

Highly efficient and stable catalyst for peroxyntirite 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 peroxyntirite decomposition. These compounds also catalyze the oxidation of ascorbic acid and the nitration of phenol by peroxyntirite.

Key words: peroxyntirite, polyoxometalates, antioxidant.

Résumé : Le nouveau polyoxomélaté substitué du cobalt, $K_7[CoAlW_{11}O_{39}] \cdot 15H_2O$ ainsi que le simple sel $CoCl_2 \cdot 6H_2O$ sont des catalyseurs efficaces pour la décomposition du peroxyntirite. Ces composés catalysent aussi l'oxydation de l'acide asorbique et la nitration du phénol par le peroxyntirite.

Mots clés : peroxyntirite polyoxomélatés, antioxydant.

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Introduction

The recent interest in peroxyntirite¹, a compound known for many years (1), was inspired by Beckman et al. (2), who suggested it was formed in vivo from NO and O₂. Under physiological conditions, ONOO⁻ is partially protonated to HOONO (pK_a = 6.8), which rapidly (k ~ 1 s⁻¹, pH = 7.0, 25°C) decomposes to highly reactive radical species in a unimolecular reaction (1-4). Peroxyntirite also reacts, with many compounds in bimolecular reactions, and it is a powerful oxidant with substantial toxicity (1-4). There is accumulating evidence for peroxyntirite 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 peroxyntirite reaction with myeloperoxidase (6.2 × 10⁶ M⁻¹ s⁻¹ at pH = 7.2) (5) inspired studies of peroxyntirite reactions with synthetic, water-soluble Fe- and Mn-porphyrin complexes (6-11). Reactions of peroxyntirite with combinations of these metalloporphyrins and antioxidants were found to be even more rapid. Recently, several nonheme catalysts for peroxyntirite decomposition were re-

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 peroxyntirite 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 peroxyntirite.

Results and discussion

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

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

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 ($k_{cat} < 1 \times 10^3$ M⁻¹ s⁻¹): $CuCl_2 \cdot 2H_2O$, $FeCl_2 \cdot 4H_2O$, $K_9[AlW_{11}O_{39}] \cdot 13H_2O$, $Na_5[AlW_{12}O_{40}] \cdot 13H_2O$, $K_7[V^{IV}AlW_{11}O_{40}] \cdot 8H_2O$, and $K_5[Co^{III}W_{12}O_{40}] \cdot 13H_2O$ (the reproducible value for the uncatalyzed rate constant, $k_o = 0.35 \pm 0.03$ s⁻¹ at pH 7.4).

The stabilities of the three most active catalysts, $CoCl_2$, $K_7[Co^{II}AlW_{11}O_{39}]$, and MnTMPyP, were evaluated by comparing their activities, indicated by experimental k_{obs} values, before and after reaction with large excesses of peroxyntirite (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 peroxyntirite decomposed and $\Delta[cat]$ is the amount of the catalytic activity lost in the catalytic reaction. The latter term

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Dedicated to Professor Brian R. James on the occasion of his 65th birthday.

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¹The term peroxyntirite is used to refer to peroxyntirite anion (O=NOO⁻) and peroxyntirous acid (ONOOH) unless otherwise indicated. The IUPAC-recommended names are oxoperoxonitrate (-1) and hydrogen oxoperoxonitrate, respectively.

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

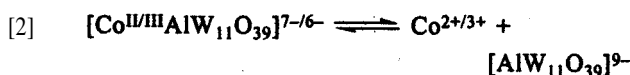
Catalyst	$k_{\text{cat}} (1 \times 10^{-4}) (\text{M}^{-1} \text{s}^{-1})$
MnTMPyP	2.1 ± 0.2
CoCl ₂	3.0 ± 0.3
K ₇ [Co ^{II} AlW ₁₁ O ₃₉]	1.9 ± 0.15

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

was calculated as $\Delta[\text{cat}] = [\text{cat}]_0(k_{\text{obs}}^{\circ} - k_{\text{obs}}^{\text{cat}})/(k_{\text{obs}}^{\circ} - k_{\text{p}})$, where [cat]₀ 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 peroxyxynitrite, and $k_{\text{obs}}^{\text{cat}}$ and k_{p} 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 peroxyxynitrite 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 K₇[Co^{II}AlW₁₁O₃₉] increases the yield of nitrite (from 11 to 23% based on peroxyxynitrite, if 20 μM of K₇[Co^{II}AlW₁₁O₃₉] is added at pH 7.4).

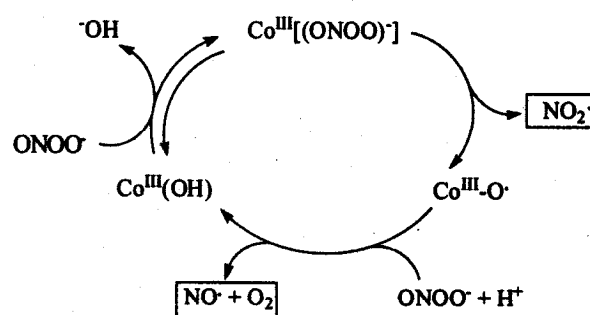
Since CoCl₂ is more active than K₇[Co^{II}AlW₁₁O₃₉], the activity of the latter could be attributed to Co²⁺ ions formed via dissociation of K₇[Co^{II}AlW₁₁O₃₉] (eq. [2]). However, the activity of a mixture of CoCl₂ and K₇[Co^{II}AlW₁₁O₃₉] was the same as the sum of the activities of each of the individual components, suggesting that K₇[Co^{II}AlW₁₁O₃₉] itself is also an active catalyst.



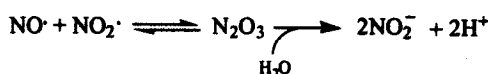
Treatment of the starting K₇[Co^{II}AlW₁₁O₃₉] with peroxyxynitrite results in its immediate oxidation to K₆[Co^{III}AlW₁₁O₃₉] (λ_{max} shifts from 560 to 680 nm). The mechanism in Scheme 1 (Proposed mechanism of peroxyxynitrite decomposition catalyzed by K₇[Co^{II}AlW₁₁O₃₉], where Co^{II} represents [Co^{II}AlW₁₁O₃₉]⁷⁻) is consistent with all the above data and is based on that proposed for catalysis of peroxyxynitrite decomposition by Fe-porphyrins (7, 11) and sulfito-bound Co(III) (*cis*-[Co(NH₃)₄(SO₃)₂]) (12): The rate-limiting step is likely to be a homolysis of the O–O bond in a Co^{III} peroxynitrito intermediate to form Co^{III}-O[•] and NO₂[•]. 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 K₇[Co^{II}AlW₁₁O₃₉], an inner-sphere mechanism cannot definitely be ruled out. Further investigations are ongoing.

The addition of ascorbic acid significantly increases the rate of peroxyxynitrite consumption in reactions catalyzed by K₇[Co^{II}AlW₁₁O₃₉], i.e., the observed second-order rate con-

Scheme 1.



Nitrite formation reaction



stant (k_{cat} , eq. [1]); increases from 1.9×10^4 to $12.7 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ 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 peroxyxynitrite decomposition. Therefore, ascorbic acid is likely to reduce the Co^{III}[(ONOO)] intermediate complex, thus accelerating the total rate.

The nitration of phenols by peroxyxynitrite 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-nitro4hydroxyphenylacetic acid (3-NO₂-4-HPA) from the nitration of 4-hydroxyphenylacetic acid (4-HPA) were quantified. The yield of 3-NO₂-HPA, and the rate of its formation, both increase with increasing K₇[Co^{II}AlW₁₁O₃₉] concentration. Based on the initial peroxyxynitrite concentration, the 3-NO₂-4-HPA yield was 7% in the absence of a catalyst and 40% in the presence of 82 μM of K₇[Co^{II}AlW₁₁O₃₉] (pH 7.4, [4-HPA] = 6.0 mM, [NaOONO]₀ = 1.1 mM). However, unlike in the catalyzed oxidation of ascorbic acid by peroxyxynitrite, 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 peroxyxynitrite decomposition have also been found to catalyze the nitration of phenols (6, 8–11, 19) likely through the coupling of phenoxy and NO₂[•] 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). Peroxyxynitrite was synthesized from H₂O₂ and nitrite in a simple flow reactor (21, 22) and its concentration was deter-

³ $k_{\text{obs}} = k_{\text{o}} + k_{\text{cat}}[\text{cat}]$, see eq. [1]

³ The reaction decelerates 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 peroxyxynitrite, and n values.

Catalyst	k'_{obs} (s^{-1}) ^a	k_{obs} (s^{-1}) ^a	$(k'_{\text{obs}} - k_{\text{obs}})/(k'_{\text{obs}} - k_0)^b$	n^c
CoCl ₂	1.85 ± 0.07	1.33 ± 0.07	0.3	~3 × 10 ³
K ₇ [CoAlW ₁₁ O ₃₉]	1.33 ± 0.05	1.30 ± 0.05	<0.03	>3 × 10 ⁴
MnTMPyP	1.10 ± 0.04	1.09 ± 0.04	<0.03	>3 × 10 ⁴

Note: All experiments carried out at 25°C. Pretreatment conditions: [NaOONO]₀ = 24 mM, [cat]₀ = 25 μM, pH 6.8, 0.16 M phosphate buffer.

^a[NaOONO]₀ = 0.4 mM, [cat]₀ = 15 μM, pH 7.4, 0.11 M phosphate buffer.

^b $k_0 = 0.35 \pm 0.03 \text{ s}^{-1}$.

^c $n = [\text{NaOONO}]_0/\Delta[\text{cat}]$; see text for details.

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

The new transition-metal-substituted POM (α -K₇[CoAlW₁₁O₃₉]•15H₂O) was prepared by metallation of the lacunary complex α -K₉[AlW₁₁O₃₉]•15H₂O (24) by Co(NO₃)₂•6H₂O. A 10 E portion of K₉[AlW₁₁O₃₉] (3.05 mmol) was stirred in water (100 mL), and a solution of Co(NO₃)₂•6H₂O (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 peroxyxynitrite 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) peroxyxynitrite 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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References

- J.O. Edwards and R.C. Plumb. *Prog. Inorg. Chem.* **41**, 599 (1994).
- J.S. Beckman, T.W. Beckman, J. Chen, P.A. Marshal, and B.A. Freeman. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 1620 (1990).
- W.A. Pryor and G.L. Squadrito. *Am. J. Physiol.* **268**, L699 (1995).
- J.S. Beckman and W.H. Koppenol. *Am. J. Physiol.* **271**, C1424 (1996).
- R. Floris, S.R. Piersma, G. Yang, P. Jones, and R. Wever. *Eur. J. Biochem.* **215**, 767 (1993).
- J.T. Groves and S.S. Marla. *J. Am. Chem. Soc.* **117**, 9578 (1995).
- M.K. Stern, M.P. Jensen, and K. Kramer. *J. Am. Chem. Soc.* **118**, 8735 (1996).
- G.G.A. Balavoine, Y.V. Geletii, and D. Bejan. *Nitric Oxide*. **1**, 507 (1997).
- G. Ferrer-Seuta, I. Batinic-Haberle, I. Spasojevic, I. Fridovich, and R. Radi. *Chem. Res. Toxicol.* **12**, 442 (1999).
- J. Lee, J.A. Hunt, and J.T. Groves. *J. Am. Chem. Soc.* **120**, 6053 (1998).
- J. Lee, J.A. Hunt, and J.T. Groves. *J. Am. Chem. Soc.* **120**, 7493 (1998).
- A.M. Al-Ajlouni, P.C. Paul, and E.S. Gould. *Inorg. Chem.* **37**, 1434 (1998).
- X. Zhang and D.H. Busch. *J. Am. Chem. Soc.* **122**, 1229 (2000).
- S. Pfeiffer, A.C.F. Gorren, K. Schmidt, E.R. Werner, B. Hansert, D.S. Böhle, and B. Mayer. *J. Biol. Chem.* **272**, 3465 (1997).
- P.K. Wick, R. Kissner, and W.H. Koppenol. *Helv. Chim. Acta*, **83**, 748 (2000).
- R.W. Hay, R. Bembi, F. McLaren, and W.T. Moodie. *Inorg. Chim. Acta*, **85**, 23 (1984).
- M.S. Ramezani, S. Padmaja, and W.H. Koppenol. *Chem. Res. Toxicol.* **9**, 232 (1996).
- J.S. Beckman, H. Ischiropoulos, L. Zhu, M. Van der Woerd, C. Smith, J. Chen, J. Harrison, J.C. Martin, and M. Tsai. *Arch. Biochem. Biophys.* **298**, 438 (1992).
- J.P. Crow. *Arch. Biochem. Biophys.* **371**, 41 (1999).
- J. Bernadou, G. Pratiel, F. Bennis, M. Girardet, and B. Meunier. *Biochem.* **28**, 7268 (1989).
- N. Hogg, V. Darley-Usmar, M.T. Wilson, and S. Moncada. *FEBS Lett.* **326**, 199 (1993).
- W.H. Koppenol, R. Kissner, and J.S. Beckman. *Methods Enzymol.* **269**, 296 (1996).
- D.S. Böhle, B. Hansert, S.C. Paulson, and B.D. Smith. *J. Am. Chem. Soc.* **116**, 7423 (1994).
- I.A. Weinstock, J.J. Cowan, E.M.G. Barbuzzi, H. Zeng, and C.L. Hill. *J. Am. Chem. Soc.* **121**, 4608 (1999).
- R.C. Plumb, J.O. Edwards, and M.A. Herman. *Analyst*, **117**, 1639 (1992).

⁵Yield: 7.31g(74%). IR (KBr pellet) (cm^{-1}): 952 (m), 935 (s), 891 (s), 798 (m), 697 (m), 533 (w), 486(w). Anal. calcd. for K₇[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.