DALTON
FULL PAPER

Kinetics and equilibrium studies on the reaction of vitamin B_{12a} with azoles†

Mohamed S. A. Hamza‡

Department of Chemistry, Faculty of Science, Ain Shams University, Abbassia, Cairo, Egypt

Received 21st March 2002, Accepted 22nd May 2002
First published as an Advance Article on the web 24th June 2002

The ligand substitution reactions of aquacobalamin (H_2Ocbl^+) were studied for pyrazole, 1,2,4-triazole and *N*-acetylimidazole as entering nucleophiles. These nucleophiles displace the coordinated H_2O molecules in H_2Ocbl^+ to form the six-coordinate product complex. The second order rate constants for the reaction of H_2Ocbl^+ with pyrazole, 1,2,4-triazole and *N*-acetylimidazole at 25 °C were found to be 35.0 ± 0.8, 24.0 ± 0.4 and 6.6 ± 1.6 M^{-1} s^{-1}, respectively. From temperature and pressure dependence studies, activation parameters ($\Delta H^{\neq}$, $\Delta S^{\neq}$ and $\Delta V^{\neq}$) for the reaction of H_2Ocbl^+ and *N*-acetylimidazole were found to be 108 ± 12 kJ mol^{-1}, +135 ± 40 J K^{-1} mol^{-1} and +7.2 ± 0.3 cm^3 mol^{-1}, respectively. In addition, $\Delta H^{\neq}$ and $\Delta S^{\neq}$ for the reaction with pyrazole were found to be 85 ± 4 kJ mol^{-1} and +69 ± 13 J K^{-1} mol^{-1} and those for 1,2,4-triazole were 90 ± 2 kJ mol^{-1} and +83 ± 6 J K^{-1} mol^{-1}. Based on the reported kinetic and activation parameters, the substitution of coordinated H_2O by these five-membered heterocyclic azoles follows an I_d mechanism in which the entering nucleophile partially participates in the transition state.

Introduction

The kinetics of the axial ligand substitution reaction of aquacobalamin (H_2Ocbl^+) and alkylcobalamins (vitamin B_{12} derivatives) have been of special interest to inorganic chemists over the last decade.[1–21] Ligand substitution reactions of vitamin B_{12a} in general follow a dissociative interchange mechanism[7–13,22,23] although claims of a dissociative mechanism have been made.[5,6,24,25] It was found that the rate of substitution of H_2O in H_2Ocbl^+ by a series of anions such as SCN^-, I^-, $S_2O_3^{2-}$, SO_3^{2-} and CN^- varies by about two orders of magnitude. However, the formation constant varies by about 11 orders of magnitude. This suggests that the substitution process is essentially dissociative.[26] Marques and co-workers [1,2] studied the kinetics of the reaction of H_2Ocbl^+ with imidazole, primary amines and some derivatives and they explained the data in terms of basicity and steric effect of these ligands. They also studied the kinetics of the substitution of H_2O by N_3^- and found that $\Delta V^{\neq}$ for the reaction equals 6.4 cm^3 mol^{-1}, which supports the operation of an interchange dissociative mechanism.[3] Furthermore, the activation enthalpies and entropies for the reaction of different anions (SCN^-, NO_2^-, $S_2O_3^{2-}$, SO_3^{2-} and HSO_3^-) showed a small difference that exists between most small anionic and neutral ligands. This was explained in the light of nucelophilic participation of these ligands in the transition state.[4] van Eldik and co-workers[7–9] found that the activation volumes for the reaction of H_2Ocbl^+ with thiourea, dimethylthiourea, 3-acetylpyridine and 4-acetylpyridine, N_3^- and HN_3 are between +4 and +10 cm^3 mol^{-1}. In the light of the activation parameters, it was suggested that these reactions proceed *via* an I_d mechanism.

One of the factors which has an effect on the ligand substitution reaction of H_2Ocbl^+ is hydrogen bonding. Hydrogen bonds play an important role in determining some of the properties of the corrinoids, such as the solubility and the formation of adducts with other hydrogen bonding molecules.[27–30] There is an interest in studying the occurrence of hydrogen bonding in iron porphyrins and cobalt corrinoids in light of the moderation of the activity of the Fe atom in haemoproteins through changes in hydrogen bonding to the coordinated imidazole of histidine.[31,32] The X-ray crystal structure and NMR spectroscopy of H_2Ocbl^+ have shown that there is an intramolecular hydrogen bond between coordinated H_2O and the O-39 carbonyl oxygen of the acetamide *c* side chain.[28–30]

There is a great interest in studying azoles which contain two or three nitrogen atoms in the five-membered ring from the bioinorganic chemistry point of view, because the imidazole group of histidine occurs as a ligand in most of the known haemoproteins.[33] The five-membered heterocycles have also been further stimulated by the success of many agricultural and medical fungicides, which inhibit steriod synthases by acting as ligands to the Fe(III)porphyrins in certain P-450 enzymes.[34,35] Imidazole derivatives and 1,2,4-triazole[36] have been considered of potential interest to coordination chemists because these compounds can be used to link studies on the metal binding properties of nitrogenous bases with information on the proton affinities in the gas phase,[37,38] in aqueous solutions and aprotic solvents,[39] and on their H-bonding capacities.[40] This paper presents the data obtained for the reaction of H_2Ocbl^+ with pyrazole, 1,2,4-triazole and *N*-acetylimidazole (N-Ac-ImH) to probe the above mentioned factors and to clarify the mechanism of these reactions.

Experimental

Materials

All the chemicals used were of analytical grade and used as received without further purification. Aquacobalamin and bis-tris buffer were purchased from Sigma. Pyrazole, N-Ac-ImH and 1,2,4-triazole were supplied by Aldrich. $HClO_4$ and $NaClO_4$ were purchased from Merck. Ultra pure water was used in the kinetic and thermodynamic measurements.

Instrumentation and measurements

The pH of the solutions was measured using a Mettler Delta 350 pH meter with a combined glass electrode. It was calibrated with standard buffer solutions at pH 4 and 7. UV-Vis spectra

---

† Electronic supplementary information (ESI) available: plot of k_{obs} *versus* [pyrazole] as a function of temperature. See http://www.rsc.org/suppdata/dt/b2/b202877h/

‡ Present address: Institute for Inorganic Chemistry, University of Erlangen-Nürnberg, Egerlandstr. 1, 91058 Erlangen, Germany. E-mail: hamza@anorganik.uni-erlangen.de

DOI: 10.1039/b202877h

were recorded on Shimadzu UV-2101 and Cary 5 spectrophotometers.

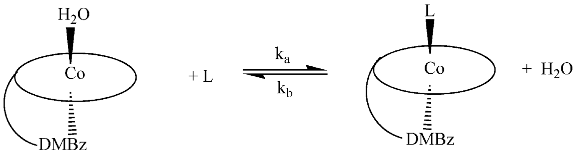
Kinetic measurements were carried out on an Applied Photophysics SX 18MV stopped-flow instrument coupled to an online data acquisition system. At least six kinetic runs were recorded under all conditions, and the reported rate constants represent the mean values. All kinetic measurements were carried out under pseudo-first order conditions, *i.e.* the ligand concentration was in at least a ten fold excess. Measurements under high pressure were carried out using a high pressure stopped-flow instrument described previously.[41] Kinetic traces were analysed with the OLIS KINFIT program (Bogart, GA).

All the instruments used during the course of this work were thermostatted to the desired temperature, ±0.1 °C. Values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were calculated from the slopes and intercepts, respectively, of plots of $\ln(k/T)$ *versus* $1/T$, and values of $\Delta V^{\neq}$ were calculated from the slope of plots of $\ln(k)$ *versus* pressure.

Equilibrium measurements: 3–5 × 10^{-5} M H_2Ocbl^+ dissolved in bis-tris buffer pH 6 ($I = 0.1$ M using $NaClO_4$), was placed in a 1.0 cm pathlength cuvette in the thermostatted cell block of the spectrophotometer for *ca.* 20 min. This solution was titrated by addition of small volumes of a concentrated stock solution of the azoles, using a Hamilton syringe. The azole solutions were prepared in the same buffer and the ionic strength was also adjusted to 0.1 M using $NaClO_4$. The titrations were carried out in duplicate and were monitored at two wavelengths where the greatest change in absorbance occurred (normally at 358 and 545 nm). The values of the equilibrium constant, K, were obtained by fitting the absorbance *versus* concentration curve to the appropriate equation, after correction for dilution (see Results and discussion).

Results and discussion

The reaction of H_2Ocbl^+ with the five-membered heterocyclic compounds (pyrazole, 1,2,4-triazole and N-Ac-ImH) have been studied in detail to investigate the effect of basicity and donor–acceptor properties of the incoming ligands, the role of H-bonding and the mechanism of these reactions. The reaction under investigation is presented in eqn. (1), where L is pyrazole, 1,2,4-triazole or N-Ac-ImH. DMBz is the 5,6-dimethylbenzimidazole moiety coordinated in the α-position of the cobalamins.



(1)

Preliminary experiments in which the UV-Vis spectrum was scanned in the range 300 to 700 nm, showed that at pH 6, pyrazole, 1,2,4-triazole and N-Ac-ImH react rapidly with H_2Ocbl^+. Equilibrium (1) was established within the time required for mixing the solutions and recording the spectra. However, another equilibrium takes place on a longer time scale (50 s) in the case of triazole (see later).

Equilibrium measurements

The values of the overall equilibrium constant K were determined spectrophotometrically (in duplicate experiments) by titrating *ca.* 3 × 10^{-5} M H_2Ocbl^+ with a concentrated stock solution of the azoles to minimize dilution effects. The UV-Vis spectrum of H_2Ocbl^+ in buffers at pH 6 shows characteristic bands at 351, 509 and 522 nm. Addition of any of these azoles used during the course of this work, shifts the γ-band in the UV-Vis spectrum to 358–359 nm or the α-band to 545–550 nm. These changes in the UV-Vis spectra are accompanied by good isosbestic points at 355, 386, 421 and 531 nm. This suggests that a simple equilibrium exists under these conditions and the aqua ligand is displaced by the azoles.

The spectrophotometric titration in the case of the azoles was monitored by following the increase in absorbance either at 358 or 545 nm or the decrease in absorbance at 351 nm, where the largest change in absorbance took place. Typical data for the reaction with pyrazole are shown in Fig. 1 as an example for one of the three azoles. The solid line represents the fit of the experimental data to eqn. (2), where A_o and A_∞ represent the absorbance at 0 and 100% formation of the product (pyrazole cobalamin), respectively, and A_x is the absorbance at any given ligand concentration [L].

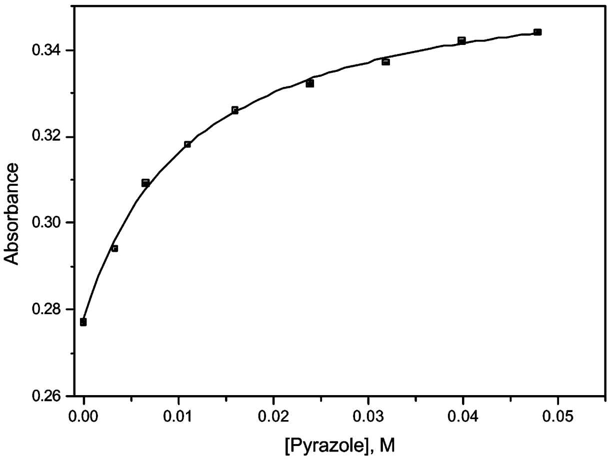


Fig. 1 Changes in absorbance at 545 nm on addition of pyrazole to H_2Ocbl^+; the solid line is a fit of the data to eqn. (2) in the text.

$$A_x = A_o + (A_\infty - A_o)K[L]/(1 + K[L]) \qquad (2)$$

The values of K and A_∞ were calculated from a non-linear least squares fit of the data to eqn. (2). The value for K(pyrazole) = 82 ± 7 M^{-1}. Moreover, the data were also analysed by plotting $\log(A_x - A_o)/(A_\infty - A_x)$ *versus* log[L], which resulted in a good linear plot with a slope of 0.97 ± 0.06 for pyrazole and 0.95 ± 0.07 in the case of N-Ac-ImH, indicating that only one ligand (L) is coordinated to the cobalt complex. The intercept of the linear plot gives the value of log K, which is in excellent agreement with the directly determined value described above.

Similar spectrophotometric titrations were carried out in the case of N-Ac-ImH and the value of K(N–Ac–ImH) was found to be 1500 ± 150 M^{-1} at 25 °C. It was difficult to determine the value of K spectrophotometrically in the case of triazole because there is an absorbance decrease at these wavelengths due to the interference from the second equilibrium (isomerization, see Kinetic measurements section). The value of K for the binding of ImH to H_2Ocbl^+ was reported to be 3.9 × 10^4 M^{-1},[42] from which it follows that the affinity of ImH for H_2Ocbl^+ is almost 26 times higher than for N-Ac-ImH and 476 times more than for pyrazole. This comparison demonstrates the effect of basicity since the $pK_{BH}{}^+$ values of pyrazole, N-Ac-ImH and ImH are 2.48, 3.6 and 7.24, respectively.[43] Good linear plots between log K for the coordination of nitrogenous bases (imidazole, pyridines and primary amines and their derivatives) *versus* $pK_{BH}{}^+$ of these bases were established in the case of Co(III)corrinoid and Fe(III)porphyrin.[44] These linear plots were used to probe the basicity and the steric effect of the nitrogenous bases.

Kinetic measurements

The reaction between 4 × 10^{-5} M H_2Ocbl^+ and excess pyrazole ([pyrazole] = 0.05–0.3 M, $I = 0.1$ M using $NaClO_4$) at pH 6 was studied at different temperatures (5–35 °C). The results are

Table 1 Kinetic data and activation parameters for the reaction of H_2Ocbl^+ with pyrazole, triazole and *N*-acetylimidazole as a function of temperature

	Pyrazole		1,2,4-Triazole		N-Ac-ImH		
Temp./°C	k_1/M^{-1} s^{-1}	k_{-1}/s^{-1}	k_1/M^{-1} s^{-1}	k_{-1}/s^{-1}	k_2/s^{-1}	K_1/M^{-1}	k_a/M^{-1} s^{-1}
5.0	2.5 ± 0.01	0.03 ± 0.002	1.5 ± 0.1	0.005 ± 0.02	0.27 ± 0.08	1.0 ± 0.4	0.27 ± 0.45
15.0	10.2 ± 0.3	0.1 ± 0.05	6.8 ± 0.2	0.05 ± 0.05	1.36 ± 0.30	1.6 ± 0.6	2.18 ± 0.63
20.0					3.02 ± 0.64	1.2 ± 0.4	3.6 ± 0.7
25.0	35.0 ± 0.8	0.4 ± 0.15	24.0 ± 0.4	0.21 ± 0.08	7.35 ± 1.57	0.9 ± 0.2	6.6 ± 1.6
35.0	92.0 ± 3.0	3.0 ± 0.5	76.0 ± 2.0	1.1 ± 0.4			
$\Delta H^{\neq}$/kJ mol^{-1}	85 ± 4	105 ± 11	90 ± 2	123 ± 7			108 ± 12
$\Delta S^{\neq}$/J K^{-1} mol^{-1}	69 ± 13	102 ± 38	83 ± 6	155 ± 25			135 ± 40

Experimental conditions: $[H_2O\ Cbl^+] = 4 \times 10^{-5}$ M, pH = 6.0, $I = 0.1$ M ($NaClO_4$).

shown in Fig. S-1(ESI), from which it follows that good linear plots with significant intercepts are obtained within the experimental error limits. Furthermore, the plots do not indicate any saturation at high [pyrazole]. This behavior can be expressed by the rate law given in eqn. (3), where k_a and k_b represent the rate constants for the forward and reverse reactions in eqn. (1), respectively. The values of k_a and k_b as a function of temperature, and the corresponding activation parameters are summarized in Table 1.

$$k_{obs} = k_a[\text{pyrazole}] + k_b \qquad (3)$$

The values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ for the forward reaction (k_a) were found to be 85 ± 4 kJ mol^{-1} and +69 ± 13 J K^{-1} mol^{-1}, respectively, whereas those for the reverse reaction (k_b) were found to be 105 ± 11 kJ mol^{-1} and 102 ± 38 J K^{-1} mol^{-1}, respectively. The significant positive values of $\Delta S^{\neq}$ obtained in this case suggest that the reaction is dissociative in nature. The values of k_a and k_b can be used to calculate K (= k_a/k_b), which turns out to be 87.5 ± 1 M^{-1} at 25 °C. The kinetically determined value of K is in very good agreement with that obtained spectrophotometrically at the same temperature, *viz.* 82 ± 7 M^{-1}.

Kinetic traces (growth in absorbance followed by decay) recorded by mixing solutions of 4×10^{-5} M H_2Ocbl^+ and 1,2,4-triazole (0.05–0.3 M) in the stopped-flow instrument at pH 6.0 (bis-tris buffer) and $I = 0.1$ M ($NaClO_4$) are shown in Fig. 2a. The observed rate constant (k_{obs}) for the first step was obtained by analyzing the kinetic trace on a shorter time scale (2 s) separately for one exponential as shown in Fig. 2b. The observed rate constant (k_{obs}) for the first step increased linearly by increasing [triazole] with a significant intercept signifying that the back reaction contributes significantly as shown in Fig. 3. The plots do not indicate any saturation at high [triazole] at the different temperatures used (5–35 °C) during this experiment. This behavior can be expressed by a rate law similar to that given in eqn. (3) in the case of pyrazole, where k_a and k_b represent the rate constants for the forward and reverse reactions in eqn. (1), respectively. The values of k_a and k_b as a function of temperature, and the corresponding activation parameters are summarized in Table 1.

The rate constants corresponding to the slower second step (obtained from a single exponential fit for a long time scale (50 s) shown in Fig. 2a), were found to be independent of triazole concentration (0.1 s^{-1}). This suggests that the triazole coordinates to Co(III) through N-1 or N-2 in a fast step since the second order rate constant obtained in the case of triazole is close to that obtained in the case of pyrazole at the same temperature. Then the second, slow step is due to a linkage isomerization and the coordination of 1,2,4-triazole through N-4 to give the stable product. It is known from the X-ray structure, vibrational and electronic spectra that 1,2,4-triazole coordinates to Co(III), Fe(III) and Mn(II) through N-4.[45–47]

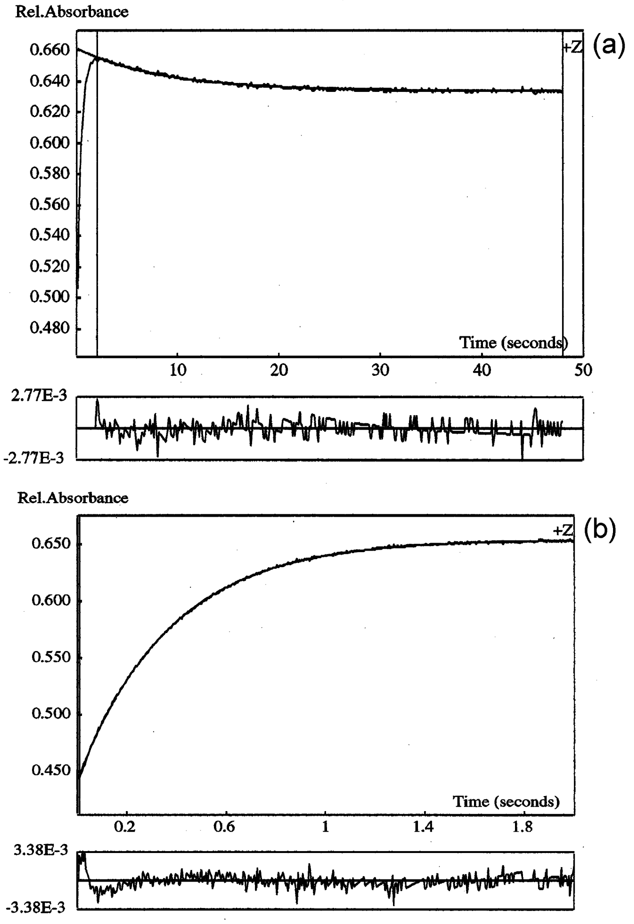
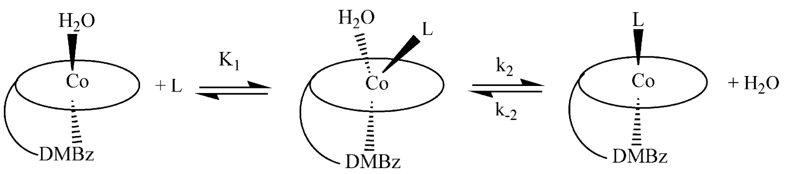


Fig. 2 (a) Typical kinetic trace for the reaction between 4×10^{-5} M H_2Ocbl^+ and 0.1 M 1,2,4-triazole, pH 6 (bis-tris buffer) and 25 °C. The trace was fitted to one exponential by following the decrease in absorbance at 550 nm for 50 s. The lower trace represents the difference between the experimental and calculated curves. (b) Typical kinetic trace for the reaction between 4×10^{-5} M H_2Ocbl^+ and 0.1 M 1,2,4-triazole, pH 6 (bis-tris buffer) and 25 °C. The trace was fitted to one exponential by following the increase in absorbance at 550 nm on a shorter time scale (2 s). The lower trace represents the difference between the experimental and calculated curves.

The values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ obtained in the case of triazole for the forward reaction (k_a) were found to be 90 ± 2 kJ mol^{-1} and +83 ± 6 J K^{-1} mol^{-1}, respectively, whereas those for the reverse reaction (k_b) were found to be 123 ± 7 kJ mol^{-1} and 155 ± 25 J K^{-1} mol^{-1}, respectively. $\Delta S^{\#}$ obtained in the case of pyrazole and triazole are significantly positive, which indicate that the reaction follows a dissociative mechanism. The value of $\Delta H^{\neq}$ for the reverse reactions in the case of pyrazole and triazole, where the value of k_b at 25 °C is small (0.4 and 0.21 s^{-1}), were found to be 105 ± 11 and 123 ± 7 kJ mol^{-1}, respectively.



(4)

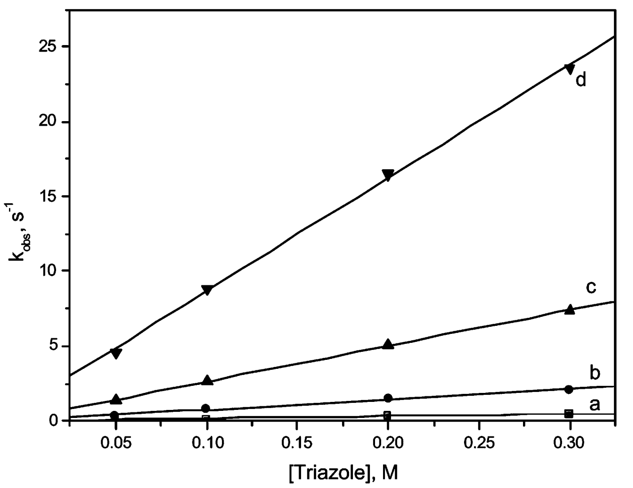


Fig. 3 Plot of k_{obs} *versus* [triazole] for the reaction between H_2Ocbl^+ and 1,2,4-triazole as a function of temperature. Experimental conditions: $[H_2Ocbl^+] = 4 \times 10^{-5}$ M, pH 6, $I = 0.1$ M ($NaClO_4$) and temperature = 5.0 (a); 15.0 (b); 25.0 (c) and 35.0 °C (d).

Interestingly, values of $\Delta H_b^{\neq}$ and k_b were found to be 86, 85 and 65 kJ mol^{-1} and 2.8, 15.2 and 37.9 s^{-1} in the case of pyridine, thiourea and dimethylthiourea, respectively.[5,8,48] This comparison shows that $\Delta H^{\neq}$ increases as the value of k_b decreases. This suggests that the reaction of H_2O with these ligands follows an I_d mechanism since the product (B_{12}–L) bond breakage will control the rate of the reverse aquation reaction (eqn. 1) and must be strongly affected by the nature and binding mode of the ligand (L).

In order to study the effect of H-bonding on the kinetics of H_2Ocbl^+ and the entering nucleophiles, the reaction of H_2Ocbl^+ and N-Ac-ImH was studied as a function of temperature at different N-Ac-ImH concentrations (0.025–0.5 M), and the results are shown in Fig. 4. This figure shows a slight curvature at high concentrations of N-Ac-ImH and negligible intercept (k_b), suggesting an irreversible reaction and the curvature as evidence for an interchange dissociative mechanism which involves ion pair formation (H-bond between N–Ac–ImH and the amide side chain of the corrin ring). The curvature shown in Fig. 4 can be interpreted in terms of an I_d mechanism outlined in reaction (4) and the corresponding rate law is shown in eqn. (5). Eqn. (5) can be simplified to eqn. (6) at low [L], where $1 + K_1[L] \approx 1$, and to eqn. (7) when k_b is negligible as in the case of N–Ac–ImH. Eqn. (6) is similar to eqn. (3) presented in the case of pyrazole and triazole where $k_a = k_2K_1$ and k_b is k_{-2}.

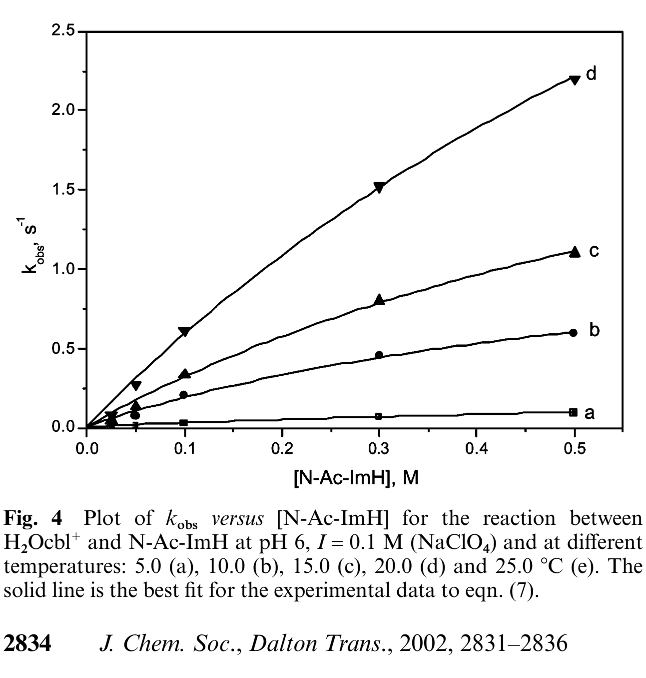


Fig. 4 Plot of k_{obs} *versus* [N-Ac-ImH] for the reaction between H_2Ocbl^+ and N-Ac-ImH at pH 6, $I = 0.1$ M ($NaClO_4$) and at different temperatures: 5.0 (a), 10.0 (b), 15.0 (c), 20.0 (d) and 25.0 °C (e). The solid line is the best fit for the experimental data to eqn. (7).

$$k_{obs} = \frac{k_2K_1[L]}{1 + K_1[L]} + k_{-2} \qquad (5)$$

$$k_{obs} = k_2K_1[L] + k_{-2} \qquad (6)$$

The experimental data obtained in the case of N-Ac-ImH were fitted to eqn. (7) using non-linear least square fitting and the resulting values of k_2, K_1 and k_a (K_1k_2, M^{-1} s^{-1}) along with the values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ are summarized in Table 1. The value of K_1 represents the formation constant for an ion pair complex, found to be 0.9 ± 0.2 M^{-1} at 25 °C.

$$k_{obs} = \frac{k_2K_1[L]}{1 + K_1[L]} \qquad (7)$$

The linear concentration dependence shown in Fig. 3 and (ESI Fig. S-1) in the case of triazole and pyrazole, respectively, and that reported earlier in the case of thioureau, dimethylthioureau, N_3^- and NO_2^-, NH_2OH[2,4,8,9] and the amine derivatives, demonstrate that the limiting rate constant obtained in the case of N-Ac-ImH can not be due to a limiting D mechanism because it would then be difficult to measure any higher rate constant for the substitution of H_2Ocbl^+ especially for stronger nucleophiles than N-Ac-ImH. The non-linear concentration dependence of k_{obs} for pyridine, 4-methylpyridine,[7,8] imidazole, methylcysteine and histamine[11,13] was ascribed to a precursor formation in terms of a dissociative I_d mechanism which is given in reaction (4) and its corresponding rate law is given in eqn. (5).

$\Delta H^{\neq}$ and $\Delta S^{\neq}$ in the case of N-Ac-ImH were found to be 108 ± 12 kJ mol^{-1} and 135 ± 40 J K^{-1} mol^{-1}, respectively. These significant positive values of $\Delta S^{\neq}$ indicate that the reaction is dissociative in nature. The effect of high pressure on the reaction of 4×10^{-5} M H_2Ocbl^+ and N-Ac-ImH was studied at pH 6, $I = 0.1$ M ($NaClO_4$), 25 °C and at two different concentrations of N-Ac-ImH. Firstly the reaction was carried out using 0.05 M N-Ac-ImH and in this case $\Delta V^{\neq}$ (k_a) was found to be 4.9 ± 0.2 cm^3 mol^{-1}. Then the reaction was repeated using 0.5 M N-Ac-ImH and in this case, $\Delta V^{\neq}$ (k_2) was found to be 7.2 ± 0.3 cm^3 mol^{-1}. In both cases, plots of ln k *versus* pressure gave good linear plots, as shown in Fig. 5, from which $\Delta V^{\neq}$ (k_a) and $\Delta V^{\neq}$ (k_2) were calculated.

If the I_d mechanism shown in eqn. (4) is considered for the reaction of H_2Ocbl^+ and N-Ac-ImH, which has the rate law expressed in eqn. (7), and at low concentration of N-Ac-ImH (0.05 M), the rate constant is $k_{obs} = k_a[L]$ where $k_a = k_2K_1$. In this case, the pressure dependence of k_a will result in $\Delta V^{\neq}(k_a) = \Delta V^{\neq}(k_2) + \Delta V(K_1)$, where $\Delta V(K_1)$ expresses the reaction volume for the formation of the intermediate ion pair complex, which will be affected by the charge of the ligand because it will determine the change in electrostriction and the associated volume increase. $\Delta V(K_1)$ is expected to be close to zero or small

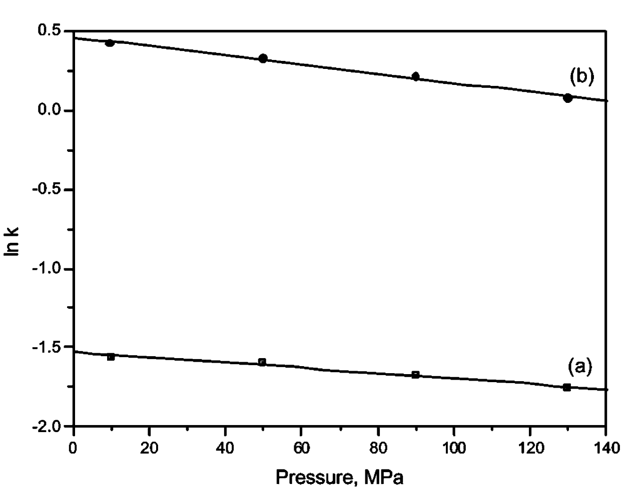


Fig. 5 Plot of ln *k versus* pressure for the reaction of H_2Ocbl^+ with *N*-acetylimidazole at pH 6, 20 °C and $I = 0.1$ M ($NaClO_4$), (a) at 0.05 M N-Ac-ImH and (b) at 0.5 M N-Ac-ImH.

and negative. In the present case, $\Delta V(K_1)$ was found to be -2.3 ± 0.4 cm^3 mol^{-1} and $\Delta V^{\neq}(k_2) = 7.2 \pm 0.3$ cm^3 mol^{-1} which suggests that the mechanism is an I_d mechanism where the entering ligand participates in the transition state. $\Delta V^{\neq}(k_2)$ for a dissociative activated interchange process should be fairly independent of the nature of L. These data are also in agreement with those reported earlier in the case of thioureau, dimethylthioureau, pyridine, 3-acetylpyridine, 4-methylpyridine, N_3^- and SCN^-. In all these cases, $\Delta V^{\neq}(k_2)$ was found to be in the range of 4–8 cm^3 mol^{-1} and the mechanism was interpreted as an I_d type.[3,7–9] The value of $\Delta V^{\neq}(k_2)$ obtained in the present study (7.2 ± 0.3 cm^3 mol^{-1}) is significantly less than that expected for a limiting D mechanism. Theoretical calculations[49–51] suggest a volume of activation $\Delta V^{\#}$ of +13.1 cm^3 mol^{-1} for a limiting D mechanism of ligand substitution involving the loss of coordinated H_2O in an octahedral complex. $\Delta V^{\neq}$ for the reaction of β-(*N*-methylimidazolyl) cobalamin with *N*-methylimidazole was reported to be +15.0 ± 0.7 and +16.8 ± 1.1 cm^3 mol^{-1} at 5×10^{-3} and 1 M *N*-methylimdazole, respectively.[20] Also, the activation volumes for the reaction of β-trifluoromethylcobalamin, β-cyanomethylcobalamin and cyanocobalamin with CN^- were found to be +14.8 ± 0.8, +12.7 ± 0.5 and +13.1 ± 0.3 cm^3 mol^{-1}, respectively.[16,17] Furthermore, $\Delta V^{\neq}$ for ligand substitution on $[Co(TMPP)(H_2O)_2]^{5+}$ and $[Co(TPPS)(H_2O)_2]^{3-}$, where TMPP = *meso*-tetrakis(4-*N*-methylpyridyl)porphine and TPPS = *meso*-tetrakis(*p*-sulfonatophenyl)porphine, was found to be +14.4 and +15.4 cm^3 mol^{-1}, respectively,[52] very close to the expected value of +13 cm^3 mol^{-1} for a limiting D mechanism.[50,51] The operation of a limiting D mechanism requires the intermediacy of a five-coordinate species or transition state.

The activation parameters $\Delta H^{\neq}$, $\Delta S^{\neq}$ and $\Delta V^{\neq}$ obtained in the case of N-Ac-ImH were found to be 108 ± 12 kJ mol^{-1}, +135 ± 40 J K^{-1} mol^{-1} and 7.2 ± 0.3 cm^3 mol^{-1}, respectively. These data along with the observed rate law presented in eqn. (7) suggest that the reaction of H_2Ocbl^+ with N-Ac-ImH indeed follows an I_d mechanism. This mechanism is accompanied by precursor formation and the value of K lies between 1–5 M^{-1} depending on the charge of the incoming ligands. The value of K obtained in the present study for the fit of the data in Fig. 5 to eqn. (7) was found to be 0.9 ± 0.2 M^{-1} at 25 °C.

By way of comparison, data in Table 1 show that k_a (M^{-1} s^{-1}) for pyrazole, triazole and N-Ac-ImH at 25 °C are 35.0 ± 0.8, 24.0 ± 0.4 and 6.6 ± 1.6 M^{-1} s^{-1}, respectively. The values for pyrazole and triazole are expected to be close since the p*K*s for pyrazole and triazole are 2.48 and 2.6, respectively. The significant decrease in $k_a = k_2K_1$ (6.6 M^{-1} s^{-1}) in the case of N-Ac-ImH as compared to the other azoles was ascribed to the possibility of H-bond formation between N-Ac-ImH and the amide side chain of the corrin ring. In the case of N-Ac-ImH, a significant curvature was obtained and the value of K for the ion pair formation was found to be 0.9 M^{-1}. Marques[2] ascribed the decrease in activation entropies and enthalpies for the reaction of H_2Ocbl^+ with a series of neutral primary amines to the possibility of H-bonding between the primary amines and the acetamide side-chains.[2] The primary amines used were classified to two classes, the first one [NH_3, $NH_2(CH_2)_3OH$, NH_2Me and NH_2Pr] has significantly larger values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ than those in the second class [NH_2OMe, NH_2OH, $NH_2CH_2CO_2Me$ and $NH_2(CH)_2OH$] which interacts by H-bonding with the acetamide side-chains of the corrin ring.[2] The second order rate constants for the substitution of H_2O by NH_2OH and CH_3NH_2 were found to be 21.5 and 1.05 M^{-1} s^{-1}, respectively, and these low values of the rate constant were ascribed to the possibility of H-bonding between the acetamide side chain of the corrin ring and the incoming ligands.[53]

The present study has shown that the mechanism for the reaction of H_2Ocbl^+ with the three azoles investigated during the course of this work is an I_d mechanism where the entering azoles participate in the transition state, and the formation constant for the formation of the ion pair was found to be 0.9 M^{-1} as in the case of N-Ac-ImH. In spite of the high value of $pK_{BH}{}^+$ for N-Ac-ImH (3.6) compared to that of pyrazole (2.48) and triazole (2.6), the rate constant (k_a = 6.6 M^{-1} s^{-1}) was found to be less than those obtained in the case of pyrazole and triazole (35 and 24 M^{-1} s^{-1}). This decrease in the rate constant was ascribed to the possibility of H-bond formation between N-Ac-ImH and the acetamide side chain of the corrin ring. This work also presents evidence for a linkage isomerization in the case of triazole, which has not been found for reactions of triazole before.

Acknowledgements

The author gratefully acknowledges Prof. Rudi van Eldik, University of Erlangen-Nürnberg, for his support and helpful discussions.

References

1 H. M. Marques, T. J. Egan, J. H. Marsh, J. R. Mellor and O. Q. Munro, *Inorg. Chim. Acta*, 1989, **166**, 249.
2 H. M. Marques, *J. Chem. Soc., Dalton Trans.*, 1991, 1437.
3 H. M. Marques, E. L. J. Breet and F. F. Prinsloo, *J. Chem. Soc., Dalton Trans.*, 1991, 2941.
4 H. M. Marques, *J. Chem. Soc., Dalton Trans.*, 1991, 339.
5 G. Stochel and R. van Eldik, *Inorg. Chem.*, 1990, **29**, 2075.
6 G. Stochel, R. van Eldik, H. Kunkely and A. Vogler, *Inorg. Chem.*, 1989, **28**, 4314.
7 F. F. Prinsloo, M. Meier and R. van Eldik, *Inorg. Chem.*, 1994, **33**, 900.
8 M. Meier and R. van Eldik, *Inorg. Chem.*, 1993, **32**, 2635.
9 F. F. Prinsloo, E. L. J. Breet and R. van Eldik, *J. Chem. Soc., Dalton Trans.*, 1995, 685.
10 H. Marques and L. Knapton, *J. Chem. Soc., Dalton Trans.*, 1997, 3827.
11 H. M. Marques, J. C. Bradley and L. A. Campbell, *J. Chem. Soc., Dalton Trans.*, 1992, **13**, 2019.
12 H. M. Marques, O. Q. Munro, B. M. Cumming and C. de Nysschen, *J. Chem. Soc., Dalton Trans.*, 1994, **3**, 297.
13 H. M. Marques, J. C. Bradley, K. L. Brown and H. Brooks, *J. Chem. Soc., Dalton Trans.*, 1993, **23**, 3475.
14 H. M. Marques, *S. Afr. J. Chem.*, 1991, **44**, 114.
15 R. Moreno-Esparza, M. Lopez and K. H. Pannell, *J. Chem. Soc., Dalton Trans.*, 1992, **11**, 1791.
16 M. S. A. Hamza, X. Zou, K. L. Brown and R. van Eldik, *Inorg. Chem.*, 2001, **40**, 5440.
17 M. S. A. Hamza, X. Zou, K. L. Brown and R. van Eldik, *Inorg. Chem.*, 2002, submitted for publication.
18 N. E. Brasch, M. S. A. Hamza and R. van Eldik, *Inorg. Chem.*, 1997, **36**, 3216.
19 (*a*) N. E. Brasch and R. J. Haupt., *Inorg. Chem.*, 2000, **39**, 5469; (*b*) N. E. Brasch, A. G. Cregan and M. E. Vanselow, *J. Chem. Soc., Dalton Trans.*, 2002, 1287.

20 A. G. Cregan, N. E. Brasch and R. van Eldik, *Inorg. Chem.*, 2001, **40**, 1430.

21 M. Wolak, A. Zahl, T. Schneppensieper, G. Stochel and R. van Eldik, *J. Am. Chem. Soc.*, 2001, **123**, 9780.

22 W. C. Randall and R. A. Alberty, *Biochemistry*, 1967, **6**, 1520.

23 W. W. Reenstra and W. P. Jencks, *J. Am. Chem. Soc.*, 1979, **101**, 5780.

24 D. Thusius, *J. Am. Chem. Soc.*, 1971, **93**, 2629.

25 F. Nome and J. H. Fendler, *J. Chem. Soc., Dalton Trans.*, 1976, 1212.

26 C. K. Ponn, *Coord. Chem. Rev.*, 1973, **10**, 1.

27 J. M. Pratt, *Inorganic Chemistry of Vitamin B12*, Academic Press, London, 1972.

28 C. Kratky, G. Farber, K. Gruber, K. Wilson, Z. Dauter, H. F. Nolting, R. Konrat and B. Krautler, *J. Am. Chem. Soc.*, 1995, **117**, 4654.

29 K. L. Brown, D. R. Evans, J. D. Zubowski and E. Valente, *Inorg. Chem.*, 1996, **35**, 415.

30 K. L. Brown and X. Zou, *Magn. Reson. Chem.*, 1997, **35**, 889.

31 R. Quinn, J. Mercer-Smith, J. N. Burstyn and J. S. Valentine, *J. Am. Chem. Soc.*, 1984, **106**, 4136.

32 G. A. Tondreau and D. A. Sweigart, *Inorg. Chem.*, 1984, **23**, 1060.

33 R. J. Sundberg and R. B. Martin, *Chem. Rev.*, 1974, **74**, 471.

34 C. J. Coulson, D. J. King and A. Wiseman, *Trends Biochem. Sci.*, 1984, **9**, 446.

35 P. A. Worthington, in *Sterol biosynthesis inhibitors*, ed. D. Berg and M. Plempel, Ellis Horwood, Chichester, UK, 1988, p. 19.

36 C. A. Hitchcock, *Biochem. Soc. Trans.*, 1991, **19**, 782.

37 R. W. Taft, F. Anvia, M. Taagepera, J. Catalan and J. Elguero, *J. Am. Chem. Soc.*, 1986, **108**, 3237.

38 M. Meot-Ner, *J. Am. Chem. Soc.*, 1988, **110**, 3071.

39 (*a*) J. Catalan and J. Elguero, *J. Chem. Soc., Perkin Trans. 2*, 1983, 1869; (*b*) J. Catalan, M. Menendez and J. Elguero, *Bull. Soc. Chim. Fr.*, 1985, 30.

40 M. H. Ibraham, P. P. Duce, D. V. Prior, D. G. Barratt, J. J. Morris and P. J. Taylor, *J. Chem. Soc., Perkin Trans. 2*, 1989, 1355.

41 R. van Eldik, W. Gaede, S. Wieland, J. Kraft, M. Spitzer and D. A. Palmer, *Rev. Sci. Instrum.*, 1993, **64**, 1355.

42 H. M. Marques, J. H. Marsh, J. R. Mellor and O. Q. Munro, *Inorg. Chim. Acta*, 1990, **170**, 259.

43 (*a*) G. D. Fasman, *Handbook of Biochemistry and Molecular Biology*, 3rd edn., CRC Press, Cleveland, OH, 1976, vol. 1 and references therein; (*b*) C. F. Kröger and W. Freiberg, *Z. Chem.*, 1965, **10**, 381; (*c*) W. P. Jencks and L. Carriuolo, *J. Biol. Chem.*, 1959, **234**, 1272.

44 (*a*) M. P. Byfield, M. S. A. Hamza and J. M. Pratt, *J. Chem. Soc., Dalton Trans.*, 1993, 1641; (*b*) M. S. A. Hamza and J. M. Pratt, *J. Chem. Soc., Dalton Trans.*, 1993, 1647; (*c*) M. S. A. Hamza and J. M. Pratt, *J. Chem. Soc., Dalton Trans.*, 1994, 1367; 1373; 1377.

45 S. Gorter and D. W. Engelfriet, *Acta Crystallogr., Sect. B*, 1981, **37**, 1214.

46 (*a*) J. G. Haasnoot, G. C. M. de Keyzer and G. C. Verschoor, *Acta Crystallogr., Sect. C*, 1983, **39**, 1207; (*b*) W. L. Driessen, R. A. G. de Graaff and J. G. Vos, *Acta Crystallogr., Sect. C*, 1983, **39**, 1635.

47 R. Van Houten, J. G. Vos and W. L. Groeneveld, *Recl. Trav. Chim. Pays-Bas*, 1979, **98**, 425.

48 A. M. van Herk, Ph.D. Thesis, Free University of Amsterdam, Netherland, 1986.

49 T. W. Swaddle, *Rev. Phys. Chem. Jpn.*, 1980, **50**, 230.

50 R. van Eldik, T. Asano and W. J. le Noble, *Chem. Rev.*, 1989, **89**, 549.

51 A. Drljaca, C. D. Hubbard, R. van Eldik, T. Asano, M. V. Basilevsky and W. J. Noble, *Chem. Rev.*, 1998, **98**, 2167.

52 J. G. Leipoldt, R. van Eldik and H. Kelm, *Inorg. Chem.*, 1983, **22**, 4146.

53 J. G. Toerien, M.Sc. Thesis, University of Witwatersrand, South Africa, 1986.