and DNA itself) in aqueous solution produces mixed-ligand complexes of the type $[Ru^{III}(edta)L]^-$, through a rapid aquo-substitution step. The $[Ru^{III}(edta)L]^-$ product complexes exhibit a characteristic ligand-to-metal charge transfer band of L in the UV region, except for L = GMP (GMP = guanosine-5′-monophosphate). Spectral data characteristic of $[Ru^{III}(edta)L]^-$ complexes are summarised in table 1.

Electrochemical measurements both by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) on $[Ru^{III}(edta)(H_2O)]^-$ in presence of L were carried out in aqueous

Table 1. Spectral and electrochemical data for $[Ru^{III}(edta)L]^-$ complexes at *p*H 5·2, μ = 0·2 M (KCl).

L	λ_{max}/nm	$E_{1/2}(Ru^{III}/Ru^{II})$/V
H_2O	283	−0·28
Adenine	292	−0·12
Adenosine	295	−0·15
Cytosine	301	−0·27
Cytidine	304	−0·27
Thymine	301	−0·26
AMP	310	−0·17
ADP	317	−0·19
ATP	320	−0·21
IMP	305	−0·23
UMP	312	−0·24
GMP (*p*H < 7·0)	323	−0·17
GMP (*p*H > 7·0)	580	−0·24
Xanthene	319	−0·17
DNA (Calf-thymus)	295	−0·15

$[Ru^{III}] = 5 \times 10^{-4}$ M; *p*H = 5·2; μ = 0·2 M; [L] = 5×10^{-3} M

solution of *p*H 5·2. In all cases, cyclic voltammogram of $[Ru^{III}(edta)L]^-$ featured a pair of quasi-reversible anodic and cathodic waves ($90 < \Delta Ep < 120$ mV). Cyclic voltammetric potentials ($E_{1/2}$) for $Ru^{III}(edta)(L)^-(edta)(L)^{2-}$ redox couple are summarised in table 1.

The spectral changes which occurred by changing the *p*H of the solution containing Ru(III)-edta and GMP are shown in figure 1 [10]. Spectral changes were found to be reversible. The absorption maximum seen at 582 nm (figure 1) is assigned to ligand-to-metal charge transfer band of coordinated GMP in its anionic form. It is well documented in the literature [11] that coordination of guanosine or adenosine bases with metal centre generally takes place through N7 site of the imidazole portion of the bases (figure 2). In the present case, it is suggested that N7 site of GMP is involved in coordination of GMP to Ru(III)-centre. Coordination of GMP through N7 enhances the acidity of N^1-H of purine portion of the GMP and at high *p*H (7·5 < *p*H < 9·0) acid dissociation N^1-H results in the formation of anionic form of coordinated GMP (figure 3). This anionic form would then be highly electron-rich in the "imidazole" portion of GMP which promote ligand-to-metal charge transfer in low-energy visible region. It was reported earlier [12] that when pyrazole or imidazole are coordinated to Ru^{III} $(NH_3)_5^{3+}$, the acidity of pyrrole N-H increases and deprotonation of pyrrole N-H produces respective pyrazolato or imidizolato complexes with ligand-to-metal charge transfer band shifted to lower energy for aqueous solution spectra. The origin of low energy band is due to the fact that the anionic form of the ligands enter considerably into π-donation towards a low spin d^5 ruthenium (III) centre. The *p*Ka value of N^1-H acid-dissociation of coordinated GMP determined spectrophotometrically [13] is 7·2 at 25°C.

At the studied *p*H range (5·0–9·0) all other DNA-bases taken here, failed to produce any colour in the visible region due to the following reasons. Adenosine bases also bind at N7 positions [11], but lack a dissociable proton on the six-membered purine ring. In cases of cytidine or thymine bases, coordination of Ru(III)-edta complex occur at N3 position with prior loss of N-H which affords no protolytic equilibria for them.

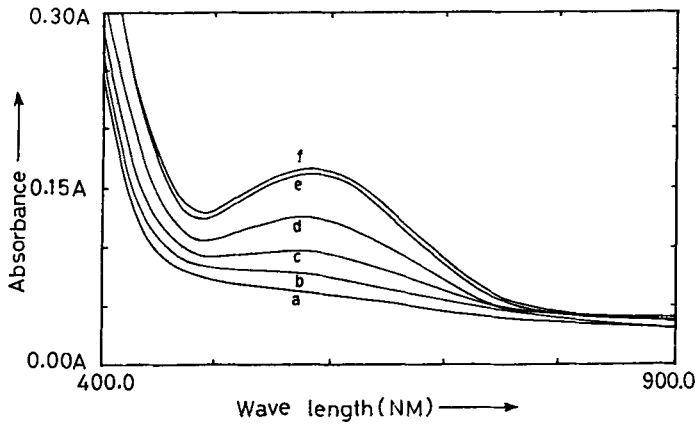

Figure 1. Spectra of Ru(III)-edta complex (in H_2O) in presence of GMP at different *p*H (adjusted by NaOH). (a) 6·16, (b) 6·54, (c) 6·96, (d) 7·45, (e) 8·9. $[Ru^{III}] = 2 \times 10^{-4}$ mol.dm^{-3}, [GMP] = 1×10^{-3} mol.dm^{-3}.

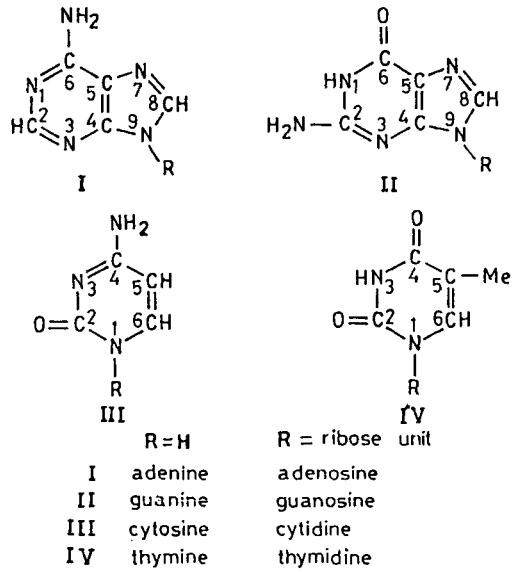

Figure 2. Schematic representation of purine and pyrimidine bases.

Spectral changes that occurred in the reaction of $[Ru^{III}(edta)(H_2O)]^-$ with L has been employed for the kinetic studies. The reaction of $[Ru^{III}(edta)(H_2O)]^-$ with L were followed as an absorbance increase and in all cases (except for adenine) the kinetic-traces were found to be single exponential. The rate of complexation of $[Ru^{III}(edta)(H_2O)]^-$ with L was found to be first order with respect to $[Ru^{III}(edta)(H_2O)]^-$. The values of observed rate constant (k_{obs}) increased linearly by increasing [L] with an appreciable intercept. On the

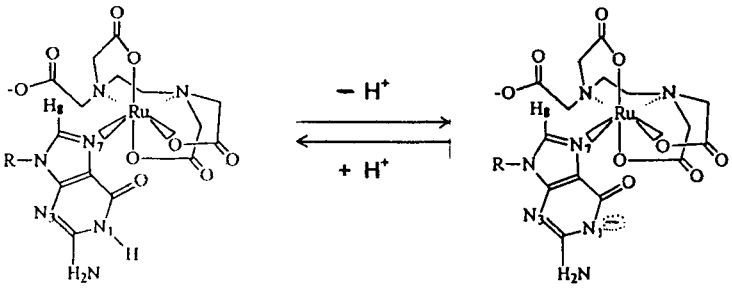

Figure 3. N^1–H acid dissociation equilibrium of coordinated GMP.

Table 2. Rate and kinetic data for the reaction

$Ru^{III}(edta)(H_2O)^- + L \underset{k_r}{\overset{k_f}{\rightleftharpoons}} Ru^{III}(edta)L^- + H_2O$

L	$k_f(M^{-1}s^{-1})$	$k_r(s^{-1})$	$K(=k_f/k_r)$	K	$k's^{-1}$
Adenine	400 ± 10	2·3	171	–	0·18
Adenosine	110 ± 4	0·77	153	168	
Cytosine	1·8 ± 0·3	0·02	75	72	
Cytidine	1·5 ± 0·4	0·03	46	40	
DNA	120 ± 4	0·85 ± 0·05		144	

$[Ru^{III}] = 5 \times 10^{-4}$ M. pH = 5·2, T = 25°C, μ = 0·2 M.

basis of the above kinetic observation the reactions of $[Ru^{III}(edta)(H_2O)]^-$ with L can be interpreted in terms of (1) for which a rate expression can be in (2).

$$[Ru^{III}(edta)(H_2O)]^- + L \underset{k_r}{\overset{k_f}{\rightleftharpoons}} Ru^{III}(edta) + H_2O \quad (1)$$

$$k_{obs} = k_f[L] + k_r \quad (2)$$

The values of k_f and k_r (determined from the slopes and intercepts of the k_{obs} *vs* (L) plots) are given in table 2.

The reaction of $[Ru^{III}(edta)(H_2O)]^-$ with adenine was characterized by [3] a rapid growth followed by a slower decay. The values of the observed rate constant for the rapid step was found to be linearly dependent on [adenine], whereas the rate constant values corresponding to the slower decay step were independent of [adenine]. On the basis of earlier reports [15,16] on the kinetics of the formation of mixed-chelate complexes of $Ru^{III}(edta)$, the present kinetic observations for L = adenine, may be interpreted in terms of a rapid formation of the mono-legated product (ligand dependent step) followed by a ring-closure step (ligand independent) in which the exocyclic NH_2 group (at C6) is coordinated to ruthenium centre by displacing a coordinated carboxylated group of edta.

Kinetics of the reaction of $Ru^{III}(edta)(H_2O)^-$ with single strand calf-thymus DNA was studied spectrophotometrically at 320 mm where appreciable absorption change

occurred[3]. Kinetics traces at various experimental conditions were found to be single exponential. This certainly indicates the absence of any other consecutive reactions.

The reaction of DNA-coordination with $[Ru^{III}(edta)(H_2O)]^-$ showed similar kinetic behaviour to that observed for other DNA-related molecules (L). The values of rate constant data are shown in table 2.

Concluding remarks

A careful analysis and comparison of spectral, electrochemical and kinetic data (tables 1 and 2) suggests that the single-strand DNA binds Ru(III)-edta complex through a straightforward and rapid aquo-substitution step. As the biological action of metallodrugs generally takes place through the covalent attachment of DNA, the results of the present studies appear to be encouraging in developing a new family of chemoselective metallodrugs in resemblance to that of *cis*$[Pt(NH_3)_2Cl_2]$[14].

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