Highly efficient and stable catalyst for peroxynitrite decomposition

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Abstract: The new cobalt substituted-polyoxometalate $K_7[CoAlW_{11}O_{39}] \cdot 15H_2O$ and the simple $CoCl_2 \cdot 6H_2O$ salt are efficient catalysts for peroxynitrite decomposition. These compounds also catalyze the oxidation of ascorbic acid and the nitration of phenol by peroxynitrite.

Key words: peroxynitrite, polyoxometalates, antioxidant.

Résumé : Le nouveau polyoxométalate substitué du cobalt, $K_7[CoAlW_{11}O_{39}]$ ·15H₂O ainsi que le simple sel CoCl₂·6H₂O sont des catalyseurs efficaces pour la décomposition du peroxynitrite. Ces composés catalysent aussi l'oxydation de l'acide ascorbique et la nitration du phénol par le peroxynitrite.

Mots clés : peroxynitrite, polyoxométalates, antioxydant.

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Introduction

The recent interest in peroxynitrite¹, a compound known for many years (1), was inspired by Beckman et al. (2), who suggested it was formed in vivo from NO and $O_2^{\bullet-}$. Under physiological conditions, ONOO- is partially protonated to HOONO (p $K_a = 6.8$), which rapidly ($k \sim 1 \text{ s}^{-1}$, pH = 7.0, 25°C) decomposes to highly reactive radical species in a unimolecular reaction (1-4). Peroxynitrite also reacts with many compounds in bimolecular reactions, and it is a powerful oxidant with substantial toxicity (1-4). There is accumulating evidence for peroxynitrite formation in vivo, which naturally has led to a search for compounds that can intercept this powerful oxidant and destroy it safely. The discovery of a fast peroxynitrite reaction with myeloperoxidase $(6.2 \times 10^6 \text{ M}^{-1} \text{ s}^{-1} \text{ at pH} = 7.2)$ (5) inspired studies of peroxynitrite reactions with synthetic, water-soluble Fe- and Mn-porphyrin complexes (6–11). Reactions of peroxynitrite with combinations of these metalloporphyrins and antioxidants were found to be even more rapid. Recently, several nonheme catalysts for peroxynitrite decomposition were re-

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- ¹The term peroxynitrite is used to refer to peroxynitrite anion (O=NOO⁻) and peroxynitrous acid (ONOOH) unless otherwise indicated. The IUPAC-recommended names are oxoperoxonitrate (-1) and hydrogen oxoperoxonitrate, respectively.
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ported (9, 12, 13); however, they appeared to be unstable and less active.

Here we report that a simple cobalt salt, $CoCl_2 \cdot 6H_2O$, and the Co-substituted polyoxometalate, $K_7[Co^{II}AlW_{11}O_{39}] \cdot 15H_2O$, catalytically decompose peroxynitrite at neutral pH (6.5–7.4) with high turnover numbers. Their activity is comparable to MnTMPyP, where TMPyP is the dianion of tetrakis(*N*-methyl-4'-pyridyl) porphyrin. All species also catalyze ascorbic acid oxidation and phenolic nitration by peroxynitrite.

Results and discussion

Peroxynitrite decay in the absence and in the presence of representative catalysts is exponential and follows the rate law in eq. [1].

[1]
$$-d[ONOO^-]/dt = k_{obs}[ONOO^-] = k_o[ONOO^-] +$$

*k*_{cat}[ONOO⁻][cat]

When k_{obs} values are plotted as a function of catalyst concentrations ([cat]) straight lines are obtained. The *y*-intercepts provide k_o and the slopes of each line provide k_{cat} . The data are summarized in Table 1. The following compounds were evaluated and found to be minimally active ($k_{cat} < 1 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$): CuCl₂·2H₂O, FeCl₂·4H₂O, K₉[AlW₁₁O₃₉]·13H₂O, Na₅[AlW₁₂O₄₀]·13H₂O, K₇[V^{IV}AlW₁₁O₄₀]·8H₂O, and K₅[Co^{III}W₁₂O₄₀]·13H₂O (the reproducible value for the uncatalyzed rate constant, $k_o = 0.35 \pm 0.03 \text{ s}^{-1}$ at pH 7.4).

The stabilities of the three most active catalysts, CoCl₂, $K_7[Co^{II}AlW_{11}O_{39}]$, and MnTMPyP, were evaluated by comparing their activities, indicated by experimental k_{obs} values, before and after reaction with large excesses of peroxynitrite (for concentrations see Table 2). The resistance of each catalyst to irreversible decomposition was estimated by $n = [NaOONO]_d/\Delta[cat]$, where $[NaOONO]_d$ is the total amount of peroxynitrite decomposed and $\Delta[cat]$ is the amount of the catalytic activity lost in the catalytic reaction. The latter term

Table 1. The rate constants (k_{cat}) of representative compounds used to catalyze peroxynitrite decomposition at 25°C.

| Catalyst | $k_{\rm cat} \ (1 \times 10^{-4}) \ ({ m M}^{-1} \ { m s}^{-1})$ |
|---|--|
| MnTMPyP | 2.1 ± 0.2 |
| CoCl ₂ | 3.0 ± 0.3 |
| K ₇ [CoAlW ₁₁ O ₃₉] | 1.9 ± 0.15 |

Note: For all experiments the pH = 7.0 ± 0.05 , 75 mM phosphate buffer, $[ONOO^-]_o = 300-400 \ \mu M$, $[cat]_o = 0-40 \ \mu M$ (0–14 μM for MnTMPyP).

was calculated as Δ [cat] = [cat]_o($k_{obs}^{\circ} - k_{obs}^{\prime})/(k_{obs}^{\circ} - k_{o})$, where [cat]_o is the initial concentration of the catalyst, k_{obs} is the decomposition rate in the presence of the catalyst that has been pretreated with a large excess of peroxynitrite, and k_{obs}° and k_{o} are the decomposition rates in the presence and the absence of the catalyst, respectively.³ The results, presented in Table 2, show that both K₇[Co^{II}AlW₁₁O₃₉] and MnTMPyP are substantially more stable than Co²⁺ (as CoCl₂).

The self-decomposition of peroxynitrite at neutral pH is known to form nitrate and nitrite, and the formation of nitrite is accompanied by dioxygen evolution in a 2:1 ratio (14). The addition of $K_7[Co^{II}AIW_{11}O_{39}]$ increases the yield of nitrite (from 11 to 23% based on peroxynitrite, if 20 µM of $K_7[Co^{II}AIW_{11}O_{39}]$ is added at pH 7.4).

Since CoCl_2 is more active than $\text{K}_7[\text{Co}^{II}\text{AlW}_{11}\text{O}_{39}]$, the activity of the latter could be attributed to Co^{2+} ions formed via dissociation of $\text{K}_7[\text{Co}^{II}\text{AlW}_{11}\text{O}_{39}]$ (eq. [2]). However, the activity of a mixture of CoCl_2 and $\text{K}_7[\text{Co}^{II}\text{AlW}_{11}\text{O}_{39}]$ was the same as the sum of the activities of each of the individual components, suggesting that $\text{K}_7[\text{Co}^{II}\text{AlW}_{11}\text{O}_{39}]$ itself is also an active catalyst.

[2]
$$[Co^{II/III}AIW_{11}O_{39}]^{7-/6-} \longrightarrow Co^{2+/3+} + [AIW_{11}O_{39}]^{9-}$$

Treatment of the starting $K_7[Co^{II}AIW_{11}O_{39}]$ with peroxynitrite results in its immediate oxidation to $K_6[Co^{III}AIW_{11}O_{39}]$ $(\lambda_{max}$ shifts from 560 to 680 nm). The mechanism in Scheme 1 (proposed mechanism of peroxynitrite decomposition catalyzed by $K_7[Co^{II}AIW_{11}O_{39}]$, where Co^{II} represents $[Co^{II}AIW_{11}O_{30}]^{7-}$) is consistent with all the above data and is based on that proposed for catalysis of peroxynitrite decomposition by Fe-porphyrins (7, 11) and sulfito-bound Co(III) (*cis*-[Co(NH₃)₄(SO₃)₂]⁻) (12).⁴ The rate-limiting step is likely to be a homolysis of the O-O bond in a Co^{III} peroxonitrito intermediate to form Co^{III}-O[•] and NO[•]₂. Previously, a Co(III) peroxynitrito complex has been isolated and characterized (15). The reaction is thought to occur by an outer-sphere mechanism. However, since the lability of Co(III) increases when its geometry is perturbed from rigorous O_h symmetry (16), as is the case in $K_7[Co^{II}AIW_{11}O_{39}]$, an inner-sphere mechanism cannot definitely be ruled out. Further investigations are ongoing.

The addition of ascorbic acid significantly increases the rate of peroxynitrite consumption in reactions catalyzed by $K_7[Co^{II}AIW_{11}O_{39}]$, i.e., the observed second-order rate con-

Scheme 1.



$$NO' + NO_2' \longrightarrow N_2O_3 \longrightarrow 2NO_2^- + 2H^+$$

stant (k_{cat} , eq. [1]), increases from 1.9×10^4 to 12.7×10^4 M⁻¹ s⁻¹ in the presence of 7.4 mM ascorbic acid at pH 7.4. However, the rate of K₆[Co^{III}AlW₁₁O₃₉] reduction by ascorbic acid is too slow to account for the observed increase in the total rate of peroxynitrite decomposition. Therefore, ascorbic acid is likely to reduce the Co^{III}[(OONO)⁻] intermediate complex, thus accelerating the total rate.

The nitration of phenols by peroxynitrite has long been known (17), and Beckman et al. (18) were the first, to our knowledge, to report a catalytic version of this process. To assess phenol nitration activity by the Co systems, the yield and the rate of 3-nitro-4-hydroxyphenylacetic acid (3-NO₂-4-HPA) from the nitration of 4-hydroxyphenylacetic acid (4-HPA) were quantified. The yield of 3-NO₂-4-HPA, and the rate of its formation, both increase with increasing $K_7[Co^{II}AIW_{11}O_{39}]$ concentration. Based on the initial peroxynitrite concentration, the 3-NO₂-4-HPA yield was 7% in the absence of a catalyst and 40% in the presence of 82 μ M of K₇[CoAlW₁₁O₃₉] (pH 7.4, [4-HPA] = 6.0 mM, $[NaOONO]_{0} = 1.1 \text{ mM}$). However, unlike in the catalyzed oxidation of ascorbic acid by peroxynitrite, the observed second-order rate constant (k_{cat} , eq. [1]) does not increase with 4-HPA concentration. The mechanism of nitration may involve a fast reduction of Co^{III}-O[•] by the phenolic species; subsequent coupling of the resulting phenoxy radical and NO₂ gives nitrophenol. Mn- and Fe-porphyrins active in peroxynitrite decomposition have also been found to catalyze the nitration of phenols (6, 8–11, 19) likely through the coupling of phenoxy and NO_2^{\bullet} radicals (19).

Experimental

MnTMPyP (ICN Biomedicals Inc.) was standardized in aqueous solution using $\epsilon_{462} = 1 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ (20). Peroxynitrite was synthesized from H₂O₂ and nitrite in a simple flow reactor (21, 22) and its concentration was deter-

⁴ The reaction decelerates at $[OH^-] = 0.4$ M, $[NaOONO]_0$; [cat] ~ 10–15, as a result of catalyst decomposition.

Table 2. The observed reaction rate constants before (k°_{obs}) and after (k'_{obs}) reaction of the catalyst with a large excess of peroxynitrite, and *n* values.

| Catalyst | $k^{\circ}_{ m obs}$ (s ⁻¹) ^a | $k_{\rm obs}^{'}$ (s ⁻¹) ^{<i>a</i>} | $(k_{\rm obs}^{\circ} - k_{\rm obs}^{'})/(k_{\rm obs}^{\circ} - k_{\rm o})^{b}$ | n ^c |
|---|--|--|---|----------------------|
| CoCl ₂ | 1.85 ± 0.07 | 1.33 ± 0.07 | 0.3 | $\sim 3 \times 10^3$ |
| K ₇ [CoAlW ₁₁ O ₃₉] | $1.33~\pm~0.05$ | 1.30 ± 0.05 | < 0.03 | $>3 \times 10^4$ |
| MnTMPyP | $1.10~\pm~0.04$ | $1.09~\pm~0.04$ | < 0.03 | $>3 \times 10^4$ |

Note: All experiments carried out at 25°C. Pretreatment conditions: $[NaOONO]_o = 24 \text{ mM}$, $[cat]_o = 25 \mu M$, pH 6.8, 0.15 M phosphate buffer.

 $^{\it a}[NaOONO]_{\rm o}$ = 0.4 mM, [cat]_{\rm o} = 15 $\mu M,$ pH 7.4, 0.11 M phosphate buffer.

 ${}^{b}k_{o} = 0.35 \pm 0.03 \text{ s}^{-1}.$

 $^{c}n = [NaOONO]_{d}/\Delta[cat];$ see text for details.

mined by UV–vis spectroscopy, $\epsilon_{302} = 1.7 \times 10^3 \ M^{-1} \ cm^{-1}$ (23).

The transition-metal-substituted new POM (α- $K_7[CoAlW_{11}O_{39}]$ ·15H₂O) was prepared by metallation of the lacunary complex α -K₉[AlW₁₁O₃₉]·15H₂O (24)by $Co(NO_3)_2 \cdot 6H_2O$. A 10 g portion of $K_9[AlW_{11}O_{39}]$ (3.05 mmol) was stirred in water (100 mL), and a solution of $Co(NO_3)_2 \cdot 6H_2O$ (0.88 g, 3.05 mmol, in 10 mL water) was added dropwise to the slurry via a pipette. The reaction mixture was stirred in a hot water bath (70-80°C) until the solution became clear. The resulting solution was dark pink (pH 6.2), and after cooling to room temperature, a red powder deposited. The product was recrystallized from a minimum of warm (60°C) water.⁵

Kinetic experiments were studied using a SF-61 stop flow instrument (Hi-Tech Scientific, U.K.). A buffer solution with the reactants and peroxynitrite diluted in aqueous NaOH were mixed and the absorbance at 302 nm was measured. The first-order reaction rate constants were determined using standard software (KISS 5.1 for Macintosh computer). The yields of nitrite and 3-NO₂-4-HPA were determined after a small amount of highly concentrated (~0.6 M) peroxynitrite solution was mixed with a buffer solution containing all other ingredients. Yields of nitrite were determined as previously described (25) and 3-NO₂-4-HPA was quantified from its absorbance at 430 nm after adjusting the pH to 10–11 (18).

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⁵Yield: 7.31 g (74%). IR (KBr pellet) (cm⁻¹): 952 (m), 935 (s), 891 (s), 798 (m), 697 (m), 533 (w), 486 (w). Anal. calcd. for K_7 [CoAlW₁₁O₃₉]·15H₂O: H 0.92, Al 0.82, Co 1.80, K 8.35, W 61.73; found: H 0.91, Al 0.88, Co 1.73, K 8.16, W 62.26.