Spectroscopic Characterization and Properties of Some Bioactive Peroxovanadium Complexes in Aqueous Solution①

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ABSTRACT Four bioactive peroxovanadium (pV) complexes bpV(ox), bpV(bipy), bpV(phen) and bpV(pic), $([VO(O_2)_2L]^{n-}$, where ligand L= oxalic acid dianion(ox), bipyridine(bipy), 1, 10-phenanthroline(phen), and pyridine-2-carboxylic acid(pic), were synthesized and characterized by ^{51}V NMR, ^{1}H NMR, ^{13}C NMR, ESI-MS, IR and elemental analysis. All ^{1}H and ^{13}C peaks were assigned by 2D ^{1}H-^{1}H COSY, HMQC and HMBC. Their stereochemical structures in solution were discussed according to the NMR signals of organic ligands. The descending stability order of complexes in aqueous solution determined by ^{51}V NMR is bpV(phen), bpV(bipy), bpV(pic) and pV(ox). The predominant decomposition patterns of these complexes were proposed on the basis of electrospray ionization MS (ESI-MS) and ^{51}V NMR. This work will facilitate the studies of interactions between pV complexes and target biomolecules in solution so as to clarify structure-function relationship of these bioactive complexes.

Keywords: peroxovanadium, characterization, solution properties, NMR, ESI-MS

1 INTRODUCTION

Peroxovanadium(pV) complexes are potent protein tyrosine phosphatase inhibitors with insulin-mimesis properties and could possibly be developed into a new kind of oral drug for the treatment of diabetes[1~3]. Recently, this kind of compound has received considerable attention. Design and synthesis of new pV compounds with safer and more effective feature has become one of the most challenging fields in vanadium chemistry. However, there are some difficulties that affect the new-drug oriented work of this category of complexes. For instance, little has been known on their

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Abbreviations: bpV(bipy), sodium (2, 2'-bipyridine) oxodiperoxovanadate, $Na[VO(O_2)_2(bipy)]\cdot 5H_2O$; bpV(phen), potassium oxodiperoxo-(1, 10-phenanthroline) vanadate, $K[VO(O_2)_2(phen)]\cdot 3H_2O$; bpV(pic), potassium picolinatooxodiperoxovanadate, $K_2[VO(O_2)_2(pic)]\cdot H_2O$; bpV(ox), oxalatooxodiperoxovanadate, $K_3[VO(O_2)_2(ox)]\cdot 2H_2O$; δ, chemical shifts unit in present paper is 10^{-6}.

structure-function relationship so far, which is closely related to their future development and application. Besides, although four typical pV complexes studied in the present paper, i.e. bpV(ox), bpV(bipy), bpV(phen) and bpV(pic), were synthesized and characterized in solid state[4~5], we have never had the reports of the crystal structures of bpV(ox)[6], bpV(pic)[5], pV(bipy)[7] and pV(phen) analog[8]. The solution chemical properties of four pV complexes are still ambiguous, since their aqueous chemistry are much more complex and variable than those in solid state. In order to obtain insights into above problems, NMR techniques combined with other spectroscopy were used in this paper. Firstly, all protons and carbons signals of four pV complexes in solution NMR spectra were assigned and other spectroscopic characterization methods were used simultaneously to investigate their solution structure. This is the necessary work for our next-step studies that will investigate the solution interactions between pV complexes and the model peptides in active center of phosphatase for further clarifying structure-function relationship of pV complexes on the basis of our last paper[9,10]. Meanwhile, it will be useful for structure elucidation and studies of new pV compounds with similar skeleton structure by NMR. Then, their properties in aqueous solution such as stability, decomposition patterns were studied by ^{51}V NMR and ESI-MS.

2 EXPERIMENTAL

2.1 Materials and preparation

The compounds V_2O_5, $NaVO_3$, H_2O_2, oxalic acid, 2, 2'-bipyridine, 1, 10-phenanthroline, pyridine-2-carboxylic acid etc. were local products of analytic grade reagent. Distilled water was used in all preparations. Four pV complexes bpV(ox), bpV(bipy), bpV(phen) and bpV(pic) were prepared by our improved synthesis methods which were described in detail as the last paper[9]. In a typical preparation procedure with bpV(ox) as an example, which was prepared by adding H_2O (20 mL) to V_2O_5(0.91 g, 5 mmol) and KOH (1.95 g) in a 125 mL Erlenmeyer flask under cooling followed by 20 mL H_2O_2(30%, w/v, solution) and oxalic acid(1.26 g, 10 mmol). The mixture was stirred for 2 h in cooled ice water bath at 0 ℃. Ethanol was then added gradually until a precipitate start to appear. The reaction mixture was filtered, filtrate was adjusted to pH 5.0, which was kept at 4 ℃ for one night to crystallize. Orange crystals obtained were re-crystallized, filtered off and washed with ethanol.

2.2 Spectroscopy

All the NMR spectra were recorded on a Varian Unity$^+$ 500 spectrometer operating at 500.0 MHz for ^{1}H, 125.7 MHz for ^{13}C and 131.4 MHz for ^{51}V at ambient room temperature, 20 ℃; ^{51}V chemical shifts were measured relative to $VOCl_3$ as

external standard at δ 0.00 with upfield shifts considered negative. Solvent for ^{51}V NMR was H_2O/D_2O (90%/10%), and that for ^{1}H and ^{13}C spectra was D_2O. DSS used as internal standard of ^{1}H chemical shifts at δ 0.00. All NMR experiments began immediately after samples were dissolved into solvent. NMR acquision parameters: for ^{51}V spectra, pulse width 3.0μs; spectra width 79 kHz; acquisition time, 0.2 s; pulse repeat time, 0.4 s, accumulation times, 400; frequency domain size, data point 32 k; for ^{13}C spectra, pulse width 6.0 μs; spectra width 50 kHz; acquisition time 0.6 s; pulse repeat time, 1.0 s, accumulation times, 4000; frequency domain size, 64 k data point. A 10 Hz line-broading factor was applied to all spectra before being transformed to the time domain. Baseline corrections were also done before internals were obtained. MS spectra were performed on Finnigan MAT LCQ ESI-MS Instrument. MS data obtained were measured immediately after pV samples were dissolved into distilled water. Elemental analysis was performed on EA1110 CHNS CE Instrument. IR spectra were recorded on a Nicolet 360 FT-IR spectrometer with samples as Nujol mulls.

3 RESULTS AND DISCUSSION

3.1 Spectroscopic Characterization of four pV complexes in aqueous solution

The results of IR of four pV complexes accorded with the previous paper[4~5]. Moreover, ^{51}V NMR, ^{13}C NMR spectra of four complexes and elemental analysis re-

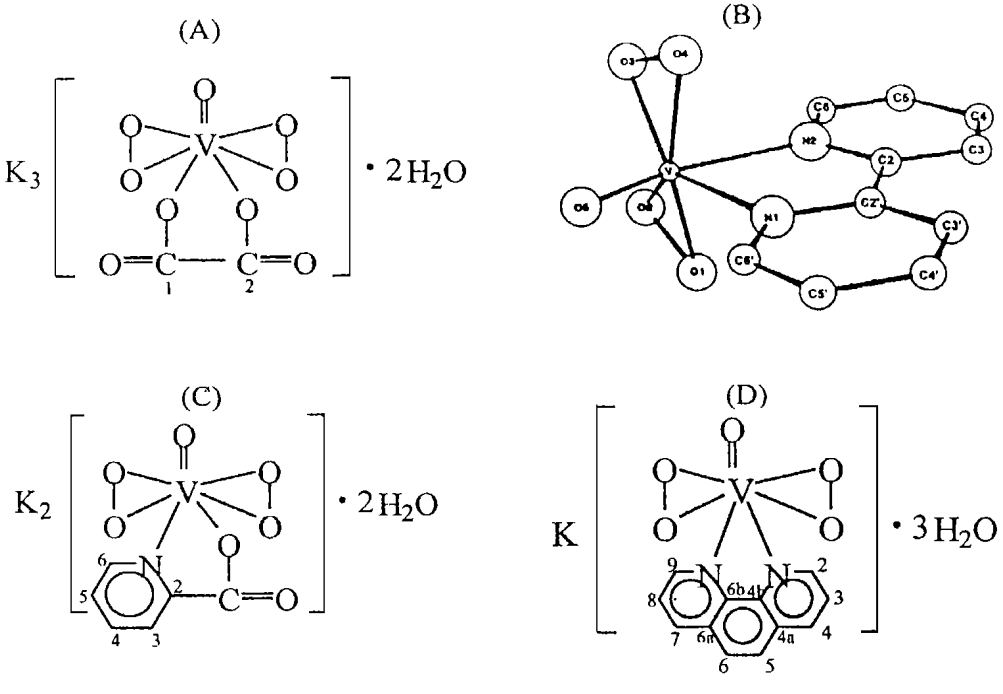

Fig. 1 Chemical structures of four pV complexes
(A) bpV(ox); (B) bpV(bipy); (C) bpV(pic); (D) bpV(phen)

ported in our last paper[9] agreed with their structures (Fig. 1) too. So, their chemical structure established as Fig. 1. In present paper, we focused on investigating their complete assignment of proton and carbon signals (See Tab. 1) based on ^{1}H-^{1}H COSY, HMQC and HMBC results. MS data were also reported as follows: bpV (ox), $K_3[VO(O_2)_2(ox)]\cdot 2H_2O$ (m/z 373.4, 100%), $[VO(O_2)_2(H_2O)]^-$ (m/z 150.4, 15%), $[VO(O_2)(H_2O)]^+$ (m/z 117.3, 2%); bpV(bipy), $Na[VO(O_2)_2(bipy)]$ (m/z 311, 100%); bpV(phen), $K[VO(O_2)_2(phen)]$ (m/z 350.3, 100%); bpV(pic), $K_2[VO(O_2)_2(pic)]\cdot H_2O$ (m/z 351.2, 100%).

Table 1. ^{1}H and ^{13}C NMR data for organic ligand of pV complexes and free organic ligand

Complexes		δ ^{1}H mult.[a](J in Hz)	δ ^{13}C[b]
bpV(bipy)	L[c]	^{1}H NMR (D_2O): 9.52(1H, d, J= 5.1 Hz, H-6'), 8.35(1H, d, J= 7.5 Hz, H-3'), 8.23(1H, t, J= 7.5 Hz, H-4'), 8.09(2H, dd, J= 5.0 Hz, 7.5 Hz, 6-H, H-3), 7.89(1H, t, J = 7.5 Hz, H-4), 7.76(1H, t, J= 5.1 Hz, H-5'), 7.32 (1H, t, J= 5.0 Hz, H-5)	^{13}C NMR(D_2O): 153.8(C-6'), 152.9(C-2'), 148.2(C-2), 145.3(C-6), 141.1(C-4'), 138.4(C-4), 125.8(C-5'), 125.1 (C-5), 122.1(C-3'), 120.3(C-3)
	F-L[d]	^{1}H NMR ($CDCl_3$): 8.68(2H, m, H-6, H-6'), 8.40(2H, m, H-3, H-3'), 7.77(2H, m, H-4, H-4'), 7.28(2H, m, H-5, H-5')	^{13}C NMR ($CDCl_3$): 156.0(C-2, C-2'), 149.0(C-6, C-6'), 136.8(C-4, C-4'), 123.6(C-3, C-3'), 121.0(C-5, C-5')
bpV(phen)	L[c]	^{1}H NMR(D2O): 9.59(1H, d, J= 4.5 Hz, H-2), 8.39(1H, d, J= 5.0 Hz, H-9), 8.07(1H, d, J= 8.0 Hz, H-7), 7.97(1H, d, J= 8.0 Hz, H-4), 7.56(1H, t, J= 6.5 Hz, H-8), 7.55(1H, t, J= 6.3 Hz, H-3), 7.22(1H, d, J= 8.5 Hz, H-6), 7.07(1H, d, J= 8.5 Hz, H-5)	^{13}C NMR(D_2O): 153.5(C-2), 146.1(C-9), 143.5(C-4b), 140.5(C-6b), 140.0(C-4), 136.7(C-7), 129.0(C-6a), 128.1(C-4a), 126.3(C-6), 125.3(C-5), 124.3(C-8), 124.0(C-3)
	F-L[d]	^{1}H NMR ($CDCl_3$): 9.15(2H, m, H-2, H-9), 8.15(2H, m, H-4, H-7), 7.68(2H, s, H-5, H-6), 7.55(2H, m, H-3, H-8)	^{13}C NMR ($CDCl_3$): 150.1(C-2, C-9), 146.1(C-4b, C-6b), 135.8(C-4, C-7), 128.4(C-4a, C-6a), 126.3(C-5, C-6), 122.9(C-3, C-8)
bpV(pic)	L[c]	^{1}H NMR(D_2O): 9.35(1H, s, H-6), 8.27(1H, br, H-4), 8.13(1H, br, H-5), 7.84(1H, s, H-3)	^{13}C NMR(D_2O): 168.6(C=O), 151.7(C-6), 150.2(C-2), 141.2(C-4), 127.4(C-3), 124.8(C-5)
	F-L[d]	^{1}H NMR ($CDCl_3$): 8.71(1H, m, H-6), 8.12 (1H, m, H-4), 7.95(1H, m, H-5), 7.57(1H, m, H-3)	^{13}C NMR ($CDCl_3$): 166.1(C=O), 149.2(C-6), 148.2(C-2), 137.2(C-4) 126.8(C-5) 124.6(C-3)
bpV(ox)	L[c]		^{13}C NMR(D_2O): 173.0(C-1), 167.6(C-2)
	F-L[d]		^{13}C NMR(D_2O): 161.3(C=O)

[a] Mult., multiplicity; m, multiplet; t, triplet; dd, doublets of doublet; d, double; br, broad; s, single. [b] The ^{13}C chemical shifts were extracted from the HMQC and HMBC (for quaternary carbons) experiments. [c] Organic ligand coordinated, [d] Free organic ligand

Complete assignments of ^{1}H NMR and ^{13}C NMR of bpV(bipy) were done as follows: firstly, the resonance at δ 9.52 in ^{1}H NMR spectra was readily assigned to

H- 6', the very downfield hydrogen due to approaching per-oxygen ring. Then, from ^{1}H-^{1}H COSY (Fig. 2a), H- 6' was coupled to hydrogen at δ 7.76 (H- 5') which was further coupled to that at δ 8.23 (H- 4'). The assignment of H- 5' and H- 4', was inferred from a group of cross-peaks at δ 9.52, 7.76, 8.23. H- 3' at δ 8.35 determined by the correlation peaks between δ 8.23 and 8.35. The splitting situation of peaks in ^{1}H NMR confirmed above assignment too. In the same way, another group of cross-peaks at δ 7.32, 7.89 and 8.09 revealed the assignment of another pyridine ring of pV(bipy) which was indicated as Tab. 1. Then, ^{13}C NMR assignment easily arose from the corresponding correlation hydrogen in HMQC spectra (Fig. 2 b), and quaternary carbons were extracted from HMBC according to adjacent assigned hydrogen. The similar method was used for assignment of bpV(phen) and bpV(pic) with the exception of easily assigned bpV(ox).

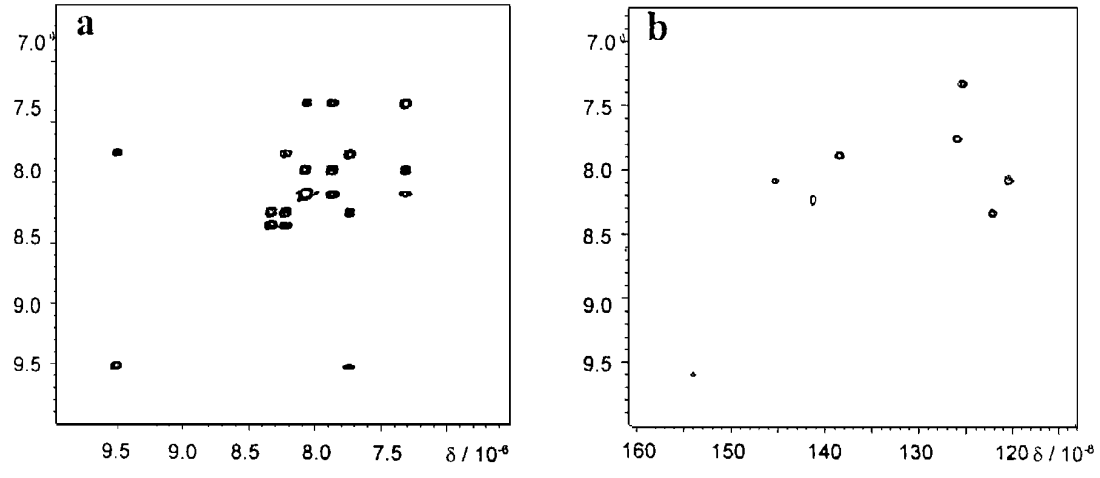

Fig. 2 ^{1}H-^{1}H COSY (a) and HMQC (b) NMR spectra for pV(bipy)

So far, there is little report of ^{1}H NMR and ^{13}C NMR data for four pV complexes until our latest paper[9]. Obvious variations were observed in their ^{1}H NMR and ^{13}C NMR spectra between the free organic ligand and coordinated ones (See Tab. 1 and Fig. 3). For example, ^{13}C NMR spectra of bpV(bipy) and bpV(phen) have 10 and 12 resonances which are twice as many as those of corresponding free ligands. Some valuable information of geometrical arrangement about pV compounds in aqueous solution can be deduced from the data. The results imply that the organic ligands array in a distorted configuration after coordination reaction. It is well known that most oxovanadate compounds exist in the form of seven-coordinated pentagonal bipyramidal geometry in solid state[11]. Fig. 1 of crystal structure of bpV(bipy) in reference [7] showed the typical steric arrangement of this family of complexes in solid state, which arrayed in a distorted pentagonal bipyramidal geometry with vanadyl oxygen O(5) and nitrogen atom N(2) from bipyridine in the axial position. The two peroxo ligands

and the other nitrogen N(1) lie in the pentagonal plane. Comparing the chemical shifts of carbon and hydrogen in NMR spectra between free organic ligand and coordinated ones of bpV(bipy) (See Tab. 1), it is obvious that chemical shifts of one of the pyridine rings adjacent to peroxo ligand (N2 located) upfield remarkably owing to being deshielded, especially for C-6' and H-6' which is the nearest to peroxo ligand. The similar case occurred to other pV complexes too. The results suggest that, in aqueous solution, the configurations of organic ligand in complexes are analogous to the solid state thus causing the asymmetrical electronic environment around the organic ligands. The doubled ^{13}C NMR resonance after complexation illustrated in Fig. 3 could be attributed to the asymmetrical electronic distribution around the bipyridine ligand after coordinating reaction. The exact assignments of all NMR signals of carbons and hydrogens for four pV compounds in solution were indicated as Tab. 1.

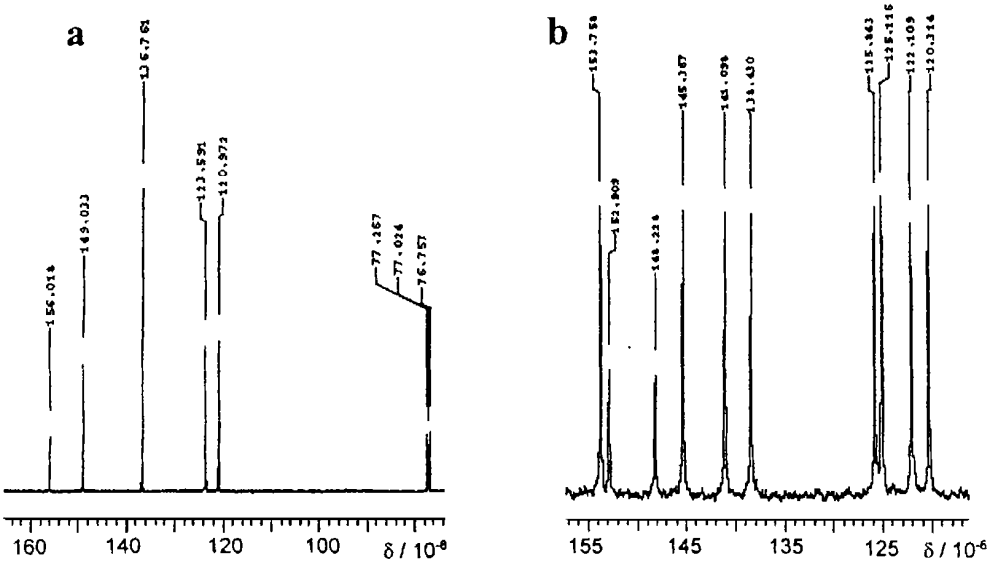

Fig. 3 ^{13}C NMR spectra for bipyridine (a) and bp(bipy) (b)

3.2 Stability and decomposition patterns of four complexes in aqueous solution

^{51}V Chemical shifts, of bpV(ox), bpV(bipy), bpV(phen) and bpV(pic) in pH 7.2 Hepes buffer aqueous solution were at δ - 732, - 746, - 742 and - 743 respectively. In order to study the stability of four pV complexes, a series of ^{51}V NMR spectra of four complexes obtained which were recorded once per 5 h going with the prolonging 48 hours. Their stability in aqueous solution was revealed by the ratio of integral area of their main peak. The results showed that bpV(ox) is most unstable among four complexes. Besides the main peak of bpV(ox) at δ - 732, two other peaks at δ - 592 and - 687 appeared immediately after bpV(ox) was dissolved in water. The integral area ratio of three peaks changed regularly with bpV(ox) concentra-

tion from 10 mmol/L to 200 mmol/L (Fig. 4), i.e. the main peak at δ – 732 augmented gradually with the increasing concentration while two other peaks at δ – 592 and – 687 decreased. As for bpV(bipy), bpV(phen) and bpV(pic), all appeared single mair peak basically unchanged within 24 h whose chemical shifts were δ – 746, – 743, – 742 respectively. However, for these three complexes, the similar situation occurred gradually when time exceeded 24 h in solution. The case indicated that their stability maintained to be ca. 24 h under this condition. Comparing the changes of the integral area ratio of the main peak of four pV complexes at the equal concentration, relative stability of four pV complexes was bpV(phen) > bpV(bipy) > bpV(pic) > bpV(ox).

Peroxovanadates have clear biochemical significance and clinical used prospect, so it is of interest to study the properties of these materials in aqueous solution at physiological pH. It is well known that oxovanadates including pV is labile and complex in aqueous solution[12, 13]. Despite previous studies on the different water-peroxovanadium clusters, the view about water-peroxovanadium is ambiguous[14–16]. Moreover, the solution properties on pure pV complexes with organic ligands were studied little. According to previous report[13, 2] on water-oxovanadates, peaks at ca. δ – 690 were assigned as water-biperoxovanadates, i.e. bpV(H_2O); and the peaks at δ – 590 to – 600 assigned as water-monoperoxovanadate, i.e. mpV(H_2O). From the stability experiment for pV complexes studied by ^{51}V NMR in our lab, it was observed that signals of bpV(H_2O) and mpV(H_2O) at corresponding chemical shifts occurred for all four complexes with time prolonging. Due to the difference of stability of four pV complexes, bpV(H_2O) and mpV(H_2O) peaks of bpV(ox) observed immediately after it was dissolved into H_2O, while it took longer time to observe the two peaks of bpV(H_2O) and mpV(H_2O) for other three pV complexes. Besides, the MS peaks of bpV(ox) at 117.3(m/z) and 150.4(m/z) also provided the evidence of the existence of bpV(H_2O) and mpV(H_2O). A recent literature[14] about

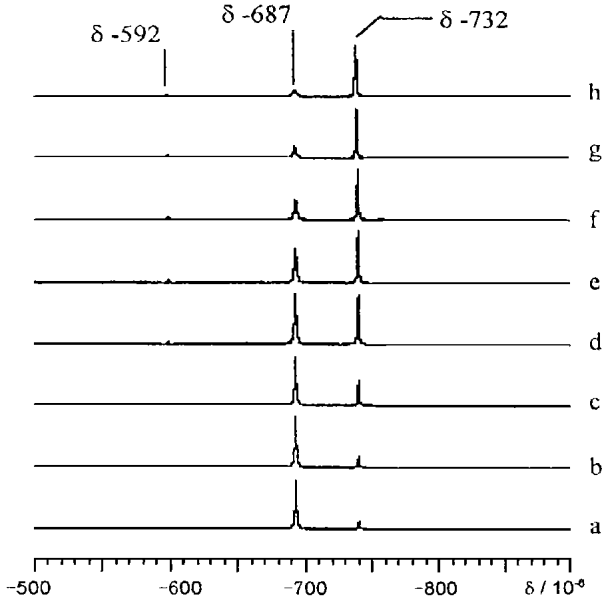

Fig. 4 ^{51}V NMR spectra for bpV(ox) in pH 7.2 Hepes buffer. a~ h: 5, 10, 25, 50, 75, 100, 150, 200 mmol/L of bpV(ox) respectively

ESI-MS of solvent-peroxovanadium provides positive support on this point too. The order of stability of four complexes is rational compared with their chemical structures so that nitrogen coordinated ligands have stronger giving-electron ability than oxygen coordinated ones thus combined the vanadium more compact. Therefore, bpV(bipy) and bpV(phen) coordinated with the organic ligand by two nitrogen atoms have more stability than bpV(pic) which coordinated by one nitrogen atom and one oxygen. Analogously, there is more stability of bpV(pic) than bpV(ox). As for more stable feature of bpV(phen) compared with bpV(bipy), it can be likely attributed to the space hinder effect of larger organic ligand of bpV(phen), which held back competitive coordination reaction induced by water molecule. Based on the experiments results mentioned above, we proposed decomposition patterns for four pV complexes in aqueous solution illustrated as Scheme 1. That is to say, firstly, water molecule competed with organic ligands to coordinate central metal vanadium, which leads to gradually leaving of the organic ligands from the complexes. Then, one peroxo-ring took off from complexes in the similar way. Actually, oxovanates properties in solution are very complicated, the two-step patterns proposed in this paper is a predominant breakdown way. After enough time in ^{51}V NMR experiments, for example, exceeding 24 h, other small peaks resulted from further decomposing can also be observed.

$$[VO(O_2)_2(\text{ligand})]^{n-} \rightleftharpoons [VO(O_2)_2(H_2O)]^- \rightleftharpoons [VO(O_2)(H_2O)]^+$$

Scheme 1. Predominant decomposition patterns of pV complexes in aqueous solution. ligand= ox, oxalic acid dianion; bipy, bipyridine; phen, 1,10-phenanthroline; pic, picolic acid.

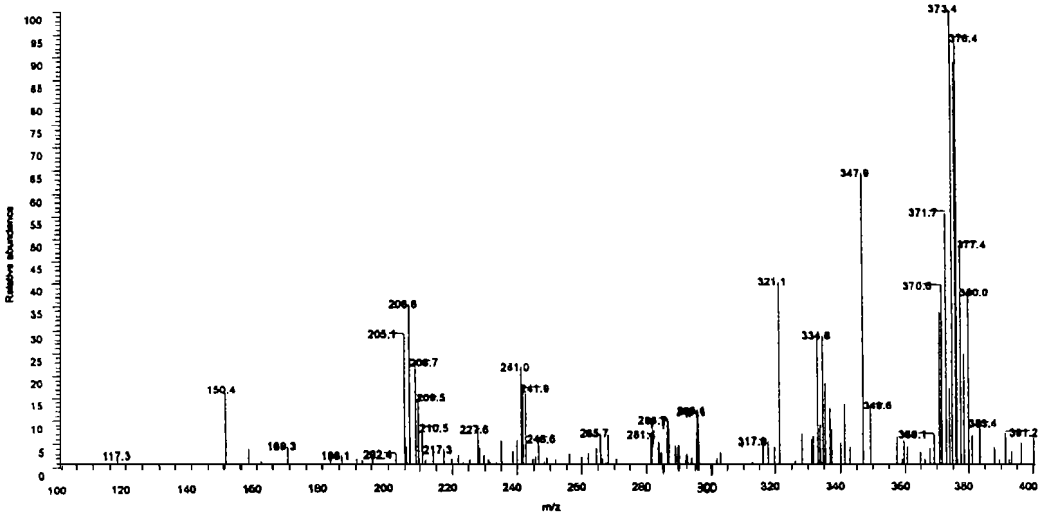

Fig. 5 ESI-MS for bpV(ox): in positive ion mode; RT= 1.26; H_2O as solvent

4 CONCLUSION

Detailed NMR assignments and characterization on four bioactive pV complexes finished mainly by means of NMR and MS spectroscopy. Their stability order and decomposition patterns in aqueous solution were also studied and discussed. The results showed their decomposing degree and speed at physiological pH related to the chemical structures and concentrations of complexes, etc. The present paper will lay a solid foundation for our in-progress studies of interactions between pV complexes with target biomolecules in solution and help to further clarify the molecular mechanism and structure-activity relationship of this new family of promising oral insulin substitute.

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