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Kinetics and mechanism of octacyanomolybdate(IV) oxidation by peroxynitrite

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A kinetic study of the oxidation of octacyanomolybdate(IV) by peroxynitrite has been performed using a stopped-flow technique. The variation of k_{obs} with the concentration of $Mo(CN)_8^{4-}$, pH and temperature have been investigated. A reaction mechanism that involves homolysis of HOONO to form •OH and $•NO_2$ radicals is proposed. Octacyanomolybdate(IV) is oxidised by HOONO (direct oxidation) and by the aforementioned radicals (indirect oxidation). The rate constants for HOONO homolysis (k_1) and direct oxidation (k_2) have been obtained along with the activation parameters of these steps.

Keywords: octacyanomolybdate(IV), peroxynitrite

Peroxynitrite ($HOONO/ONOO^–$) plays important roles in biological and physical chemistry. For example, in biological systems peroxynitrite is formed by diffusion-controlled reaction ($k = 6.7 \times 10^9$ $M^{-1}s^{-1}$)[1] between superoxide ($\cdot O^-_2$) and nitric oxide (·NO) in tissues or cells that simultaneously generate these two radicals.[2] Peroxynitrite has been proposed as a potential pathogenic agent in human diseases[3,4] and as an important microbicidal agent generated by phagocytic cells associated with the host defense system.[5,6]

The peroxynitrite anion and its conjugate acid are strong oxidants whose chemistry is poorly understood. The peroxynitrite anion is relatively unreactive, whereas peroxynitrous acid (HOONO, $pK_a = 6.8$) is an unstable and weak acid that can isomerise to nitrate (at a rate of 1.3 $s^{–1}$ at 25 °C)[7,8] or decompose to nitrite and oxygen.[9,10] Isomerisation to nitrate is the major pathway in acidic media.

Peroxynitrous acid can oxidise a wide range of ions and molecules including $I^–$,[11] $CN^–$,[11] sulfhydryles,[12] dimethyl sulfoxide,[13,14] methionine,[15] DNA,[16] ascorbic acid,[17] and also hydroxylate and nitrate aromatic compounds.[18–20] The oxidation of the various substrates by HOONO can take place directly, as in the case of $I^–$,[11] or indirectly,[21] although most substrates appear to react by both pathways simultaneously.[22] The indirect pathway involves the decomposition of HOONO to give a highly reactive intermediate species, which subsequently oxidises substrates or hydroxylates and nitrates aromatic compounds. The structure and reactivity of this intermediate have been debated at length. The high reactivity has been attributed to: (a) *trans*-HOONO;[23] (b) an intermediate structurally related to the transition state formed in the isomerisation of HOONO to nitric acid;[7] (c) the nitronium ion, NO^+_2, formed by heterolytic cleavage of the O–O bond;[8] (d) a caged radical pair[8] or free radicals, formed by homolytic cleavage of the O–O bond.[18,24,25]

We now describe a study of the kinetics of the oxidation of $Mo(CN)_8^{4–}$ by peroxynitrite. It has been reported that the rate of this reaction is independent of substrate concentration.[9] We have observed, however, that the oxidation rate increases slightly with the initial concentration of $Mo(CN)_8^{4–}$, suggesting that a mixed direct and indirect oxidation takes place.

Materials and methods

Chemicals

All chemicals were of analytical grade and were used as received. Solutions were prepared with deionised water obtained using an OSMO BL-6 deioniser from SETA Purification Systems.

Potassium octacyanomolybdate(IV) dihydrate, $K_4Mo(CN)_8{\cdot}2H_2O$, was prepared by reduction of $Na_2MoO_4{\cdot}2H_2O$ by KBH_4 in a potassium cyanide medium, following the method of Leipoldt *et al.*:[26] $Na_2MoO_4{\cdot}2H_2O$ (36 g, 0.15 mol), KCN (175 g, 2.7 mol) and KBH_4 (18 g, 0.33 mol) were dissolved in water (300 ml). Concentrated acetic acid (140 ml) was added to the solution over 1 hour with stirring. The solution changed from colourless to green and then to yellow. The solution was then heated for 20 minutes on a water bath in order to complete the reaction. The solution was allowed to cool and ethanol (500 ml) was added to precipitate the $K_4Mo(CN)_8{\cdot}2H_2O$. The product had a greenish colour. The crude product (approx. 30 g) was dissolved in water (25 ml) and the resulting solution was boiled for 10 minutes with animal charcoal. The solution was filtered and ethanol (60 ml) was added in order to precipitate the product. A yellow crystalline product was obtained, $K_4Mo(CN)_8{\cdot}2H_2O$, which has an absorption maximum at $\lambda = 363$ nm ($\varepsilon = 204 M^{-1}cm^{-1}$) and a shoulder at 410 nm ($\varepsilon = 123$ $M^{–1}cm^{–1}$). $K_3Mo(CN)_8{\cdot}2H_2O$ formed by the oxidation of $K_4Mo(CN)_8{\cdot}2H_2O$ by alkaline peroxynitrite solution has an absorption maximum at $\lambda = 388$ nm ($\varepsilon = 1200 M^{-1}cm^{-1}$).

Peroxynitrite was prepared using the method of Leis *et al.*[27] This method is based on the direct formation of the peroxynitrite anion in basic media by reacting the strong nucleophile $HOO^–$ with 2-ethoxyethyl nitrite, eqn (1)

$$RONO + HOO^– \rightarrow ROH + ONOO^– \qquad (1)$$

The reaction must be carried out with equimolecular amounts of H_2O_2 and 2-ethoxyethyl nitrite in an alkaline medium to avoid the presence of H_2O_2 in the final solution. The preparation of 100 ml of a peroxynitrite stock solution (0.016 M) was performed as follows: 0.109 M aqueous H_2O_2 (15 ml) was mixed with 2 M NaOH (15 ml) and water (70 ml). 2-Ethoxyethyl nitrite (0.2 ml, 0.195 g) [prepared from 2-ethoxyethanol and sodium nitrite at acidic pH[28]] was then added to the reaction mixture. After 5 minutes a yellow solution of peroxynitrite ($\lambda_{max} = 302$ nm, $\varepsilon = 1670$ $M^{–1}$ $cm^{–1}$) was obtained. Yields greater than 94% (determined spectrophotometrically) were obtained. The stock solution was stored at –18 °C to avoid decomposition. The solutions used in each experiment were prepared by dilution from the stock solution (final pH, 11–12). The peroxynitrite concentrations of these solutions were determined by measuring the absorbance at 302 nm.

At neutral pH the buffer solutions used were purged with argon in order to remove atmospheric CO_2. At pH ≤ 5, however, buffer solutions were not purged because the formation of the adduct[29] $ONOOCO^-_2$, by reaction between $ONOO^-$ and CO_2, does not compete with the self-decomposition of peroxynitrite due to the low concentration of $ONOO^-$.

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Kinetic measurements

The kinetic measurements were carried out using an SX-18 MV Sequential Stopped-Flow apparatus from Applied Photophysics. The self-decomposition of peroxynitrite was followed at 302 nm by mixing peroxynitrite, at pH 12, with 0.2 M acetate or phosphate buffers at various pHs. The $Mo(CN)_8^{4-}$ oxidation was followed by measuring the absorbance of $Mo(CN)_8^{3-}$ formed, at 388 nm. Equal volumes of peroxynitrite, at pH 12, and 0.2 M acetate or phosphate buffer containing the reductant were mixed. $Mo(CN)_8^{4-}$ absorbs at 388 nm and so we used as a blank a solution of $Mo(CN)_8^{4-}$ at the same pH and concentration as the sample used in the oxidation reaction. The pH of the reaction mixture was measured at the end of the oxidation. The optical path was either 1 or 0.2 cm. Apparent rate constants for both peroxynitrite self-decomposition and oxidation of $Mo(CN)_8^{4-}$ were determined by nonlinear least-squares fitting of the stopped-flow data to a single exponential function with a nonzero offset (first-order reaction kinetics). The oxidations of $Mo(CN)_8^{4-}$ were carried out using concentrations of reductant in excess of that of peroxynitrite. Reported values are the average of at least five separate determinations. The temperature was kept constant, with a stability of ± 0.05°C, using a thermostat (Selecta, Model Ultraterm 200). Release of O_2 due to peroxynitrite decomposition and substrate oxidation were measured with a YSI-5000 dissolved oxygen meter in conjunction with a YSI-5010 BOD probe. The electrode was calibrated in air by placing the probe in air at 100% relative humidity at a temperature of 21 °C. The determination of nitrate and nitrite as products of the peroxynitrite decomposition in the presence or absence of substrate was performed by HPLC using a Perkin-Elmer series 4 liquid chromatograph (column: MFE®-Sil amino 5µm 150 × 4.6 mm).

Results

Peroxynitrite self-decomposition was studied over the pH range 4.0–10.1. The observed first-order rate constant, k_{obs}, as a function of pH can be described by the equation proposed by Keith and Powell[30]

$$k_{obs} = k_{HA}[H^+]/([H^+] + K_a) \qquad (2)$$

where k_{HA} is the first-order rate constant for peroxynitrous acid decomposition and K_a is the ionisation constant for peroxynitrous acid. By fitting the k_{obs} – $[H^+]$ data, Fig. 1, to eqn (2) we obtained the values $k_{HA} = 1.63 \pm 0.01 s^{-1}$ and $pK_a = 6.8 \pm 0.1$ at 27 °C. These values are in good agreement with those in the literature.[7,12] In addition, k_{obs} was found to be independent of initial peroxynitrite concentration over the concentration range 2×10^{-4} to 10×10^{-4} M. The dependence of k_{obs} on the temperature was studied in the range 14–47 °C. The Arrhenius equation was applied and gave values of $E_a = 84 \pm 2$ kJ mol^{-1} and $A = (8 \pm 6) \times 10^{14}$ at pH 5. The Eyring equation gave values of $\Delta H^\# = 85 \pm 2$ kJ mol^{-1} and $\Delta S^\# = 31 \pm 7$ Jmol^{-1} K^{-1}.

The observed pseudo-first-order rate constants, k^S_{obs}, for the oxidation of $Mo(CN)_8^{4-}$ were found to be greater than those for the self-decomposition of peroxynitrite over the pH range 4.0–7.2. The increase in the observed rate constant was more appreciable at pH 5 than at higher pH values (Fig. 1). By fitting the data k^S_{obs}–$[H^+]$, Fig. 1, to the equation

$$k^S_{obs} = k^S_{HA}[H^+]/([H^+] + K_a) \qquad (3)$$

we obtained the rate constant for peroxynitrite decomposition in the presence of 1.0×10^{-2} M $Mo(CN)_8^{4-}$. The values found were $k^S_{HA} = 1.81 \pm 0.02$ s^{-1} and $pK_a = 6.7 \pm 0.1$ at 27 °C.

The variation of the ionic strength over the range 0.1–0.6 M upon addition of sodium chloride did not influence k^S_{obs}.

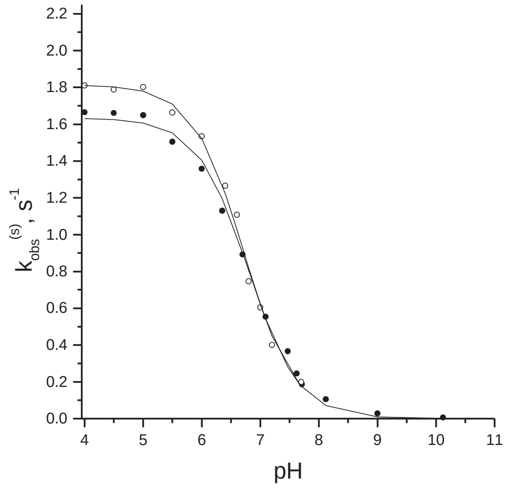


Fig. 1 Influence of pH on the observed rate constant of (○) octacyanomolybdate(IV) (0.01 M) oxidation and (•) peroxynitrite (8×10^{-4} M) decomposition, both at 27 °C.

The value of k^S_{obs} was also found to be independent of the initial concentration of peroxynitrite over the concentration range 2×10^{-4} M to 10×10^{-4} M. The addition of either nitrate or nitrite over the concentration range 2.5×10^{-4} to 12.5×10^{-4} M, at pH 5, had no influence on k^S_{obs} . Furthermore, the rate constant k^S_{obs} did not vary with the initial concentration of octacyanomolybdate, $[Mo(CN)_8^{4-}]_0$, at very low concentrations in the range 0–2 × 10^{-3} M (data not shown). However, it was found that as the reductant concentration was increased, k^S_{obs} varied linearly with $[Mo(CN)_8^{4-}]_0$ – as can be seen from Fig. 2. The *y*-intercept of this plot was identical, within experimental error, to the rate constant for peroxynitrite decomposition in the absence of $Mo(CN)_8^{4-}$. The dependence of k^S_{obs} on temperature was studied at pH 5 in the range 14–47 °C at eight different initial concentrations of octacyanomolybdate(IV) (Fig. 2). The observed activation energy decreased slightly from 84 kJ mol^{-1} to 79 kJ mol^{-1} on increasing $[Mo(CN)_8^{4-}]_0$ from 0 to 0.06 M.

Under the conditions $[HOONO]_0 = 8 \times 10^{-4}$ M, $[Mo(CN)_8^{4-}]_0 = 1 \times 10^{-2}$ M, $T = 22.5$ °C and $p = 709$ mmHg we observed the formation of 6.8 mg/l of O_2 at pH 8 during the oxidation of $Mo(CN)_8^{4-}$, whereas at pH 5 an insignificant yield of O_2 was observed.

The yield for the oxidation of octacyanomolybdate(IV), defined as the ratio $[Mo(CN))_8^{3-}]/[HOONO]_0$, was close to 0.30 and was independent of the initial concentration of

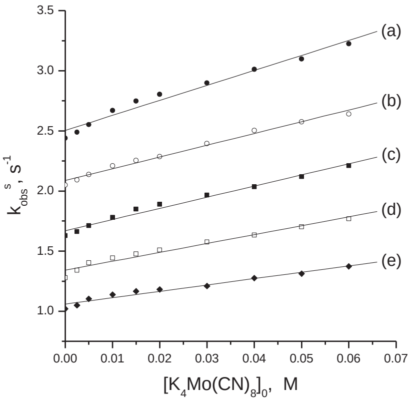
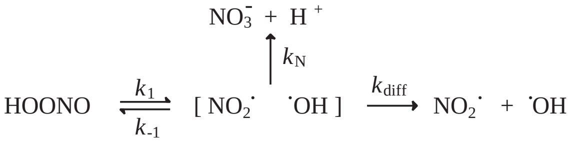


Fig. 2 Variation of the observed rate constant as a function of temperature and octacyanomolybdate(IV) concentration. Peroxynitrite (8×10^{-4} M) was added to the substrate in 0.1 M acetate buffer, pH 5.0: at (a) 31 °C; (b) 29 °C; (c) 27 °C; (d) 25 °C and (e) 23 °C.

octacyanomolybdate(IV) in the range 0–2 × 10^{-3} M. However, on increasing the reductant concentration the yield increased hyperbolically and tended towards a constant value of 0.80.

Discussion

Two mechanisms have mainly been used to explain the oxidation reactions of peroxynitrite. The first mechanism involves homolysis of HOONO to form ·OH and $\cdot NO_2$ radicals (k_1),[31,32] which are the oxidants. These radicals are initially in a solvent cage, *i.e.* surrounded by solvent molecules. This caged radical pair can then diffuse out of the cage to give free radicals (k_{diff}), re-form to give HOONO (k_{-1}), or form nitric acid (k_N). In general, it is assumed that only free radicals can be scavenged since the reaction of radicals with scavengers is too slow in comparison with the recombination of two radicals near one another. Since caged radicals cannot be scavenged, this mechanism can explain the irreducible yield of nitrate that is formed in the presence of very high concentrations of radical scavengers



$$\begin{tikzcd} & \mathrm{NO_3^-} + \mathrm{H^+} & \\ \mathrm{HOONO} \arrow[r, shift left, "k_1"] & {[\,\mathrm{NO_2^{\cdot}}\quad {}^{\cdot}\mathrm{OH}\,]} \arrow[l, shift left, "k_{-1}"] \arrow[u, "k_{\mathrm{N}}"'] \arrow[r, "k_{\mathrm{diff}}"] & \mathrm{NO_2^{\cdot}} + {}^{\cdot}\mathrm{OH} \end{tikzcd} \qquad (4)$$

The second mechanism involves an energetic isomer of peroxynitrous acid, $HOONO^*$, which is formed in a steady state[3] and has hydroxyl radical-like reactivity. This highly reactive intermediate can either isomerise to NO^-_3 or react with $ONOO^-$ to give NO^-_2 and O_2. This mechanism can also explain the irreducible yield of nitrate in the presence of hydroxyl radical scavengers, since $HOONO^*$ would be less reactive than the hydroxyl radical itself.

In our attempt to explain the reactivity of HOONO toward octacyanomolybdate(IV) we chose the first mechanism because many observations reported in the literature, as well as some results described here, support the cage mechanism. Merenyi and co-workers,[31,32] by means of thermodynamic and thermokinetic arguments, concluded that the reactive species' are a mixture of ·OH and $\cdot NO_2$ free radicals. Gerasimov and Lymar[33] determined that free ·OH and $\cdot NO_2$ radicals are formed as intermediates during the decay of HOONO using inorganic radical scavengers. Furthermore, the homolysis of HOONO to ·OH and $\cdot NO_2$ free radicals is supported by the fact that the frequency factor obtained in the present work for the decay of HOONO is of the same order of magnitude as those found for the homolysis of peroxides in the gas phase and in nonpolar organic solvents.

The $\cdot NO_2$ radical produced in reaction (4) dimerises and hydrolyses through the following reactions:

$$\cdot NO_2 + \cdot NO_2 \rightleftharpoons N_2O_4 \qquad (5)$$

$$N_2O_4 + H_2O \longrightarrow NO_2^- + NO_3^- + 2H^+ \qquad (6)$$

for which $k_5 = 4.6 \text{ x } 10^8$ $M^{-1}s^{-1}$, $k_{-5} = 6.9 \times 10^3$ s^{-1} and $k_6 = 1 \times 10^3$ M^{-1} s^{-1}.[34]

Also, ·OH produced in reaction (4) can oxidise NO^-_2 as follows:

$$\cdot HO + NO_2^- \longrightarrow HO^- + \cdot NO_2 \qquad (7)$$

with a rate constant of $k_7 = 1 \times 10^{10}$ M^{-1} s^{-1}.[32]

Moreover, in the presence of reductant both ·OH and $\cdot NO_2$ radicals oxidise $Mo(CN)_8^{4-}$ through the additional reactions:

$$Mo(CN)_8^{4-} + \cdot OH \longrightarrow Mo(CN)_8^{3-} + OH^- \qquad (8)$$

$$Mo(CN)_8^{4-} + \cdot NO_2 \longrightarrow Mo(CN)_8^{3-} + NO_2^- \qquad (9)$$

The sigmoidal shape of the plot k^S_{obs}/pH stems from the existence of an equilibrium such as,[10] where the peroxynitrite anion is rapidly protonated to give its conjugate acid. The decrease in the observed rate constant with pH suggests that HOONO rather than $ONOO^-$ is the reactive species.

$$H^+ + {}^-OONO \rightleftharpoons HOONO \qquad (10)$$

In basic media, moreover, the superoxide ion produced in the reaction between $ONOO^-$ and ·OH is reduced to $\cdot NO_2$ and produces O_2

$$HO\cdot + {}^-OONO \longrightarrow H^+ + NO_2^- + \cdot O_2^- \qquad (11)$$

$$\cdot O_2^- + H^+ \rightleftharpoons \cdot HO_2 \qquad (12)$$

$$\cdot O_2^- + \cdot NO_2 \longrightarrow O_2 + NO_2^- \qquad (13)$$

for which $k^{11} = 4.8 \times 10^9$ M^{-1} s^{-1}, [35] $pK_{12} = 4.8$ and $k_{13} = 4.5 \times 10^9$ M^{-1} s^{-1}.[36]

Also, octacyanomolybdate(V) formed in reactions (8) and (9) can oxidise O^-_2 to produce O_2.

$$Mo(CN)_8^{3-} + O_2^- \longrightarrow Mo(CN)_8^{4-} + O_2 \qquad (14)$$

The presence of $\cdot O^-_2$ in the medium has been confirmed by studying the decomposition of HOONO in the presence of tetranitromethane, TNM. At basic pH values the characteristic absorption band at 360 nm was observed due to the $C(NO_2)^-_3$ formed through reaction (15).

$$C(NO_2)_4 + \cdot O_2^- \longrightarrow C(NO_2)_3^- + NO_2 + O_2 \qquad (15)$$

for which $k_{15} = 2 \times 10^9$ M^{-1} s^{-1}.[37]

At pH 5, however, the concentration of $ONOO^-$ is very low, so the formation of $\cdot O^-_2$ through reaction[11] is negligible and $C(NO_2)^-_3$ will not be formed – a situation that has been observed experimentally. The scheme involving eqns (10)–(14) explains the insignificant yield of O_2 observed in an acidic medium (pH 5) for $Mo(CN)_8^{4-}$ oxidation and the formation of O_2 found at pH 8.6. The bimolecular rate constants corresponding to reactions (8) and (14) are $k_8 = 5.8 \times 10^9$ M^{-1} s^{-1} (pH = 6.5)[38] and $k_{14} = 5.7 \times 10^4$ M^{-1} s^{-1} (pH = 2), respectively.[39]

The linear increase in the oxidation rate upon increasing the initial concentration of $Mo(CN)_8^{4-}$ suggests the existence of direct oxidation. Reactions (8) and (9) represent the indirect oxidation while reaction (16) followed by fast reaction (9) represents the direct oxidation. Hence, it can be proposed that a mixed direct and indirect oxidation takes place.

$$Mo(CN)_8^{4-} + ONOOH \xrightarrow{k_2} Mo(CN)_8^{3-} + OH^- + \cdot NO_2 \qquad (16)$$

By applying the steady state approximation to the short lived cage $[NO_2\cdot\ \cdot OH]$, we obtained the rate constant, k^S_{obs}, for the first-order disappearance of peroxynitrite.

$$k^s_{obs} = \left[\frac{k_1 (k_N + k_{diff})}{(k_{-1} + k_N + k_{diff})} + k_2 [Mo(CN)_8^{4-}]_0 \right] \left(\frac{[H^+]}{K_a + [H^+]} \right) \qquad (17)$$

where the disappearance of peroxynitrite was the sum of the anion and its conjugate acid.

The rate constant for the decomposition of HOONO in the presence of the substrate would be

$$k^{s}_{HA} = \frac{k_1(k_N + k_{diff})}{(k_{-1} + k_N + k_{diff})} + k_2[Mo(CN)_8^{4-}]_0 \qquad (18)$$

where the first addend is the rate constant for the indirect reaction, which is equal to the observed rate constant for the decomposition of HOONO in the absence of radical scavengers, k_{HA}.

The rate constants for both the direct reaction, k_2, and the indirect reaction, k_{HA}, were obtained from the slope and *y*-intercept of the linear plot k^S_{HA} *vs* $[Mo(CN)_8^{4-}]_0$. The values found for these constants were 7.9 M^{-1} s^{-1} and 1.7 s^{-1}, respectively, at 27 °C. The rate constant k_2 is very close to that reported for the direct oxidation of $Fe(CN)_6^{4-}$, which is 8.2 M^{-1} s^{-1} at 22 °C.[33] The values of k^S_{HA} were obtained by dividing k^S_{obs} by 0.98, which is the value of $[H^+]/(K_a + [H^+])$ at pH 5. The oxidation yield obtained when the direct oxidation is negligible is 30%, and so $k_N \approx 2.3\ k_{diff}$. As both rate constants k_N and k_{-1} are approximately equal because the reaction of ·OH + ·NO_2 yields approximately equal amounts of ONOOH and NO^-_3 + H^+,[32] then $k_{HA} = 0.6\ k_1$ and k_1 would be 2.9 s^{-1} at 27 °C. The variation of k^S_{obs} with the initial octacyanomolybdate(IV) concentration was studied at five different temperatures and the results give the variation of k_1 and k_2 with temperature. We found that both constants obey Arrhenius (Fig. 3) and Eyring laws. The activation parameters for both homolysis of HOONO and direct oxidation are shown in Table 1. The Arrhenius preexponential factor, A, found for HOONO homolysis is close to 6 × 10^{15} s^{-1}, which is the approximate value calculated for unimolecular O–O bond cleavage in HOONO, in the gas phase, using thermokinetic properties.[14] Moreover, A is in accordance with the expected value of $10^{16\pm1}$ for the unimolecular homolysis reactions.[8] This result supports the cage mechanism over the HOONO* mechanism. Direct oxidation of $Mo(CN)_8^{4-}$ by peroxynitrous acid may occur by an outer-sphere electron transfer reaction. The activation entropy reflects the bimolecular nature of the reaction and is a typical entropy change for bringing the reactants to an encounter pair with no interaction, which is usually around –8 cal mol^{-1} K^{-1}. The activation energy is within the range 13–18 kcal mol^{-1}, calculated theoretically by the concerted oxidation of amines, sulfides and alkenes by peroxynitrous acid.[40]

Table 1 Preexponential factors and activation parameters for HOONO homolysis and direct oxidation of octacyanomolybdate(IV)

	HOONO homolysis	Direct oxidation
A/s^{-1} or $M^{-1}s^{-1}$	$(5 \pm 2) \times 10^{14}$	$(15 \pm 1) \times 10^{11}$
Ea/kJ mol^{-1}	82 ± 1	65 ± 2
$\Delta H^{\neq}$/kJ mol^{-1}	79 ± 1	63 ± 2
$\Delta S^{\neq}$/kJ mol^{-1} K^{-1}	28 ± 3	–20 ± 4

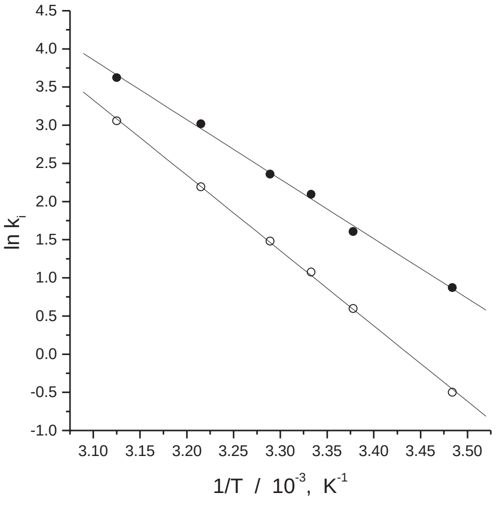


Fig. 3 Arrhenius plot of (○) HOONO homolysis constant, *i* = 1, and (•) direct oxidation constant, *i* = 2.

Conclusion

The oxidation of octacyanomolybdate(IV) by peroxynitrite involves homolysis of HOONO to form ·OH and ·NO_2 radicals. The substrate is oxidised by these radicals (indirect reaction) and by HOONO (direct reaction). The rate constants of HOONO homolysis and direct oxidation have been obtained along with the activation parameters for these steps.

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