

Experimental and theoretical studies of the dehydration kinetics of two inhibitor-containing half-sandwich cobalt(II) complexes

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Abstract

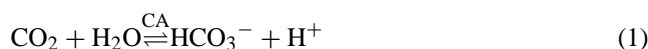
Two inhibitor-containing half-sandwich cobalt(II) complexes $[\text{Tp}^{\text{Me}}\text{CoX}]$ ($[\text{Tp}^{\text{Me}}] = \text{hydrotris}(3,5\text{-dimethylpyrazolyl})\text{borate}$; $\text{X}^-: \text{N}_3^-$ (**1**), NCS^- (**2**)) have been synthesized and characterized. The structure of **2** was determined by X-ray crystallographic analysis. The dehydration kinetic measurements of HCO_3^- catalyzed by the cobalt(II) complexes are performed by the stopped-flow techniques at $\text{pH} < 7.9$. The five-coordinated aqua complex must be the reactive catalytic species in the catalyzed dehydration reaction and the rate-determining step is the substitution of the labile water molecule by HCO_3^- . The acid dissociation constants (expressed as $\text{p}K_a$ values) for **1** and **2** are 7.9 and 7.8, respectively, which are obtained by pH titration. The dehydration rate constant k of the rate-determining step of **2** ($95.9 \text{ M}^{-1} \text{ s}^{-1}$) is lower than that of **1** ($129 \text{ M}^{-1} \text{ s}^{-1}$), which can be correlated with a higher activation energy barrier, this being the origin of the variation in the inhibition ability between N_3^- and NCS^- . The inhibition ability of NCS^- is strong than that of N_3^- , which is also confirmed by the decrease in effective atomic charges of the Co(II) ions as revealed by the theoretical calculations.

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Keywords: Homogeneous catalysis; Dehydration kinetics; Stopped-flow techniques; Cobalt; Inhibitor

1. Introduction

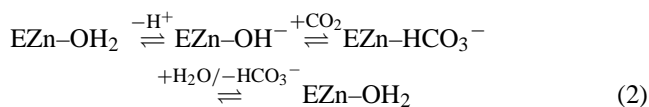
The carbonic anhydrases (CA) are zinc-containing metalloenzymes that catalyze the reversible hydration of carbon dioxide and dehydration of bicarbonate as given in (1) [1–9].



Since CO_2 is either the starting point for photosynthesis or the endpoint of substrate oxidation, carbonic anhydrases are known to be ubiquitous, occurring in animals, plants and several bacteria. CA has high turnover numbers, i.e. the number of substrate molecules transformed per unit time by each enzyme molecule [10]. For example, in the presence of human carbonic anhydrase II (HCA II), the maximal CO_2 turnover number is $\approx 10^6 \text{ s}^{-1}$, whereas the uncatalyzed reaction is kinetically slow around physiological pH ($k \approx 10^{-1} \text{ s}^{-1}$). Therefore, the nature of the active site has been a topic of

great interest. X-ray diffraction studies reveal that the active site environment of HCA II can be divided into hydrophilic and hydrophobic halves. The hydrophilic half contains the proton acceptor group His-64 and partially ordered water molecule. The ordered water molecules in the active cavity are thought to be important for the intramolecular proton transfer from the zinc-bound water to His-64. In the hydrophobic half, a “deep” water molecule close to the zinc center is thought to be important for CO_2 recognition [11].

The most widely accepted catalytic cycle for CA is the so-called zinc-hydroxide mechanism as summarized in (2) [1–9]. The catalytic activity is characterized by $\text{p}K_a$



value of ca. 7.0, which means that the coordinated water molecule ionizes to a coordinated hydroxide around neutral pH. Hence, the hydration of CO_2 is dominant above $\text{pH} = 7$, while the dehydration of HCO_3^- is observed below $\text{pH} = 7$. It has been postulated that this $\text{p}K_a$ value represents that of

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the coordinated water molecule on the Zn(II) ion center that participates in the catalytic reaction. The catalytic activities of CA are greatly hindered in the presence of inhibitors, which are classified as two types: the metal complexing inorganic anions (e.g. CN^- , SCN^- , N_3^- , I^- , HS^- , etc.) and the unsubstituted sulphonamides possessing the general formula RSO_2NH_2 (R: aryl, hetaryl, perhaloalkyl) [12–15]. The first type of inhibitor was important for understanding the detailed catalytic and inhibitory mechanisms, whereas the second led to the development of several classes of pharmacological agents. Despite of the numerous papers in recent years focusing on the investigation of structure and properties of the sulphonamide inhibitors [16–24], studies of the dehydration kinetics of CA and CA models containing different inorganic ions are relatively few.

We have previously reported the crystal structure of new model complexes for CA by using Trofimenko's hydrotris(pyrazolyl)borate ligand system (Tp^{R}) [25,26]. The trigonally capping Tp^{R} has the advantage over other ligand systems in mimicking the coordination environment of CA made by three histidyl residues [27–29]. It is noteworthy that the zinc ions in the active site of CA can be replaced by different transition metal ions. It has been reported that cobalt(II) substituted-CA retains ca. 50% enzyme catalytic activity of unsubstituted-CA. It is so far the highest activity obtained among different metal ion substituted-CA. Therefore, in view of the model studies of bicarbonate dehydration kinetics, it is generally accepted to use cobalt(II) complexes as CA and substituted-CA mimics. In this paper, we have synthesized two inhibitor-containing half-sandwich cobalt(II) complexes $[\text{Tp}^{\text{Me}}]\text{CoX}$ ($[\text{Tp}^{\text{Me}}]$: hydro-tris(3,5-dimethylpyrazolyl)borate; X^- : N_3^- (**1**), NCS^- (**2**)) and have focused on both experimental and theoretical evaluations of their bicarbonate dehydration kinetic properties.

2. Experimental

2.1. Materials and methods

All the starting chemicals used in this work were of analytical reagent and used without further purification, unless otherwise stated. Hydrotris(3,5-dimethylpyrazolyl)borate (Tp^{Me}) was synthesized according to the literature method [30], and the purity was confirmed by IR, MS-FAB, and elemental analysis. The indicator bromocresol purple (Sigma) and the biological buffer Tris (tris[hydroxymethyl]amino-methane, Sigma) were purchased and used as received. All solutions were prepared using double distilled water that was boiled for more than 2 h prior to use to remove the dissolved CO_2 . The concentrations of the indicator, Tris, and NaClO_4 were kept at 2.5×10^{-4} , 7.0×10^{-2} , and 7.5×10^{-2} M, respectively. The pH titration technique was carried out according to the previous related literature reports [31,32].

Elemental analyses for C, H, and N were carried out on a Perkin-Elmer analyzer at the Institute of Elemento-Organic Chemistry, Nankai University. Infrared spectra on KBr pellets were recorded on a Shimadzu IR-408 spectrophotometer in the range of 4000–600 cm^{-1} . UV-Vis spectra in methanol were recorded on a Shimadzu UV-2101 spectrophotometer in the range of 1000–200 nm. Mass spectrum was performed at the Central Laboratory of Nankai University on VG ZAB-HS spectrometer.

2.2. Synthetic procedures

Caution! The perchlorate salts in this study are all potentially explosive and should be handled with care.

Synthesis of $[\text{Tp}^{\text{Me}}]\text{CoN}_3$ (**1**): An aqueous solution (1 ml) of NaN_3 (0.039 g, 0.6 mmol) was added to a methanol solution (5 ml) of $\text{Co}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (0.073 g, 0.2 mmol). The mixture was stirred at room temperature for 2 h. $\text{K}[\text{Tp}^{\text{Me}}]$ (0.067 g, 0.2 mmol) in methanol solution (10 ml) was added dropwise to the above mixture and stirred for another 10 h. Dark green microcrystals were filtered and recrystallized from methanol (0.056 g, yield 71%). Anal. for **1**, calcd. for $\text{C}_{15}\text{H}_{22}\text{BCoN}_9$: C, 45.25; H, 5.57; N, 31.66. Found C, 45.07; H, 5.65; N, 31.58. IR (KBr pellet) 2500 (B–H), 2070 cm^{-1} ($\text{N}\equiv\text{N}$).

Synthesis of $[\text{Tp}^{\text{Me}}]\text{Co}(\text{NCS})(\text{CH}_3\text{OH})_2$ (**2**): The same procedure used for **1** was employed. From $\text{Co}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (0.073 g, 0.2 mmol), KSCN (0.058 g, 0.6 mmol), and $\text{K}[\text{Tp}^{\text{Me}}]$ (0.067 g, 0.2 mmol) and was obtained **2** (0.074 g, yield 77%). Purple single crystals of **2** suitable for X-ray diffraction analysis were obtained from slow evaporation of the filtrate. Anal. for **2**, calcd. for $\text{C}_{18}\text{H}_{30}\text{BCoN}_7\text{O}_2\text{S}$: C, 45.20; H, 6.27; N, 20.49. Found C, 45.04; H, 6.45; N, 20.39. IR (KBr pellet) 2500 (B–H), 2120 ($\text{C}\equiv\text{N}$) cm^{-1} .

2.3. X-ray crystallographic analysis of **2**

A single crystal of **2** with approximate dimensions of 0.30 mm \times 0.25 mm \times 0.20 mm was mounted on a glass fiber. A full data collection were performed with graphite monochromatized Mo $\text{K}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) on a Bruker SMART 1000 diffractometer equipped with a CCD camera. Cell parameters were determined from a least-squares refinement in the range $2.20^\circ < \theta < 25.03^\circ$ at 293(2) K. The intensity data of 8119 reflections were measured up to $\theta_{\text{max}} = 25.03^\circ$ using a ω - 2θ scan technique in the hkl range -15 to 13 , -10 to 14 , -14 to 17 . Some doubly measured reflections were averaged, $R_{\text{int}} = 0.0953$, resulting in 4089 independent reflections of which 2132 were considered as observed with the $I > 2\sigma(I)$ criterion. The absorption correction was performed empirically [33]. The structure was solved by direct methods using SHELXS-97 [34], and refined by the full-matrix least-squares method on F^2 using SHELXL-97 [35]. All non-hydrogen atoms were refined anisotropically, while hydrogen atoms were set in calculated positions and treated as riding atoms with

Table 1
Crystallographic data for **2**

Empirical formula	C ₁₈ H ₃₀ BCoN ₇ O ₂ S
Formula weight	478.29
Crystal dimension (mm ³)	0.30 × 0.25 × 0.20
Crystal color	Purple
Temperature (K)	293(2)
Crystal system	Monoclinic
Space group	P2 ₁ /c
<i>a</i> (Å)	13.418(15)
<i>b</i> (Å)	12.036(12)
<i>c</i> (Å)	14.565(16)
β (°)	95.92(2)
<i>V</i> (Å ³)	2340(4)
<i>Z</i>	4
ρ_{calcd} (Mg m ⁻³)	1.358
<i>F</i> (000)	1004
μ (mm ⁻¹)	0.851
Measured reflections	8119
Independent reflections	4089 (<i>R</i> _{int} = 0.0953)
Independent reflections (<i>I</i> > 2 σ (<i>I</i>))	2132
<i>R</i> ^a	0.0569
<i>Rw</i> ^b	0.1142
GOF	0.896

$$^a R = \sum ||F_o| - |F_c|| / \sum |F_o|$$

$$^b Rw = (\sum w(F_o^2 - F_c^2)^2 / \sum (F_o^2)^2)^{1/2}; w = 1/[\sigma^2(F_o^2) + (0.0975P)^2 + 0.0000P], \text{ where } P = (F_o^2 + 2F_c^2)/3.$$

a common fixed isotropic thermal parameter. The final cycle of the refinement was based on 275 refined parameters to give *R* = 0.0569 and *Rw* = 0.1142. The largest peak and hole in the final difference map were 0.354 and -0.523 e Å⁻³, respectively. The relevant data of the X-ray crystallographic analysis are given in Table 1.

2.4. Dehydration kinetics

All kinetic measurements were made with a Union Giken RA-401 stopped-flow spectrophotometer equipped with a Union Giken RA-451 rapid-scan attachment in order to determine the change in absorption of the reaction mixture directly after mixing under a certain light wavelength. To increase the solubility of the cobalt(II) complexes, 70% ethanol/H₂O (v/v) mixed solution was used. Temperature was maintained by using a Union Giken RA-454 superthermostat, and temperature accuracy is within 0.1 K. The pH values in this work were all directly measured by means of a Beckman Φ 71 pH meter. The ionic strength of all test solutions was adjusted to 0.11 M with the aid of NaClO₄ for the dehydration reaction. HCO₃⁻ solutions (7.5 × 10⁻³ M) were freshly prepared from NaHCO₃ and used within 10 h. The most appropriate pH value and light wavelength to study the reaction for this buffer-indicator pair (Tris with bromocresol purple) are ca. 7.0 and 595 nm, respectively, which are revealed by UV-Vis absorption spectra. Therefore, the reaction is monitored at 595 nm wavelength with a tungsten lamp as the light source. The apparent first-order dehydration rate constants (*k*_{obs}^d) were obtained with at least three half-lives and represent the average of the best

three runs. Reproducibility of the values of *k*_{obs}^d was better than ±5%.

2.5. Theoretical calculations

During the dehydration process of HCO₃⁻, the nucleophilic attack of the free HCO₃⁻ on the Co(II) ion of the model complex is affected by the effective atomic charge of the Co(II) ion. To obtain information on the variation in the effective atomic charge of the Co(II) ion by coordinating different inhibitors (N₃⁻/NCS⁻), we have carried out theoretical calculations of **1** and **2** at the UHF level, using a lan12dz basis set for the Co atoms and the corresponding basis set for each of the other atoms. This basis set consisted of the 6-31G** basis functions for H, B, C, N, O, and S [36] and the Los Alamos effective core potential (ECP) for Co with the outermost core orbitals included in the valence description [37–39]. The geometric parameters employed in these calculations were based on the X-ray crystallographic data in this work. All calculations were carried out using the Gaussian-98 package [40].

3. Results and discussion

3.1. Structure of the model complexes

Since the active site of CA is of the composition [His]₃Zn(II)-OH₂, where [His]₃ are three histidyl residues, a rational approach towards obtaining synthetic analogues is to use tridentate ligands in mimicking the protein ligation [41]. Trofimenko's hydrotris(pyrazolyl)borate ligand system [Tp^R], in which three pyrazolyl groups are attached to a common tetrahedral or trigonal pyramidal center, has the advantage over other ligand systems in mimicking the coordination environment of CA. Although half-sandwich metal(II) complexes of Tp^R ligands with bulky substituents (R) are easily formed, it is still a key problem to obtain half-sandwich metal(II) complexes of Tp^R ligands with small substituents, such as Tp^{Me}. Following the reported methods [42], we have succeeded in the preparation of [Tp^{Me}]CoX (X⁻: N₃⁻, NCS⁻) by the reaction of Co(ClO₄)₂·6H₂O and K[Tp^{Me}] in the presence of excess X⁻.

Single crystals of **2** are carefully grown and then analyzed by X-ray diffraction. The molecular structure diagram of **2** is shown in Fig. 1. Selected bond lengths and angles are listed in Table 2. Despite of the coordinated inhibitor NCS⁻ and two solvent methanol molecules, the complex [Tp^{Me}]Co shows a pseudo-C_{3v} symmetry. The coordination of the Co(II) ion in **2** is to three nitrogen atoms of Tp^{Me}, one nitrogen atom of NCS⁻, and two oxygen atoms of CH₃OH forming an octahedron geometry with the N(1)-Co(1)-N(7), N(3)-Co(1)-O(1), and N(5)-Co(1)-O(2) bond angles being 172.38, 175.28, and 175.84°, respectively. This coordination environment of the Co(II) ion is very similar to that of

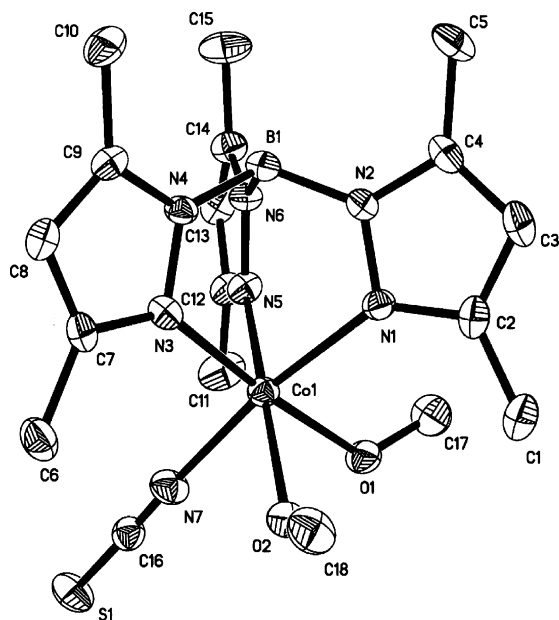


Fig. 1. The molecular structure diagram (30% probability) of **2**. Hydrogen atoms are omitted for clarity.

the active site of cobalt(II) substituted HCA II. The average Co–N(Tp^{Me}) bond length is 2.12 Å, which is comparable with 2.11 Å in HCA II [11]. The Co–N(NCS[−]) and average Co–O(CH₃OH) bond lengths are 2.076 and 2.182 Å, respectively. These crystallographic parameters of [Tp^{Me}]Co are

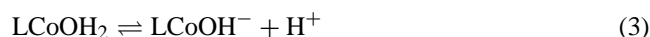
Table 2
Selected bond lengths (Å) and angles (°) for **2**

Co(1)–N(7)	2.076(5)
Co(1)–N(3)	2.109(4)
Co(1)–N(5)	2.116(4)
Co(1)–N(1)	2.136(4)
Co(1)–O(1)	2.165(4)
Co(1)–O(2)	2.198(4)
S(1)–C(16)	1.641(6)
N(7)–C(16)	1.146(6)
N(7)–Co(1)–N(3)	98.49(17)
N(7)–Co(1)–N(5)	96.96(17)
N(3)–Co(1)–N(5)	87.57(16)
N(7)–Co(1)–N(1)	172.38(16)
N(3)–Co(1)–N(1)	87.71(16)
N(5)–Co(1)–N(1)	87.68(16)
N(7)–Co(1)–O(1)	85.92(17)
N(3)–Co(1)–O(1)	175.28(15)
N(5)–Co(1)–O(1)	90.23(16)
N(1)–Co(1)–O(1)	88.03(15)
N(7)–Co(1)–O(2)	80.47(15)
N(3)–Co(1)–O(2)	96.03(15)
N(5)–Co(1)–O(2)	175.84(14)
N(1)–Co(1)–O(2)	94.53(14)
O(1)–Co(1)–O(2)	86.32(14)
C(17)–O(1)–Co(1)	129.5(3)
C(18)–O(2)–Co(1)	132.4(3)
C(16)–N(7)–Co(1)	167.9(5)
N(7)–C(16)–S(1)	177.0(5)

comparable with those of our previously reported [Tp^{Me}]Ni [25].

3.2. Dehydration kinetics

The acid–base behavior of the cobalt(II) complexes in 0.1 mol l^{−1} NaNO₃ at 298 K has been investigated through pH titration. From the titration curve of the Tp^{Me}–Co binary system we can establish that two H₂O-adducted species LCoOH₂⁺ and LCoOH are formed in the pH range of 6–8 as shown below (L = the ligands Tp^{Me} and X[−]). The two



complexes dissociate protons in slightly alkaline solution. The p*K*_a values of **1** and **2** are 7.9 and 7.8, respectively. The lower p*K*_a value of **2** than **1** indicates a decrease of the effective atomic charge of the cobalt(II) ions, which facilitates the protonation of the water molecules that coordinate to the cobalt(II) ions. We therefore attribute this protonation to the coordinated water molecule. The catalytic activity of Co(II) complexes is characterized by p*K*_a value. The dehydration kinetic measurements of HCO₃[−] should be carried out in the case of pH < p*K*_a to avoid the interference of the reverse hydration reaction especially at higher pH.

As mentioned in the experimental section, remarkable changes in absorption spectra were observed in our dehydration kinetics measurements when HCO₃[−] was added to the reaction mixture, whereas the hydrogen ion concentration was maintained constant by means of buffer solutions. The steady-state absorption spectra of the cobalt(II) complexes **1** and **2** before and after the addition of HCO₃[−] in the buffer–indicator pair (Tris with bromocresol purple) solution are shown in Fig. 2. It is easy to note that the absorption at ca. 595 nm changes remarkably, which gives us the opportunity to perform kinetics measurements by monitoring the variation of the absorption during the reaction. That is, the dehydration rate *V* can be obtain by the measurement of d*A*/ε*dt*. Hence, we are also able to later compare the catalytic activities of those cobalt(II) complexes with those of zinc(II) complexes, since CA is zinc-containing metalloenzymes.

Under our experimental conditions, the dehydration rate increases linearly with the increase of [HCO₃[−]]. This means the dehydration is first-order in [HCO₃[−]], which indicates that the dehydration rate follows the rate law

$$V = \frac{-d[\text{HCO}_3^-]}{dt} = k_{\text{obs}}[\text{HCO}_3^-] \quad (4)$$

where *k*_{obs} is the apparent first-order dehydration rate constant. *k*_{obs} should include all the catalytic species, such as the cobalt(II) complexes, acid and some other species. Therefore, *k*_{obs} can be written in the following form (complex: Co(II) complex)

$$k_{\text{obs}} = k_{\text{cat}}[\text{complex}]^m + k_{\text{H}}[\text{H}^+]^n \quad (5)$$

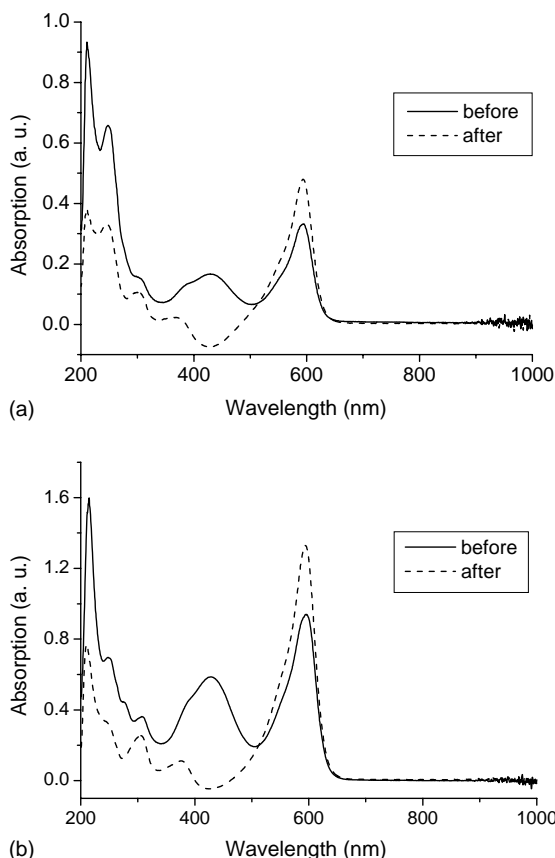


Fig. 2. The steady-state absorption spectra of the cobalt(II) complexes **1** (a) and **2** (b) before and after the addition of HCO_3^- in the buffer-indicator pair solution.

where k_{cat} and k_{H} are the catalytic rate constants of the cobalt(II) complex and H^+ , respectively and m and n are constants. When the observed apparent dehydration rate constants were plotted against the complex concentrations at a given pH, we can obtain the k_{cat} and m values. Typical kinetic traces observed for different concentrations of **1** and **2** catalyzed dehydration of HCO_3^- at $\text{pH} = 7.014$ (≈ 7.0 , the most appropriate pH value to study the reaction as denoted in the experimental section) are shown by the plot of k_{obs} versus [complex] in Fig. 3. It is evident that the apparent dehydration rate constants k_{obs} vary linearly with the total complex concentrations showing a first-order dependence on cobalt(II) complex ($m = 1$). Regression of k_{obs} versus [complex] for the two complexes, from the slope, we have

$$k_{\text{cat}} = 114 \text{ M}^{-1} \text{ s}^{-1}, \quad \text{for } \mathbf{1}$$

$$k_{\text{cat}} = 82.4 \text{ M}^{-1} \text{ s}^{-1}, \quad \text{for } \mathbf{2}$$

The catalytic activity of **2** is lower than **1** indicating that the inhibition ability of the small inorganic ion NCS^- is stronger than that of N_3^- . The effect of free Co(II) ions on the reaction was also checked at the same pH value, but no catalysis was observed. In related experiments, we have also checked the bicarbonate dehydration kinetics catalyzed by

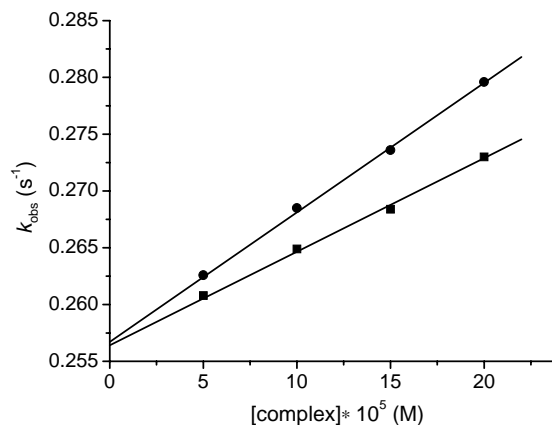


Fig. 3. Plot of k_{obs} vs. [complex] for the catalyzed dehydration of HCO_3^- . Experimental conditions: $\text{pH} = 7.014$; $[\text{HCO}_3^-] = 7.5 \times 10^{-3} \text{ M}$; ionic strength = 0.11 M ; [buffer] = $7.0 \times 10^{-2} \text{ M}$; [indicator] = $2.5 \times 10^{-4} \text{ M}$; temperature = 25.0°C . Key: (●) **1**; (■) **2**.

half-sandwich zinc(II) complexes. We found that the k_{obs} values of these cobalt(II) complexes are comparable with those of corresponding zinc(II) complexes, this indicating the suitability of using cobalt(II) complexes as CA model compounds.

In Fig. 3, the intercept values are not equal to zero, which means that in the absence of the cobalt(II) complexes, the dehydration reaction does take place. That is, H^+ and/or solvent can catalyze HCO_3^- dehydration. Plot of k_{obs} in the absence of Co(II) complexes versus different $[\text{H}^+]$ is given in Fig. 4. It is evident that first-order dependence on $[\text{H}^+]$ has been observed. In this case,

$$k_{\text{obs}}k_{\text{H}}[\text{H}^+] + k_0 \quad (6)$$

least-square fit gives

$$k_{\text{H}} = 1.84 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}, \quad k_0 = 7.93 \times 10^{-2} \text{ s}^{-1}$$

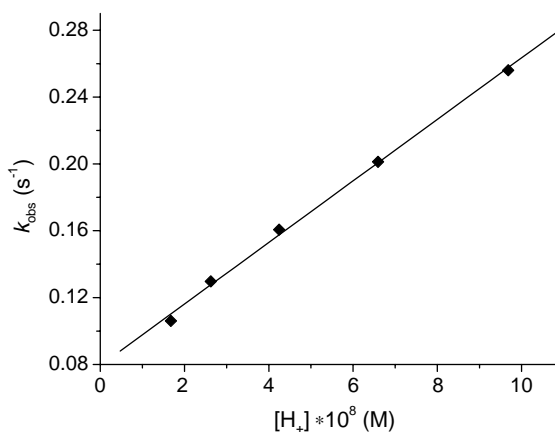


Fig. 4. Plot of k_{obs} vs. $[\text{H}^+]$ for the uncatalyzed dehydration of HCO_3^- . Experimental conditions: $[\text{HCO}_3^-] = 7.5 \times 10^{-3} \text{ M}$; ionic strength = 0.11 M ; [buffer] = $7.0 \times 10^{-2} \text{ M}$; [indicator] = $2.5 \times 10^{-4} \text{ M}$; temperature = 25.0°C .

In Fig. 4, the intercept is not zero, which shows that besides k_H , there is a slow reaction with $k_0 = 7.93 \times 10^{-2} \text{ s}^{-1}$. The small value of k_0 is probably due to the solvolysis of bicarbonate. This means that HCO_3^- can be hydrolyzed by solvolysis, but the reaction rate is low. From the above results, it has been found that the reaction rate is of first-order with respect to cobalt(II) complex and hydrogen ion concentrations. Therefore, we have the following kinetic equation

$$V = k_{\text{obs}}[\text{HCO}_3^-] = (k_{\text{cat}}[\text{complex}] + k_H[\text{H}^+] + k_0)[\text{HCO}_3^-] \quad (7)$$

3.3. Catalytic mechanism

The dehydration reaction of HCO_3^- catalyzed by the Co(II) complexes can be account for in terms of the mechanism outlined in Fig. 5, which are based on the principle that only the aqua complex can react with HCO_3^- in order to catalyze the dehydration reaction ($\text{X}^-: \text{N}_3^-/\text{NCS}^-$) [1–9]. Seen from this figure, a solvent water molecule coordinates to the Co(II) ions of **1** and **2**, respectively, to complete a five-coordinated aqua complex showing a trigonal bipyramidal coordination geometry. This five-coordinated H_2O -adduct is considered as the reactive catalytic species in the dehydration of HCO_3^- . In this mechanism, it is assumed that the produced bicarbonate complex is unstable and rapidly releases CO_2 . It is reasonable to assume that no stable bicarbonate complex is formed since the release of CO_2 was observed during the reaction. In addition, no evidence whatsoever could be found for the presence of such a complex. Hence, the rate-determining step of the dehydration reaction must be the substitution of the labile water molecule by HCO_3^- , which nucleophilic attacks on the Co(II) ion of the model complex. The overall mechanism of dehydration of HCO_3^- catalyzed by the model complexes can be therefore stated as follows: in the case of $\text{pH} < \text{p}K_a$, the aqua complex exhibits the catalytic activity on the dehydration of HCO_3^- in which the rate-determining step is the substitution of the labile water molecule by HCO_3^- , followed by the rapid decarboxylation of the coordinated bicarbonate molecule as found for many model bicarbonate complexes.

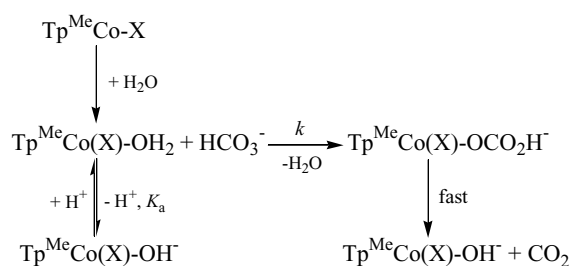


Fig. 5. The proposed mechanism of the dehydration reaction of HCO_3^- catalyzed by the cobalt(II) complexes with Tp^{Me} ligand.

As have been pointed out, the observed apparent dehydration rate constant is of first-order with respect to the total complex concentration

$$k_{\text{obs}} = k_{\text{cat}}[\text{complex}] + k_H[\text{H}^+] + k_0 \quad (8)$$

From the following stoichiometric equation (L: the ligands Tp^{Me} and X^-)

$$[\text{complex}] = [\text{LCoOH}_2] + [\text{LCoOH}^-] \quad (9)$$

we have

$$[\text{complex}] = [\text{LCoOH}_2] \left[1 + \frac{K_a}{[\text{H}^+]} \right] \quad (10)$$

According to the above proposed mechanism,

$$k_{\text{obs}} = k[\text{LCoOH}_2] + k_H[\text{H}^+] + k_0 \quad (11)$$

where k is the dehydration rate constant of the rate-determining step. Combination of the Eqs. (10) and (11),

$$k_{\text{obs}} = \frac{k[\text{complex}][\text{H}^+]}{[\text{H}^+] + K_a} + k_H[\text{H}^+] + k_0 \quad (12)$$

Comparison of the Eqs. (8) and (12), we therefore have

$$k_{\text{cat}} = \frac{k[\text{H}^+]}{[\text{H}^+] + K_a} \quad (13)$$

Using the K_a values of **1** and **2** previously obtained by pH titration, we can calculate the dehydration rate constants of the rate-determining step

$$k = 129 \text{ M}^{-1} \text{ s}^{-1}, \quad \text{for } \mathbf{1}$$

$$k = 95.9 \text{ M}^{-1} \text{ s}^{-1}, \quad \text{for } \mathbf{2}$$

The higher the k value, the lower the activation energy barrier. Therefore, the origin of the variation in the inhibition ability between the two inhibitors N_3^- and NCS^- can be correlated with changes in the activation energy barriers of the rate-determining step. The present results have proved that cobalt(II) complex with Tp^{R} ligand system can serve as a good CA model that catalyze the bicarbonate hydrolysis. From k_{cat} and k values, we can conclude that different small inorganic ions that coordinated to the Co(II) ions can influence the catalytic activity of their cobalt(II) complexes. This may be due to the variation of the effective atomic charges of the Co(II) ions, which is confirmed by the following theoretical calculations.

3.4. Theoretical calculations

It is evident from the above experimental measurements that the inhibition ability of NCS^- is stronger than that of N_3^- . During the dehydration process of HCO_3^- , the nucleophilic attack of the free HCO_3^- on the Co(II) ion of the model complex is affected by the effective atomic charge of the Co(II) ion. In this case, the nitrogen atom of the inhibitor ($\text{N}_3^-/\text{NCS}^-$) that coordinates to the Co(II) ion serves as the electron donor. Low effective atomic charges caused

by stronger donor inhibitors is disadvantageous to the nucleophilic attack of the free HCO_3^- ion on the Co(II) ion. To obtain more information on the variations in the effective atomic charge of the Cu(II) ion by coordinating different inhibitors ($\text{N}_3^-/\text{NCS}^-$), we have carried out theoretical calculations of **1** and **2** at the UHF level, using a lanl2dz basis set for the Co atoms and the corresponding basis set for each of the other atoms. The total atomic charges have been found to be 1.195 and 0.684 for the Co(II) ions of **1** and **2**, respectively. Therefore, we can conclude that the electrons from the N donor atom of NCS^- are more delocalized on the Co(II) ion leading to the decrease of the effective atomic charge of the Co(II) ion, which can rationalize the variations in inhibition ability between two inorganic ions N_3^- and NCS^- .

4. Conclusion

In summary, we have presented here both experimental and theoretical evaluations toward the comparison of the inhibition ability between two small inorganic ions N_3^- and NCS^- in the dehydration of HCO_3^- catalyzed by two half-sandwich cobalt(II) complexes. The Co(II) complexes in our kinetic measurements exhibit a catalytic activity for the dehydration of HCO_3^- at $\text{pH} < 7.9$. The five-coordinated aqua complex must be the reactive catalytic species in the catalyzed dehydration reaction and the rate-determining step is the substitution of the labile water molecule by HCO_3^- . Results from kinetic measurements show that the dehydration rate constant k of the rate-determining step of **2** is lower than that of **1**, which can be correlated with a higher activation energy barrier, this being the origin of the variation in the inhibition ability between N_3^- and NCS^- . The inhibition ability of NCS^- is strong than that of N_3^- , which is also confirmed by the decrease in effective atomic charges of the Co(II) ions as revealed by theoretical calculations in this work. These results would be a helpful richness to the kinetics and mechanism study of hydration and dehydration reactions catalyzed half-sandwich metal complexes.

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