Synthesis and Spectroscopic Characterizations of an Insulinomimetic Peroxovanadate Complex in Aqueous Solution†

CAI, Shu-Hui[a](蔡淑惠) YU, Xian-Yong[b](于贤勇) CHEN, Zhong*[,a,b](陈忠)
WAN, Hui-Lin[b](万惠霖)

[a] *Department of Physics, State Key Laboratory for Physical Chemistry of Solid Surface, Xiamen University, Xiamen, Fujian 361005, China*

[b] *Department of Chemistry, State Key Laboratory for Physical Chemistry of Solid Surface, Xiamen University, Xiamen, Fujian 361005, China*

The reaction system of diperoxooxalatovanadate {$K_3[VO(O_2)_2(C_2O_4)]\cdot H_2O$, bpV(Oxa)} and imidazole was studied in an aqueous solution by 1D multinuclear (^1H, ^{13}C and ^{51}V) NMR, 2D NMR diffusion ordered spectroscopy (DOSY), and variable temperature NMR techniques. It was shown that DOSY was a useful tool for the study of a mixture. All of the ^1H and ^{13}C NMR signals of the peroxovanadate (V) complexes were assigned. The NMR experimental results indicated that a new complex was formed through the coordination interactions between bpV(Oxa) and imidazole. The newly-formed complex is stable under the experimental condition. Electrospray ionization mass spectrometry (ESI-MS) provides positive evidence to support the conclusion.

Keywords diperoxovanadate, imidazole, synthesis, DOSY, NMR, ESI-MS

Introduction

Peroxovanadate (pV) compounds were reported to represent a new kind of powerful insulinomimetic agents because of their biological activity.[1,2] They can induce a response both *in vitro* and *in vivo*, and may be developed into a new kind of oral drugs for diabetes in future.[3] Recently, interest has been increasing both in coordination chemistry and in biological mechanism of peroxovanadate compounds as antidiabetic agents.[4] The chemistry of peroxovanadate compounds under physiological condition and the synthesis of new peroxovanadate compounds are among the most challenging fields. Due to their great insulinomimetic potency, diperoxovanadate imidazole complexes have received significant interest.[3,4] Although a crystal of diperoxovanadate imidazole complex has been synthesized by the reaction of V_2O_5, H_2O_2 and imidazole,[3,4] the reaction of imidazole with diperoxooxalatovanadate {$K_3[VO(O_2)_2(C_2O_4)]\cdot H_2O$, bpV(Oxa)[1]}, which is an important intermediate, has not been reported previously. In this paper, the interaction between bpV(Oxa) and imidazole was investigated by NMR and ESI-MS.

^{51}V NMR is an important method in characterizing and analyzing vanadium (V) compounds.[5-12] By using a reference scale, ^{51}V NMR techniques could be sufficiently advanced in studying structures and coordination environments of known or unknown vanadium(V) compounds.[13,14] Several NMR techniques including 1D multinuclear (^1H, ^{13}C and ^{51}V) NMR, 2D NMR diffusion ordered spectroscopy (DOSY),[15] and variable temperature NMR were adopted to obtain insights into the reaction system of bpV(Oxa) and imidazole. An insulinomimetic peroxovanadate complex was found to form through the coordination interactions between bpV(Oxa) and imidazole. All ^1H and ^{13}C signals of the newly-formed complex were assigned. In addition, electrospray ionization mass spectrometry (ESI-MS) of the reaction system was also performed, which is a gentle ionization method and has been successfully applied in the analysis of many vanadium compounds, including water/ethanol solution of peroxovanadate.[16]

Experimental

Materials and preparation

$K_3[OV(O_2)_2(C_2O_4)]\cdot H_2O$ (the structural sketch of its anion in solid state was shown in Fig. 1a) was prepared and characterized according to the method reported previously.[17,18] The compounds H_2O_2, NaCl, oxalic acid, imidazole (Imi) and V_2O_5 were the analytic grade reagents of the First Shanghai Reagent Plant. Deuterium oxide (D_2O) was the product of Beijing Chemical Plant. EtOH and wa-

* E-mail: chenz@jingxian.xmu.edu.cn; Fax: +86-592-2189426

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ter were redistilled. The ionic medium was chosen to represent physiological condition (0.150 mol/L NaCl D_2O solution, pH 3—7, aerobic atmosphere, ambient temperature).

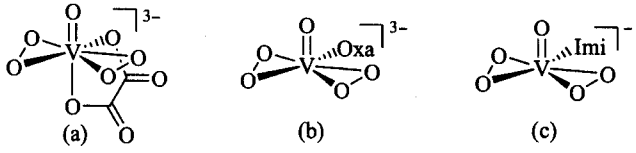

Fig. 1 Structural sketch of (a) bpV(Oxa) anion in solid state, (b) bpV(Oxa) anion in aqueous solution, and (c) anion of the newly-formed complex.

Spectroscopy

All NMR spectra were recorded on a Varian Unity plus 500 spectrometer operating at 500.4 MHz for ^1H NMR, 125.7 MHz for ^{13}C NMR and 131.4 MHz for ^{51}V NMR. Solvent for ^1H NMR, ^{13}C NMR and ^{51}V NMR spectra was D_2O. DSS [3-(trimethylsilyl)-propanesulfonic acid sodium salt] was used as an internal reference for ^1H NMR and ^{13}C NMR chemical shifts. ^{51}V NMR chemical shifts were measured relative to the external standard $VOCl_3$ with upfield shifts considered negative. Signal-to-noise ratios were improved by a line-broadening factor of 10 Hz in the Fourier transformation of all ^{51}V NMR spectra. DOSY was recorded by using a z-gradient probe, which delivers a maximum gradient strength of 30 G/cm. The gradient compensated stimulated echo spin lock (BPPSTE)[19] was used to acquire DOSY spectra. The typical experimental parameters for an ^1H DOSY spectrum are as follows: gradient duration $\delta = 2$ ms, gradient strength $G = 30$ G/cm, diffusion delay $\Delta = 400$ ms, and time interval between $\pi/2$ and π pulses $\tau = 1.3$ ms. Diffusion coefficient in NMR was generally achieved by stepwise ramping up of the amplitudes of pulsed field gradients (PFGs), and the diffusion times were optimized for every experiment. The typical time required for a 2D ^1H DOSY spectrum was approximately 0.8 h (16 scans, relaxation delay 4 s). Reference deconvolution and baseline correction were used to compensate experimental imperfections for all DOSY spectra.[19] The spectrum editing for different components was performed by selecting a specific diffusion row which corresponds to a 1D ^1H NMR spectrum of the individual component.

ESI-MS was measured by a Finnigan MAT LCQ instrument with the following parameters: sheath gas (N_2) 30 mL/min, aux gas (N_2) 4 mL/min, capillary temperature 175 ℃, discharge voltage 2.52 kV, spray voltage 3.5 kV, capillary voltage −4.0 kV, and scan range m/z 50—800. Flow rate of mobile phase was set to 0.8 mL/min. The solutions of the reaction system of bpV(Oxa) and imidazole were introduced by direct injection. The diperoxovanadate solutions were prepared by dissolving bpV(Oxa) (5×10^{-3} and 5×10^{-4} mol/L, respectively) in CH_3CH_2OH/H_2O (1 : 1, $V : V$). Imidazole (one, three, and five molar equivalents, respectively) was added immediately after bpV(Oxa) had been dissolved completely.

Results and discussion

Reaction processes monitored by ^{51}V NMR and ^1H NMR

The reaction procedure of bpV(Oxa) and imidazole was probed by ^{51}V and ^1H NMR. There are two peaks in the ^{51}V NMR spectrum of bpV(Oxa) solution, locating at δ −692 and −738, respectively. The peak at δ −738 is assigned to bpV(Oxa), and the peak at δ −692 is assigned to bpV(D_2O).[18,20] When imidazole was added to bpV(Oxa) (0.2 mol/L), a new single peak appeared at δ −748 with a half-height-width about 120 Hz. Increasing equivalent of imidazole from 0.2 to 2.0 with an increment of 0.2 per step, it was found that the intensity of δ −748 peak increased and almost reached its maximum after imidazole reached 1.0 equivalent due to its stronger coordination capacity, as shown in Fig. 2. In the mean time, both the intensities of the signals at δ −692 and −738 decreased gradually. Some small peaks at δ −732 and −763 which were assigned to $V(O_2)_3^-$ and $[(VO(O_2)_2)(OH)]^{3-}$ respectively were also found.[21,22]

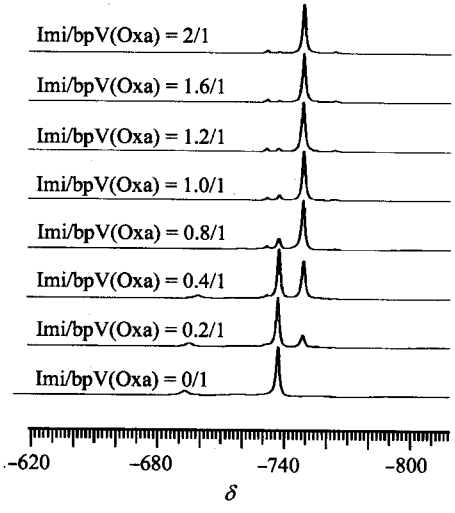

Fig. 2 ^{51}V NMR spectra of bpV(Oxa) (0.2 mol/L) and imidazole with different molar ratios in solution.

According to the previous report,[4] the above experimental results imply the formation of a new peroxovanadate complex through stronger coordination interaction of imidazole to vanadium of bpV(Oxa), compared to the coordination interaction of oxa^{2-} or D_2O to vanadium under the condition of near physiological pH. The peak at δ −748 was assigned to the new complex formed by V—N bond, as shown in Fig. 1c.

The experimental results shown in Fig. 2 demonstrate that the concentration of imidazole influences the equilibrium of the reaction system. How about the influence of pH variation? Take the system of bpV(Oxa) and imidazole

with 1 : 2 molar ratio in solution as an example. When 0.2, 0.4, 0.6, 0.8 and 1.0 equivalent DCl were gradually added to the system respectively, the ^{51}V peak at δ −748 was almost unchanged. However, in the ^{1}H NMR spectra, the peaks at δ 7.14 and 7.80 belonging to the free imidazole gradually moved to δ 7.47 and 8.64 respectively, as shown in Fig. 3. The results of ^{1}H and ^{51}V NMR spectra of the solution with 1.0 equivalent DCl agreed with those in previous report.[4] These experimental results imply that the protonation site is located far away from the vanadium center. The peaks at δ 7.47 and 8.64 were assigned to the imidazolium counterion, in agreement with the results obtained from ^{1}H and ^{13}C NMR spectra (next section).[4]

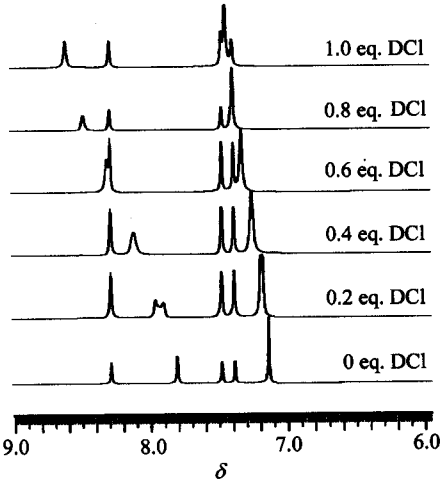

Fig. 3 ^{1}H NMR spectra of bpV(Oxa) (0.2 mol/L) and imidazole with 1 : 2 molar ratio and different molar ratios of DCl.

Variable temperature ^{51}V NMR experiments were carried out to see the effect of temperature on the equilibrium of the reaction system. The results obtained from the system of bpV(Oxa) and imidazole with 5 : 2 molar ratio are shown in Fig. 4. Four conclusions can be drawn from Fig. 4: (1) The quantity of the newly-formed complex increases and the chemical shifts of all peaks move toward down field when the temperature increases. (2) The trend is reversed when the temperature decreases. It is still unknown why at the same temperature, the spectrum obtained from lowering temperature is slightly different from that obtained from

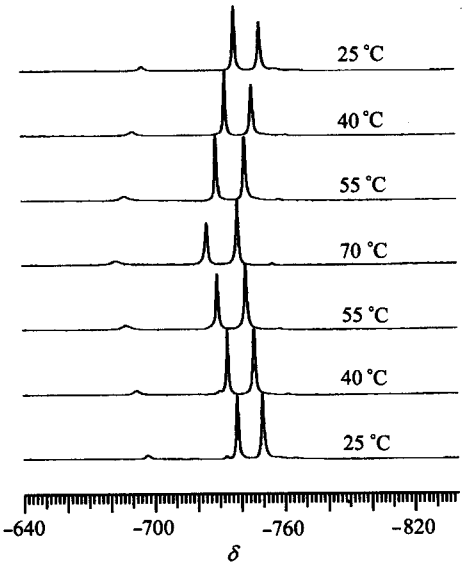

Fig. 4 Variable temperature ^{51}V NMR spectra of bpV(Oxa) (0.2 mol/L) and imidazole with 5:2 molar ratio in D_2O solution. From bottom to top, the temperature increases, then decreases.

increasing temperature. (3) The newly-formed complex is stable in the temperature range of 25—70 ℃. (4) The change of the temperature affects the chemical shift of vanadium compounds in solution.[23]

The ^{51}V NMR spectra of the system of bpV(Oxa) and imidazole with 1:2 molar ratio were also recorded every 12 h at room temperature. It shows that the newly-formed complex is stable during the experimental time (a week). Therefore, the spectra recorded in our experimental conditions such as ^{1}H 2D DOSY and ^{13}C NMR spectra are creditable.

Identification and assignment of ^{1}H *and* ^{13}C NMR *spectra*

The ^{1}H and ^{13}C NMR spectra of the system of bpV-(Oxa) (0.2 mol/L) and imidazole with 1:2 molar ratio, and the system of bpV(Oxa), imidazole and DCl with 1:2:1 molar ratio were measured. The results are listed in Table 1.

It is noted that both the coordinated oxalate of bpV-(Oxa) and free oxalate could produce a δ_C 175.9 peak in solution. The ratio of δ 170.5 to 175.9 peak areas in ^{13}C NMR spectrum of bpV(Oxa) is smaller than 1, indicating

Table 1 Assignment of ^{1}H and ^{13}C NMR spectra of imidazole, $[OV(O_2)_2(Imi)]^-$, $[HImi]^+$, bpV(Oxa) and oxalate in the reaction system

	Imidazole[a]		$[OV(O_2)_2(Imi)]^-$ [a,b]		$[HImi]^+$ [b]		bpV(Oxa)[a,b]	Oxalate
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_C	δ_C
C-2	7.80	138.5	8.30	139.5	8.64	136.1		
C-4	7.14	124.2	7.49	129.1	7.47	121.7		
C-5	7.14	124.2	7.40	120.7	7.47	121.7		
$O_2CCO_2^{2-}$							170.5 175.9	175.9

[a] In the system of bpV(Oxa) (0.2 mol/L) and imidazole with 1:2 molar ratio; [b] in the system of bpV(Oxa) (0.2 mol/L), imidazole, and DCl with 1:2:1 molar ratio.

that the δ 175.9 peak comes not only from free oxalate, but also from bpV(Oxa), *i.e.*, the chemical environment of one carbon atom in bpV(Oxa) is similar to that of the carbon atoms in the free oxalate. Therefore, we suggest that one of the two V—O bonds between vanadium and oxalate in crystal structure has been broken in solution, *i.e.*, the coordination number of vanadium is six when bpV(Oxa) is dissolved in solution, as shown in Fig. 1b.

2D ^{1}H DOSY *spectrum of the reaction system*

The system of bpV(Oxa) and imidazole with 1:2 molar ratio in solution was taken as an example for 2D ^{1}H DOSY investigation (Fig. 5). In 2D DOSY spectrum, the x-axis shows chemical shift, and the y-axis indicates diffusion coefficient. Fig. 5 illustrates the presence of two components. The component with slower diffusion rate (indicated by the solid line) is the newly-formed species. The trace of the solid line (Fig. 5b) corresponds to the "net" 1D ^{1}H NMR spectrum of the new component. The chemical structure of the new component could be identified and characterized accordingly. The trace of the dashed line in the DOSY spectrum (Fig. 5c) shows two peaks of free imidazole.

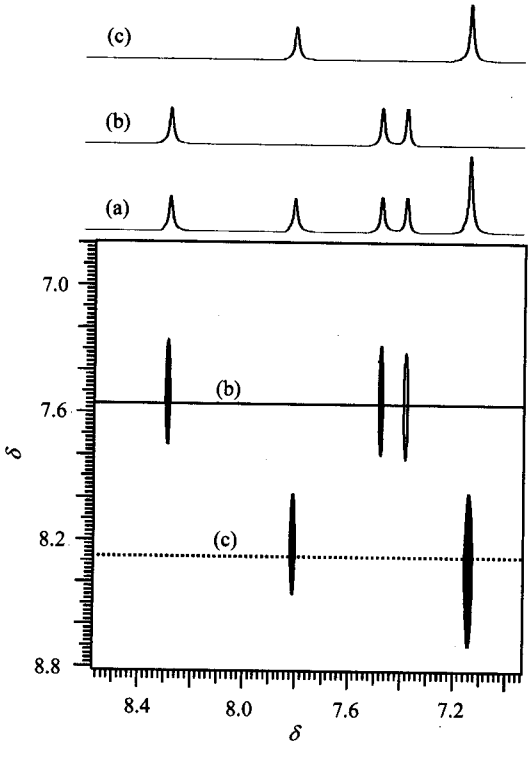

Fig. 5 ^{1}H DOSY spectrum of bpV(Oxa) (0.2 mol/L) and imidazole with 1:2 molar ratio in D_2O solution. (a) Mixed system; (b) coordinated imidazole; (c) free imidazole.

ESI-MS *study of the reaction system*

ESI-MS techniques were used to study the reaction system of bpV(Oxa) and imidazole in negative ion mode.[16] Most of the observed peaks could be attributed to vanadium species in different coordination spheres at higher m/z value. For example, the mass spectrum of the reaction system of bpV(Oxa) (5×10^{-3} mol/L) and imidazole with 1:1 molar ratio gives $[VO_3(H_2O)]^-$ (m/z 117, 30%), $[OV(O_2)_2]^-$ (m/z 131, 100%), $[VO_3(EtOH)]^-$ (m/z 145, 20%), $[VO_3(EtOH)(H_2O)]^-$ (m/z 163, 40%), $[OV(O_2)_2(Imi)]^-$ (m/z 199, 50%), and $[(VO_3)OV(O_2)_2+H]^-$ (m/z 232, 10%) (Fig. 6).

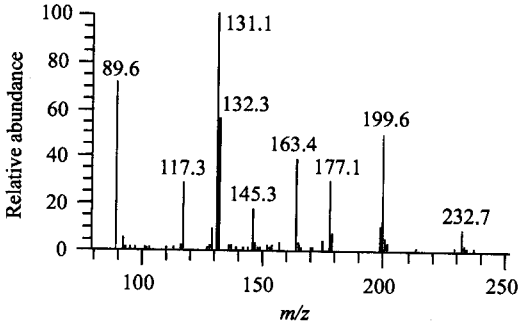

Fig. 6 ESI-MS spectrum of bpV(Oxa) (5×10^{-3} mol/L) and imidazole with 1:1 molar ratio in D_2O solution.

Table 2 listed the main results of ESI mass spectra of bpV(Oxa) (5×10^{-3} and 5×10^{-4} mol/L) and imidazole with 1:1, 1:3 and 1:5 molar ratios in solution. It shows that the intensities of $[OV(O_2)_2(Imi)]^-$ species increase with the addition of imidazole, indicating that the more the equivalent of imidazole, the more the target species $[OV(O_2)_2Imi]^-$. Keeping the molar ratio between bpV(Oxa) and imidazole unchanged, the reduction of the concentration of bpV(Oxa) decreases the relative intensity of the species $[OV(O_2)_2(Imi)]^-$. This is because that, under low imidazole concentration, the solvent molecule has more chance to attack metal atom, which leads to the lower $[OV(O_2)_2(Imi)]^-$ concentration. The ESI-MS results provide a positive evidence for the formation of the species $[OV(O_2)_2(Imi)]^-$ in the reaction system, in agreement with NMR results.

Reaction modes in the mixture

After analyzing and comparing ^{1}H, ^{13}C and ^{51}V NMR spectra of bpV(Oxa), imidazole and their mixtures with different molar ratios, we suggested the most possible reaction modes among them as follows: (1) The bpV(Oxa) dissolves in the solution and forms the $[OV(O_2)_2(Oxa)]^{3-}$ species with six-coordinated vanadium. This coordinatively unsaturated (coordination number is six instead of seven) vanadium species is probably related to its reactivity. (2) The imidazole competitively coordinates to vanadium, accompanied by the leaving of oxalate from complex. This produces a new species $[OV(O_2)_2(Imi)]^-$. This new species is six-coordinated, in agreement with the X-ray crystal structures of $HImi[OV(O_2)_2(Imi)]$.[4] (3) Partial bpV(Oxa) is hydrolyzed and forms species $[OV(O_2)_2(D_2O)]^-$ which is further substituted by

Table 2 Relative abundances of $[OV(O_2)_2]^-$ and $[OV(O_2)_2(Imi)]^-$ in ESI mass spectra of systems of bpV(Oxa) and imidazole with different molar ratios in solution

c(bpV(Oxa)) (mol/L)	Species	m/z	Relative abundance (%)		
			bpV(Oxa):Imi = 1:1	bpV(Oxa):Imi = 1:3	bpV(Oxa):Imi = 1:5
5×10^{-3}	$[OV(O_2)_2]^-$	131	100	43	8
	$[OV(O_2)_2(Imi)]^-$	199	50	100	100
5×10^{-4}	$[OV(O_2)_2]^-$	131	100	100	68
	$[OV(O_2)_2(Imi)]^-$	199	13	58	100

imidazole and forms $[OV(O_2)_2(Imi)]^-$. (4) When DCl is added drop by drop to the reaction system, the imidazolium counterion forms since imidazole is the strongest proton acceptor in the system.

Conclusions

In this paper, an insulinomimetic peroxovanadate complex $[OV(O_2)_2Imi]^-$ was produced in near physiological pH solution through competitive coordination between oxalate of bpV(Oxa) and imidazole. Normally, the bond between V and O is weaker than the bond between V and N due to the weaker electron-donation ability of oxygen. In bpV(Oxa), oxalate coordinates to vanadium by oxygen. Therefore, it can be replaced by imidazole which coordinates to vanadium by nitrogen.

Several spectral techniques especially DOSY and ESI-MS were used to obtain direct information of the structure and the chemistry of peroxovanadates in solution without chemical separation. It is shown that DOSY is a useful tool in analyzing and characterizing mixtures. This technique may be used to study components of mixture *in vivo* without destroying compounds.

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