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Kinetics and equilibria for the axial ligation of bromomethyl (aqua)cobaloxime with pyridines – Isolation characterization and DNA binding

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Abstract. The kinetics and equilibria for the axial ligation of pyridine and substituted pyridines to bromomethyl(aqua)cobaloxime have been measured spectrophotometrically in aqueous solutions of ionic strength l·0 M (KC1) at 25°C as a function of *p*H. The binding constants and rate of formation increase in the order 4-NH_2Py > 4-EtPy > 4-MePy > Py > 2-NH_2Py > 2-EtPy. The data have been interpreted based on the basicity of the ligand, π-back bonding from Co(III) $\rightarrow$ L and hard and soft interactions. The rate of substitution of H_2O varies with the pKa of the incoming ligand, thus establishing the existence of nucleophilic participation of the ligand in the transition state. We have investigated the DNA binding of bromomethyl(aqua)cobaloxime with DNA. Bromomethyl(ligand)cobaloximes were isolated and characterized by elemental analysis, IR and NMR (^{1}H, ^{13}C) spectra.

Keywords. Cobaloximes; kinetics and equilibria; DNA-binding.

1. Introduction

Cobaloximes are complexes containing the *bis* (dimethylglyoximato) cobalt(III) moiety, $Co(DH)_2^+$ (DH = mono anion of dimethylglyoxime). These simulate the reactions of vitamin-B_{12} and accepted as coenzyme B_{12} model compounds. Homolytic cleavage of the Co–C bond is the key step in the mechanism of action of many enzymes.[1] It is widely believed that structural and conformational changes in coenzyme B_{12} lead to acceleration in Co–C bond cleavage rates.[2,3] The study of simple models of the B_{12} coenzyme, such as the cobaloximes, has furnished a significant amount of data[4,5] that have provided a foundation for understanding the behaviour of cobalamins.[6] Randaccio *et al.*[7–9] compared the properties of rhodoximes and cobaloximes on the basis of electronic and steric effects. These studies were useful in understanding the mechanism of Co–C bond cleavage in the vitamin B_{12} coenzyme.

Since binding of cobaloximes is closely related to the structural and binding characteristics of corrin systems involved in biological mechanisms and in continuation of our work,[10–13] we report here the isolation of bromomethyl(ligand) cobaloximes, equilibria and kinetics of the axial ligation of the bromomethyl(aqua) cobaloximes with pyridine and substituted pyridines. We have also investigated the interaction of bromomethyl cobaloxime with CT DNA.

2. Materials and methods

Pyridine, 4-Amino Pyridine, 4-Ethyl Pyridine, 4-Methyl Pyridine, 2-Amino Pyridine and 2-Ethyl Pyridine were obtained in highest purity from Acros. KC1, HPLC grade methanol, acetic acid, HC1, phosphoric acid, formic acid were obtained from Fluka. Dipotassium hydrogen phosphate, potassium dihydrogen phosphate, potassium phosphate, *tris* (hydroxymethyl)aminomethane (*Tris*), sodium acetate, potassium hydroxide were obtained from Acros. Double distilled, deionized water was used throughout.

To maintain appropriate *p*H 0·2 M buffers of HC1 (0–1·5 *p*H), KH_2PO_4 and H_3PO_4 (2·0 *p*H), HCOOH and KOH (2·5–3·0 *p*H), CH_3COOH and CH_3COONa (3·5–5·5 *p*H), K_2HPO_4 and KH_2PO_4 (6·0–8·0 *p*H),

*For correspondence

Tris and HC1 (8·5–9·0 *p*H), K_2HPO_4 and K_3PO_4 (9·5–11·5 pH) are used.

Alkyl(aqua)cobaloximes were prepared by the procedure of Brown *et al.*[14] All manipulations were performed under minimal illuminations due to photolability of carbon-cobalt bond.[6] These alkyl (aqua)cobaloximes are photolabile, particularly in solution. *p*H measurements were made with a Digisun digital *p*H meter equipped with a combined glass electrode. The electrode was standardized at two *p*H values (*p*H = 4 and *p*H = 9·2) with standard buffer solutions. UV and visible spectra were recorded on a Hitachi U-3410, the sample compartment of which is provided with a thermostat and the concentrations of bromomethyl(aqua)cobaloxime (0·001 M) was fixed at 436 nm. For axial ligation single wavelength measurements were made on Elico single beam spectrophotometer SL 171 model. The sample compartment of which was thermostated at 25 ± 0·1°C.

$[BrCH_2Co(DH)_2L]$ complexes were isolated by mixing 1 : 3 ratio of bromomethyl (aqua) cobaloxime and the desired base ligand (L) in ethanol. This mixture was heated at 40–50°C by constant stirring for 1–2 h. Then added minimum amount of distilled water to the above solution to get the precipitation and allowed it to settle. The resulting precipitate of yellow powder was filtered, washed with distilled water, 95% ethanol and ether and dried *in vacuo*. Yields were 70–80%. ^{1}H NMR spectra were recorded on varian Gemini 200 MHz NMR spectrometer. Samples were prepared by dissolving in DMSO-d_6. Infrared spectra were obtained on Perkin Elmer FTIR-1605 spectrometer using KBr pellets. The molar conductivities of 0·001 M solution of the complexes in methanol show that they are non-electrolytes.

3. Equilibrium measurements

Apparent binding constant (K_{app}) values were determined by spectrophotometric measurements at 436 nm for the axial ligation of bromomethyl (aqua)cobaloxime, as a function of *p*H. In a 3 ml cuvette solutions containing $[CH_2BrCo(DH)_2(H_2O)]$, an appropriate buffer (0·2 M) to maintain *p*H, KC1 to maintain ionic strength (l·0 M) and varying concentrations of ligand were taken in a cell block maintained at 25 ± 0·1°C. Solutions were allowed to equilibrate at 25°C in the spectrophotometer cell block for at least 15 min prior to addition of cobaloxime.

Final absorbance readings were taken after equilibrium was established as indicated by the time independence of the readings. For such experimental set-ups, at a given *p*H (1) is applied.

$$\Delta A = \Delta A_{max}\,[L]_f/(1/K_{eq} + [L]_f), \quad (1)$$

where ΔA is the absolute value for difference in absorbance between solutions containing cobaloxime and added ligand (L) and solutions containing only cobaloxime at the same concentration. ΔA_{max} is the maximum absorbance change thus obtained at high [L] at which the aqua complex is completely converted to the product. The data were analysed[15] by a least-squares fit to the rearranged form of (1) to give (2).

$$\Delta A = \Delta A_{max} - \{1/K_{eq}\,\Delta A/L_f\}, \quad (2)$$

$$[L]_f = [L]_{tot} - (C_T\,\Delta A/\Delta A_{max}). \quad (3)$$

$[L]_f$ is calculated from (3) using measured value of ΔA_{max}, where $[L]_{tot}$ is the total concentration of added ligand and C_T is the total concentration of cobaloxime. ΔA is plotted as a function of $\Delta A/[L]_f$ and the slope is $-1/K_{eq}$.

3.1 *Kinetic measurements*

Kinetic measurements were made for the binding of ligand to bromomethyl(aqua)cobaloxime. For each ligand L, at various *p*H, first order rate constants (k_{obs}) was determined from the absorbance measurements at the same wavelength used for K_{eq} determinations under pseudo-first order condition with L, in at least 10 fold excess over cobaloxime concentration (0·00125 mol dm^{-3}).

Progress of the reaction was monitored by measurements of the change in the absorbance on addition of bromomethyl(aqua)cobaloxime to a 3 ml cuvette which contained known concentration of ligand at appropriate *p*H in the thermostated (25 ± 0·1°C) cell compartment.

First order rate constants (k_{obs}) were obtained by least-squares fit of the data to (4)

$$\ln(A_t - A_\alpha) = k_{obs}t. \quad (4)$$

where A_t, is the absorbance at time t and A_α is the final absorbance.

Second order rate constants, k_{on}, at a given *p*H for a given ligand were obtained from the slopes of least squares fits of the data to (5)

Table 1. 1R* and ^{1}H NMR# spectral data for bromomethyl (ligand)cobaloximes – [$BrCH_2Co(DH)_2L$].

Sl. no.	Complex [$BrCH_2Co(DH)_2L$] where L =	Co–N (N of L)	υ Co–N (N of DH)	υ N–O	υ CH_3	υ C=N	υ H–O…H	^{1}H NMR Data
1	H_2O Water	–	510·0	1085·0 1231·0	1376·0 1438·0	1572·0	1774·0	2·26 (*s*), 3·38 (*s*)
2	C_5H_5N Pyridine	452·6	514·6	1088·5 1234·0	1366·4 1442·9	1559·9	1735·0	2·18 (*s*), 3·50 (*s*), 7·42 (*t*) 7·85 (*m*), 8·50 (*d*)
3	$C_5H_4N–CH_3$ 4-Methylpyridine	456·4	515·3	1089·1	1369·3 1234·4	1559·3 1438·0	733·2	215 (*s*), 2·40 (*s*), 3·58 (*s*) 7·15 (*d*), 8.40 (*d*)
4	$C_5H_4N–C_2H_5$ 4-ethyl pyridine	456·8	518·9	1092·7 1237·1	1366·7 1427·2	1558·2	1730·2	1·15 (*t*), 2·16 (*s*), 2·45 (*q*) 3·50 (*s*), 7·10 (*d*), 8·35 (*d*)
5	$C_5H_4N–NH_2$ 4-Amino pyridine	450·2	512·0	1087·1 1239·0	1365·4 1431·2	1560·4	1735·4	2·12(s), 3·45 (*s*), 5·65 (*bs*), 6·45 (*d*), 7·85 (*d*)
6	$C_5H_4N–C_2H_5$ 2-Ethyl pyridine	450·2	513·0	1087·1 1233·4	1365·4 1437·2	1560·4	1735·4	1·22 (*t*) 2·20 (*s*), 2·84 (*q*) 3·52 (*s*), 7·20 (*t*), 7·31 (*d*), 7·70 (*t*), 8·60 (*d*)
7	$C_5H_4N–NH_2$ 2-Amino pyridine	451·0	514·0	1088·2 1233·6	1367·2 1432·6	1560·2	1735·2	2·18 (*s*), 3·54 (*s*), 4·67 *bs*) 6·33 (*s*), 6·94 (*t*), 7·23 (*t*), 7·0 (*t*)

Recorded as KBr discs and values in cm^{-1}. #in ppm relative to TMS and solvent used DMSO-d^6

$$k_{obs} = k'_{on}\,[L]_t + k_{off}, \quad (5)$$

where $[L]_t$ is the total concentration of L present. Values of k'_{on}, the second-order ligation rate constants with respect to free ligand was calculated[16] from $k_{on} = k'_{on}/\alpha_L$, where $\alpha_L = K_d/(K_d + [H^+])$; K_d = dissociation constant of the ligand.

4. Results and discussion

The IR data for bromomethyl(aqua)cobaloxime and bromomethyl(ligand)cobaloxime are presented in table 1. The disappearance of peak at 3072 cm^{-1} and appearance of peak at 450 cm^{-1} υ(Co–N) indicate the formation of bromomethyl (pyridine)cobaloxime by replacing H_2O. The characteristic absorption bands due to dimethyl glyoximato ligands in these cobaloximes do not shift largely by the change of axial ligands. This suggests that the strength of the Co–N bonds in the equatorial position is not much affected by the change of the axial ligands because, the Co–N (equatorial) bonds are very strong due to Co $\rightarrow$ N=C π bond.

These results can be interpreted as follows. The coordination of more electron donating base to Co(III) causes the increase in electron density in Co(III) which facilitates the back donation from Co(III) to the nitrogen atoms of dimethyl glyoximato ligands resulting in the increase in electron densities in C=N and N–O bonds. The increase in electron density in N–O bonds causes the stronger hydrogen bridges of O–H…O and higher frequency shifts of N–O stretching vibrations. The facilitated back donation from cobalt to nitrogen atoms of dimethylglyoxime lowers the C=N stretching frequency. As the electron donating power of ligand increases, the binding constant (K_{eq}) increases and the binding constants (K_{eq}) follows the order 4-NH_2Py > 4-EtPy > 4-MePy > Py.

The electronic spectra of bromomethyl(aqua) cobaloximes in MeOH shows spin allowed $^1A_1g \rightarrow {}^1T_1g$ transition[17] in the region ~ 22,000 cm^{-1} due to CH_2Br^- to Co(III) σ donation. This band disappears or the intensity drastically decreases in bromomethyl (ligand) cobaloximes. The electronic spectra of $BrCH_2Co(DH)_2OH_2$, $BrCH_2Co(DH)_2Py$, $SCNCo(DH)_2Py$ and CN $Co(DH)_2Py$ are compared. In case of $BrCH_2$ complexes only intense absorbance band at 22,000 cm^{-1} is observed. No band observed for SCN or CN complexes indicating the band at 22,000 cm^{-1} is due to CH_2Br^- to Co(III) σ donation. The charge-transfer spectra of the trans [$BrCH_2Co(DH)_2L$] complexes show a band at 41,000 cm^{-1} due to intra-ligand π–π* transition of

Table 2. ^{13}C NMR$^{\#}$ Spectral data for bromomethyl(ligand)cobaloximes – $[BrCH_2Co(DH)_2L]$.

Sl. no.	Complex $[BrCH_2Co(DH)_2L]$ where L =	eq CH_3	C=N	CH_2Br	Pyridine carbons			
					C_2, C_6	C_3, C_5	C_4	R
1	Water	13.10	149.92	41.42	–	–	–	–
2	C_5H_5N pyridine	12·95	150·96	46·10	153·81	130·42	141·60	–
3	C_5H_4N–CH_3 4-methyl pyridine	12·78	150·34	44·80	153·62	129·80	154·86	22·34 (CH_3)
4	C_5H_4N–C_2H_5 4-Ethyl pyridine	12·60	150·68	45·92	153·40	128·64	155·70	28·70 (CH_2) 13·25 (CH_3)
5	C_5H_4N–NH_2 4-amino pyridine	12·32	150·94	46·20	153·12	108·40	158·30	–

Dissolved in DMSO-d^6 and values in ppm relative to TMS

Table 3. Psuedo first order rate constants at different pH for the formation and dissociation of $[BrCH_2Co(DH_2)OH_2]$ at 25°C in aqueous solution, ionic strength 1·0 M KCl.

k_{obs} (s^{-1})			k_{off} (s^{-1})			k_{obs} (s^{-1})			
*p*H	Py	MePy	pH	Py	MePy	C : L	[L] × 10^{-2}	Py × 10^{-2}	MePy × 10^{-3}
						1 : 10	1·00	0·8	1·53
4·0	8·00 × 10^{-3}	7·00 × 10^{-3}	2·0	1·54 × 10^{-2}	–	1 : 15	1·50	1·10	2·50
						1 : 20	2·00	1·47	3·93
4·5	1·20 × 10^{-2}	1·10 × 10^{-2}	2·5	1·04 × 10^{-2}	–	1 : 25	2·50	1·71	6·29
						1 : 30	3·00	1·92	–
5·0	1·78 × 10^{-2}	1·20 × 10^{-2}	3·0	8·20 × 10^{-3}	9·00 × 10^{-3}	1 : 35	3·50	2·10	–
5·5	2·00 × 10^{-2}	1·22 × 10^{-2}	3·5	–	7·00 × 10^{-3}	k'_{on}		0·0052	0·0031
6·0	2·10 × 10^{-2}	–	4·0	–	6·00 × 10^{-3}	α		0·0299	0·0074
6·5	2·14 × 10^{-2}	–				k_{on} (dm^3 mol^{-1} s^{-1})		0·1739	0·4189

$[BrCH_2Co(DH_2)OH_2]$ 1·25 × 10^{-3} and $[L]_T$ 1·25 × 10^{-2}

the coordinated dimethylglyoxime.[18] A band occurring at 27,500 cm^{-1} is assigned to the pyridine → Co(LMCT) and that at 46,000 cm^{-1} is to the $d\pi$(Co) → π^* (DH) (MLCT) transition.[19] The σ (DH) → σ^*Co (LMCT) is masked by the intense short wavelength bands of bromomethyl(ligand) cobaloximes.[20]

In all these complexes ^1H NMR shows a sharp singlet at around 2·10–2·20 ppm corresponding to the equatorial methyl protons of dimethyl glyoxime and a singlet ~3·50 ppm corresponding to CH_2Br protons. In the pyridine free ligand the $H_2(H_6)$, $H_3(H_5)$) and H_4 proton chemical shifts appear at 7·60 ppm, 7·25 ppm and 7·75 ppm, respectively. Coordination of pyridine to Co(III) center of bromomethylcobaloxime render the $H_2(H_6)$ protons shift to farthest down field and observed at 8·50 ppm. The chemical shifts observed at 7·42 ppm and 7·85 ppm for $H_3(H_5)$ and H_4 protons respectively. All the other complexes follows the same trend of downfield shift and the data are presented in table 1. In the ^{I3}C NMR spectra, the peak around 13·0 ppm corresponds to the four equatorial methyl groups of dimethyl glyoxime as shown in table 2. The C=N group chemical shifts observed around 150·0 ppm. The chemical shifts for the pyridine and substituted pyrdines are in the aromatic region as expected and observed in the down field when compared to free ligands.

Figure 1 shows the association kinetics and as the time increases the absorbance decreases indicating the formation of complex. Figure 2 shows the increase in absorbance with increase in time, this is because the complex $[BrCH_2Co(DH)_2L]$ dissociates and forms $[BrCH_2Co(DH)_2(OH_2)]$ with increase in time.

If we compare the (K_{eq}) binding constants[21,22] of various ligands binding with $BrCH_2Co(DH)_2OH_2$ they are in the order $K_{OH} < K_{2\text{-}EtPy} < K_2\text{-}NH_2Py < K_{py} < K_{4\text{-}Mepy} < K_{4\text{-}EtPy} < K_4\text{-}NH_2Py < K_{lmd} < K_{1\text{-}Melmd} << K_{CN}$ has shown in table 3. This trend is in accordance with the basicity. Except 2-EtPy and 2-NH_2 Py all other ligands binding to $BrCH_2Co(DH)_2OH_2$ follow the bascitiy order. Though 2-EtPy and 2-NH_2Py are

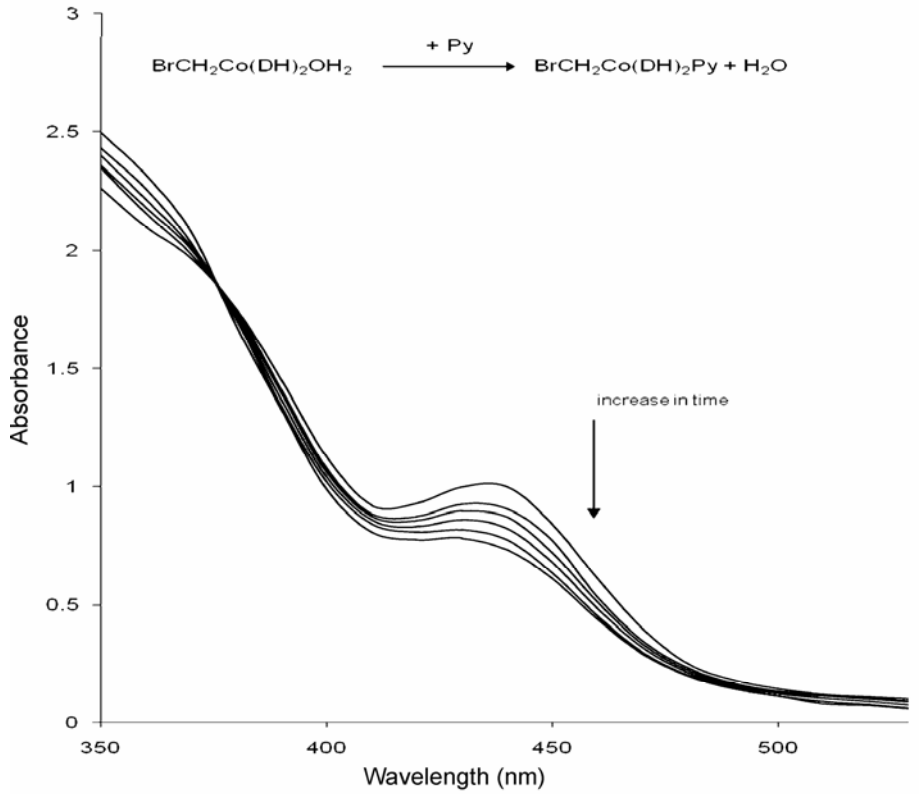

Figure 1. UV-Visible scan: Kinetics of association of $BrCH_2Co(DH)_2OH_2$ with pyridine at *p*H = 4·0 and 25°C. Isosbestic point = 380 nm.

more basic than Pyridine, they form less stable complexes than Pyridine. This is due to steric hindrance caused by the substituent (ethyl or amino group) at C_2 of Pyridine. In addition, much higher stability of CN^- compared to pyridine and imidazole is due to more basic and π accepting ability of cyanide than imidazole and pyridine.

The plot of pseudo first order rate constant k_{obs}, against ligand concentration was linear with very small intercept indicating that some dissociation accompanies the complex formation, and k_{off} increases with increase in *p*H.

When k_{obs} verses the concentration of the entering ligand were plotted, the k_{obs} increases with increasing ligand concentration over the whole ligand concentration range.

$$BrCH_2Co(DH)_2H_2O \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} BrCH_2Co(DH)_2 + H_2O \quad (6)$$

$$[BrCH_2Co(DH)_2] + L \overset{K_{eq}}{\rightleftharpoons} [BrCH_2Co(DH)_2\,L]. \quad (7)$$

Evidence for the existence of pentacoordinate species among alkyl cobinamides and alkylcobaloximes is given by Firth *et al*.[23,24] Because of the delocalization of electron density from unsaturated equatorial ligand (DMG) on to the Co(III) center. This induces partial Co(II) character and account for higher kinetic lability. All this support the D (dissociation) mechanism shown in above equation.

Figure 3 shows the pseudo-first order rate constant for the formation (k_{obs}) and dissociation k_{off} as a function of *p*H. For pyridine k_{obs} increase slowly up to 5·5 *p*H and then steady and not much increase in k_{obs} with increase in *p*H. Similar trends were observed in the binding studies, as the *p*H increases K_{eq} increases and at higher *p*H K_{eq} is almost constant.

Second order rate constants k'_{on} have been calculated to compare the rate constants of pyridine and 4-methyl pyridine for the formation of complexes with $BrCH_2Co(DH)_2OH_2$ from the slopes of the pseudo-first order rate constants as a function of ligand concentration. The pH independent second order rate constants were caluculated by dividing

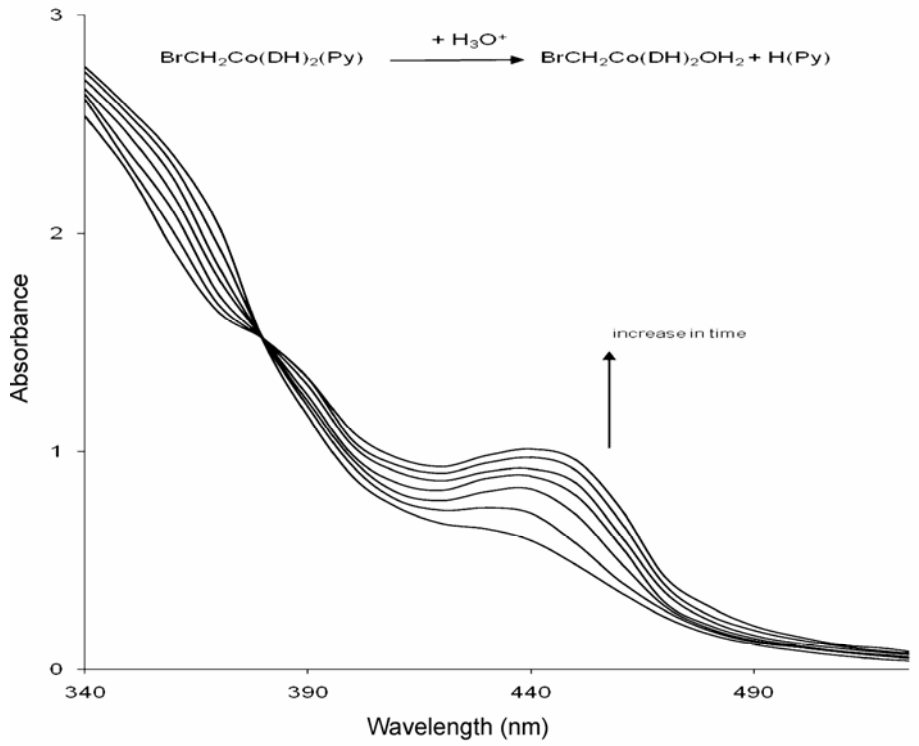

Figure 2. UV-Visible scan: Kinetics of dissociation of $BrCH_2Co(DH)_2Py$ into $BrCH_2Co(DH)_2OH_2$ at *p*H = 2·5. Isosbestic point = 380 nm.

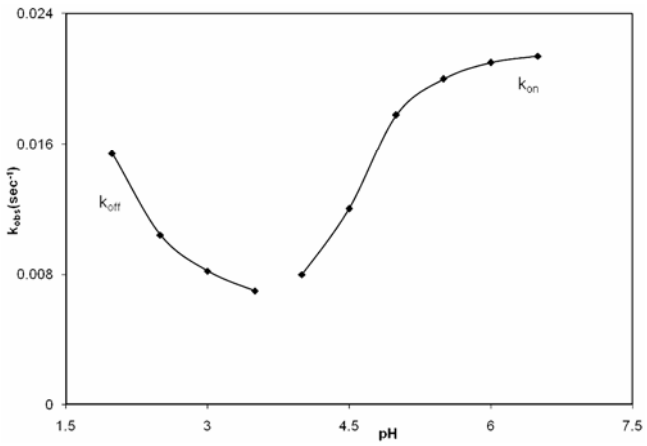

Figure 3. Dependance of *p*H on pseudo-first order rate constants for the formation and dissociation of $[BrCH_2Co(DH)_2(Py)]$ at 250°C and ionic strength 1·0 M KCl.

k'_{on} with α_L ($k_{on} = k'_{on}/\alpha_L$) where α_L is the degree of dissociation of the ligands at a given *p*H. The k_{on} of 4-MePy > Py. This order of k_{on} is in accordance with the basicity of the ligands.

5. DNA binding studies

5.1 *Absorption studies*

The application of electronic absorption spectroscopy in DNA-binding studies is one of the most useful techniques.[25,26] Complex binding with DNA in the groove mode usually results in hypochromism and bathochromism. The extent of the hypochromism commonly parallels the groove binding strength. The absorption spectra of the complex $[CH_2BrCo(DH)_2(OH_2)]$ in the absence and presence of calf thymus DNA in *tris* buffer are illustrated in figure 4. In the UV region, the intense absorption bands observed for Co(III) complexes are attributed to intraligand $\pi - \pi^*$ transition of the coordinated groups. Addition of increasing amounts of CT DNA results in hypochromism and a moderate bathochromic shift of the UV spectrum of the complex $[CH_2BrCo(DH)_2(OH_2)]$. To quantitatively determine binding strength of the Co(III) complex, the intrinsic binding constants K_b of the complex with CT DNA were determined according to below given equation[27] Through a plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ vs [DNA],

where [DNA] is the concentration of DNA in base pairs. Where ε_a is the extinction coefficient observed for the MLCT absorption band at given DNA concentration, ε_f is the extinction coefficient of the complex in the absence of DNA, ε_b is the extinction coefficient of the complex fully bound to DNA. In the plots [DNA]/($\varepsilon_b - \varepsilon_f$) vs. [DNA], K_b is given by the ratio of slope to intercept. Intrinsic binding constants K_b of $1{\cdot}8 \times 10^4\ M^{-1}$ were obtained from the decay of the absorbance. The binding constants indicate that the complex binds strongly to the DNA.

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/(K(\varepsilon_b - \varepsilon_f)). \quad (8)$$

Binding of $CH_2BrCo(DH)_2Py$ with CT-DNA also measured and compared with aqua complex. $CH_2BrCo(DH)_2Py$ binds strongly ($K = 1{\cdot}03 \times 10^5\ M^{-1}$) to DNA when compared to aqua complex. This indicates that the pyridine ligand may partially intercalate in between base pairs.

5.2 *Fluorescence spectroscopic studies*

The complex $[CH_2BrCo(DH)_2(OH_2)]$, exhibits luminescence in *tris* buffer (*p*H 7·0) at room temperature with a maximum at 387 nm. Binding of the complex to DNA was found to increase the fluorescence intensity. The emission spectra of the complex in the absence and presence of CT DNA are shown in figure (5).

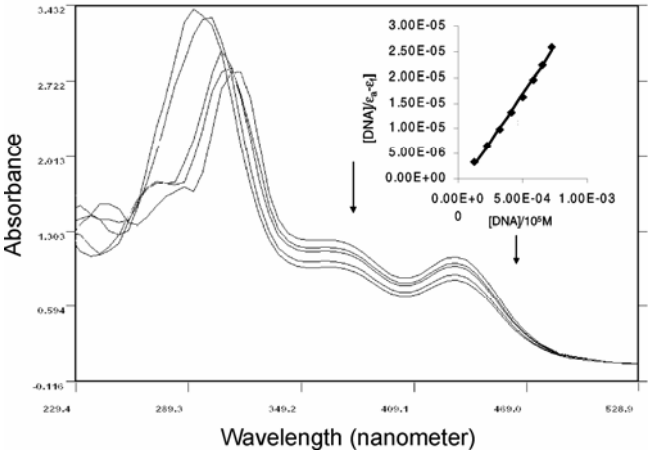

Figure 4. Absorption spectra of complex in *tris*-HCl buffer on addition of CT DNA in absence (top) and presence of CT DNA (lower) the [Co] = 10 μm; [DNA] = 0–126 μm. Inset: plots of relative intensity vs [DNA]/[Co] for the titration of DNA with Co(III) complex.

This observation is further supported by the emission quenching experiments using $[Fe(CN)_6]^{4-}$ as quencher. The ion $[Fe(CN)_6]^{4-}$ distinguish between differentially bound Co(III) species and positively charged free complex ions readily as the ions are quenched by $[Fe(CN)_6]^{4-}$. The complex bound to DNA can be protected from the quencher, because highly negatively charged $[Fe(CN)_6]^{4-}$ would be repelled by the negative DNA phosphate backbone, hindering the quenching of the emission of the bound complex. The method essentially consists of

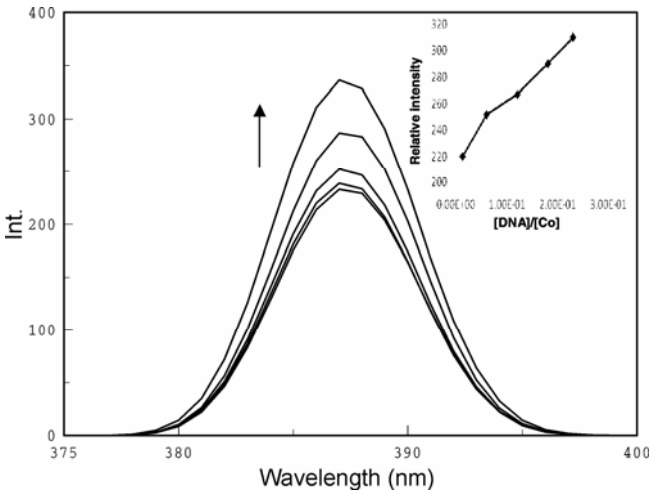

Figure 5. Fluorescence Emission spectra of complex in *tris*-HCl buffer fluorescence intensity increases on increasing CT DNA concentrations (5 μl, 10 μl, 15 μl, 20 μl of DNA addition). Inset: Plots of relative integrated emission intensity vs [DNA]/[Co].

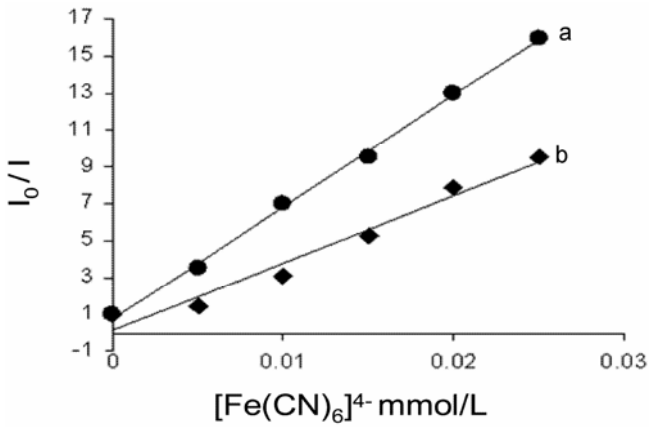

Figure 6. Emission quenching of Co(III) complex + DNA with ferrocyanide (a), quenching of Co(III) complex along with ferrocyanide (b), [Co] = 2 μmol/cm^{-3}, [DNA]/[Co] = 40.

titrating a given amount of DNA-metal complex with increasing the concentration of $[Fe(CN)_6]^{4-}$. The ferro-cyanide quenching curves for this complex in the presence and absence of CT DNA are shown in figure 6. The complex is protected by DNA hence quenching is low in presence of DNA. The absorption and fluorescence spectroscopy studies determine the binding of complex with DNA.

6. Conclusions

The binding of Pyridines to $BrCH_2Co(DH)_2OH_2$ follow the basicitiy order. 2-Et-Pyridine and 2-NH_2-Pyridine are more basic than pyridine but they form less stable complexes due to steric hindrance of the substituent at C_2 of Pyridine. D mechanism was proposed for the substitution of H_2O by L. The binding of $BrCH_2Co(DH)_2OH_2$ to DNA was monitored by absorbance and fluorescence spectroscopy techniques. This complex binds to DNA moderately through groove mode.

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