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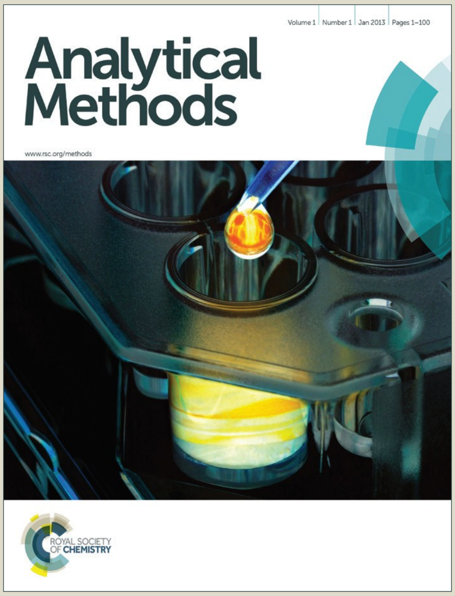

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Investigation on Structural characterization and reversible oxygenation mechanism of oxygenated cobalt-3-(4-thiazolyl)-L-alanine and cobalt-Histidine complexes using electrospray tandem mass spectrometry

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The reversibility of dioxygen uptake by a complex is highly dependent on the stability of its superoxo- or dinuclear peroxo-intermediates. It is important to characterize oxygenated intermediates for the mechanistic investigation of reversible dioxygen uptake by complexes. Although the oxygenated complexes can be investigated by conventional technique, the oxygenated species can only be identified indirectly. Thus new techniques for direct differentiation of the oxygenated species are required to prove the existence of oxygenated species in complexes. In this paper, the electrospray mass spectrometry (ESI-MS) method was developed to directly identify oxygenated species. The newly reported cobalt-3-(4-thiazolyl)-L-alanine (Co-Tza) and the well-known cobalt-histidine (Co-His) complexes were used as targets. Fragmented ions of the dinuclear oxygenated complexes of Co-Tza and Co-His in aqueous samples were evident on ESI-MS spectra. The ESI mass spectra of Co-Tza and Co-His suggested the formation of dinuclear peroxo complexes, $[Co^{III}L_2]_2(O_2)$, and the spectrum of Zn-His only showed the fragments of a mononuclear $Zn(His)_2$ complex. The results also revealed that in the electrospray positive ion detection mode, the Co-Tza and Co-His complexes had similar fragmentation patterns, resulting likely from the similarities in ligand structure and coordination modes between the complexes. The fragmented structure of sodium and potassium adducts of Co-Tza and Co-His were almost identical, indirectly confirming the rationality of the proposed fragmentation patterns. For comparison, theoretical modeling computations and volumetry were used to examine the oxygenation of Co-Tza. The results revealed that ESI-MS can be a useful and powerful technique for characterizing oxygenated complexes and investigating oxygenation mechanisms.

Introduction

To better understand the reversible oxygenation mechanisms of natural oxygen carriers, such as hemoglobin and myohemoglobin, synthetic reversible oxygen

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carriers are of interest as model compounds. A variety of synthetic dioxygen complexes have been proposed, prepared and studied.[1-3] Among these complexes, a cobalt-histidine complex (Co-His) has attracted considerable attention because it is one of the few synthetic compounds that can well reversibly uptake dioxygen in aqueous solutions at room temperature.[4,5] Based on this unique property, we proposed that its coordination structure plays an important role in the reversible oxygenation process and we synthesized a number of *N*-containing heteroaromatic ligands whose structures are analogous to histidine. The oxygenation properties of their cobalt complexes were then studied.[6-8] These Co complexes could undergo 500-3000 oxygenation-deoxygenation cycles during several days of continuous oxygenation reaction. One of these complexes is cobalt-3-(4-thiazolyl)-L-alanine complex (Co-Tza), which had excellent reversibility for the uptake of dioxygen, similar to Co-His. Figure 1 presents the chemical structures of the Tza and His ligands. Because of their excellent reversibility of uptaking dioxygen, characterizing their structures is important for the study of reversible oxygenation mechanisms.

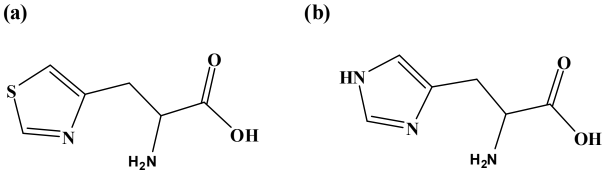

Figure 1. Chemical structures of Tza (a) and His (b)

To better understand the binding mode of the absorbed dioxygen in these complexes, a weight-loss method[9] and headspace gas chromatography[10] were developed in our lab to determine the contents of dioxygen in the oxygenated complexes. The results demonstrated that although the oxygenated cobalt complexes can release gas when heated, the oxygenated species can only be identified indirectly. Thus a new method for direct differentiation of the oxygenated species is required to prove the existence of oxygenated species in complexes.

So far, the methods used to investigate dioxygen complexes include spectrophotometry,[11] pH potentiometric methods,[12] oxygen electrodes,[13] gas volumetry,[14,15] stripping voltammetry,[16] electronic spin resonance and infrared spectroscopy.[17]

ESI-MS is a soft ionization method that minimizes fragmentation, generally leaving species unaltered. It also allows for a direct acquisition of mass spectra from aqueous samples. Because of these properties, ESI-MS is well-suited for investigating the coordination behavior of transition metal complexes. Since new millennium, ESI-MS has been used to study the structural features and stability of transition metal complexes, emerging as a very powerful technique among many available methods.[18-24] The speciation of an imidazolate-bridged copper (II)-zinc (II) complex[25] and the coordination behavior of dacarbazine with transition metal ions[26] have been investigated by ESI-MS.

As mentioned above, the electrospray tandem mass spectrometry (ESI-MS) could be a direct means to detect the oxygenated species, however only a few literatures reported to investigate dioxygen complexes by MS since it is difficult to detect the

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oxygenated species as they are unstable under the ESI-MS conditions. Jason Love et al[27] used ESI-MS as an auxiliary means to confirm the existence of oxygenated species in their complexes, and they successfully detected and stabilized for the first time the oxygenated precursor ion $[Co_2(\mu\text{-}O_2)(L)]H^+$, and they further undertook CID studies to investigate the stability and fragmentation pathways of $[Co_2(O_2)(L)]H^+$ and its monooxygenated analogue $[Co_2(O)(L)]H^+$.

We tried to use ESI-MS to detect the oxygenated species in the oxygenation of histidine-cobalt complex and its aqueous samples separately adsorbing oxygen at early time and continuously adsorbing oxygen for 24 hours were taken for comparison. The fragment ions of the oxygenated complexes were confirmed by its sub-fragment ion by further fragmentation[28]. On this basis, we have detected the oxygenated species and investigated the evolution of oxygenation of the ethylenedianmine[29] and triethylenetetramine[30] cobalt complex by ESI-MS.

Although the oxygenated species could be successfully stabilized and detected by ESI-MS, the techniques are still in a primary stage and needs to be developed; meanwhile it provides the possibility to study the reversible oxygenation mechanism of Co complexes by ESI-MS.

It is known that，during oxygenation, a dioxygen molecule binds to Co (II) resulting in an oxidative addition in which a partial electron transfer from the metal center toward dioxygen occurs. This leads to the formation of a mononuclear superoxo- or a dinuclear peroxo- intermediate, with a $Co:O_2$ ratio of 1:1 or 2:1.[2,31-33] The ability of a complex to reversibly uptake dioxygen is highly dependent on the stability of the superoxo- or dinuclear peroxo- intermediate. Therefore, it is necessary to identify the presence of oxygenated intermediate in reversible oxygenation mechanism study.

Since Tza and His are both tridentate ligands (L), in complexes of a divalent transition metal (M) the species ML_2 is predominant and they produce the characteristic mass of the complex. When these complexes react with dioxygen to form dinuclear oxygenated complexes, the dinuclear oxygenated complexes formed have masses higher than that of ML_2. Thus the unoxygenated and oxygenated species in the same complex have different masses which should be observed using ESI-MS.

Based on this assumption, in this work the ESI-MS method was proposed to directly investigate the reversible oxygenation mechanism of Co-Tza and Co-His complexes. Fragment ions of the dinuclear oxygenated complexes were identified from aqueous samples of Co-Tza and Co-His complexes. In comparison with the well-known Co-His complex oxygenation from the literature, theoretical modeling computations–and volumetry were used to examine oxygenation of the Co-Tza complex. Based on these results, the structures and fragmentation mechanisms of the oxygenated species were deduced.

Experimental

Materials and reagents

Histidine (His) was purchased from Sigma (St. Louis, MO, USA).

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3-(4-thiazolyl)-L-alanine (Tza) was purchased from Shanghai Hanhong Chemical Co., Ltd (Shanghai, China). HPLC gradient grade acetonitrile was obtained from Fisher Scientific (Fair Lawn, New Jersey, USA). Cobalt acetate ($Co^{II}(Ac)_2{\cdot}4H_2O$), zinc acetate ($Zn(Ac)_2{\cdot}2H_2O$), sodium hydroxide (NaOH) and potassium hydroxide (KOH), all p.a. grade, were obtained from Zhiyuan Chemical Reagent Co., Ltd (Tianjin, China). Ultrapure water was produced from a PURELAB Ultra purification system (ELGA, High Wycombe, UK).

Synthesis of cobalt-3-(4-thiazolyl)-L-alanine and cobalt-histidine complexes

Cobalt-3-(4-thiazolyl)-L-alanine (Co-Tza) and cobalt-histidine (Co-His) complexes were prepared under nitrogen in an oxygen-free MBraun UNILab glove box. Co-Tza and Co-His were synthesized by adding solutions of 3-(4-thiazolyl)-L-alanine (34.4 mg, 0.2 mmol) and histidine (31.0 mg, 0.2 mmol) in water (5 mL) to solutions of $Co(Ac)_2{\cdot}4H_2O$ (24.9 mg, 0.1 mmol) in water (5 mL), respectively. The pH values were then adjusted to pH 8 using sodium hydroxide or potassium hydroxide to prepare their sodium or potassium salts, respectively. The synthesized complexes were transferred to test tubes in the glove box and fixed to the top of a glass burette filled with a known amount of oxygen. The Co-Tza and Co-His complexes were analyzed by mass spectrometry before and after oxygen exposure for 24 hours, respectively.

Synthesis of zine-histidine complex

Zine-histidine complex was prepared without the use of glove box. Zn-His was synthesized by adding solution of histidine (31.0 mg, 0.2 mmol) in water (5 mL) to solution of $Zn(Ac)_2{\cdot}2H_2O$ (21.9 mg, 0.1 mmol) in water (5 mL). The pH value was adjusted to pH 8 using sodium hydroxide.

Theoretical simulation

Theoretical calculations were carried out using a G03 quantum chemistry program package.[34] Full geometry optimization computations were performed using the B3LYP method. The 6-31G(d) basis set was used for C, H, N, O and S atoms, and the LanL2DZ effective core potential was applied to metal atoms.

Volumetry

In the glove box, equal volumes of aqueous solutions of Co (II) (0.02 mol L^{-1}) and Tza (0.04 mol L^{-1}) were mixed in conical flasks.[14] The resulting aqueous solution was rapidly placed in an eudiometer that had been filled with dioxygen in advance. Measurements of changing volumes of dioxygen with time were performed until the volume of dioxygen was constant. The final dioxygen volume was then recorded.

Tandem mass spectrometric and chromatographic conditions

Mass spectrometry was performed using a Waters Quattro Premier XE mass spectrometer equipped with an electrospray ionization source (Micromass, Manchester, UK). The mass analyzer was operated in the positive ionization mode for

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all analytes. The optimized parameters were: the source temperature was 120 °C; the desolvation temperature was 450 °C; the capillary voltage was 3.5 kV; the desolvation gas flow was 400 L h^{-1}; the cone voltage was 60 V; the cone gas flow was 50 L h^{-1}; the collision energy was 25 eV and the multiplier was 650 V. The mass spectra collected during a full scan in the positive ion mode were obtained by scanning over the mass range *m/z* 150–900. Nitrogen (99.99% purity) was used as the desolvation and nebulization gas, and ultrapure argon (99.99% purity) was used as the collision gas. Full scan and MS/MS mass spectra were obtained by flow injection analysis (FIA) of individual aqueous solutions at 0.01 mol L^{-1}. Acetonitrile/water (1:1 *v/v*) was used as the mobile phase.

Results and discussion

Mass spectrometry analysis of 3-(4-thiazolyl)-L-alanine and histidine

The positive ESI ion mode is selected because it is more sensitive and can produce better fragmentation patterns for the Tza and His ligands. The positive ion mass spectrum of Tza is presented in Figure 2a. The full scan mass spectrum revealed that a *m/z* 173.00 was the base peak for Tza, corresponding to the protonated molecular ion $[M+H]^+$. Along with the base peak, other fragment ions at *m/z* 126.93, 194.99 and 210.98 corresponding to $[M+H\text{-}HCOOH]^+$, $[M+Na]^+$ and $[M+K]^+$, respectively, were also observed.

Figure 2b presents the positive ion ESI mass spectrum of His. The base peak of the protonated molecular ion $[M+H]^+$ was observed at *m/z* 156.00 along with a peak corresponding to $[M+H\text{-}HCOOH]^+$ at *m/z* 109.98. Fragment ions at *m/z* 177.99 and 193.99 corresponding to $[M+Na]^+$ and $[M+K]^+$, respectively, were also observed.

In this study, the ionic species underwent little fragmentation and provided useful structural information on Tza and His.

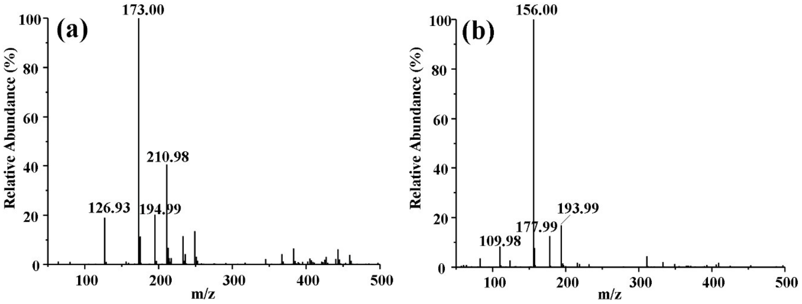

Figure 2. Full scan positive ion electrospray mass spectra of Tza (a) and His (b)

Mass spectrometry analysis of cobalt-3-(4-thiazolyl)-L-alanine and cobalt-histidine complexes prior to oxygen exposure

Figure 3 presents the full scan mass spectra of the cobalt-3-(4-thiazolyl)-L-alanine (Figure 3a) and cobalt-histidine (Figure 3b) complexes prior to oxygen exposure. From Figure 3a, the ESI mass spectrum of Co-Tza in the positive ion mode displays a series of characteristic ions at *m/z* 424.00, 402.04, 271.02, 194.95 and 172.92, corresponding to $[CoL_2+Na]^+$ (L=Tza-H), $[CoL_2+H]^+$, $[2M+H\ \text{-}CHNH_2COOH]^+$,

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$[M+Na]^+$ and $[M+H]^+$, respectively. Figure 3b presents the positive ion ESI mass spectrum of Co-His, where the base peak from $[CoL_2+Na]^+$ (L=His-H) was observed at *m/z* 390.06. Other fragment ion peaks at *m/z* 472.06 and 199.95 from $[CoL_2+CH_3COO+2Na]^+$ and $[L+2Na]^+$, respectively, were also apparent.

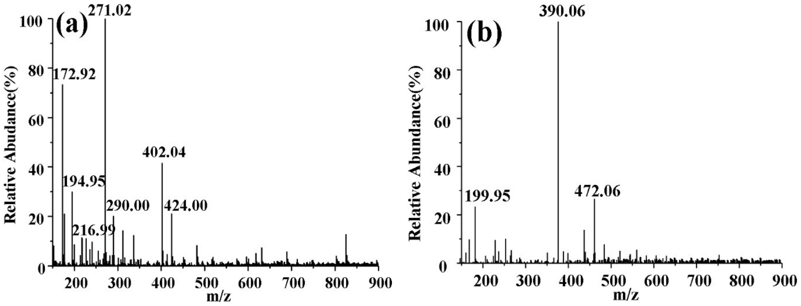

Figure 3. Full scan positive ion electrospray mass spectra of the Co-Tza (a) and Co-His (b) complexes prior to exposure to oxygen

The molecular formulas of Co-Tza and Co-His were determined from the presence of quasi-molecular ion peaks $[CoL_2+Na]^+$ and $[CoL_2+H]^+$ in the positive ion ESI-MS. Based on these peaks, it can be concluded that the Co-Tza and Co-His complexes, prior to oxygen exposure, should be the bis-ligand, CoL_2, species.

Mass spectrometry analysis of oxygenated cobalt-3-(4-thiazolyl)-L-alanine and cobalt-histidine

Continuous oxygenation studies of Co-Tza and Co-His have demonstrated that both complexes can reversibly uptake dioxygen over 3 days and a suitable timeframe for forming stable oxygenated complexes was approximately 24 hours.[35] Based on these studies, samples of Co-Tza and Co-His that had been exposed to pure dioxygen for 24 hours were analyzed using ESI-MS. Figure 4 shows the positive ion ESI mass spectra of Co-Tza (Figure 4a) and Co-His (Figure 4b) after oxygen exposure.

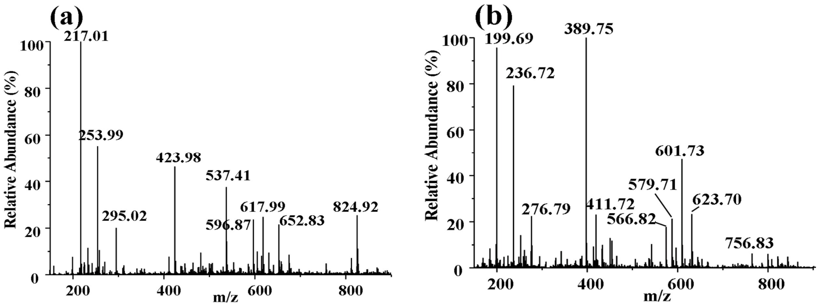

Figure 4. Full scan positive ion electrospray mass spectra of the oxygenated Co-Tza (a) and Co-His (b) complexes

From Figure 4a, the ESI mass spectrum of Co-Tza in the positive ion mode displays a series of characteristic ions at *m/z* 824.92, 652.83, 596.87, 423.98, 253.99 and 217.01. The intense ion peak at *m/z* 423.98 was attributed to the bis-ligand species ion $[CoL_2+Na]^+$. The abundance of the CoL_2 ion peak observed in the MS spectrum indicated that it was not fragmented. Several fragment ions with *m/z* values greater than 423.98 were observed.

A key ion was observed at *m/z* 824.92, a mass nearly twice of that CoL_2, and was

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attributed to the oxygenated dinuclear species $[Co_2(L_4\text{-}2O)O_2+Na]^+$. This suggests that a dinuclear peroxo-complex exists after oxygenation. In this complex, two CoL_2 are linked by a bridging dioxygen molecule and form the oxygenated complex (Figure 7) and during fragmentation two ligands in the complex lose an O atom respectively from their carboxylic functional group. The complex could then lose one neutral ligand molecule (Tza) to produce another dinuclear peroxo-complex, $[Co_2(L_3\text{-}HO_2)O_2+Na]^+$, corresponding to the fragment at *m/z* 652.83. MS/MS experiments on the $[Co_2(L_4\text{-}2O)O_2+Na]^+$ and $[Co_2(L_3\text{-}HO_2)O_2+Na]^+$ ions both indicated the loss of one neutral ligand to produce the most abundant ions, $[Co_2(L_3\text{-}HO_2)O_2+Na]^+$ at *m/z* 652.77 and $[Co_2(L_2\text{-}H_2O_2)O_2+Na]^+$ at *m/z* 480.86. Along with the ions at *m/z* 824.92 and 652.83, another dinuclear peroxo-complex ion at *m/z* 596.87 corresponding to $[Co_2(L_3\text{-}C_3H_7O_2N)O_2+Na]^+$ was also observed. The abundance of dinuclear peroxo-complexes was relatively low, suggesting that the oxygen bridge between the two cobalt centers was relatively unstable.

The capacity of the complexes for incorporating dioxygen and ratios of complex to dioxygen were determined using volumetry. The volumetry results are presented in Table 1.

The results in Table 1 show that 1 mmol of the Co complex in aqueous solution incorporated approximately 12 mL of dioxygen rapidly, indicating that two complexes can accept one molecule of dioxygen in aqueous solution, consistent with the Co/O_2 ratio reported for Co-His dioxygen complexes.[5]

Based on our volumetry experiments, Co-Tza and Co-His absorbed oxygen in a ratio of Co:O_2 = 2:1, demonstrating that the oxygenated complex consisted of a dinuclear species, which supports the MS results and is in a good agreement with previous results.[1,2]

The positive ion mode ESI-MS for the Co-His complex also demonstrated a series of characteristic ions at *m/z* 756.83, 601.73, 389.75, 236.72 and 199.69 that were attributed to $[Co_2(L_4\text{-}2O)O_2+Na]^+$, $[Co_2(L_3\text{-}HO_2)O_2+Na]^+$, $[CoL_2+Na]^+$, $[Co(L\text{-}HO)+H_2O+Na]^+$ and $[L+2Na]^+$ (L=His-H), respectively. The peroxo complex will be neutral. Thus the peroxo complex will need to be protonated/Na/K under the electrospray conditions. These Na-fragmentation patterns indicated that the oxygenated species were peroxo forms.

The fragmentation pattern was similar to that of the Co-Tza complex, due to similarities in their structures and coordination modes. Among the fragment ions, the bis-ligand $[CoL_2+Na]^+$ ion at *m/z* 389.75 was the base peak for the Co-His complex. The ions at *m/z* 756.83 and 601.73 were from the oxygenated dinuclear Co-His complex.

From the ESI-MS/MS results, it is obvious that the Co-Tza and Co-His complexes produce a series of identical fragment ions $[Co(L\text{-}HO)+H_2O+Na]^+$, $[CoL_2+Na]^+$, $[Co_2(L_3\text{-}HO_2)O_2+Na]^+$ and $[Co_2(L_4\text{-}2O)O_2+Na]^+$. The ESI mass spectra of the oxygenated Co-Tza and Co-His complexes suggest the formation of dinuclear peroxo-complexes, $[Co^{III}L_2]_2(O_2)$, where the nitrogen atoms of the imidazole (thiazolyl) rings have been removed from the cobalt atoms to allow the formation of the peroxo bridge. According to the mass spectra, possible fragmentation mechanisms

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for the complexes during ESI-MS were proposed (Schemes 1 and 2). The results demonstrate that the oxygenated Co-Tza and Co-His complexes can easily be distinguished using tandem mass spectrometry. The results suggested a dinuclear peroxo-structure, in agreement with previous research.[36]

During ESI-MS measurements, neutral complexes will sometimes appear in the gas phase as protonated species at *m/z* = (M+1) or as adducts of Na^+ at *m/z* = (M+23) and K^+ at *m/z* = (M+39). In this study, the positive ion mode ESI-MS for the Co-Tza and Co-His complexes revealed a series of sodium species ($[Co(L\text{-}HO)+H_2O+Na]^+$, $[CoL_2+Na]^+$, $[Co_2(L_3\text{-}HO_2)O_2+Na]^+$ and $[Co_2(L_4\text{-}2O)O_2+Na]^+$). The sodium adducts of the complexes were detected because the solution pH was adjusted using a dilute NaOH solution. To confirm these observations, KOH was used to adjust the pH of samples in place of NaOH during the synthesis of the Co-Tza and Co-His complexes. A series of the same fragments, with potassium adducts, were observed and were the dominant signals from ESI-MS. The sodium adduct peaks from Co-Tza that had high-intensities corresponding to $[CoL_2+Na]^+$ (*m/z* 423.98), $[Co_2(L_3\text{-}HO_2)O_2+Na]^+$ (*m/z* 652.83) and $[Co_2(L_4\text{-}2O)O_2+Na]^+$ (*m/z* 824.92), completely disappeared and were replaced by those of the potassium adducts $[CoL_2+K]^+$ (*m/z* 440.00), $[Co_2(L_3\text{-}HO_2)O_2+K]^+$ (*m/z* 669.03) and $[Co_2(L_4\text{-}2O)O_2+K]^+$ (*m/z* 841.16) (Figure 5). Similarly, the sodium adduct peaks from Co-His that had high intensities corresponding to $[Co(L\text{-}HO)+H_2O+Na]^+$ (*m/z* 236.72), $[CoL_2+Na]^+$ (*m/z* 389.75) and $[Co_2(L_3\text{-}HO_2)O_2+Na]^+$ (*m/z* 601.73), were also completely replaced by those of the potassium adducts $[Co(L\text{-}HO)+H_2O+K]^+$ (*m/z* 252.96), $[CoL_2+K]^+$ (*m/z* 406.05), and $[Co_2(L_3\text{-}HO_2)O_2+K]^+$ (*m/z* 618.05) (Table 2). These results confirm that ESI-MS is a reliable analysis technique.

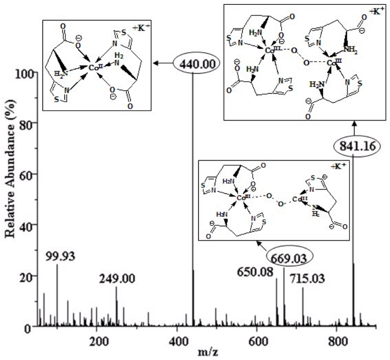

Figure 5. Full scan positive ion electrospray mass spectrum of Co-Tza depicting a series of potassium adduct peaks when the Co-Tza complex was synthesized using KOH

Mass spectrometry analysis of zine-histidine complex

Figure 6 presents the mass spectrum of the zinc-histidine (Zn-His) complex. The natural abundance of Zn is 100% ^{64}Zn, 57.4% ^{66}Zn, 8.4% ^{67}Zn, 38.7% ^{68}Zn, and 1.2% ^{70}Zn. The natural abundance of oxygen atoms is 100% ^{16}O, 0.037% ^{17}O and 0.205% ^{18}O. Therefore, the complex containing one Zn showed the relative abundances of M (*m/z* 395.08) to be 100%, M+2 (*m/z* 397.01) to be 57.7%, M+4 (*m/z* 399.05) to be 40.5%, M+3 (*m/z* 397.97) to be 8.4% and M+6 (*m/z* 401.07) to be 1.2%. The three

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observed ions at *m/z* 177.93, 199.95 and 377.10 corresponding to $[M+Na]^+$ (M=His), $[L+2Na]^+$ (L=His-H) and $[2M+L+2H\text{-}CO_2\text{-}HCOOH]^+$, respectively, were all the fragment ions of His ligand. The intense ion peak at *m/z* 395.08 was attributed to the bis-ligand species ion $[ZnL_2+Na]^+$. None of fragment ions with *m/z* values greater than 401.07 was observed. This shows that none of dinuclear complex exists after Zn-His oxygenation. The MS results provide good supporting evidence that Zn-His can not uptake dioxygen.

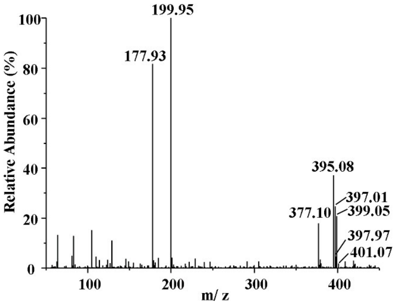

Figure 6. Full scan positive ion electrospray mass spectra of the Zn-His complex

The comparison study was conducted on the oxygenated cobalt complexes (Co-Tza and Co-His) and zinc complex (Zn-His) since only cobalt complex has a very high affinity to dioxygen in oxygen atmosphere. The ESI-MS results showed that in mass spectrum the fragment ions of higher *m/z* values corresponding to the dinuclear oxygenated species were observed for Co-TZA and Co-His while for Zn-His only fragment ions corresponding to ZnL_2 were observed. The MS results confirmed that the dinuclear oxygenated complex existed in the Co-Tza and Co-His complexes.

This study demonstrates that the mass spectra obtained are representative of the existing species and the molecular structures of species can be determined based on information obtained from ESI-MS experiments.

Theoretical simulations

Structural models of the studied compounds are presented in Figure 7. For the $Co(Tza)_2$, we used two geometric isomeric forms, trans-Tza and cis-Tza as the starting structures for optimization. The results of optimization showed that the quartet quartet and doublet spices, the quartet corresponds to high spin complex, it cannot turn into a stable structure, while the doublet spices corresponds to the low spin state complexes and it exists as a stable species. $[Co(Tza)_2](O_2)$ was assigned as doublet, which corresponds to the superoxide complexes. The multiplicity of $[Co(Tza)_2]_2(O_2)$ was assigned as singlet, which corresponds peroxy bridge form; or as triplet, which corresponding superoxide bridge form. And the calculation results showed the peroxy bridge forms are more stable species, which are in good agreement with the results of MS analysis.

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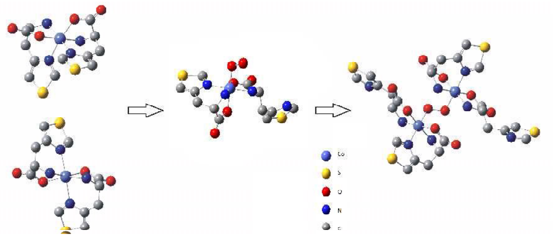

Figure 7. Theoretical structural evolution of the Co(II)-Tza complex during oxygenation

Figure 7 shows the molecular structure evolution of the Co^{II}-Tza complex during oxygenation, where the evolution was calculated using theoretical simulations.[34] It is apparent from the structural optimization that the oxygenated Co-Tza and Co-His complexes were dinuclear peroxo-species $[Co^{III}L_2]_2(O_2)$ and the high-spin state of the superoxide form will result in a longer Co-O bond length (1.909 Å), which weakens the bond energy and makes dissociation easier. The O-O bond in dioxygen was only stretched from 1.207 Å to 1.304 Å because of the weak interaction between the Co^{II}-Tza complex and dioxygen. This is possibly the reason for the oxygenation reversibility of the Co-Tza complex and the reason that superoxide species cannot be identified in the MS spectrum. In the calculated configuration, thiazolyl can serve as a leaving group to provide a vacancy for coordinating dioxygen. According to our theoretical calculations, dinuclear peroxo-complexes could be confirmed. The proposed configuration is stabilized by a hydrogen bond (2.510-2.677 Å) between the O atom of dioxygen molecule and the N atom of amino group.

Conclusions

In this work, electrospray mass spectrometry was investigated as a possible technique for the detection and identification of dinuclear species of Co-Tza and Co-His complexes and for the investigation their oxygenation mechanisms. The results demonstrated that the oxygenated Co-Tza and Co-His complexes were dinuclear peroxo-species $[Co^{III}L_2]_2(O_2)$. It was also observed that in the electrospray positive ion detection mode, the fragmentation patterns of Co-Tza were similar to those of Co-His because of similarities in the ligand structures and coordination modes. The fragment structures of sodium and potassium adducts were similar for both Co-Tza and Co-His, indirectly confirming the rationality of the proposed fragmentation pattern. Theoretical modeling computations and volumetry were also used to examine the oxygenation of the Co-Tza complex. Results from this study indicate that ESI-MS can be a useful and powerful technique for characterizing oxygenated complexes and for studying oxygenation mechanisms.

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References

1 E. C. Niederhoffer, J. H. Timmons, A. E. Martell, *Chem. Rev.*, 1984, **84**, 137-203.

Analytical Methods Accepted Manuscript

2 C. Comuzzi, A. Melchior, P. Polese, R. Portanova, M. Tolazzi, *Inorg. Chem.*, 2003, **42**, 8214-8222.

3 L. Q. Hatcher, M. A. Vance, A. A. Narducci Sarjeant, E. I. Solomon, K. D. Karlin, *Inorg. Chem.*, 2006, **45**, 3004-3013.

4 J. D. Wang, E. Collange, D. J. Aymes, M. R. Paris, R. Fournaise, *Bull. Soc. Chim. Fr.*, 1994, **131**, 37-44.

5 J. Simplicio, R. G. Wilkins, *J. Am. Chem. Soc.*, 1967, **89**, 6092-6095.

6 R. P. Tao, Unpublished M. S. Thesis, Xinjiang University, 2011.

7 F. Yue, N. Song, Y. Huang, J. D. Wang, Z. F. Xie, H. Q. Lei, X. C. Zhang, P. Fu, R. P. Tao, X. Chen, M. S. Shi, *Inorg. Chim. Acta*, 2013, **398**, 141-146.

8 X. C. Zhang, F. Yue, Y. Huang, P. Fu, X. Cheng, J. D. Wang, *Chem. J. Chinese Universities*, 2012, **33**, 1370-1375.

9 F. R. Xiao, L. Chen, J. D. Wang, *Acta Chim. Sin.*, 2006, **64**, 1517-1522.

10 W. Wang, S. Feng, J. D. Wang, *J. Chromatogr. A*, 2008, **1193**, 142-145.

11 Y. Hayashi, T. Kayatani, H. Sugimoto, M. Suzuki, K. Inomata, A. Uehara, Y. Mizutani, T. Kitagawa, Y. Maeda, *J. Am. Chem. Soc.*, 1995, **117**, 11220-11229.

12 A. E. Martell, R. J. Motekaitis, Wiley-VCH Verlag GmbH, New York, 2nd edn., 1992.

13 J. D. Wang, Y. Wang, Y. Zhang, Q. J. Song, H. Zhang, Y. Lu, X. S. Li, *Chinese J. Appl. Chem.*, 1998, **15**, 97-99.

14 M. Calvin, C. H. Barkelew, *J. Am. Chem. Soc.*, 1946, **68**, 2267-2273.

15 E. W. Hughes, W. K. Wilmarth, M. Calvin, *J. Am. Chem. Soc.*, 1946, **68**, 2273-2278.

16 E. G. Jäger, J. Knaudt, M. Rudolph, M. Rost, *Chem. Ber.*, 1996, **129**, 1041-1047.

17 D. H. Busch, N. W. Alcock., *Chem. Rev.*, 1994, **94**, 585-623.

18 J. Anichina, X. Zhao, D. K. Bohme, *J. Phys. Chem. A*, 2006, **110**, 10763-10769.

19 N. G. Tsierkezos, D. Schröder, H. Schwarz, *Int. J. Mass Spectrom.*, 2004, **235**, 33-42.

20 N. G. Tsierkezos, M. Diefenbach, D. Schröder, H. Schwarz, *Inorg. Chem.*, 2005, **44**, 4969-4978.

21 M. L. Reyzer, J. S. Brodbelt, *J. Am. Soc. Mass Spectrom.*, 2000, **11**, 711-721.

22 A. A. Shvartsburg, *J. Am. Chem. Soc.*, 2002, **124**, 12343-12351.

23 M. Y. Combariza, A. M. Fahey, A. Milshteyn, R. W. Vachet, *Int. J. Mass Spectrom.*, 2005, **244**, 109-124.

24 J. Anichina, D. K. Bohme, *Int. J. Mass Spectrom.*, 2007, **267**, 256-262.

25 I. Szilágyi, I. Labádi, K. Hernadi, I. Pálinkó, N.V. Nagy, L. Korecz, A. Rockenbauer, Z. Kele, T. Kiss, *J. Inorg. Biochem.*, 2005, **99**, 1619-1629.

26 C. G. Hartinger, M. G. Ferri-Mendoza, A. A. Nazarov, B. K. Keppler, *Polyhedron*, 2006, **25**, 1971-1978.

27 M. Volpe, H. Hartnett, J. W. Leeland, K. Wills, M. Ogunshun, B. J. Duncombe, C. Wilson, A. J. Blake, J. McMaster, J. B. Love, *Inorg. Chem.,* 2009, **48**, 5195–5207.

28 J. H. Fu, J. F. Li, P. Fu, F. Yue, X. L. Zhang, J. D. Wang, *Chem. J. Chinese U.,* 2011, **32**, 1483-1487.

29 J. F. Li, J. H. Fu, P. Fu, X. L. Zhang, R. Zhu, J. D. Wang, *Chinese J. Inorg. Chem.*, 2011, **27**, 2225-2230.

30 J. F. Li, J. H. Fu, C. X. Wang, H. Li, J. D. Wang, *Chinese J. Inorg. Chem.*, 2015, **31**, 673-680.

31 T. Gajda, B. Henry, J. J. Delpuech, *Inorg. Chem.*, 1997, **36**, 1850-1959.

32 G. J. Gainsford, W. G. Jackson, A. M. Sargeson, *Aust. J. Chem.*, 1986, **39**, 1331-1336.

Analytical Methods Accepted Manuscript

33 W. R. Harris, R. C. Bess, A. E. Martell, T. H. Ridgway, *J. Am. Chem. Soc.*, 1977, **99**, 2958-2963.

34 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, and J. A. Pople，Gaussian 03, Revision E.01, Gaussian, Inc., Wallingford, CT, 2004.

35 N. Song, Unpublished M. S. Thesis, Xinjiang University, 2010.

36 S. A. Bedell, A. E. Martell. *J. Am. Chem. Soc.* 1985, **107**, 7909-7913.

Analytical Methods Accepted Manuscript

Table 1. Volumetry results (T = 25.0±0.1 °C, C_{Tza} = 0.04 mol L^{-1}, $C_{Co}{}^{2+}$ =0.02 mol L^{-1})

Sample volume (mL)	50.0	60.0	80.0	100.0
Taken oxygen volume (mL)	12.1	14.3	19.8	24.6

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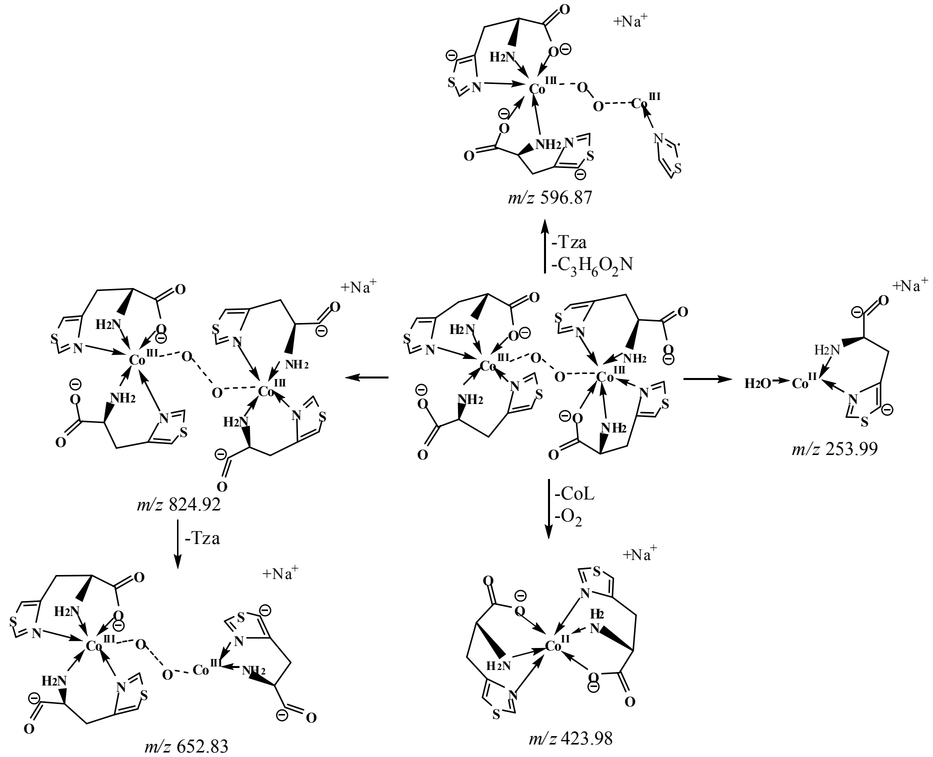

Scheme 1. Proposed fragmentation patterns of oxygenated Co-Tza

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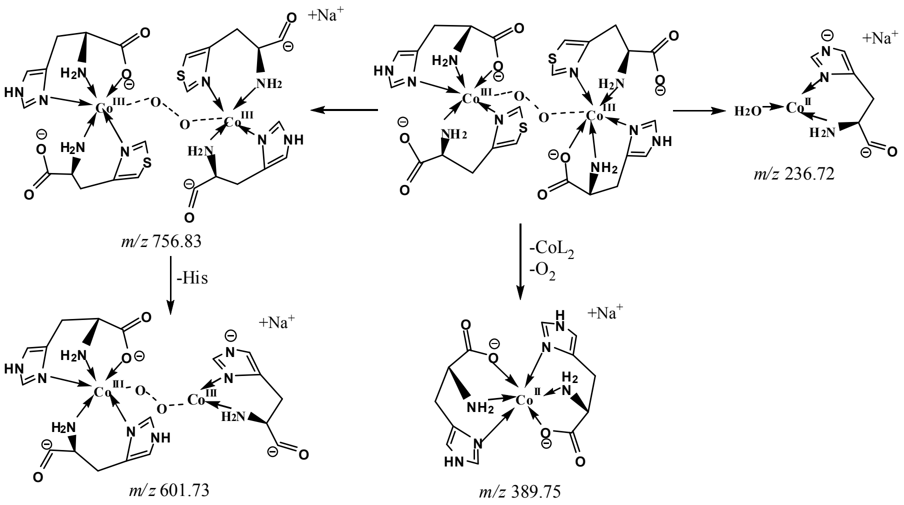

Scheme 2. Proposed fragmentation patterns of oxygenated Co-His

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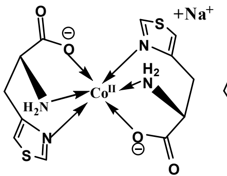
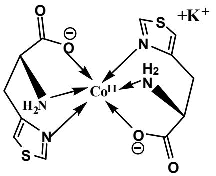
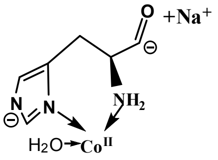
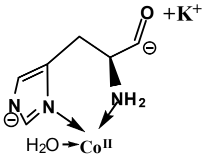
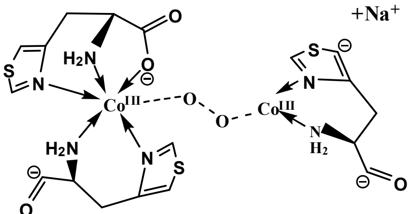
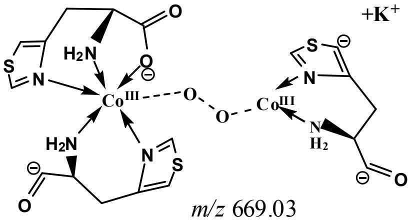
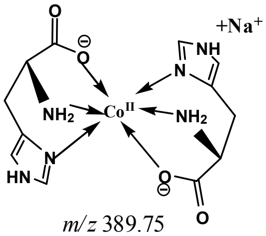
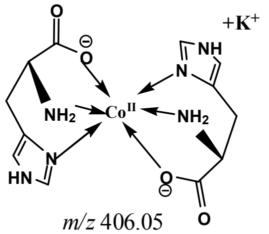
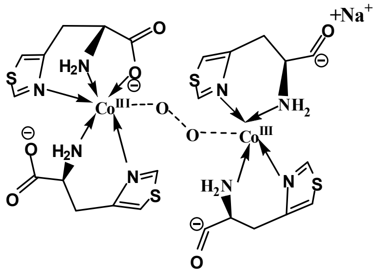
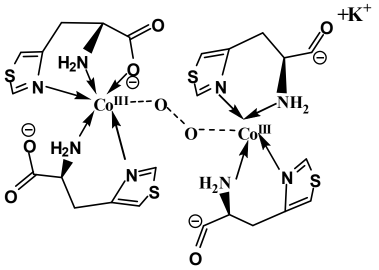
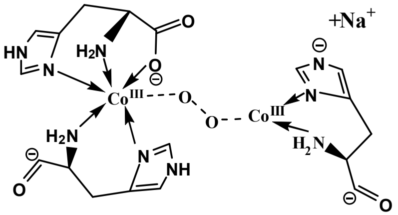
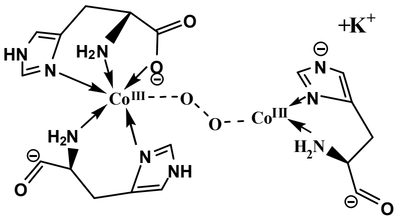
Table 2. Comparisons between the structures of the sodium and potassium adducts of oxygenated Co-Tza and Co-His complexes

Co-Tza	Co-His
 m/z 423.98  m/z 440.00	 m/z 236.72  m/z 252.96
 m/z 652.83  m/z 669.03	 m/z 389.75  m/z 406.05
 m/z 824.92  m/z 841.16	 m/z 601.73  m/z 618.05

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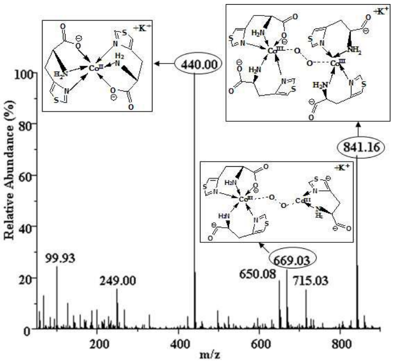

A new ESI-MS method was developed to identify oxygenated species in Co-Tza formed by the uptake of dioxygen. The structures and fragmentation mechanisms of the Co-Tza and Co-His were deduced.

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