

## Kinetics and binding studies on the reaction of cyanopropyl(aquo)cobaloxime with CT-DNA and amines

P Pallavi<sup>a</sup>, Penumaka Nagababu<sup>a</sup>,  
J Naveena Lavanya Latha<sup>b</sup> & S Satyanarayana<sup>a,\*</sup>

<sup>a</sup>Department of Chemistry, Osmania University,  
Hyderabad 500 007, India

Email: ssnarasani@yahoo.com

<sup>b</sup>Centre for Cellular and Molecular Biology,  
Hyderabad 500 007, India

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The ligand substitution reactions of cyanopropyl (aquo) cobaloxime have been studied with various amines such as methylamine, ethylamine, butylamine, pentylamine, hexylamine, cyclopropylamine, cyclohexylamine and cyclopentylamine, as entering nucleophiles. The entering nucleophile displaces the molecule *trans* to the cyanopropyl group to form the six-coordinated complex, [CNCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Co(DH)<sub>2</sub>L]. The rate constants have been determined by varying the pH and concentration of the ligand under pseudo first order conditions. Equilibrium constants have been determined as a function of pH, for the binding of amines to [CNCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Co(DH)<sub>2</sub>L]. Study shows that the entering nucleophile participates in the transition state, and hence, SN<sup>1</sup> mechanism is proposed. Antimicrobial activity of cyanopropyl(aquo)cobaloxime on *E. coli* has been studied. The interaction of cyanopropyl(aquo) cobaloxime with CT DNA has been studied spectrophotometrically and binding constant has been calculated.

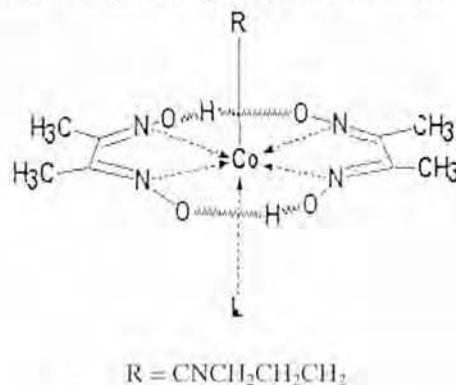
Vitamin B<sub>12</sub> has attracted the attention of chemists, as it is the only vitamin that has a metal in its structure. An intriguing aspect of B<sub>12</sub> is the high stability of the cobalt-carbon bond at the axial position in coenzyme B<sub>12</sub>. The activity of the coenzyme is the homolytic cleavage of the Co-C bond for the catalytic reactions. Model complexes have been synthesized to provide better insight into the mechanistic details of coenzyme B<sub>12</sub>. The cobalt-carbon cleavage is influenced by the distraction of the *corrin* ring and also by the substitution of the *trans* ligand at the  $\alpha$ -position, i.e., dimethylbenzimidazole, by various nucleophiles<sup>1,2,3</sup>. However, the mechanistic aspects have not been studied.

Substitution behaviour of aquacobalamin, in which reversible displacement of water by the entering nucleophile takes place, has been of great interest<sup>4,5</sup>. Marques and co-workers<sup>6</sup> have given evidence for

I<sub>d</sub> mechanism. Eldik and co-workers<sup>7</sup> have reported the kinetic aspects of cobaloximes using azoles as ligands.

To highlight the influence of *trans* and *cis* ligands bound to cobalt(III) on the Co-C bond, model complexes that contain Co(III) bound to equatorial ligands and also bound to two *trans* axial ligand were synthesized and studied. Studies by using various equatorial ligands such as dimethylglyoxime, glyoxime, imine and amines have been studied<sup>8,9</sup>. Cobaloximes play efficient models of coenzyme B<sub>12</sub>. Thus, our studies are confined to cobaloximes.

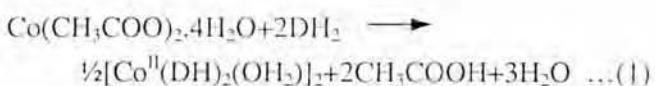
We have previously studied cobaloximes with different ligands at the  $\alpha$  and  $\beta$ -positions, the kinetics and binding aspects and their influence on Co-C bond<sup>10-13</sup>. We report herein the binding and kinetics of cyanopropyl(aquo)cobaloxime with primary and cyclic amines such as methylamine, ethylamine, butylamine, cyclopropylamine, cyclopentylamine and cyclohexylamine. We have also investigated the interaction of metal complex with CT DNA.

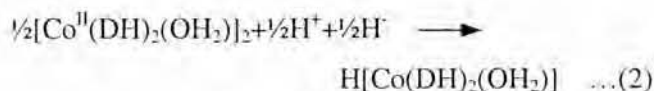


### Experimental

Dimethylglyoxime was purchased from Sigma Chemicals. KCl, HPLC grade methanol, acetic acid, HCl, phosphoric acid cobalt acetate, sodium borohydride were purchased from Fluka. Deionised water was used throughout. The reactions were carried out in dim light due to sensitivity of the Co-C bond to light.

The complex was prepared as previously reported by Brown *et al.*<sup>14</sup> (Eqs 1-3).





All experiments were performed under minimal illuminations due to photolability of organocobalt bond.

The binding and kinetic studies were carried out using Elico double beam spectrophotometer (model BL-198). The sample compartment temperature was maintained at  $25^\circ\text{C} \pm 1^\circ\text{C}$ .

The apparent dissociation constants ( $K_{\text{app}}$ ) were determined for the axial ligation of cyanopropyl (aquo)cobaloxime with different ligands. By taking fixed concentration of the complex and varying ligand concentration the absorbance was recorded. Solutions containing cyanopropyl(aquo)cobaloxime, an appropriate buffer (0.2 M) to maintain pH, KCl to maintain ionic strength (1.0 M) and varying concentrations of ligand were taken in 3 ml cuvette and allowed to equilibrate in a thermostat cell holder at  $25^\circ\text{C} \pm 0.1^\circ\text{C}$  for 15 mins prior to addition of cobaloxime.

Absorbances were recorded and the apparent equilibrium constants were calculated from the plot of  $\Delta A/[L]_f$  versus  $\Delta A$ . Thus, for each ligand,  $K_{\text{app}}$  was calculated using Eq. 4.

$$\Delta A = \Delta A_{\text{max}} [L]_f / (1/K_{\text{app}} + [L]_f) \quad \dots(4)$$

The least square fit of the above equation after rearrangement is given by Eq. 5

$$\Delta A = \Delta A_{\text{max}} - \{1/K_{\text{app}}(\Delta A/[L]_f)\} \quad \dots(5)$$

where  $\Delta A$  = difference in absorbance between solutions containing only cobaloxime with and without ligand and,  $\Delta A_{\text{max}}$  = maximum difference in absorbance recorded at high ligand concentration.

$[L]_f$ , the unbound ligand concentration was calculated from Eq. 6.

$$[L]_f = [L]_T - (C_T \Delta A / \Delta A_{\text{max}}) \quad \dots(6)$$

where  $[L]_T$  is the total volume of ligand added, and,  $C_T$  is the total concentration of cyanocobaloxime.

The pH independent equilibrium constants were then calculated from Eq. 7.

$$K_{\text{eq}} = K_{\text{app}} / \alpha_L \quad \dots(7)$$

where  $\alpha_L$ , the fraction of ligand as free base, was calculated from Eq. 8.

$$\alpha_L = K_a / (K_a + [\text{H}^+]) \quad \dots(8)$$

#### Kinetic studies

We have investigated the ligand substitution reactions of cyanopropylcobaloxime with amines at  $25^\circ\text{C}$ . The reaction rates were determined by maintaining pseudo-first order conditions by taking 10-fold excess of ligand concentration with respect to the complex concentration. The kinetics was studied at 450 nm by varying the concentration of the ligand and using appropriate buffer at pH than that of  $pK_a$  of ligand. The absorbance was monitored at  $\lambda_{\text{max}}$  450 nm.

The first rate constants ( $k_{\text{obs}}$ ) were obtained by least square fits of the data to Eq. 9.

$$\ln A_t - \ln A_\infty = k_{\text{obs}} t \quad \dots(9)$$

where  $A_t$  is absorbance at time  $t$  and  $A_\infty$  is the final absorbance.

#### Results and discussion

The ligand substitution reaction of cyanopropyl aquocobaloxime with amines is shown by the reaction given below.



UV-vis scan of cyano(aquo)propylcobaloxime with varying concentration of the ligand is given in Fig. 1. Depending on the  $pK_a$  values of the ligands, the binding studies were made in the pH range above and below the  $pK_a$  values. The  $K_{\text{app}}$  values were determined as a function of pH.

The dependence of  $K_{\text{app}}$  for ligation of cyano(aquo)propylcobaloxime with amines upon the pH is given in Fig. 2. Up to  $pK_a$  of the ligand,  $\log K_{\text{app}}$  increases with pH but above  $pK_a$ ,  $\log K_{\text{app}}$  is independent of pH. It was observed that the  $K_{\text{app}}$  value below the  $pK_a$  value is very low due to protonation of ligand. As the pH increases, the ligand gets deprotonated and binds strongly to Co(III) and  $K_{\text{app}}$  increases.

The equilibrium constants with respect to linear chain amines follows the order: methylamine < ethylamine < butylamine < pentylamine < hexylamine. With cyclic amines, it increases in the order: cyclopropylamine < cyclopentylamine < cyclohexylamine.

As basicity increases, stability constant increases in cycloamines and linear chain amines. Though cycloamines are more basic than straight chain

compounds they form less stable complexes because of steric crowding. The bulky group attached to nitrogen causes hinderance to binding of nitrogen to cobalt(III). The steric crowding dominates the  $\sigma$  donor ability of the ligand.

The rate of ligand substitution is  $pH$  dependent. The rate of the reaction increases drastically near the  $pK_a$  of the ligand. The slope of the plot of  $k_{obs}$  versus concentration of the ligands (Table 1) gives second order rate constant,  $k'_{on}$  at a given  $pH$ .

The slopes of the least square fit of the Eq. 10 gives the second order rate constant.

$$k_{obs} = k'_{on} [L]_T + k_{off} \quad \dots(10)$$

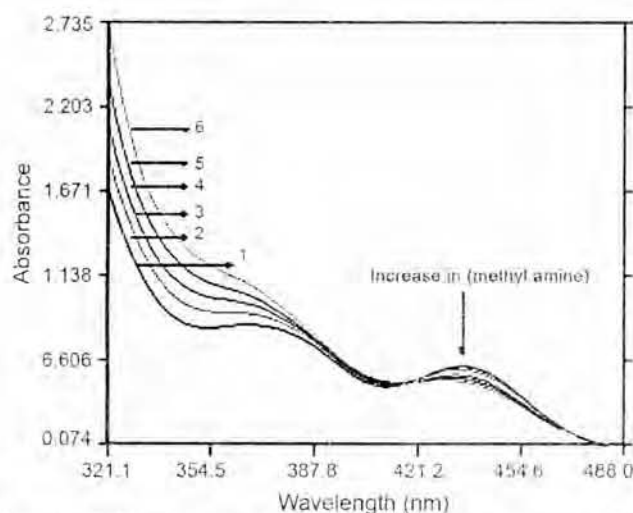


Fig. 1 — UV-vis spectra of binding of cyanopropyl(aquo) cobaloxime with varying concentration of methylamine [ $pH = 10$ ; temp =  $25^\circ C$ , isosbestic point = 470 and 420 nm. [Curve 1, 10  $\mu L$ ; curve 2, 20  $\mu L$ ; curve 3, 0  $\mu L$ ; curve 4, 40  $\mu L$ ; curve 5, 60  $\mu L$ ; curve 6, 50  $\mu L$ ].

$[L]_T$  = Total ligand concentration

The  $pH$  independent second order rate constants,  $k_{on}$  were obtained by using the Eq. 11.

$$k_{on} = k'_{on} / \alpha_L \quad \dots(11)$$

As the  $pH$  increases, the  $k_{obs}$  increases and the deprotonated form of the ligand is readily available at higher  $pH$ .

The second order rate constants increases as the nucleophilicity of the ligand increases. This is in accordance with the order of  $K_{eq}$  values. The studies on cobalt complexes and adenosylcobaloxime provide evidences for the mechanism of substitution to be dissociative<sup>15</sup> ( $I_i$  or  $D$ ). In view of the evidence presented above for the existence of pentacoordinate

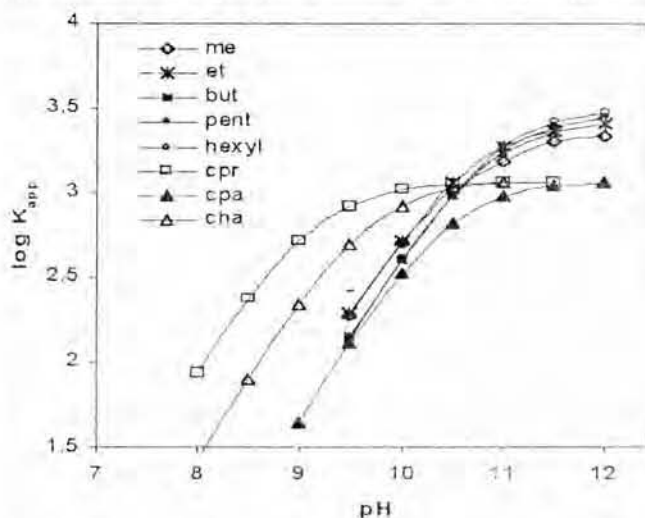


Fig. 2 — Plot of  $\log k_{app}$  versus  $pH$ , for the axial ligation of cyanopropyl(aquo)cobaloxime by different amine ligands at  $25^\circ C$ .

Table 1 — Dependence of the rate constants, ( $k_{obs}$ ) for the axial ligation of cyanopropyl (aquo) cobaloxime, on the concentration of the ligand (L) at  $25^\circ C$

M:L	$k_{obs}(s^{-1})$			M:L	$k_{obs}(s^{-1})$		
	Simple amines				Cyclic amines		
	Butylamine	Pentylamine	Hexylamine		Cyclopropylamine	Cyclopentylamine	Cyclohexylamine
1:10	$4 \times 10^{-5}$	$5.2 \times 10^{-5}$	$3.0 \times 10^{-5}$	1:10	$5.2 \times 10^{-6}$	$9.5 \times 10^{-5}$	$3.8 \times 10^{-5}$
1:15	$5 \times 10^{-5}$	$8.4 \times 10^{-5}$	$6.7 \times 10^{-5}$	1:15	$1.4 \times 10^{-5}$	$1.8 \times 10^{-4}$	$8.4 \times 10^{-5}$
1:20	$6 \times 10^{-5}$	$1.5 \times 10^{-4}$	$1.4 \times 10^{-4}$	1:20	$2.4 \times 10^{-5}$	$2.2 \times 10^{-4}$	$3.3 \times 10^{-4}$
1:25	$7 \times 10^{-5}$	$2.2 \times 10^{-4}$	$2.2 \times 10^{-4}$	1:25	$3.4 \times 10^{-5}$	$3.2 \times 10^{-4}$	$5.6 \times 10^{-4}$
1:30	$9 \times 10^{-5}$	$2.8 \times 10^{-4}$	$3.0 \times 10^{-4}$	1:30	$4.8 \times 10^{-5}$	$4.6 \times 10^{-4}$	$8.1 \times 10^{-4}$
$k_{on}$	0.0118	0.0132	0.0139	$k_{on}$	0.0404	0.0198	0.0021
$pH$	7.5	7.5	8.0	$pH$	8.5	8.0	6.0
$\alpha$	$5.62 \times 10^{-4}$	$4.89 \times 10^{-4}$	$1.35 \times 10^{-3}$	$\alpha$	$6.87 \times 10^{-3}$	$3.79 \times 10^{-3}$	$1.54 \times 10^{-2}$
$k_{on}$	20.996	26.99	32.63	$k_{on}$	5.88	5.227	0.4196

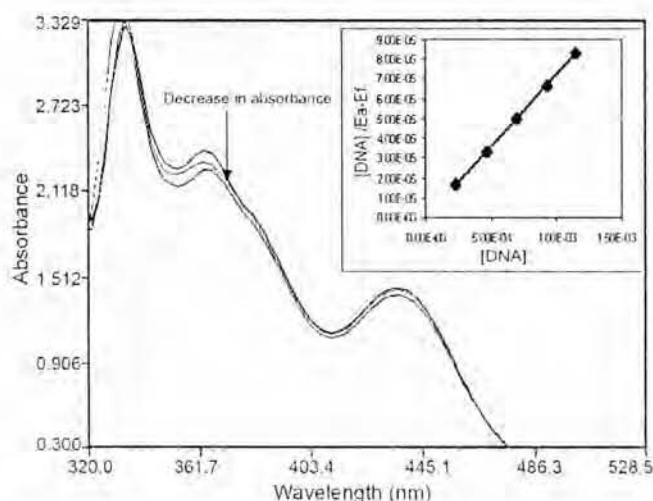


Fig. 3 — Absorbance of  $[\text{CNCH}_2\text{CH}_2\text{CH}_2\text{Co}(\text{DH})_2\text{L}]$  decreases on addition of DNA.

alkylcobaloximes and the ligation kinetic studies, both on alkyl cobalt complexes and on cobaloxime complexes<sup>16,17</sup> with other equatorial ligand system, an  $\text{S}_{\text{N}}1$  mechanism is suggested.

#### Antimicrobial activity

The above complexes have wide antimicrobial activity and therefore it is more appropriate to study the antimicrobial activities of these complexes against *E. coli*.  $\text{DH}_5 \infty$  strain of *E. coli* cells were inoculated on agar plates with Whatman no. 1 filter paper disc (1 mm dia.) loaded with the complexes (0-50  $\mu\text{m/L}$ ).

It was observed that the studied complexes inhibit the growth of *E. coli*. The antimicrobial activity has been found to be concentration and substitute dependent as the zone of inhibition increases with increase in concentration of the complex (5 mm dia. for 20  $\mu\text{m/L}$ ).

#### DNA binding

The absorption of metal complex in presence of CT DNA decreases as compared to that in the absence of DNA (Fig. 3) indicating that metal complex bind to DNA. In order to quantitatively compare the binding strength of the complex  $[\text{CNCH}_2\text{CH}_2\text{CH}_2\text{Co}(\text{DH})_2\text{OH}_2]$ , the intrinsic binding constants  $K$  of the complex

with CT DNA was determined according to the Eq. 12 by a plot of  $[\text{DNA}]/(\Sigma\epsilon_{\text{b}} - \Sigma\epsilon_{\text{f}})$  versus  $[\text{DNA}]$ .

$$[\text{DNA}]/(\Sigma\epsilon_{\text{b}} - \Sigma\epsilon_{\text{f}}) = [\text{DNA}]/(\Sigma\epsilon_{\text{b}} - \Sigma\epsilon_{\text{f}}) + 1/(K(\Sigma\epsilon_{\text{b}} - \Sigma\epsilon_{\text{f}})) \dots (12)$$

Due to the lability of the aquo group in cyanopropyl(aquo)cobaloxime, it is likely that coordination of cobalt(III) to the site on the DNA occurs with concomitant loss of water from the cobalt.

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