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Nitric Oxide Dioxygenation Reaction by Oxy-Coboglobin Models: In-situ Low-Temperature FTIR Characterization of Coordinated Peroxynitrite

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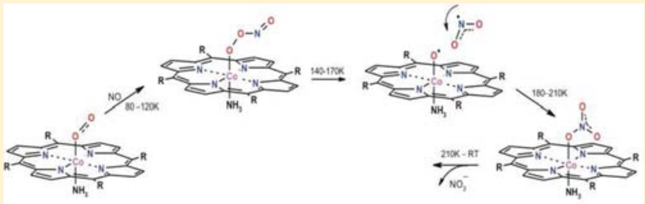
Ⓢ *Supporting Information*



ABSTRACT: The oxy-cobolglobin models of the general formula $(NH_3)Co(Por)(O_2)$ (Por = *meso*-tetra-phenyl and *meso*-tetra-*p*-tolylporphyrinato dianions) were constructed by sequential low temperature interaction of NH_3 and dioxygen with microporous layers of Co−porphyrins. At cryogenic temperatures small increments of NO were introduced into the cryostat and the following reactions were monitored by the FTIR and UV−visible spectroscopy during slow warming. Upon warming the layers from 80 to 120 K a set of new IR bands grows with correlating intensities along with the consumption of the $\nu(O_2)$ band. Isotope labeling experiments with $^{18}O_2$, ^{15}NO and $N^{18}O$ along with DFT calculations provides a basis for assigning them to the six-coordinate peroxynitrite complexes $(NH_3)Co(Por)(OONO)$. Over the course of warming the layers from 140 to 170 K these complexes decompose and there are spectral features suggesting the formation of nitrogen dioxide NO_2. Upon keeping the layers at 180−210 K the bands of NO_2 gradually decrease in intensity and the set of new bands grows in the range of 1480, 1270, and 980 cm^{-1}. These bands have their isotopic counterparts when ^{15}NO, $^{18}O_2$ and $N^{18}O$ are used in the experiments and certainly belong to the 6-coordinate nitrato complexes $(NH_3)Co(Por)(\eta^1{-}ONO_2)$ demonstrating the ability of oxy coboglobin models to promote the nitric oxide dioxygenation (NOD) reaction similar to oxy-hemes. As in the case of Hb, Mb and model iron-porphyrins, the six-coordinate nitrato complexes are not stable at room temperature and dissociate to give nitrate anion and oxidized cationic complex $Co(III)(Por)(NH_3)_{1,2}$.

■ INTRODUCTION

Nitric oxide (NO) has been well characterized as an important regulatory molecule in mammalian biology.[1] The interaction of NO with ferro- and ferri-heme proteins plays central roles in cardiovascular regulation and neurotransmission.[2] While submicromolar NO concentrations are sufficient to perform these functions, overproduction of nitric oxide can result in harmful consequences mainly due to formation of the peroxynitrite anion with further decomposition to aggressive free radicals.[3]

It is commonly accepted that heme proteins are the principal agents responsible for the NO scavenging.[2−4] Under anaerobic conditions, NO detoxification occurs via a 2-electron reduction of two NO molecules to N_2O by NO reductases (NORs). In many bacteria and archaea, this NO-reductase reaction is catalyzed by diiron proteins.[4e,f] Under aerobic conditions, the main part for NO scavenging by red cells involves its direct reaction with HbO_2 leading to formation of nitrate anion and the ferric form of the protein, metHb. It is suggested that this process proceeds through the formation of coordinated peroxynitrite that undergoes homolytic O−O bond cleavage forming ferryl species and free radical nitrogen dioxide NO_2. Subsequent attack of the ferryl O-atom by NO_2 through its nitrogen atom produces O-bound nitrate, decay of which results in metHb and nitrate anion.[4]

Although all researchers are prone to think that the first stage of the reaction is the formation of coordinated peroxynitrite, at this moment there is no strong experimental evidence of its observation. Kinetics studies by Herold et al. using rapid scanning UV−vis spectroscopy at 20 °C followed the reactions of NO with HbO_2 and with oxy-myoglobin (MbO_2) and demonstrated the formation of transients with ms lifetimes.[5] Subsequently, Olson et al.,[6] using EPR to probe rapid-freeze quenched (RFQ) samples from the NO reaction with HbO_2, reported formation of a high-spin Fe(III) species on this same time scale. These intermediates were assigned as high-spin ferri-heme peroxynitrite complexes on the basis of their electronic spectra;[5] however, Goldstein et al.[7] reported an intermediate with analogous spectral properties from the reaction of ferryl myoglobin with NO_2 and concluded that it was the nitrato analog $Fe(III)(ONO_2)$. Notably, resonance Raman spectral

Received: June 19, 2012
Published: August 10, 2012

dx.doi.org/10.1021/ja305774v | *J. Am. Chem. Soc.* 2012, 134, 13861−13870

(RRS) studies of the ms intermediate formed by reaction of NO with MbO_2 in 3 °C alkaline solution and trapped by RFQ identified this as the nitrato, not the peroxynitrito, complex.[8]

A spectroscopic study was recently carried out in our laboratory to probe the viability of a $Fe^{III}(OONO)$ intermediate in the reaction of the six-coordinate oxy-heme model complex $(NH_3)Fe(Por)(O_2)$ with NO in low-temperature sublimed solids.[9] Surprisingly at temperatures as low as 80−100 K the six-coordinate nitrato complexes $(NH_3)Fe(Por)(\eta^1\text{-}ONO_2)$ were formed without spectral detection of any intermediates. In particular, peroxynitrite complexes were not observed, and this result implied that, if formed, isomerization of this putative intermediate to the nitrato product was quite facile even under these conditions.

In this work we returned to this problem by using Co-porphyrins as the model compounds. In studies of hemoproteins, the technique of replacement of the neutral iron porphyrin prosthetic group with different metalloporphyrins has been very useful,[10] especially with Co-porphyrins that possess dioxygen binding ability, but contrary to EPR silent Fe(II)-porphyrins (d^6) these can be studied by EPR due to single odd-electron configuration (d^7). The EPR studies of Co hemoproteins provided many important insights for understanding the electronic and geometric structures, which were not possible for the diamagnetic deoxy and oxy iron hemoproteins. With regard to the observation of peroxynitrite complexes, the results obtained for the Co-containing systems were more promising.[11] Koppenol and co-workers reported[12] the synthesis of the tris(tetraethyl-ammonium)-pentacyanoperoxy nitritocobaltate(III) complex by the reaction of NO with pentacyanodioxygencobaltate(II). They explained its stability with the presence of cyano groups diminishing the Lewis acid character of the metal. Theopold and co-workers attempted to isolate a peroxynitrito complex from the reaction of the dioxygen complex of tris(pyrazolyl)borate-cobalt(II) with NO in tetrahydrofuran or toluene solution.[13] At temperatures below −61 °C an intermediate was observed, which may have been the desired peroxynitrito complex. Eventually the nitrito and nitrato complexes were obtained in this study.

Although Co-substituted hemoproteins and Co-porphyrin models reversibly bind O_2, to the best of our knowledge there are not studies devoted to their potential ability to promote nitric oxide dioxygenation (NOD) reaction. In this paper we addressed this problem by application of the sublimed layers methodology[14] that is based on the preparation of the porous layered solids by the vacuum sublimation of metal *meso*-tetraarylporphyrinato complexes onto a substrate cooled by liquid nitrogen (LN_2). The facile reactions of these heme models with volatile reactants can be studied in detail using FTIR and optical spectroscopy. The power of this technique draws from the ability to investigate such processes under carefully controlled and tunable conditions, especially temperature.

In this study, the reaction of nitric oxide (NO) with specially constructed porous layered solids of the oxy-coboglobin models $(NH_3)Co(Por)(O_2)$ was monitored by FT-IR and UV−visible spectroscopy from 80 K to room temperature. It was found for the first time that the NOD reaction proceeds also with coboglobin models. For this system, fortunately, the formation of the six-coordinate nitrato complexes $(L)Co(Por)(\eta^1\text{-}ONO_2)$ proceeds at much higher temperatures (beginning at 180 K), and at lower temperatures it was possible to detect the intermediates that were characterized by FT-IR spectroscopy with the use of experiments with isotopically labeled $^{18}O_2$, ^{15}NO and $N^{18}O$ species. These data are supported by the predictions of DFT calculations that give support to the belief that we were able to register the FTIR spectra of coordinated peroxynitrite for the first time.

It should be mentioned here that as valuable as model compounds Co-porphyrins are, they cannot be considered equal with heme systems. For example, Co promotes/stabilizes superoxo character of corresponding dioxygen adducts to a much higher extent than Fe, Co(III) has a higher preference toward six-coordination than Fe(III), Co(III) is more kinetically inert than Fe(III), etc.[10b] All this can lead to different mechanisms and characteristics of intermediate species along reaction pathways. However, despite the observed intermediates being more related to the chemistry of cobalamins and their interactions with nitrogen oxides species, the spectral information obtained here will be very useful for analogous studies performed with iron−porphyrins and related systems.

■ EXPERIMENTAL SECTION

Co(TPP) and Co(TTP) were synthesized using the literature methods.[15] Before the experiment they were additionally purified by column chromatography with dry alumina using reagent grade chloroform as the eluent. NO (^{15}NO, $N^{18}O$) was purified by passing it through KOH pellets and a cold trap (dry ice/acetone) to remove the higher nitrogen oxides and trace quantities of water. The purity was checked by IR measurements of the layer obtained by slow deposition of NO onto the cold substrate of the optical cryostat (77 K). The IR spectrum did not show the presence of N_2O, N_2O_3 or H_2O. ^{15}NO with 98.5% enrichment was purchased from the Institute of Isotopes, Republic of Georgia, and was purified by the same procedures. $N^{18}O$ was obtained by the reduction of $N^{18}O_2$ over metallic Hg in an evacuated flask. $N^{18}O_2$ was synthesized according to the following procedure. The 1:2 mixture of N_2 and $^{18}O_2$ gases (Cambridge isotopic laboratories) was introduced into an evacuated glass balloon provided with inserted electrodes. Upon continuous electric discharge, the colorless gas mixture turned to reddish-brown, indicating the formation of $N^{18}O_2$.

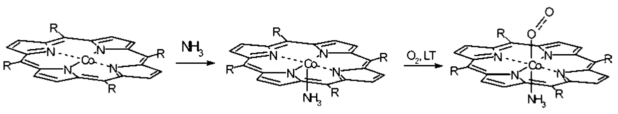
Sublimed layers of Co(TPP) (Co(TTP)) were obtained on the cold (77 K) KBr support of an optical cryostat according to the published procedure.[14] Then about 0.3 Torr equivalent NH_3 was added at LN_2 and the layer was annealed at 120−130 K temperatures, at which the formation of $Co(Por)(NH_3)$ was completed. A few tenths of a torr of O_2 was then introduced into the cryostat at LN_2 and annealed to assist the formation of desired mixed dioxygen complexes $(NH_3)Co(Por)(O_2)$. Both of these processes can be controlled while monitoring by FTIR spectroscopy according to the shifts of some of the porphyrin bands. After obtaining the desired six-coordinate dioxygen complexes, the cryostat was pumped out at maximally high temperatures until the mixed complex was intact, then the layer was cooled by LN_2 and small increments (∼0.1 Torr equivalent) of NO were stepwise introduced into the cryostat. The layer was then slowly warmed to room temperature and the FTIR or UV−visible spectra were obtained at various controlled temperatures measured by a thermocouple. The UV−visible spectra were taken after the FTIR measurements of the same samples confirmed the identity of each species. The FTIR and UV−visible spectra were acquired on a Nexus (Thermo Nicolet Corporation) in the spectral range 400−4000 cm^{-1} with a resolution 2 cm^{-1} and Specord M-40 (Carl Zeiss, Jena) or Helios γ (Thermo Nicolet Corporation) spectrometers in the range of 350−900 nm, respectively.

Computational analysis was carried out with Spartan '06 software running under a Windows operating system. Equilibrium geometries for the low-spin, ground state derivatives of $(NH_3)Co(P)(OONO)$ (P = porphinato dianion) were first determined by DFT (BP/6-31G*) methods and IR frequencies calculated from the resulting structures. In the case of the cis forms of $(NH_3)Co(P)(OONO)$ and $(NH_3)Co(TPP)(OONO)$, the OONO dihedral angle was locked at 0.0° in the determination of the equilibrium geometry and IR spectrum. The relative energies of the *cis* and *trans* isomers were determined from the Spartan '06 thermodynamics program based upon geometries determined by DFT methods and IR frequencies determined by DFT methods for the $(NH_3)Co(P)(OONO)$ models. Likewise, DFT methods were used with Spartan '10 for determining equilibrium geometries, IR frequencies and thermodynamics parameters for the *cis* and *trans* (OONO) isomers of $(NH_3)Co(TPP)(OONO)$.

RESULTS AND DISCUSSION

Construction of Six-coordinate Oxy-coboglobin Models. To construct the six-coordinate dioxygen complexes of porphyrins with *trans* ammonia ligand $(NH_3)Co(Por)(O_2)$ the strategy demonstrated in Scheme 1 was applied. In contrast to iron–porphyrins, the first axial ligand binding constant K_1 in Co–porphyrins is much greater than that of the second ligand K_2.[16] This allows for obtaining the five-coordinate complexes by the reaction of small quantities of NH_3 with amorphous layers of Co-porphyrins.

Scheme 1



R-phenyl, p-tolyl

As monitored by the vacuum gauge, low pressures of NH_3 (~0.3 Torr equivalent) were supplied into the cryostat at low temperatures and the layers were allowed to be warmed until certain temperatures, at which the formation of five-coordinate complexes was completed. FTIR spectra served as a reliable tool for monitoring this process, since some porphyrin bands underwent noticeable shifts upon the $Co(Por) + NH_3 \rightarrow Co(Por)(NH_3)$ transition.

The spectral changes observed upon the formation of $Co(Por)(NH_3)$ complexes are reproduced in the Figure S1a,b (Supporting Information). Intense porphyrin bands of Co(TPP) in the vicinity of 1000 cm^{-1} shift to lower frequencies and change their relative intensities. The same occurs with the single porphyrin band of Co(TTP) in this spectral range. Notably, when six-coordinated dioxygen complexes $(NH_3)Co(Por)(O_2)$ were formed upon exposition of this species under O_2 at low temperatures, these bands shifted back to their initial positions. It is reasonable to suggest that these spectral changes upon NH_3 binding are connected with the out-of-plane motion of the Co center in the five-coordinate complex, followed by a shift back of the Co center into the plane of the porphyrin when O_2 binds. Such out-of-plane motion perturbs the overlapping between metal d_π- and porphyrin e_g*-orbitals resulting in changes of energy of some porphyrin vibrations.

After formation of the five-coordinate ammonia complexes, $Co(Por)(NH_3)$, a few tenths Torr of O_2 ($^{18}O_2$) was supplied into the cryostat at LN_2 and the layer was allowed to warm under O_2 atmosphere to a certain temperature at which the intensity of $\nu(O_2)$ band, which characterizes the dioxygen complex $(NH_3)Co(Por)(O_2)$, reached maximum intensity. The layer was then cooled to LN_2 and the O_2 excess was pumped out. Spectrally controlled pumping was continued upon layer warming until the temperature (~120 K), at which the coordinated O_2 was still intact in vacuo. This procedure was applied to eliminate the traces of O_2 adsorbed in the layer.

Figure 1 demonstrates the spectral changes upon the reaction of O_2($^{18}O_2$) with $Co(TTP)(NH_3)$. The intense $\nu(O_2)$ band of coordinated dioxygen at 1141 cm^{-1} appeared in spectra and shifts to 1073 cm^{-1} upon use of $^{18}O_2$ in perfect agreement with that expected for a perturbed diatomic molecule.

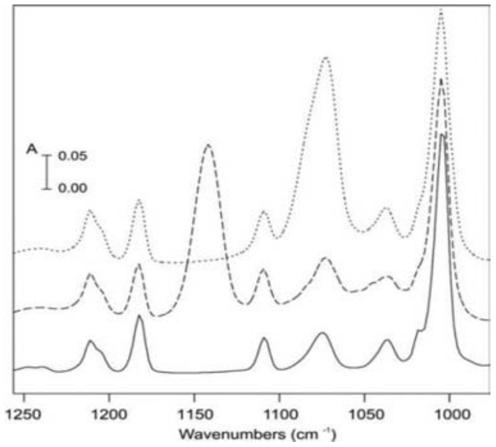


Figure 1. FTIR spectra in the range of $\nu(O_2)$ stretching for Co(TTP) (solid line), $(NH_3)Co(TTP)(O_2)$ (dashed line) and $(NH_3)Co(TTP)(^{18}O_2)$ (dotted line) at 80 K.

The same bands for $(NH_3)Co(TPP)(O_2)$ appear at 1139 and 1072 cm^{-1} (Supporting Information, Figure S2). This result indicates the formation of six-coordinate dioxygen complexes, since the $\nu(O_2)$ in the five-coordinate complex with Co(TPP) appears at 1278 cm^{-1} in an Ar matrix[17] and 1251 cm^{-1} in sublimed layers.[18] The absence of the O_2 band in the 1250 cm^{-1} range also indicates that there are no unligated Co-porphyrin molecules in the layer. The disposition of $\nu(O_2)$ in the six-coordinate mixed complexes are indicative for an end-on bound superoxo ligand.[19a] Upon formation of the 5-coordinate ammonia complexes $Co(Por)(NH_3)$ both Soret and Q bands in the UV–visible spectra undergo red shifts and new weak band emerges as a shoulder at about 590 nm (Supporting Information, Figure S3, dashed). Further bathochromic shifts are observed upon formation of the six-coordinate dioxygen complexes with well separated two bands in the Q-band region (Figure S3, dotted) that manifest the formation of six-coordinate dioxygen complexes with the trans electron donor ligand.[19b]

Reaction of NO with Six-coordinate Dioxygen Complexes. FTIR Characterization of an Intermediate. After constructing the six-coordinate dioxygen complexes, $(NH_3)Co(Por)(O_2)$, their low-temperature interaction with NO was spectrally monitored upon slow warming of the layers. The introduction of NO into the cryostat was carried out stepwise by small increments (~0.1 Torr) of preliminary cooled NO, and new portions were introduced only after spectral changes indicated the consumption of the previous one.

It is necessary to note that the FTIR spectrum of Co(TTP) is more convenient for the spectral study performed in this investigation. Particularly, the band connected with the ring stretching vibration of tolyl groups in the vicinity of 1600 cm^{-1} has a very low intensity and the interval between 700 and 800 cm^{-1} is relatively free of the bands compared to TPP-derivative.

As will be shown below, both of these ranges give important information about intermediates formed in the course of the NO reaction with the six-coordinate dioxygen complexes. For this reason, the spectral data are listed for Co(TTP) derivative and those for Co(TPP) are represented in the Supporting Information (Table S1).

The spectral changes observed upon the reaction of NO with layered $(NH_3)Co(TTP)(O_2)$ are demonstrated in Figure 2.

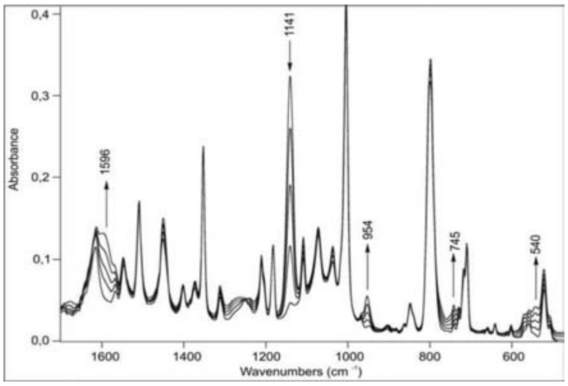


Figure 2. FTIR spectral changes accompanying the reaction of small NO increments with the layered $(NH_3)Co(TTP)(O_2)$ upon its warming from 80 to 120 K.

The band of coordinated dioxygen at 1141 cm^{-1} decreases in intensity and a set of new bands grow with correlating intensities in the vicinity of 1600, 950, 740, and 540 cm^{-1} that belong to an intermediate that is fairly stable until 140 K, but after further warming begins to decompose.

The aforementioned bands had their analogs when $^{18}O_2$, ^{15}NO and $N^{18}O$ isotopomers were used in the experiments. These data are summarized in Table 1 together with calculated values of stretching frequencies assuming formation of the O-coordinated peroxynitrite as a most plausible scenario for the interaction of NO with $(NH_3)Co(Por)(O_2)$ at very low temperatures. In calculations that were performed for all isotopic species, the relative values were given more credence than the absolute frequencies. The new bands in aforementioned spectral ranges were assigned as follows.

The high frequency band in the vicinity of 1600 cm^{-1} is sensitive to both isotopomers of nitrogen oxide (^{15}NO, $N^{18}O$) and therefore should be assigned to the moiety containing both these atoms. The position of this band lies in the range characteristic for the double bond and the values of isotopical shifts are close to the values predicted by DFT calculations for $\nu(N{=}O)$ of coordinated peroxynitrite within a few cm^{-1} (see Table 1). In the peroxynitrite anion obtained by photolysis of KNO_3 in an Ar matrix, this mode is observed at 1444 cm^{-1} for the *cis* isomer and at 1528 cm^{-1} for *trans* K(OONO).[20] In the IR spectrum of peroxynitrite anion as tetramethylammonium salt this vibration is disposed presumably at 1496 cm^{-1} (nujol mull).[21] The uncertainty here is connected with the obscuring by the bands of the tetramethylammonium cation. Noteworthy, in the peroxynitrous acid, HOONO, this band is disposed at 1703 cm^{-1},[22] so the covalent bonding of peroxynitrite's terminal oxygen increases the $\nu(N{=}O)$ band by about 200 cm^{-1} as a result of the greater energy separation in the two modes associated with the nitrogen−oxygen stretches. In our system, this band occupies an intermediate position between those of peroxynitrite anion and peroxynitrous acid, the behavior that should actually be anticipated upon coordination with transition metal, in which the bonding exhibits partially ionic character. It is noteworthy that the value of 1621 cm^{-1} was reported by Koppenol and coauthors for the frequency of this mode in the sodium pentacyanoperoxynitritocobaltate(III) $Na_3[Co(CN)_5(OONO)]$.[12]

The band in the 950 cm^{-1} region is sensitive to the oxygen labeling in the dioxygen moiety (Table 1) and upon ^{18}O substitution it undergoes more than 30 cm^{-1} low-frequency shift. Upon ^{15}N substitution this band was shifted by only 8 cm^{-1}, indicating relatively little involvement of the N atom in this mode (Figure 3). Disposition of this band is typical for peroxo compounds and the magnitudes of the isotopic shifts indicate that this band involves O−O stretching to a great extent. Analogous vibrational modes were observed at 952, 932, and 915 cm^{-1} in the K(OONO),[20] $N(CH_3)_4(OONO)$[21] and $Na_3[Co(CN)_5(OONO)]$,[12] respectively. For peroxynitrous acid in an Ar matrix this vibration was found at 952 and 957 cm^{-1} in different matrix sites and reveals 4−5 cm^{-1} downshifts in the ^{15}N-containing species.[22a] The 5 cm^{-1} downshift of this band was also observed for $N(CH_3)_4(OO^{15}NO)$[21] relative to the natural abundance compound. The same pattern is observed in our case. The analysis above provides reason

Table 1. Infrared Frequencies and Their Assignment for the Co-OONO Group of the Intermediate Proposed to be $(NH_3)Co(TTP)(OONO)$[a]

	ν (N=O), $[\Delta\nu] = \nu - \nu_{isotope}$		ν_s(O−O−N), δ(O−N=O), $[\Delta\nu] = \nu - \nu_{isotope}$		ν_a(O−O−N), δ(O−N=O), $[\Delta\nu] = \nu - \nu_{isotope}$		ν (Co−O), $[\Delta\nu] = \nu - \nu_{isotope}$	
adduct	exp.	comp.	exp.	comp.	exp.	comp.	exp.	comp.
$(NH_3)Co(TTP)(OONO)$	1596	1678	954	967	740	711	544	560
$(NH_3)Co(TTP)(^{18}O^{18}ONO)$	1594 [2]	1677 [1]	924 [30]	930 [37]	∼710 [30]	686 [25]	522 [22]	536 [24]
$(NH_3)Co(TTP)(OO^{15}NO)$	1564 [32]	1647 [31]	946 [8]	960 [7]	737 [3]	708 [3]	542 [2]	559 [1]
$(NH_3)Co(TTP)(^{18}O^{18}O^{15}NO)$	1564 [32]	1646 [32]	914 [40]	922 [45]	∼710 [30]	683 [27]	523 [21]	536 [24]
$(NH_3)Co(TTP)(^{18}O^{18}ON^{18}O)$	1555 [41]	1635 [42]	916 [38]	925 [42]	∼707 [33]	681 [30]	522 [22]	536 [24]
cis-$(NH_3)Co(P)(OONO)$		1702		910		796		575
trans-$(NH_3)Co(TPP)(OONO)$		1665		967		704		565
cis-$(NH_3)Co(TPP)(OONO)$		1673				798		573

[a] Calculations are performed for the $(NH_3)Co(P)(OONO)$, (P = porphynato dianion) that has an equilibrium geometry with the OONO ligand in the *trans* orientation (dihedral angle = 174.44°), except where noted for this *cis* OONO isomer in which the dihedral angle was fixed at 0.0°. Calculations were also performed for $(NH_3)Co(TPP)(OONO)$ for both the *cis* and *trans* isomers. For the *cis* isomer the dihedral angle was fixed at 0.0°, and for the *trans* isomer it was calculated as 175.48°.

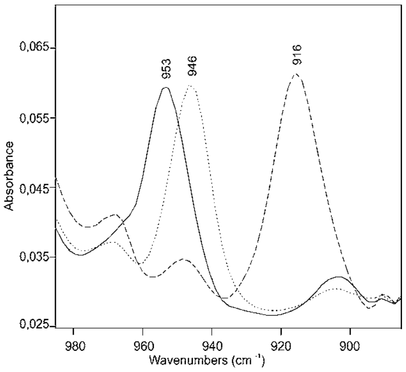


Figure 3. FTIR spectra in the range of predominantly ν(O−O) vibrations of intermediate proposed to be $(NH_3)Co(TTP)(OONO)$ species for the experiments with O_2/NO (solid), $O_2/^{15}NO$ (dotted), $^{18}O_2/N^{18}O$ (dashed).

enough to assign the 954 cm^{-1} band to preferentially O−O stretching of O−O−N═O group.

DFT computations were used to determine frequencies of the O−O−N═O ligand with isotopic substitution. The equilibrium geometries of the ligand were first determined for the porphine model system as $(NH_3)Co(P)(OONO)$. In this case, the *cis* isomer was 12 kJ/mol more stable than the *trans* isomer at 77 K. With the actual $(NH_3)Co(TPP)(OONO)$, steric factors played an important role in the ligand geometry since the *cis* isomer was found to be 56 kJ/mol less stable than the *trans* isomer. Further computations to determine the relative isotopic shifts were carried out using the simpler porphine model system. These computational studies show that O−O stretching band just mentioned could better be described as a symmetrical O−O−N stretch mixed with the O−N═O bending mode.

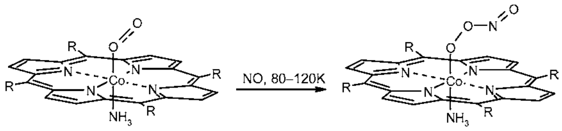
In the spectra of Co(TTP) derivatives, a band appears at about 740 cm^{-1}, the growth of which correlates with intensities of the aforementioned bands and is similar to the band at about 950 cm^{-1} revealing weak and strong sensitivity to ^{15}NO- and $^{18}O_2$-labeling isotopomers, respectively. For the Co(TPP) complexes, this band is masked by the intense TPP bands and can be registered only in difference spectra. DFT calculations predict that in this spectral range the asymmetric O−O−N stretch mixed with O−N═O bending mode should be disposed. Experimental observations are in accordance with this prediction.

The next band in the set of bands that grow with correlating intensities upon consumption of dioxygen in the six-coordinate complexes $(NH_3)Co(Por)(O_2)$ is disposed in the low-frequency range in the vicinity of 540 cm^{-1}. It displays noticeable sensitivity to $^{18}O_2$ substitution and is almost insensitive to ^{15}N labeling (Table 1). Computations predict the existence of the Co−O stretching mode in this spectral region. For characterization of the metal−oxygen bond in the oxy-globins and related model compounds the IR spectroscopy is rarely used most likely because of the low intensity of the corresponding bands. In this study we also were not able to detect in the FTIR spectra the ν ($Co-O_2$) band in $(NH_3)Co(Por)(O_2)$ complexes. In contrast the Resonance Raman spectroscopy gives valuable information about vibrations of metal−oxygen moiety.[23] It reveals the band of $\nu(Co-O_2)$ at 527 cm^{-1} for the $Co(II)(TpivPP)(1,2\text{-}Me_2Im)(O_2)$, 516 cm^{-1} for the $Co(II)(TpivPP)(N\text{-}MeIm)(O_2)$ and 540 cm^{-1} for oxy meso CoMb. The observation of an isotopically sensitive band at frequencies close to those mentioned above together with DFT predictions gives the basis to suggest that addition of NO to coordinated O_2 leading to the formation of single O−O peroxo bond intensifies the Co−O vibration allowing its observation in the FTIR spectra.

In the spectral range of this study (400−4000 cm^{-1}), we did not observe any isotopically sensitive bands that could be assigned to the uncoupled ν(N−O) of coordinated peroxynitrite. According to DFT calculations, this vibration should lay at 436 cm^{-1}. Taking that into account and also the fact that most of the calculated frequencies were increased compared with the experimentally observed ones, it is likely that it may be disposed near the spectral limit of our measurement. This low value indicates that the bond between N═O and O−O groups is rather weak. Such weak bonding was also predicted for peroxynitrous acid, for which, according to the *ab initio* calculation at the RMP2/6-31G* level, the ν(N−O) stretching is disposed at 439 cm^{-1},[24] which was out of the spectral range of measurements performed by Lee and coauthors.[22]

Dioxygen binding with Co-porphyrins is weak and O_2 releasing could not be completely excluded. This may lead to NO autoxidation that in turn should result in formation of different N_xO_y species. Therefore the thorough literature analysis of the IR spectra of such compounds has been performed. Due to low temperature conditions of this experiment special attention was attracted to the thermally unstable adducts that were mainly studied by matrix isolation spectroscopy. This analysis shows that the set of bands observed in this work for the intermediate does not match to the spectra of any N_xO_y species, IR spectra of which are described in literature.[24,25] We believe, therefore, that the intermediate formed at the initial stage of the reaction between NO and $(NH_3)Co(Por)(O_2)$ is the six-coordinate peroxynitrite complex $(NH_3)Co(Por)(OONO)$ as is demonstrated on the Scheme 2.

Scheme 2



Its equilibrium geometry determined by DFT (BP/6-31G*) is shown in Figure S7 (Supporting Information) and has a *trans* OONO dihedral angle of 174.44°. It is necessary to stress that formation of a peroxynitritometal complex via the reaction of a dioxygenmetal ion with nitrogen monoxide is in most cases inferred and not directly observed.[11]

For completeness, it should be added here that according to the indirect experimental evidence and computational work the peroxynitrite can be not only O-bound,but also be N-coordinated[11b−h] or an O,O′ chelating[11i,j] ligand. For ruling out these possibilities, the following experimental and computational works were carried out. By sequential reactions of NO and NH_3 vapors on the sublimed layers of Co-porphyrins, the thermally unstable six-coordinate complexes $(NH_3)Co(Por)(NO)$ were obtained.[19c] Introduction into the cryostat a few tenths of a torr of O_2 at 80 K and slow warming of the substrate to 120 K did not lead to the appearance of new bands in the FTIR spectra manifesting that no intermediate is formed. Hence the hypothesis of an N-coordinated peroxynitrite should be ruled out, at least in our experimental conditions. On the other hand, the DFT calculations for the chelating peroxynitrite gives a set of IR bands completely different from those observed in our study. Particularly, for such a mode of binding, the nitrosyl ν(NO) is predicted to be disposed at about 1300 cm^{-1} which is incompatible with the experimental observations.

Decomposition of the Peroxynitrite Intermediate. After the bands of intermediate reach their maxima and all coordinated dioxygen in $(NH_3)Co(Por)(O_2)$ is spent the cryostat was pumped out at temperatures (about 150 K), at which the intermediate still intact. This allows pumping out the unreacted NO and some part of the NH_3 excess. Upon further warming of the layer to 170 K the bands at 953, 740, and 540 cm^{-1} disappear. During this process the band in the vicinity of 1600 cm^{-1} undergoes a small high frequency shift but only slightly decreases in intensity. Simultaneously, a weak band grows at about 775 cm^{-1} as a low-frequency shoulder of the intense porphyrin band at about 800 cm^{-1} and is seen more clearly in the ^{15}N-labeled experiments (Figure 4a,b).

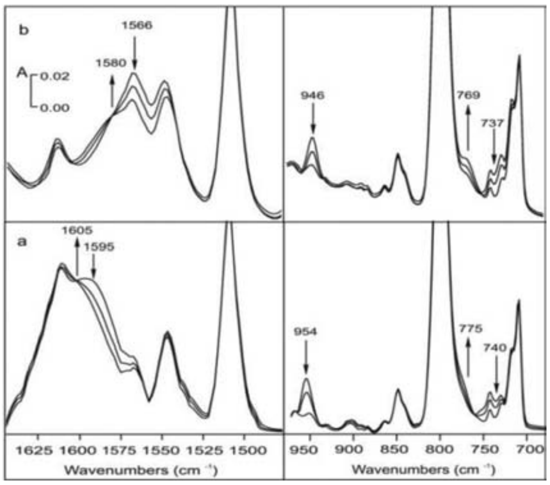
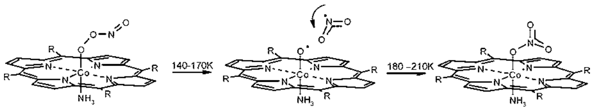


Figure 4. FTIR spectra demonstrating decomposition of peroxynitrite intermediate upon warming the layers of (a) $(NH_3)Co(TTP)(OONO)$ and (b) $(NH_3)Co(TTP)(OO^{15}NO)$ from 150 to 170 K.

These bands with the isotopic counterparts in the experiments with ^{15}N- and ^{18}O-labeled compounds most likely belong to the $\nu_a(NO_2)$ and δ(ONO) of nitrogen dioxide NO_2 monomer. The frequency of the first band is somewhat lower, and the frequency of the second one is somewhat higher than reported for the NO_2 monomer in the matrices.[25a] We think that these deviations are connected with the H-bonding of nitrogen dioxide with coordinated or free NH_3 that is still present in the layer. As a general rule, hydrogen bonding lowers the frequency of stretching vibrations, since it lowers the restoring force, but increases the frequency of bending vibrations since it produces additional restoring force.[26] It should be added that the frequency of the nitrogen dioxide monomer reveal considerable dependence on the environment. The $\nu_a(NO_2)$, for example, appears at 1611 cm^{-1} in Ar and at 1624 cm^{-1} in a carbon dioxide matrix.

The results obtained can be interpreted in terms of homolytic disruption of O—O bond, that should lead to the formation of NO_2 and Co oxo species $(NH_3)(Por)Co{=}O \leftrightarrow (NH_3)(Por)Co{-}O^{\bullet}$ as is shown on the Scheme 3. According to DFT calculations the ν(Co═O) vibration should appear at 691 cm^{-1} and at 660 cm^{-1} for ^{18}O-labeled analog. However, we did not find an isotopically sensitive band in this spectral range. It probably has a very low intensity and is obscured by the bands of porphyrin itself. What can be reliably confirmed for this stage of reaction is the breaking of the O—O peroxide bond that is weak and readily undergoes homolysis for cobalt alkylperoxides.[27]

Scheme 3



It is worth noting that the decomposition of the intermediate assigned to coordinated peroxynitrite does not lead to immediate formation of six-coordinate nitrato complexes (see below) but proceeds through the formation of the second intermediate with disrupted peroxide bond, that supports the idea about the formation of the caged $(NH_3)(Por)Co{-}O^{\bullet}/^{\bullet}NO_2$ pair.

Formation of the Six-coordinate Nitrato Complexes $(NH_3)Co(Por)(\eta^1\text{-}ONO_2)$. Further slow warming of the layer from 180 to 210 K leads to the gradual disappearance of the species characterized by the bands at about 1600 and 778 cm^{-1} (1565 and 769 cm^{-1} in the experiments with ^{15}NO), with concomitant growth of three new bands in the vicinity of 1485, 1270, and 985 cm^{-1} (Figure 5a,b). These bands have their isotopic counterparts at about 1450, 1245, and 970 cm^{-1} when ^{15}NO was used in the experiments. The frequencies of the new species formed in the 180—210 K temperature interval in the experiments with the natural abundance O_2 and NO and different ^{18}O- and ^{15}N-containing isotopomers with their various combinations are summarized in Table 2. The spectra of three such systems are demonstrated in Figure 6.

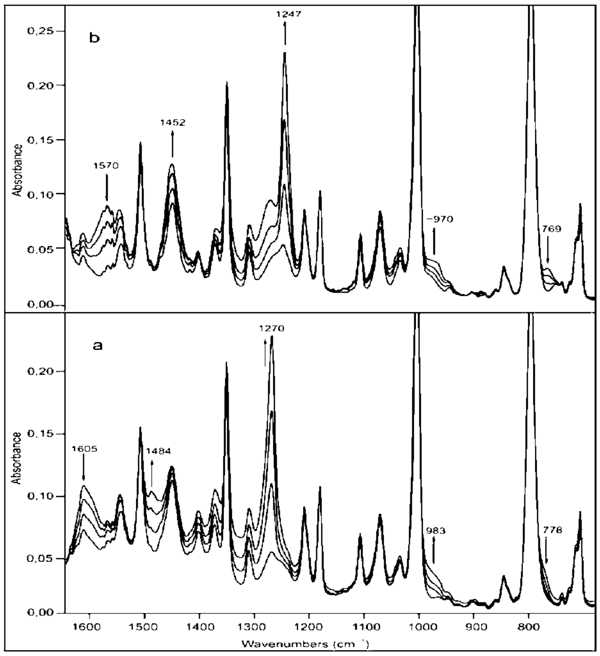


Figure 5. FTIR spectral changes upon warming of (a) $(NH_3)Co(TTP)(O_2)$+NO and (b) $(NH_3)Co(TTP)(O_2)+^{15}NO$ systems from 180 to 210 K.

The positions of these bands, their relative intensities and isotopic shifts are close to those observed in the six-coordinate

Table 2. Infrared Frequencies (cm^{-1}) of Nitrato Group in the Six-coordinate Nitrato Complexes of Co- and Fe-TPP (Data in Parentheses Are Given for TTP Derivatives)

compounds	$\nu_a(NO_2)$ (m)	$\nu_s(NO_2)$ (s)	ν(O−N) (w)	ref.
$(NH_3)Co(TPP)$ (η^1-ONO_2)	1484 (1485)	1270 (1269)	~983 (983)	this work
$(NH_3)Co(TPP)$ (η^1-$O^{15}NO_2$)	1459 (1460)	1248 (1247)	~970 (970)	this work
$(NH_3)Co(TPP)$ (η^1-$^{18}ON^{18}O_2$)	1456 (1460)	1244 (1243)	942 (935)	this work
$(NH_3)Co(TPP)$ (η^1-$^{18}ON(^{18}O)O$)	1465 (1470)	1252 (1254)	953 (955)	this work
$(NH_3)Co(TPP)$ (η^1-$^{18}O^{15}N(^{18}O)O$)	1437 (1438)	1228 (1229)	946 (946)	this work
$(NH_3)Fe(TPP)$ (η^1-ONO_2)	1499 (1496)	1268 (1268)	938 (936)	9
$(NH_3)Fe(TPP)$ (η^1- $^{18}ON(^{18}O)O$)	1481 (1482)	1252 (1253)	913 (914)	9
$(NH_3)Fe(TPP)$ (η^1-$O^{15}NO_2$)	1472 (1469)	1249 (1249)	925 (925)	9
$(NO)Fe(TPP)$ (η^1-ONO_2)	1505	1265	969	28a
$(NO)Fe(TPP)$ (η^1-$O^{15}NO_2$)	1472	1246	954	28a
$(THF)Fe(TPP)$ (η^1-ONO_2)	1491 (1486)	1280 (1277)	~997 (~995)	28b
$(THF)Fe(TPP)$ (η^1-$O^{15}NO_2$)	1457 (1454)	1258 (1258)	986 (984)	28b

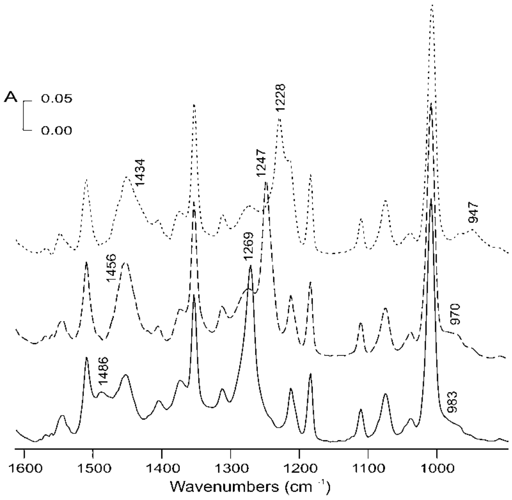


Figure 6. FTIR spectra of 3 different $(NH_3)Co(TTP)(O_2)$ + NO systems after warming to 210 K and high vacuum pumping: $(NH_3)Co(TTP)(O_2)$ + NO (solid line), $(NH_3)Co(TTP)(O_2)$ + ^{15}NO (dashed line), $(NH_3)Co(TTP)(^{18}O2)$ + ^{15}NO (dotted line).

nitrato complexes of iron-porphyrins with trans electron-donor ligands[9,28] and can be unambiguously assigned to the six-coordinate nitrato complexes $(NH_3)Co(Por)(\eta^1{-}ONO_2)$ (see discussion below). In these compounds the nitrate group is coordinated through the one of oxygen atoms in monodentate fashion. The three IR-active stretching modes expected for this structure would be a low-frequency ν(O−N) band for the coordinated oxygen and two(symmetric and asymmetric) modes for the uncoordinated NO_2 fragment. Therefore, the bands in the vicinity of 1485, 1270, and 985 cm^{-1} should be assigned to the vibrations that predominantly belong to $\nu_a(NO_2)$, $\nu_s(NO_2)$ and ν(O−N) stretching of coordinated nitrato group.

The stable nitrato complexes of metalloporphyrins characterized until now are the complexes where the nitrate is a sole axial ligand.[29] In mononuclear complexes, the nitrate ligand can be bound with metal by one or two oxygen atoms forming mono- or bidentate complexes. In metalloporphyrins the mode of coordination depends both on the nature of metal and porphyrin substituents. For example, in $Fe(TPP)(\eta^2\text{-}O_2NO)$ the symmetric bidentate coordination is realized,[30] while in $Fe(OEP)(\eta^1\text{-}ONO_2)$ coordination occurs in monodentate fashion.[31] In $Co(OEP')(\eta^2\text{-}O_2NO)$ (OEP′-modified octaethylporhyrinato ligand) the nitrate is bound in symmetric bidentate form,[32] while in $Mn(TPP)(\eta^1\text{-}ONO_2)$ monodentate coordination is realized.[33] Vibrational spectroscopy can be useful for differentiation of these modes of binding in nitrato complexes of transition metals. In the bidentate form, two high frequency stretching bands {ν(N═O) and $\nu_a(NO_2)$} of comparable intensities are more separated than $\nu_a(NO_2)$ and $\nu_s(NO_2)$ vibrations upon monodentate coordination.[34]

This pattern is also operational in the nitrato complexes of metalloporphyrins. It has been shown that coordination of the sixth ligand to $Fe(TPP)(\eta^2\text{-}O_2NO)$ forming the thermally unstable six-coordinate complexes $(L)Fe(TPP)(\eta^1\text{-}ONO_2)$ (L = NO, THF)[28a,b] is accompanied with bidentate−monodentate isomerization. This process leads to the low frequency shift of the high frequency band at about 1530 cm^{-1} for 30−40 cm^{-1} and noticeable decreasing in its intensity, while the second high frequency band in the vicinity of 1270 cm^{-1} increases in intensity with very slight frequency shifting.

As mentioned above, the six-coordinate nitrato complexes of metalloporphyrins with biologically important Fe, Co and Mn metals are not stable at ambient conditions and were spectrally characterized only for the iron−porphyrins at low temperatures.[9,28,35] In the complexes obtained by low-temperature interaction of electron donor ligands with the $Fe(TPP)(\eta^2\text{-}O_2NO)$, the following bands were reported for the nitrato group in $(L)Fe(TPP)(\eta^1\text{-}ONO_2)$: 1505 m, 1266 s and 978 w and 1490 m, 1278 s and ~997 w cm^{-1} for the NO[28a] and THF[28b] *trans* ligands correspondingly. These data together with that of the six-coordinate nitrato complexes of iron-porphyrins with trans ammonia ligand $(NH_3)Fe(Por)(\eta^2\text{-}O_2NO)$ obtained by the NOD-reaction[9] show the bands of coordinated nitrate lie in the same spectral range with the same relative intensities and closely related values of isotopical shifts (see Table 2) as was observed in this work for the Co-derivatives. Additional evidence for the formation of six-coordinate nitrato complexes with the *trans* ammonia ligand $(NH_3)Co(TPP)(\eta^1\text{-}ONO_2)$ is the presence in spectra of the stretching bands of coordinated NH_3 in the high frequency range 3450−3250 cm^{-1} that is free of other spectral features. They undergo low-frequency shifts from the values of $\nu_a(NH_3)$ and $\nu_s(NH_3)$ of free NH_3 (Figure S5, Supporting Information) as usually occurs upon ammonia coordination.[34] In the 1270 cm^{-1} spectral range a band appears (Figure 6) as a high frequency shoulder of the intense $\nu_s(NO_2)$ band in the spectra of natural abundance nitrato complex, but is well separated in the spectra of isotopical species (Figure 6, dashed and dotted lines). This band represents the deformation $\delta_s(NH_3)$ mode of coordinated ammonia[34] and was seen also in the spectra of Fe-derivatives.[9] The electronic absorption spectra of Co-porphyrins also undergo noticeable changes upon formation of both the six-coordinate dioxygen and nitrato complexes as can be seen in Figure 7.

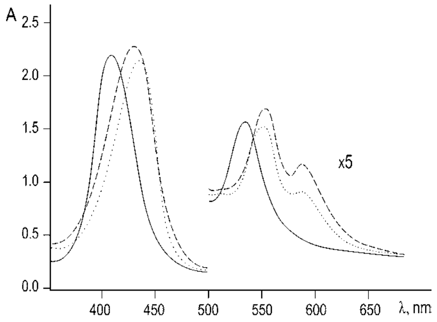


Figure 7. UV−visible spectra of thin layers containing Co(TTP) (solid line) $(NH_3)Co(TTP)(O_2)$ (dashed line) and $(NH_3)Co(TTP)(\eta^1\text{-}ONO_2)$ (dotted line). The spectra were taken after FTIR measurements of the same samples confirming the identity of each species.

The Soret band of Co(TTP) at 409 nm undergoes a red shift to 434 and 439 nm upon formation of $(NH_3)Co(TTP)(O_2)$ and $(NH_3)Co(TTP)(\eta^1\text{-}ONO_2)$ indicating an increasing metal oxidation state. In its turn the single band of Co(TTP) at 535 nm splits into two components disposing at 558 and 597 nm for $(NH_3)Co(TTP)(O_2)$ and 556 and 596 nm for $(NH_3)Co(TTP)(\eta^1\text{-}ONO_2)$. Absorption spectra of nitrato-complexes obtained in this paper are very close to those available in literature for tervalent cobalt complexes of porphyrins.[36]

The discussion above clearly indicates that Co-porphyrins similar to heme-analogues also promote the NOD-reaction but at much higher temperatures, allowing the observation of transient species formed in the course of this biologically important transformation.

Further Transformations of Six-coordinate Nitrato Complexes upon Warming. The six-coordinate nitrato-complexes $(NH_3)Co(Por)(\eta^1\text{-}ONO_2)$ are not thermally stable and decompose after warming the systems to room temperature. Spectral changes accompanying this process are demonstrated in the Figure 8. The bands of coordinated nitrato group disappear and new broad band overlapping with the porphyrin band at 1350 cm^{-1} grows in the vicinity of 1370 cm^{-1}. This band has its isotopic analogs in the experiments with ^{15}N (Figure 9) and ^{18}O containing species and unambiguously should be assigned to the degenerate asymmetric stretching mode ν_3 of the nitrate anion.[34] Sustaining this interpretation a weak isotopically sensitive band also grows in the vicinity of 830 cm^{-1} where the out-of-plane deformation mode ν_2 of NO_3^- anion is disposed.[34]

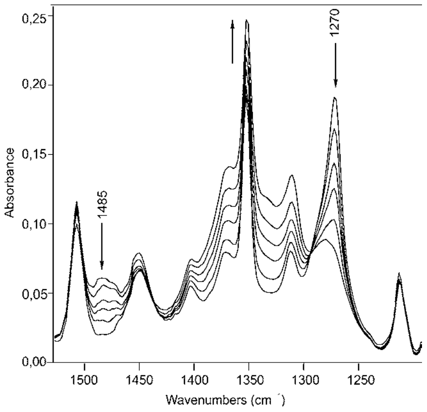


Figure 8. FTIR spectra demonstrating decomposition of six-coordinate nitrato complex $(NH_3)Co(TTP)(\eta^1\text{-}ONO_2)$ with elimination of NO_3^- upon warming the layered solid from 210 K to RT.

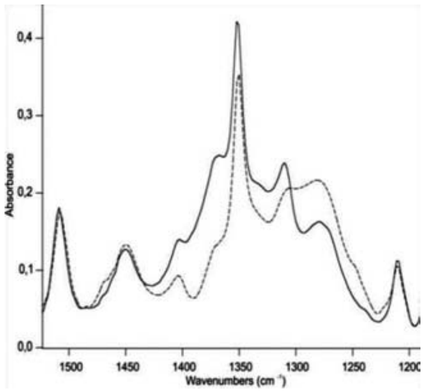
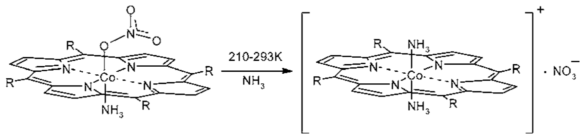


Figure 9. FTIR spectra of the products of NOD reaction at room temperature $Co^{III}(TTP)(NH_3)_{1,2}\bullet NO_3^-$ (solid line), $Co^{III}(TTP)(NH_3)_{1,2}\bullet ^{15}NO_3$ (dashed line).

The doubly degenerate in the bare ion ν_3 band is not a single band but has complex shape that is certainly connected with the H-bonding of NO_3^- with NH_3 (coordinated or solvated) that lowers the D_{3h} symmetry of nitrate-anion and removes the degeneracy of ν_3 mode. Such splitting was described in the literature for the NO_3^-/H_2O system. Depending on the quantity of the H_2O molecule per an NO_3^- ion and the symmetry of H-bonding, the different characteristics of ν_3 splitting into two components was observed with separation of the bands as much as 43 cm^{-1} in some cases.[37] Obviously the same pattern should be observed for the NO_3^-/NH_3 system.

The results obtained clearly indicate that six-coordinate nitrato-complexes of Co-porphyrins decompose at room temperature to nitrate-anion and amino-complexes of Co(III)-porphyrins. In contrast to the Co(II) species the six-coordinate amino-complexes of Co(III)-porphyrins much more stable[36] and there is uncertainty in our experiments if the cationic complex formed is mono- or bis-ligated $\{Co(III)(Por)(NH_3)$ or $Co(III)(Por)(NH_3)_2\}$. The NH_3 stretching vibrations in the 3250−3450 cm^{-1} range are disposed at higher wavenumbers for uncoordinated NH_3 compared to those of coordinated ammonia and the spectral features indicate that there is some quantity of uncoordinated NH_3 in the layer in the course of decomposition of nitrato-complexes. Since UV−visible spectra undergo very minor changes upon transition from six-coordinate nitrato complexes to ammonia complexes of Co(III)-porphyrins (Figure S6, Supporting Information), we think that the coordination number of Co(III)-ion did not change and so favor the formation of six-coordinate ammonia complex (Scheme 4). The species eventually formed in the course of NOD reaction is fairly stable and high-vacuum pumping upon warming the system to 350 K does not reveal noticeable spectral changes. The result obtained in this work is similar to that for heme-containing globins[4] and modeling iron-porphyrin systems,[9] in which the NOD-reaction results in the formation of nitrate anion and oxidized hemes.

Scheme 4



CONCLUSIONS

As shown by FTIR and UV−visible spectroscopy NO reacts with layered oxy-coboglobin model compounds, (NH$_3$)Co(Por)(O$_2$), at low temperatures giving the six-coordinate nitrato complexes (NH$_3$)Co(Por)(η^1-ONO$_2$) thereby demonstrating the ability of these systems to promote nitric oxide dioxygenation (NOD) reaction. In contrast to the iron porphyrin analog (NH$_3$)Fe(Por)(O$_2$), in which NOD-reaction proceeds at temperatures lower than 100 K without spectral observation of intermediates,[9] the formation of nitrato complexes by Co-derivatives occurs at much higher temperature (180 K) giving the opportunity to follow the sequence of reactions at lower temperatures. The first transient formed at very low temperatures (80−120 K) and characterized by FTIR spectroscopy is believed to be the putative six-coordinate peroxynitrite complex (NH$_3$)Co(Por)(η^1-OONO). This conclusion was made based on the experiments with labeled ^{15}NO, N^{18}O and ^{18}O$_2$ species and predictions provided by DFT computations. In the temperature interval 140−170 K this intermediate decomposes and there is spectral evidence of NO$_2$ formation. This should be interpreted as homolytic O−O bond cleavage and formation of a caged radical pair (NH$_3$)Co(Por)-O$^•$ and $^•$NO$_2$. Beginning at 180 K three new bands in the vicinity of 1480, 1270, and 980 cm^{-1} grow manifesting formation of the six-coordinate nitrato complexes. These complexes are not stable at room temperature and eliminate nitrate anion to form cationic Co(III)-porphyrin complexes ligated by ammonia.

The overall NOD-reaction with oxy-cobalt models mimics the reaction promoted by hemes and the results of this study favor the mechanism of this transformation offered by numerous researchers[4] but never unambiguously demonstrated by direct experimental observations.

ASSOCIATED CONTENT

Ⓢ Supporting Information

Figure S1. FTIR spectral changes accompanying the formation of (NH$_3$)Co(Por). Figure S2. FTIR spectra of Co(TPP), (NH$_3$)Co(TPP)(O$_2$) and (NH$_3$)Co(TPP)(^{18}O$_2$) in the range of ν(O$_2$) vibration. Figure S3. UV−visible spectra of layered Co(TPP), Co(TPP)(NH$_3$) and (NH$_3$)Co(TPP)(O$_2$). Figure S4. UV−visible spectra of layered Co(TPP) and (NH$_3$)Co(TPP)(OONO) and (NH$_3$)Co(TPP)(ONO$_2$). Figure S5. FTIR spectra of (NH$_3$)Co(TTP)(η^1-ONO$_2$) in the range of NH$_3$ stretching vibrations. Figure S6. Visible spectra of (NH$_3$)Co(TTP)(η^1-ONO$_2$) and Co(III)(TTP)(NH$_3$)$_2$·NO$_3^-$. Figure S7. Equilibrium geometry of the trans-(NH$_3$)Co(TPP)(OONO) derivative as obtained from DFT (BP/6-31G*) optimization. Table S1. Infrared frequencies and their assignment for the Co-OONO group of the intermediate supposed to be (NH$_3$)Co(TPP)(OONO). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the State Committee of Science RA (Grant #11-1d052) and Armenian National Science and Education Fund (ANSEF) based in New York (Project PS-Cheminorg −2520).

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