Nucleophilic Participation of Incoming Ligands in the Transition State of Substitution Reactions of Aquocobalamin: Kinetics of the Reaction with Imidazole and its Derivatives

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Abstract

The kinetics of the reaction of aquocobalamin (vitamin B_{12a}) with imidazole, 1-methyl imidazole, 1,5-dimethyl imidazole, 4(5)-methyl imidazole, 2-methyl imidazole, imidazole 4(5)-lactic acid, histamine, and histidine were studied as a function of pH at 25 °C, 1.0 M ionic strength (KCl) by UV–Vis spectrophotometry. 2-Methyl imidazole and, by analogy, the 4-substituted tautomers of assymmetrically substituted imidazoles, compared to imidazoles where tautomerism is impossible (1-methyl imidazole, 1,5-dimethyl imidazole), inconsequential (imidazole), or where the 5-substituted tautomer is the greatly predominant species in solution (neutral histamine, anionic histidine), replaces H_2O in B_{12a} insignificantly slowly due to steric repulsion between the annular substituent and the corrin ring. The rate of substitution of H_2O varies linearly with the pK_a of the incoming ligand, L (L = anionic histidine, neutral histamine, 1-methyl imidazole, 1,5-dimethyl imidazole) establishing the existence of nucleophilic participation of L in the transition state. The positive deviation of imidazole itself from this series is attributed to the possibility of its reaction through two annular coordination sites. The negative deviation of 4(5)-methyl imidazole, imidazole 4(5)-lactic acid, cationic histamine and neutral histidine from the linear relationship found is attributed to the presence of two tautomeric forms in solution and provides a means of estimating values of the tautomerisation constant, K_T, for these species.

Introduction

The cobalt corrinoids (derivatives of vitamin B_{12}) contain a Co ion, formally in the +3 oxidation state, coordinated by four nitrogen atoms of the macrocycle, corrin [1]. In the cobalamins, the lower (α) coordination site is occupied by 5,6-dimethylbenzimidazole (Bz), which is bonded to an aminopropanol side-chain of the corrin ring by an α-glycosidic link through a ribose and phosphate. The upper (β) coordination site may be occupied by a variety of ligands; in aquocobalamin (vitamin B_{12a}) this site is occupied by a water molecule (or hydroxide, pK_{Co} = 8.10 at 1.0 M ionic strength [2, 3]).

The inorganic chemistry of vitamin B_{12} and its derivatives [1, 4] continues to attract considerable attention and the ligand substitution reactions of aquocobalamin (here, for convenience, abbreviated Bz-Co-H_2O, i.e. showing only the axial ligands, and neglecting the overall charge) has been the subject of a number of investigations. Randall and Alberty [5, 6] and Thusius [7, 8] established that the substitution rates for B_{12a} with various incoming ligands, L (L = Br^-, I^-, SCN^-, NCO^-, $S_2O_3^{2-}$, N_3^-, SO_3^{2-}, HSO_3^-, CN^-) vary by about two orders of magnitude, whereas formation constants vary by about 11 orders of magnitude. Together with the observation that the log of the rate of aquation of Bz-Co-L increases linearly with the log of the equilibrium constant for eqn. (1), this led to the view that the mechanism of the ligand substitution reactions of B_{12a} is essentially dissociative [9].

$$\text{Bz-Co-L} + H_2O \rightleftharpoons \text{Bz-Co-}H_2O + L \quad (1)$$

Imidazole, however, was found to react significantly more slowly than other ligands studied (27 M^{-1} s^{-1}; cf. 0.1–7.1 × 10^3 M^{-1} s^{-1}) [6] which led to the suggestion that the mechanism of ligand substitution of B_{12a} was not a simple uni-molecular release of water followed by fast binding of the incoming ligand [6].

Reenstra and Jencks [2] studied the kinetics of the reaction of B_{12a} with cyanide in detail and established that both HCN and CN^- react with the Co(III) centre. They argued that the intimate mechanism of the reaction is essentially I_d, and that there may be a small component of nucleophilic participation by the incoming ligand in the transition state. More recently [3], this reaction was reinvestigated both as a function of pH and temperature. The

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transition state involving CN^- is enthalpically stabilised by 25 kJ mol^{-1} relative to that involving HCN, which provides strong evidence for nucleophilic participation of the incoming ligand in the rate-determining step of the reaction.

The variability in the ligand substitution rates may be due to other factors; for example, the second-order rate constant for substitution of H_2O in B_{12a} by NH_2OH and CH_3NH_2 is 21.5 and 1.05 M^{-1} s^{-1}, respectively [10] and may reflect the influence of hydrogen bonding between the acetamide side-chains of the corrin ring, which point towards the β face, and the incoming ligand.

We report here the results of an investigation into the rates of substitution of H_2O in B_{12a} by a series of closely related ligands, L, all derivatives of imidazole, and establish that, at least as far as these ligands are concerned, L participates in the transition state of the reaction.

Experimental

Materials

Imidazole (Aldrich) was recrystallised twice from hot toluene. 2-Methyl imidazole (Fluka) was found by capillary GC (on a 25 m Carbowax 20M column) to be contaminated by approximately 0.4% imidazole, a fact readily verified by fluorescence spectroscopy since imidazole, but not 2-methyl imidazole, fluoresces at 385 nm when irradiated at 324 nm. The crude material was repeatedly recrystallized from hot toluene until no trace of imidazole was found by GC, and there was no significant fluorescence in the samples. 4(5)-Methyl imidazole (Aldrich) was purified by vacuum sublimation. 1,5-Dimethyl imidazole was prepared as described by Pyman [11]. This isomer was separated from the 1,4-isomer by repeated fractionation through a 7 cm column packed with glass helices. GC analysis showed that contamination of the 1,4-isomer in the final distillate was ~2%. Histamine, histidine, imidazole 4(5)-lactic acid (Sigma) and 1-methyl imidazole (Aldrich) were used without further purification. Water was distilled twice in an all-glass Büchi Fontavapor 285 double-distillation still, and further purified by passage through a Millipore MilliQ system (18 MΩcm). Hydroxocobalamin was purchased from Roussel and found by HPLC to be >99% pure.

Methods

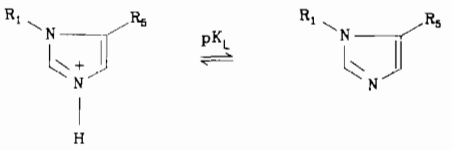
The kinetics of ligand substitution of B_{12a} were studied between pH 5.5 and 10.5 using phosphate, tris/HCl and bicarbonate buffers (all 0.1 M), as appropriate. Ionic strength was maintained at 1.0 M with KCl. Reactions were performed under pseudo first-order conditions; they were initiated by addition of 100 μl B_{12a} (to a final concentration of between 50 and 100 μM) to 2.4 ml of a solution containing the ligand (concentration at least 10 times greater than the B_{12a} concentration), buffer and KCl, in a 1 cm pathlength cuvette which had reached thermal equilibrium (25.0 ± 0.1 °C) in the constant temperature cell block of a Cary 2300 spectrophotometer, and followed by observing the increase in absorbance at 358 nm, the γ-band maximum of the imidazole complex. At any given pH, usually five ligand concentrations were used, and varied by a factor of 10–100. Absorbance changes were monitored for >five half times, and pseudo first-order rate constants were obtained from a linear least-squares fit of $\ln(A_\infty - A)$ versus time; the computer programme used utilises an iterative procedure to find the optimum value of A_∞ for the best straight line. Second-order rate constants were then determined from the linear relationship between the pseudo first-order rate constant and the ligand concentration using standard linear regression methods. The pH of all solutions was determined using a Metrohm 605 pH meter and a Metrohm series EA 6.0203 micro combination glass electrode calibrated against standard buffers, all maintained at 25.0 ± 0.1 °C. This equipment was also used for the determination of the acid dissociation constants of the ligands.

The acid dissociation constants of all ligands were determined at 25.0 ± 0.1 °C by titration of *c.* 25 mM ligand solution in KCl (to a total ionic strength of 1.0 M) using standardised 1.0 M HCl or NaOH solutions (Merck), as appropriate. The electrode response (mV) as a function of titrant concentration was analysed using the computer programme EQUILIBRIA [12], from which acid dissociation constant values were obtained.

The fitting of the experimental data to non-linear equations was done with a computer programme using a Newton–Raphson procedure employing Marquardt's algorithm.

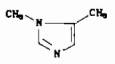
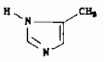
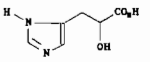
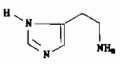
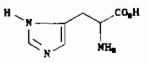
Results

Imidazoles undergo protonation of the endocyclic N atom, with acid dissociation constants, pK_L, in the range 6–8, according to eqn. (2).



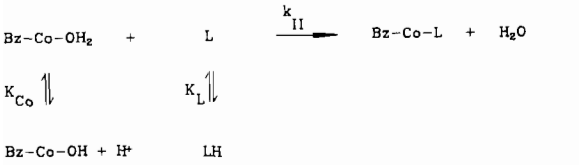
(2)

In addition, the side chain, R_5, may also have an ionisable group (pK_R). The values of all acid dissociation constants in each imidazole derivative studied (**I–VIII**) were determined potentiometrically, and the results are shown in Fig. 1.

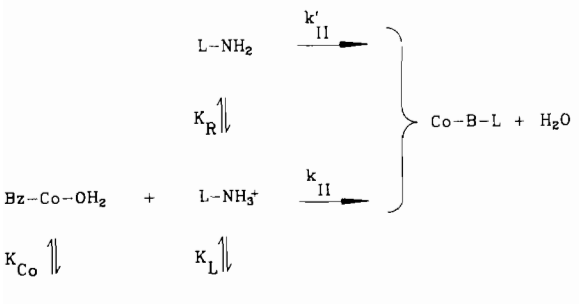
I:	Imidazole		7.241 ± 0.008
II:	1–Me imidazole		7.209 ± 0.005
III:	1,5–diMe imidazole		7.973 ± 0.009
IV:	4(5)–Me imidazole		7.763 ± 0.002
V:	2–Me imidazole		7.908 ± 0.003
VI:	imidazole–4(5)–lactic acid		2.988 ± 0.007[a] 7.386 ± 0.006[b]
VII:	histamine		6.412 ± 0.004[b] 9.957 ± 0.004[c]
VIII:	histidine		1.792 ± 0.007[a] 6.231 ± 0.007[b] 9.160 ± 0.007[c]

a:carboxylate
b:endocyclic N
c:exocyclic amine

Fig. 1. Structure and acid dissociation constants, pK_L, of imidazole and its derivatives. pK_L determined potentiometrically at 25.0 ± 0.1 °C, μ = 1.0 M (KCl); see text.



Scheme 1.



Scheme 2.

The plots of the pseudo first-order rate constant as a function of ligand concentration gave good straight lines with intercepts which, with the exception of 2-methyl imidazole ($9 \pm 3 \times 10^{-4}$ s^{-1}), were either very close to, or statistically indistinguishable from, the origin, i.e. the rate constant for aquation of the imidazole complex is in most cases very slow.

It is well-established that hydroxocobalamin is substitution-inert [2, 3, 13]. Furthermore, only the ligand, L, with a deprotonated endocyclic N, will displace H_2O from the Co(III) ion of aquocobalamin. Hence, the experimentally observed second-order rate constant, k_{II}^{obs}, corresponding to reaction (3) for which the rate law is given in eqn. (4) where $[Co_T] = [Bz\text{-}Co\text{-}OH_2] + [Bz\text{-}Co\text{-}OH]$ and $[L_T] = [L] + [LH]$, is related to the true second-order rate constant, k_{II}, for substitution of H_2O by the ligand, L, as shown in Scheme 1 if the functionality R_5 has no ionisable group in the operating pH range (5.5–10.5), or in Scheme 2 if it does.

$$Co_T + L_T \rightleftharpoons Bz\text{-}Co\text{-}L \tag{3}$$

$$\frac{d[Bz\text{-}Co\text{-}L]}{dt} = k_{II}^{obs} \times [Co_T] \times [L_T] \tag{4}$$

The relevant acid dissociation and rate constants are defined in the two Schemes.

Of the imidazoles studied (Fig. 1), **I–VI** are governed by Scheme 1; it is readily shown that the rate law is given by eqn. (5) and that k_{II}^{obs} and k_{II} are related by eqn. (6).

$$\frac{d[Bz\text{-}Co\text{-}L]}{dt} = \frac{k_{II}[Co_T][L_T]}{(1 + [H^+]/K_L)(1 + K_{Co}/[H^+])} \tag{5}$$

$$k_{II} = k_{II}^{obs}\left(1 + \frac{[H^+]}{K_L}\right)\left(1 + \frac{K_{Co}}{[H^+]}\right) \tag{6}$$

For **VII** and **VIII** (Fig. 1), Scheme 2 applies. Since the state of ionisation of the substituent R_5 affects the reaction rate (see below), the rate law involves two terms (eqns. (7) and (8)),

$$\frac{d[Bz\text{-}Co\text{-}L]}{dt} = [Co]_T[L]_T\left[\frac{[H^+]}{[H^+] + K_{Co}}\right] \times \left[\frac{k_{II}}{(1 + [H^+]/K_L + K_R/[H^+]} + \frac{k_{II}'}{(1 + [H^+]/K_R + [H^+]^2/K_L K_R)}\right] \tag{7}$$

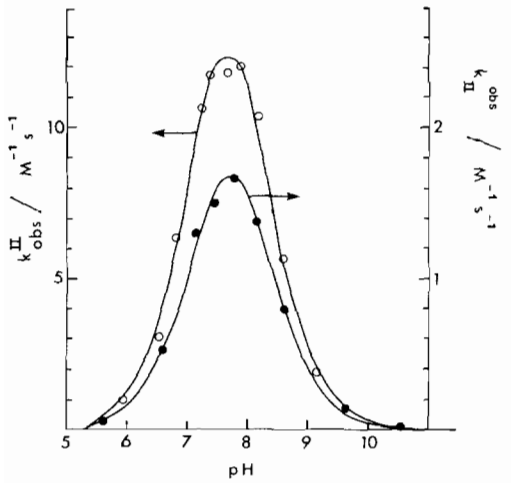


Fig. 2. Dependence of k_{II}^{obs} (eqn. (2)) on pH for reaction of imidazole (○) and imidazole 4(5)-lactic acid (●) with aquocobalamin at 25.0 °C, μ = 1.0 M (KCl).

$$k_{II}^{obs} = \left[\frac{[H^+]}{[H^+] + K_{Co}}\right]\left[\frac{k_{II}}{(1 + [H^+]/K_L + K_R/[H^+])} + \frac{}{(1 + [H^+]/K_r}\right] \quad (8)$$

Full pH profiles of the reaction rate were determined for imidazole and imidazole 4(5)-lactic acid, where Scheme 1 is applicable, and for histamine and histidine, where Scheme 2 is applicable. The results are given in Figs. 2 and 3, where the theoretical curves were fitted utilising either eqns. (6) or (8), as appropriate.

The good agreement between the experimental results and the theoretical curve in both Figures serves to validate Schemes 1 and 2. The result obtained for imidazole is in reasonable agreement with the previously reported value of 27 ± 6 M^{-1} s^{-1} [6]. For the other ligands, for which Scheme 1 is applicable, the reaction rate was determined only at selected pH values, and eqn. (6) applied to obtain the values of k_{II}. These results are given in Table 1. All kinetic results are summarised in Table 2.

Discussion

Using both standard framework molecular models, and molecular mechanics procedures using the computer programme ALCHEMY (Tripos Associates, St. Louis, MO) and the force field we have recently developed for studying steric factors controlling the structure of metalloporphyrins [14], we found severe steric strain between substituents at the 2 position of a coordinated imidazole and the corrin ring. The slow rate of substitution of H_2O in B_{12a}

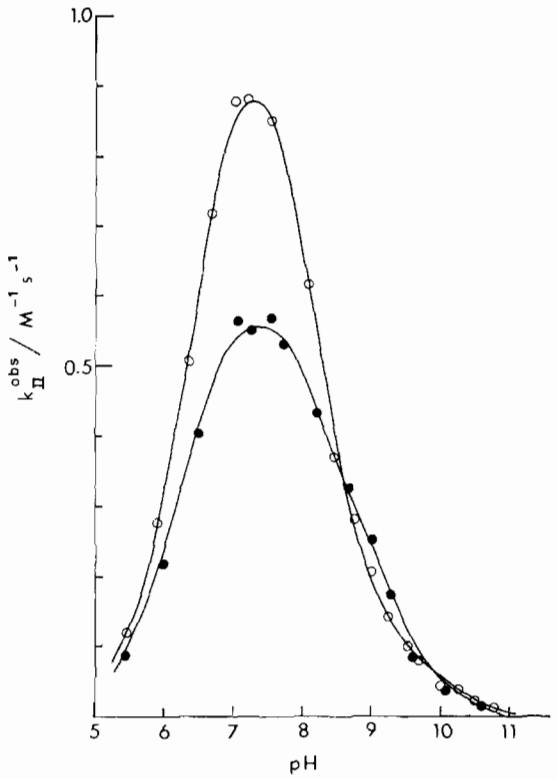
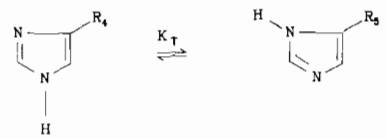


Fig. 3. Dependence of k_{II}^{obs} (eqn. (2)) on pH for reaction of histamine (○) and histidine (●) with aquocobalamin at 25.0 °C μ = 1.0 M (KCl).

by 2-methyl imidazole, compared to other imidazoles (Table 2), is attributed to this steric factor. Unsymmetrically substituted imidazoles are known to undergo rapid annular tautomerism as shown in eqn. (9) [15–18], where electron-donating and electron-withdrawing groups favour the 5- and 4-substituted isomers, respectively [15].



4–substituted isomer 5–substituted isomer (9)

1-Methyl imidazole and 1,5-dimethyl imidazole exist only in one tautomeric form; tautomerism in imidazole itself, and 2-methyl imidazole, is inconsequential because of symmetry. Although tautomeric interconversion is usually very rapid [16], hydrogen bonding between the annular NH group and a H bond acceptor in a side chain has been demonstrated to slow down the interconversion rate in benzimidazoles [19]. Monocationic histamine is reported to exist in aqueous solution as a 4:1 mixture

TABLE 1. Observed (k_{II}^{obs}) and derived (k_{II}) rate constants for reaction of some substituted imidazoles with aquocobalamin at 25.0 °C, μ = 1.0 M (KCl)

Ligand	pH	k_{II}^{obs} [a] (M^{-1} s^{-1})	k_{II} [b] (M^{-1} s^{-1})	$\bar{k}_{II}$ (M^{-1} s^{-1})
1-Methyl imidazole	6.254 ± 0.004	1.63	16.6	
	7.635 ± 0.005	8.85	16.3	
	9.105 ± 0.003	1.49	16.8	16.6 ± 0.3
1,5-Dimethyl imidazole	6.033 ± 0.004	0.262	23.3	
	7.510 ± 0.005	4.98	24.4	
	9.033 ± 0.005	2.14	22.3	23.3 ± 1.1
4(5)-Methyl imidazole	6.004 ± 0.003	0.151	8.89	
	7.622 ± 0.008	2.84	9.02	
	9.078 ± 0.003	0.817	9.00	8.97 ± 0.07
2-Methyl imidazole	6.103 ± 0.007	3.94×10^{-4}	0.0258	
	7.660 ± 0.005	6.97×10^{-3}	0.0263	
	9.005 ± 0.004	2.66×10^{-3}	0.0260	0.0260 ± 0.003

[a]Defined by eqn. (2). [b]See Scheme 2.

TABLE 2. Summary of the rate constants for reaction of substituted imidazoles with aquocobalamin at 25.0 °C, μ = 1.0 M (KCl)

Ligand	k_{II} [a] (M^{-1} s^{-1})	k'_{II} [a] (M^{-1} s^{-1})
Imidazole	21.2 ± 0.7	
1-Methyl imidazole	16.6 ± 0.3	
1,5-Dimethyl imidazole	23.4 ± 1.1	
4(5)-Methyl imidazole	8.97 ± 0.07	
2-Methyl imidazole	0.0260 ± 0.0003	
Imidazole 4(5)-lactic acid	3.35 ± 0.08	
Histamine	1.14 ± 0.01	7.51 ± 0.36
Histidine	0.65 ± 0.01	4.42 ± 0.13

[a]See Schemes 2 and 3 for definition of rate constants.

with the 4-substituted species favoured [20]; the neutral molecule exists exclusively as the 5-substituted tautomer, as found on its crystallisation from benzene [21]. These experimental results are in agreement with *ab initio* and INDO MO calculations [22] which show that although the 4-tautomer of monocationic histamine is more stable than the 5-tautomer, on neutralisation the equilibrium shifts in favour of the latter by between about 20 and 30 kJ mol^{-1}. Reynolds *et al.* [23] used ^{13}C NMR to show that in basic solution, but below the pK_R of the pendent amino group, neutral histidine exists predominantly in the 4-tautomeric form. By analogy with observations with substituted benzimidazoles [19], it is reasonable to presume that intramolecular hydrogen bonding (Fig. 4) is responsible for favouring the 4-tautomer when the amino group of the side-chain of histamine and histidine is protonated, but the 5-tautomer when it is deprotonated. In imidazole 4(5)-lactic acid, intramolecular hydrogen bonding (Fig. 4) could lead to stabilisation of either the 4- or the 5-tautomer (the pK_a of the hydroxyl group is >13 since no influence of this group was observed in the titration with NaOH). Since a hydrogen bond in which O is the hydrogen donor and N the acceptor is about 2 kJ mol^{-1} more stable than one in which N is the donor and O the acceptor [24], the 4-tautomer may be expected to be somewhat favoured.

In Fig. 4, k_{II} is plotted as a function of pK_L (the acid dissociation constant of the annular N atom) of the imidazoles **I–VIII**.

A good straight line ($R^2 = 0.996$) is obtained through the points for anionic histidine, neutral histamine, 1-methyl imidazole and 1,5-dimethyl imidazole, i.e. those species in which there is no tautomerism, the 5-substituted imidazole is the only one present, or the 5-tautomer is by far the more favoured species in solution. Imidazole reacts somewhat faster, probably because (making the reasonable assumption that the rate of tautomerisation is much faster than the rate of attack on Co(III)) there are two annular sites available for reaction. For this reason, imidazole was excluded from the data used for calculating the straight line.

The results of Fig. 5 establish that the rate of substitution of water by the incoming ligand depends linearly on the base strength of the ligand, which is strong evidence that, at least for this range of structurally related ligands, there is nucleophilic participation of the incoming ligand in the transition state. This provides further and more conclusive experimental evidence for this conclusion previously suggested by the difference in rate of substitution of H_2O by HCN and CN^- [3].

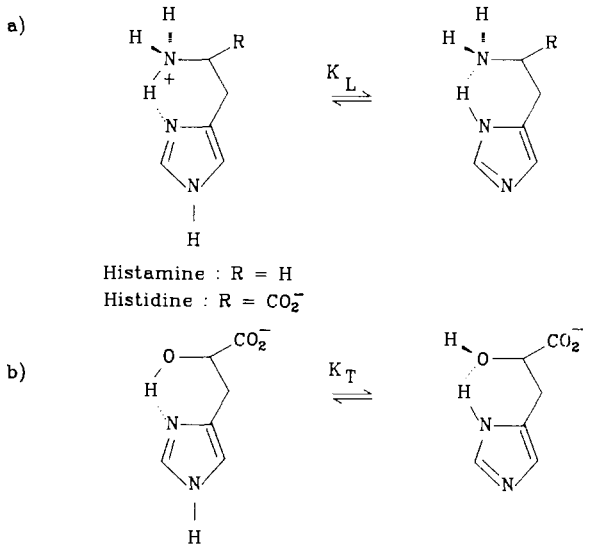


Fig. 4. Intramolecular hydrogen bonding in (a) cationic and neutral histamine and neutral and anionic histidine; and (b) in imidazole lactic acid.

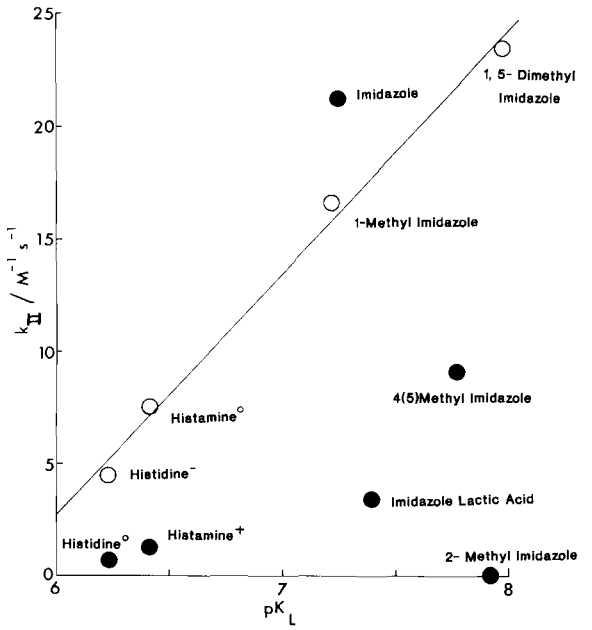


Fig. 5. Dependence of k_{II} on the acid dissociation constant, pK_L, of the annular N atom. The straight line through the open circles gives $R^2 = 0.996$.

As Reenstra and Jencks [2] have correctly pointed out, even a small amount of nucleophilic participation of the incoming ligand in the transition state will result in a significant rate increase if the intimate mechanism of the reaction is I_d. It is difficult to believe that a small Co(III) ion, especially embedded within such a bulky macrocycle, could expand its coordination sphere to such an extent for the reaction to proceed via a true associative mechanism.

TABLE 3. Tautomerisation constant, K_T, for substituted imidazoles estimated from deviation of the linear relationship between rate of reaction with aquocobalamin and annular N acid dissociation constant

	K_T [a]	ΔG [b] (kJ mol^{-1})	K_T [c]	Reference
Histidine° [d]	6.9	−4.8	~4	23
Histamine⁺ [d]	5.2	−4.1	~4	20
4(5)-Methyl imidazole	1.4	−0.8	0.6[e]	
Imidazole 4(5)-lactic acid	4.3	−3.6		

[a]Defined for the reaction (5-tautomer) ⇌ (4-tautomer). [b]Based on the value of K_T at 25.0 °C, μ = 1.0 M (KCl). [c]K_T reported, referenced in last column. [d]With protonated amino side-chain. [e]Based on the relationship log K_T = $3.2\sigma_m$ [15] and $\sigma_m = -0.07$ for methyl [25].

What the present work does demonstrate is that there is *some* participation of the incoming ligand in the transition state so that the rate of the reaction is sensitive to the donor power of the ligand. Whether this is sufficient to describe the mechanism as I_a is debatable, as there is no sharp distinguishing line between the two.

Comparison of the rate of substitution of H_2O by 1-methyl imidazole and 2-methyl imidazole shows that the rate of reaction of 4-tautomers is insignificant compared to that of 5-tautomers. Hence, deviation from the linear relationship illustrated in Fig. 5 provides an estimate of the position of the tautomerisation equilibrium; deduced values of the tautomerisation constant, K_T, defined by eqn. (10), are listed in Table 3.

$$K_T = [\text{4-tautomer}]/[\text{5-tautomer}] \quad (10)$$

The results for K_T obtained from this approach are in reasonable agreement with previous estimates for histidine and histamine, and the value for 4(5)-methyl imidazole is compatible with that obtained using Charton's application of the Hammett equation of the problem of imidazole ring tautomerism [15]. The K_T values for imidazole 4(5)-lactic acid verifies the suggestion made above that the 4-tautomer is somewhat favoured over the 5-tautomer because of the more stable hydrogen bond formed when O is the hydrogen donor and N the hydrogen acceptor.

Conclusions

This study has provided experimental evidence for the nucleophilic participation of at least some entering ligands in the transition state in the ligand substitution reactions of aquocobalamin. The study has also provided an apparently novel method for estimating the equilibrium constants for annular

tautomerism of imidazoles; however, because of the number of parameters invoked compared with the number of data, this conclusion can only be regarded as tentative.

Acknowledgement

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