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A *trans* influence study in propyl (aquo)cobaloxime by imidazoles and amino acids

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Abstract. Substitution reactions of propyl cobaloxime with imidazole, substituted imidazoles, histidine, histamine, glycine and ethyl glycine ester are carried out as a function of pH. Trends in the formation constants are explained based on the steric hindrance, extent of *p*-bonding and *s*-donor capacity of the incoming ligand. Molecular mechanics is used to theoretically determine the bond length and bond strain values by MM2 parametrization and these are correlated with the experimental data.

Keywords. Spectrophotometry; equilibrium constants; *trans* influence; molecular mechanics; Co–C bond.

1. Introduction

Adenosyl cobalamin (Adocbl) is an essential cofactor for at least 17 different enzymatic systems.[1–4] A key to the reactivity of Adocbl is in the cleavage of the biologically rare Co–C bond. Exactly how Adocbl-dependent enzymes accomplish rate acceleration [10^{12}-fold acceleration] is still not well understood.[5,6] In recent years, there were several attempts to replicate them in experimental model systems with varying success.[7–10] Among the vitamin B_{12} models, organocobaloximes are noteworthy because of their ability to accommodate a wide variety of metal-bound alkyl groups containing a large number of different types of substituents.[11] There has been continual interest to examine the steric *cis* and *trans* effects in these cobaloximes to study the factors influencing the Co–C bond cleavage. The strength of the Co–C *s*-bond depends on several factors. These are the equatorial ligands which influence the redox potential of the central cobalt ion; the character of the ligand in the trans position to the R group; the character of the substituents on the aliphatic residue R; steric hindrance which stems from the equatorial ligand structure and from the substituents on R.

The present paper describes the equilibrium and molecular mechanistic studies performed for propyl (aquo)cobaloxime with a series of ligands to unravel the differences in Co–C and Co–N bond lengths.

2. Materials and methods

Imidazole (Imd), 1-methyl imidazole (1-Meimd), 2-methyl imidazole (2-Meimd), 1,2 dimethyl imidazole (1,2-Dimeimd), 2-ethyl imidazole (2-Etimd) (Acros) and histidine (Histd), histamine (Hisamn), glycine (Gly), ethyl glycine ester (Etglyest) and alkylating agents (Sigma–Aldrich) were used as purchased. Potassium dihydrogen phosphate, potassiumphosphate, *tris*(hydroxymethyl)-aminomethane (Tris) were obtained from Acros. Propyl (aquo) cobaloximes were prepared according to earlier reported procedure.[12] All manipulations were performed under minimal illuminations due to photolability of organocobalt bond.[13]

pH values were determined with a Digisun digital pH meter equipped with a combination glass electrode. The electrode was standardized at two pH values (4·0 and 9·2). UV–visible spectra were recorded on a Hitachi U-3410 spectrophotometer. Throughout the study, the concentration of propyl (aquo)cobaloxime was maintained as 0·001 M and absorption was fixed at 436 nm. Axial ligation kinetics was monitored by an Elico single beam spectrophotometer SL 171 model, the sample compartment of which was thermostatted at 25 ± 0·1°C.

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3. Results and discussion

3.1 *Trans influence studies*

A *trans* influence study includes investigations of all possible steric and electronic changes detectable in *trans* ligands. Cobaloximes have been the preferred systems for such studies in octahedral systems. Usually, the *trans* ligand is varied, and changes in the *cis* equatorial dioxime ligand are monitored. For this, apparent equilibrium constants for the axial ligation of alkyl(aquo)cobaloximes were determined spectrophotometrically. Solutions containing propyl cobaloxime, an appropriate buffer (0·2 M) to maintain pH, KCl to maintain ionic strength (1·0 M) and varying concentrations of ligand are taken in 3 ml cuvettes and allowed to equilibrate in the thermostatted cell compartment holder at 25 ± 0·1°C for 15 min prior to the addition of propyl cobaloxime:

$$K_{app} = \frac{[CH_3CH_2CH_2Co(DH)_2L]}{[CH_3CH_2CH_2Co(DH)_2OH_2][L]_{free}}. \quad (1)$$

For a given pH, K_{app} is calculated from the experimental data as below,

$$\Delta A = \Delta A_{max}\,[L]_f/[(1/K_{app} + [L]_f], \quad (2)$$

where ΔA is the difference in absorbance between solutions containing cobaloxime with added ligand (L) and solutions containing only cobaloxime at the same concentration, ΔA_{max} is the maximum absorbance change thus obtained at high $[L]_T$, and $[L]_f$ is the unbound ligand concentration. The data are analysed by a least-squares fit to a rearranged form of (2) to give,

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

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

$[L]_f$ is calculated from (3) using the measured value of ΔA and ΔA_{max}, $[L]_T$ is the total concentration of added ligand and C_T is the total concentration of cobaloxime.

From the UV–Vis spectra (figure 1) of $C_3H_7Co(DH)_2OH_2$ recorded for varying concentrations of histidine, it is evident that as the concentration of histidine increases, absorbance decreases. Values of K_{app} are evaluated from the least-squares fit of (4) and the slope is $-1/K_{app}$. The pH independent binding constant K_{eq} is calculated from the relation $K_{eq} = K_{app}/a_L$, where $a_L = Ka/(Ka + [H^+])$, Ka is the dissociation constant of the ligand. Table 1 summarizes the values of equilibrium constants and K_{app} for the reaction of all the ligands with propyl (aquo) cobaloxime. Logarithmic plots of log K_{app} vs pH are shown in figure 2, from which it is obvious that as the pH increases, the K_{app} value increases and after a certain value of pH, they become independent of pH. Affinities of the ligands follow the order, 1-Meimd > Imd > Histd > Hisamn > Gly > Glyest > 2-Meimd > 1,2Dimeimd > 2-Etimd.

The order of these ligands may be explained by considering the HSAB principle, basicity of ligands and their ability of *p*-bonding and *s*-donation. The series of imidazoles and substituted imidazoles follow the order, 1-Meimd > Imd > 2-Meimd > 1,2dimeimd > 2-etimd. For 1-meimd and Imd the formation constants are high for higher p*Ka* values i.e. they follow the basicity order. From 2-meimd to 2-etimd, steric hindrance at C_2 position of the imidazole plays a role and they do not follow the basicity order. pH dependence plots of log K_{app} for imidazole reveal that, initially as the pH increases, log K_{app} increases indicating that imd free base is the only ligating species. With further increase in pH, K_{app} reaches maximum value as the availability of free imidazole is maximm at higher pH values and for further rise in pH, K_{app} is pH independent.

If we consider the amino acid series, they fall in the order, Histd > Hisamn > Gly > Etglyest. In the case of histamine and histidine, there is no increase in K_{app} at pH above the p*Ka* of the ligand indicating that the binding is through the endocyclic nitrogen. If it binds through NH_2 group at higher pH, there should be an increase in K_{app} even at higher pH.

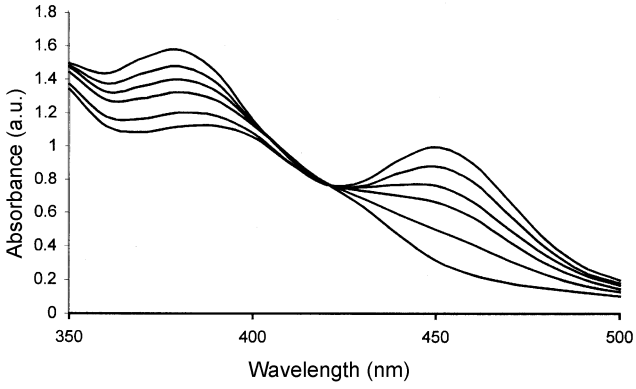

Figure 1. Binding of $[C_3H_7Co(DH)_2OH_2]$ with varying concentrations of histidine at pH 7·5 and 25°C.

Table 1. Formation constants (log K_{app} values) for the $[C_3H_7Co(DH)_2L]$ complexes.

pH	Hisamn	Histd	Gly	Etglyest	1-Meimd	Imd	2-Meimd	2-Etimd
4·5	2·28	2·5						
5·0	2·4	2·7			1·47	1·9		
5·5	2·56	2·89			2·05	1·95		
6·0	2·79	3·1			2·58	2·0		
6·5	3·0	3·2			3·01	2·46		
7·0	3·17	3·25			3·2	2·82	1·3	0·382
7·5	3·3	3·3		2·0	3·3	3·06	1·4	0·818
8·0	3·4	3·31	1·51	2·18	3·35	3·1	1·5	1·163
8·5	3·44	3·33	1·99	2·22	3·4	3·12	1·62	1·358
9·0	3·445	3·35	2·44	2·3			1·66	1·45
9·5			2·78	2·35			1·69	1·5
10·0			2·9	2·35			1·72	1·52
10·5			2·995	2·35				
11·0			3·0	2·37				
11·5								
K_{eq}	1765	2190	1846	180	2316	1761	51	32

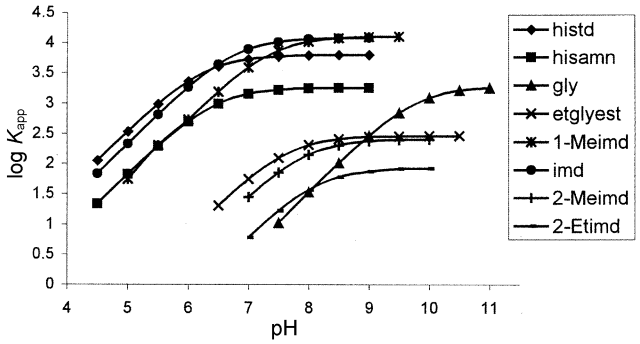

Figure 3. Dependence of log K_{app} on the pH for the axial ligation of $C_3H_7Co(DH)_2OH_2$ by different ligands at 25°C.

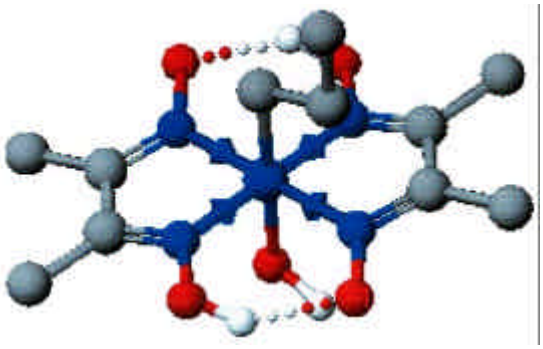

Figure 3. A ball and stick representation of the minimum energy structure of $[C_3H_7Co(DH)_2H_2O]$ (centre dark blue: cobalt, light blue: nitrogen; red: oxygen, grey: carbon, white: hydrogen).

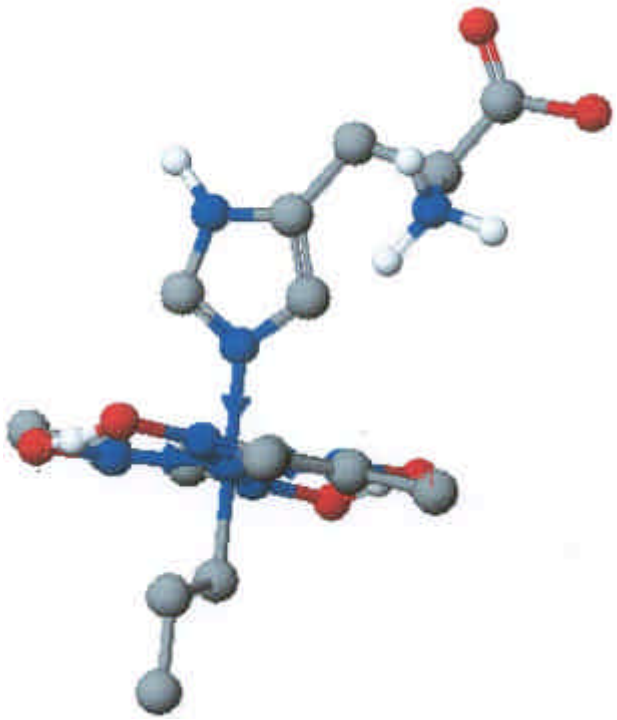

Figure 4. A ball and stick representation of the minimum energy structure of $[C_3H_7Co(DH)_2Histd]$ obtained by MM calculations. Colour codes as in figure 3.

With histidine, coordination is through the nitrogen of the imidazole ring, though there is a possibility of COO^- and NH_2 coordination, the NH_2 is mostly protonated below pH 8·0, hence not available for bind

Table 2. Bond lengths and bond strain values (in parentheses) obtained from molecular mechanics studies with propyl(aquo) cobaloxime.

	Aquo	Histd	Hisamn	Gly	Etglyest	Imd	1-Meimd	2-Meimd	2-Etimd
Co–N(1)	1·940 (0·198)	1·942 (0·219)	1·942 (0·206)	1·943 (0·227)	1·942 (0·201)	1·942 (0·217)	1·941 (0·210)	1·944 (0·251)	1·945 (0·254)
Co–N(2)	1·942 (0·201)	1·943 (0·232)	1·942 (0·217)	1·944 (0·237)	1·942 (0·227)	1·943 (0·220)	1·943 (0·229)	1·944 (0·244)	1·944 (0·248)
Co–N(3)	1·934 (0·100)	1·937 (0·133)	1·936 (0·122)	1·937 (0·136)	1·936 (0·137)	1·936 (0·121)	1·936 (0·127)	1·937 (0·140)	1·938 (0·147)
Co–N(4)	1·934 (0·105)	1·935 (0·127)	1·935 (0·115)	1·936 (0·122)	1·935 (0·118)	1·936 (0·122)	1·936 (0·117)	1·936 (0·132)	1·938 (0·147)
Co–C	1·954 (0·183)	1·956 (0·205)	1·956 (0·206)	1·956 (0·209)	1·955 (0·203)	1·955 (0·198)	1·955 (0·196)	1·956 (0·206)	1·956 (0·210)
Co–N	–	1·935 (0·100)	1·934 (0·099)	1·929 (0·118)	1·929 (0·112)	1·934 (0·103)	1·934 (0·102)	1·949 (0·332)	1·951 (0·370)
N(1)–C(1)	1·262 (0·003)	1·262 (0·004)	1·262 (0·004)	1·262 (0·003)	1·262 (0·002)	1·262 (0·003)	1·262 (0·004)	1·262 (0·004)	1·262 (0·003)
N(2)–C(2)	1·262 (0·003)	1·261 (0·002)	1·262 (0·004)	1·262 (0·003)	1·262 (0·003)	1·262 (0·004)	1·262 (0·003)	1·262 (0·005)	1·262 (0·004)
N(3)–C(3)	1·261 (0·001)	1·261 (0·003)	1·261 (0·002)	1·261 (0·001)	1·261 (0·002)	1·261 (0·001)	1·261 (0·001)	1·261 (0·001)	1·262 (0·003)
N(4)–C(4)	1·261 (0·002)	1·261 (0·002)	1·261 (0·002)	1·261 (0·002)	1·262 (0·002)	1·262 (0·002)	1·262 (0·002)	1·261 (0·001)	1·26 (0·001)

N(1), N(2), N(3), N(4) are the nitrogen atoms that bond with the cobalt mimicking the corrin ring system
C(1), C(2), C(3), C(4) are the carbon atoms attached to the nitrogens present in the entity mimicking the corrin ring system
C (carbon atom) in the propyl cobaloxime and N (nitrogen atom) in the ligand that bond with the cobalt atom

ing. Though histamine is slightly more basic than histidine, histidine forms a more stable complex than histamine because histidine is a better *p*-acceptor than histamine.

$K_{Gly} > K_{Etglyest}$ may be explained based on the basicity of the ligands, as both are *s*-donors. Glycine is more basic than glycine ester and hence forms more stable complexes than glycine ester. If we compare the pH-dependent binding plots of glycine and glycine ester in both cases K_{app} increases with increase in pH and after a certain pH value they become pH-independent.

"Soft" (or class b) character has been assigned to alkyl cobaloximes.[14–16] This is evident from the greater ligand affinity of imidazole[17–21] histidine or histamine compared to "hard" glycine or ethyl glycine ester. Further, softness may be directly related to the ability of a cobalt complex to stabilize a carbon-cobalt bond as seen in the cobaloximes.

The order of stability of the complexes may be attributed to the ability of imidazoles or histidine or histamine to accept electrons into higher-energy unfilled *p** anti-bonding orbitals through *dp–pp* back-bonding. But glycine and ethyl glycine ester cannot accept electrons in either fashion. A reverse order for the dependence of $RCo(DH)_2L$ stability on ligand basicity among the two series of ligands, aromatic (histamine, histidine, imidazole and substituted imidazoles) and aliphatic (glycine and ethyl glycine ester) is not unexpected owing to the following reasons.

(i) An increase in basicity is associated with increased ability for *s*-donation;
(ii) An increase in basicity is associated with the decreased ability of the aromatic ligands to function as *p*-acceptors. Hence, though histamine is more basic than histidine it forms slightly less stable complexes than histidine.

3.2 *Molecular mechanistic studies*

Molecular mechanics is a tool of increasing importance for structural investigations of coordination and organometallic chemistry.[22–25] In molecular mechan-

ics, mathematical equations are used to simulate all the components that contribute to the strain energy of a molecule, which is then minimized to find a low energy conformation. E_s, E_t, E_b and E_{vdw} were calculated using standard MM protocols.

In the present work, we have performed geometry optimization using MM2 parameterization provided in the Bio Med CACache 5·02 software. Figures 3 and 4 are the ball and stick representations of propyl (aquo) and propyl(histd)cobalximes respectively generated in the work space of the software. Bond-length and bond-strain values are then evaluated and compared. Table 2 illustrates the values that are obtained performing MM2 parameterization and are in confirmation with the binding data.

4. Conclusions

In this study we have observed that the formation constants follow the trend, 1-Meimd > Imd > Histd > Hisamn > Gly > Glyest > 2-Meimd > 1,2Dimeimd > 2-Etimd. This is explained based on the *ð*-bonding and basicity of the ligands (pK_a values). Though 2-meimd to 2-etimd are basic than Imd, they form less stable complexes due to steric hindrance. Co(III) is soft and hence binds more strongly to histidine and histamine as compared to glycine and ethyl glycine ester. From the molecular mechanics studies, the effect of incoming ligand on the Co–C bond is revealed.

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