COBALT(II) COMPLEX BINDING WITH COPOLYMER AS A PROFICIENT CATALYST FOR OXIDATION OF CATECHOL

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Abstract

The metallopolymer cobalt(II) complex of a simple pyridine- and amide-containing copolymer (Co^{II}-P_9) was prepared and characterized with IR and ^1H NMR. The ^1H NMR spectrum of the copolymer indicates that the average repeating unit of the copolymer P_9 is vinyl pyridine:acrylamide = 5:1. The IR spectra suggest that cobalt is bound to the polymer through the pyridine nitrogen atom and the acrylamide does not participate in the binding. The oxidation of catechol by Co^{II}-P_9 in the presence of 7.5 mM H_2O_2 exhibits enzyme-like pre-equilibrium kinetics, yielding $k_{cat} = 0.026 \pm 0.3$ s^{-1}, dissociation constant $K' = 1.9 \pm 0.6$ mM, and $k_{cat}/K' = 13.7$ M^{-1}s^{-1}. The inert 4,5-dichlorocatechol is also oxidized by Co^{II}-P_9 in absence of H_2O_2 to afford rate constants of $k_{cat} = (3.1 \pm 0.2) \times 10^{-4}$ s^{-1} and $K' = 0.7 \pm 0.1$ mM. This study has introduced a simple catalase-like system to explore the mechanism of Co(II)-centered redox chemistry and its application.

Key words; Catechol, oxidase, copolymer, vinyl pyridine, inhibition

Introduction

The oxidation of organic substrates with molecular oxygen under mild conditions is of great interest for industrial and synthetic processes, especially from economical and environmental points of view [1]. Although the reaction of organic compounds with dioxygen is thermodynamically favored it is kinetically hindered due to the triplet ground state of O_2. The synthesis and investigation of functional model complexes for metalloenzymes with oxidase or oxygenase activity is therefore of great interest for the development of new and efficient catalysts for oxidation reactions.

Catechol oxidase, tyrosinase, and polyphenol oxidases are analogous metalloenzymes which oxidize phenolic compounds to the corresponding quinones in the presence of oxygen. Catechol oxidase exclusively catalyzes the oxidation of catechols to quinones without acting on tyrosine [2]. This reaction is of great importance in medical diagnosis for the determination of the hormonally active

catecholamines adrenaline, noradrenaline and dopa [3]. There have been great efforts to synthesize model complexes as functional or structural models for catechol oxidases or related copper containing enzymes [4- 9].

Transition metal complexes have been widely studied in the area of bio-inorganic, bio-organic, and catalytic chemistry [10–13]. Cobalt complexes are interesting compounds in the field of catalysis not only because they are relatively inexpensive, but also because of the rich spectroscopy of the cobalt(II) center [14]. Cobalt-based catalysts are important and widely used materials in the industrial process, including hydrogenation, hydrotreating, and combustion processes [15].

In the field of biomimetic dioxygen carriers, the ability of Schiff base-cobalt complexes to bind dioxygen reversibly has been extensively examined since the late 30's, when Tsumaki discovered that the Co(Salen) complex could carry dioxygen reversibly [16]. Over the past few decades many Schiff base-cobalt complexes were synthesized and their thermodynamic and kinetic oxygenation properties characterized [17–21]. Recently, we have prepared and investigated a copolymer (P_1) of 4-vinyl pyridine (4Vp) and acrylamide (Ac) with an average repeating unit (RU) of $4Vp_3Ac_1$ which has been demonstrated to bind metal ions with a stoichiometry of metal:RU = 1:1 [22]. The copper complex of P_1 (Cu^{II}-P_1) has been demonstrated to show significant hydrolytic [22] and oxidative activities [23]. Hence we have demonstrated that metal-binding polymers are a versatile and tunable framework for the design of novel catalysts. Herein, the preparation of a copolymer analogous to P_1 and the oxidative activity of its cobalt (II) complex are described. The significant catechol oxidation activity of this complex suggests that this complex can serve as a model for catechol oxidase. The primary intention is to elucidate trends in reactivity that might be valuable for the use of this class of metallopolymer complexes in oxidation catalysis.

Experimental Section

The reagents 4-vinylpyridine was obtained from Across Organics (Fair Lawn, NJ). Acrylamide was from BioRad (Richmond, CA); HEPES, chelex resin, 3-methyl,2-benzothiazolinone hydrazone (MBTH), catechol (Cat) and 4,5 dichlorocatechol (DCC) were from Sigma-Aldrich (St Louis, MO). The buffer solutions were treated with Chelex resin to remove any trace amount of metal ions.

Polymer synthesis: Copolymers of 4-vinylpyridine (4Vp) and acrylamide (Ac) were prepared according to published procedures [24] by mixing freshly distilled 4-vinylpyridine and recrystalized acrylamide in DMF, then followed by

polymerization with the initiator 2,2′-azoisobutyronitrile (AIBN). The copolymers were precipitated in ethyl acetate, then filtered and dried in a vacuum oven at 40°C. The formation of the polymer was verified with a Bruker ADX 250 NMR spectrometer. A total recycle time of ~10 s was used to ensure complete relaxation of the ^{1}H NMR signals. Infrared absorption spectra in the range (600–4000 cm^{-1}) were measured on a Nicolet Avatar 320 FTIR spectrophotometer at University of South Florida, USA. CsI was usually used with dried Nujol as mulling agent for the solid compounds. Occasionally, spectra were recorded using potassium bromide discs. Calibration of the frequency reading was made with a polystyrene film.

Reactivity studies: The initial rates of the oxidation of different concentrations of catechol and 4,5 dichlorocatechol ranging from 0.2 to 5.0 mM by 20 µM Co^{II}-**P$_9$** (in terms of metal concentration) in buffered aqueous solution (50% water/methanol) at pH 8.0 in the presence of H$_2$O$_2$ were determined on a Varian Cary 50 spectrophotometer. The oxidation of the catechol and 4,5-dichlorocatechol can be monitored at 500 nm by the use of 3-methyl,2-benzothiazolinone hydrazone (MBTH) as an ortho-quinone indicator [25].

Results and discussion

Vinyl pyridine and acrylamide were chosen for the preparation of metallopolymers based on the ability to bind transition metals (pyridine moiety) and form H−bonding through the amide group, thus may loosely mimic the active site environment of various oxidoreductase enzymes.

An average of three integrations of ^{1}H-NMR spectra of the copolymer **P$_9$** was used for the calculation of the copolymer composition. The monomer composition of the copolymer can be calculated from the ratio of these integrals, which are proportional to the number of the protons that contribute to the peaks. Figure 1 shows the ^{1}H-NMR spectrum of the copolymer dissolved in d$_6$ DMSO. Peaks at 8.2, and 6.6 ppm are supposed to be due to two pyridine protons in the *o*-position and two pyridine protons in *m*-position. These two peaks are also supposed to be equally integrated.

The difference in the integration of these two peaks (0.19) is due to overlapping of NH$_2$ with the two pyridine protons (Fig. 1A). This assumption is proposed, based on the following evidences; the difference in the integration between the two peaks disappears upon deuteration (Fig. 1B), supporting the idea that this (0.19) is attributed to NH$_2$ amide. This data is also confirmed by subtracting the deuterated spectrum from the original one showing two peaks due to this difference (Fig.1C),

which is attributable to NH$_2$. The ^{1}H-NMR spectral data suggest that the monomer ratio of the copolymer is 5: 1 of 4Vp : Ac i.e. the copolymer repeating unit (RU) contains 4Vp$_5$Ac$_1$. The molecular weight of this copolymer is found to be more than 50 kilo Dalton, based on membrane cut off experiment.

Metal binding and catalyst stoichiometry: The pyridine- and acrylamide-containing copolymer family has been previously determined to bind various metal ions; however, detailed investigation of the coordination chemistry and catalytic properties was lacking [26] until a recent investigation on Cu^{II} binding and hydrolytic activity of a copolymer of RU = 4Vp$_3$Ac$_1$ [22]. The IR spectrum of the copolymer **P$_9$** exhibits two bands at 3378 and 3180 cm^{-1} which are attributed to overlapping water molecules with N−H (asymmetric and symmetric) of NH$_2$. The band at 1600 cm^{-1} is assigned to β NH$_2$ [27]. $\nu_{(C=O)}$ stretching band is observed at 1670 cm^{-1}, while the $\nu_{(C-H)}$ signal aliphatic band appears at 2931 cm^{-1}[28]. The two bands at 1487 and 1450 cm^{-1} are attributed to $\nu_{(C=N)}$ [29]. The lower intensity of carbonyl band at 1671 cm^{-1} and β NH$_2$ at 1598 cm^{-1} as well as the appearance of NH$_2$ at lower wavenumbers 3334 and 3168 cm^{-1} in the IR spectrum of **P$_9$** in Nujol indicating that the NH$_2$ amide and carbonyl are taking part in hydrogen bonding [30-32].

On comparing the IR spectrum of the Co(II) complex (Co^{II}-**P$_9$**) to that of the copolymer, that data show that the copolymer binds to Co(II) through nitrogen atom of vinyl pyridine and the carbonyl group doesn't participate in the binding. This assumption was confirmed by disappearing one of the $\nu_{(C=N)}$ bands and shifting the other one to higher wavenumber. The bands at 1670 and 1600 cm^{-1} due to $\nu_{(C=O)}$ and β NH$_2$, respectively, remain at the same positions. The position of bands at 3184 and 2928 cm^{-1} attributable to N−H symmetric stretching of NH$_2$ and aliphatic $\nu_{(C-H)}$ [29] remain unchanged. The electronic spectrum of the solid deep blue Co^{II}-P9 dissolved in DMSO on heating displays three bands at 610, 630 (sh) and 665 nm in the visible region. The band at 665 nm is attributed to ^{4}A$_2$ → ^{4}T$_1$ (P) transition and clearly indicates that the geometry around Co(II) is tetrahedral geometry [33]. All these data together with the cobalt content of Co^{II}-**P$_9$** (9.1 wt. %) suggest the binding of cobalt (II) to at most four vinyl pyridine nitrogen atoms.

In order to determine the metal-to-polymer stoichiometry for catalysis, 40 μM RU of the polymer in an aqueous methanol solution (50% methanol in 50 mM HEPES buffer at pH 8.0) was titrated with an aqueous solution of Co^{II} and the catalytic activity of the metal complex toward the oxidation of 1.0 mM catechol in

presence of 10 mM H_2O_2 (the reactivity studies are discussed below) has been checked at pH 8.0 and 25 °C. A gradual increase in activity with Co^{II} addition was observed until two equivalents of Co^{II} was added with respect to [RU] to reach a plateau, indicating a 2:1 stoichiometry for metal binding to each RU necessary for catalysis. This activity profile can be best fitted to the Hill equation (Eq. 1) [34] with a Hill coefficient of $\theta = 1.93$ and $r^2 = 0.99$ (Fig. 2, solid trace), but not to a quadratic equation for 2:1 binding mode ($r^2 = 0.97$; Fig. 2, dashed trace). The affinity constant for the binding of Co^{II} to the copolymer is calculated to be $(1.79 \pm 0.7) \times 10^5$ M^{-1}. This observation reveals an interesting metal binding property of the polymer wherein *cooperativity in activity is induced* upon metal binding. Cooperativity is a unique property of a number of proteins and enzymes, such as hemoglobin, which is not usually observed in simple chemical systems. This observation suggests that metallopolymers may serve as good functional models for metalloproteins and metalloenzymes, despite the heterogeneous nature of copolymers in their composition and structure.

Oxidation of catechol: Oxidation of catechol to produce quinone is a two-electron process. The reaction can be followed by monitoring the absorbance of the red adduct formed by *o*-quinone with MBTH at 500 nm ($\varepsilon = 3.25 \times 10^4$ M^{-1} cm^{-1}) [25]. To ensure that the activity is due to the metallopoymer, the copolymer and cobalt(II) ion have been used as controls in the oxidation of the catechol. The copolymer (**P₉**) showed a negligible activity toward the oxidation of catechol, while the cobalt (II) ion showed activity five times lower than that of the metallopolymer. In order to gain further information about the mechanism of the oxidative activity of the Co^{II}-**P₉** and its interaction with H_2O_2, catechol has been used as a substrate to provide detailed kinetic information. The oxidation rates of catechol by 20 µM Co^{II}-**P₉** in term of [Co^{II}] at different concentrations of catechol have been determined in the presence of different amounts of H_2O_2. The rate of catechol oxidation is found to be nonlinear, reaching a plateau at high catechol concentrations which suggest an enzyme-like pre-equilibrium kinetics. This kinetics can be described as the binding of catechol with the catalyst Co^{II}-**P₉** to form a transient substrate-catalyst complex, followed by the conversion of the bound substrate into products (Eq. 2). The rate law for this reaction can be obtained with steady-state approximation similar to the Michaelis-Menten kinetics in enzyme catalysis. The rate law for this reaction mechanism can be expressed as in (Eq. 3), wherein $K' = (k_{-1} + k_{cat}) / k_1$ is the dissociation constant of the (Co^{II}-**P₉**)-S complex similar to the Michaelis constant in enzyme kinetics. The reaction in the presence of saturation amount of H_2O_2 (7.5 mM) produces a first-order rate constant $k_{cat} = 0.026 \pm 0.03$ s^{-1} ($t_{1/2} = 26.6$

s), The apparent dissociation constant K′ = 1.9 ± 0.6 mM and a significant catalytic efficiency k_{cat}/K′ = 13.7 $M^{-1}s^{-1}$ as the second order rate constant (Fig. 3), While k_{cat} value of 4.62×10^{-3} s^{-1} is obtained in the absence of H_2O_2. The catalysis shows 5.5 $\times 10^4$ times rate increase in terms of the first-order rate constant (k_{cat}/k_o, wherein k_o = 4.74×10^{-7} s^{-1} is the rate constant for the uncatalyzed reaction) this catalyst shows only 5.5 times lower than what has been reported with Cu^{II} β-amyloid [35].

The oxidation of catechol as a function of H_2O_2 also shows a saturation pattern at high concentrations, indicating direct binding of this oxidant to the active metal center. Therefore, both catechol and H_2O_2 are considered to be substrates. For a bi-substrate binding mechanism, the binding of two substrates (catechol and H_2O_2) to the active center of Co^{II}-**P₉** can be described in terms of the Hanes equation (Eq. 4) [36]. Hanes analysis was used to calculate the apparent and intrinsic dissociation constants (Fig. 4). It is important to determine the rates at varying amounts of H_2O_2 with a fixed concentration of catechol and *vice versa*. Figures 5A and 5B show the conversion of the plot in Figure 4 to a secondary plot of the slope (1/V_{max}) and y–intercept (K_{app}/V_{max}) as a function of 1/[H_2O_2], affording K′$_{(cat)}$ = 2.73 mM, $K_{i(cat)}$ = 2.58 mM, K′ $_{(H2O2)}$ = 4.30 mM, and $K_{i(H2O2)}$ = 3.23 mM. The K′/K_i ratio is 1.06 for catechol and 1.33 for H_2O_2 which indicate the bindings of these two substrates are not mutually exclusive and the presence of only a small cooperativity between the two substrates [37].
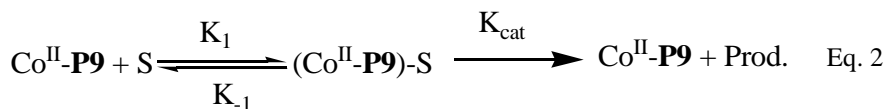
To investigate the efficiency of the catalyst Co^{II}-**P₉** on the oxidation of less activated catechols, 4,5-dichlorochatechol (DCC) has been used as a substrate. The rate of oxidation of various amounts of DCC by 20 μM Co^{II}-**P₉** in terms of [Co^{II}] in the absence of H_2O_2 reaches a plateau (Fig. 6) indicating pre-equilibrium mechanism, which yields a quite significant first-order rate constant k_{cat} = (3.1 ± 0.2) $\times 10^{-4}$ s^{-1} and K′ = 0.70 ± 0.09 mM. The oxidation of DCC in the presence of 7.5 mM H_2O_2 affords k_{cat} = (1.03 ± 0.03) $\times 10^{-2}$ s^{-1} and K′ = 2.1 ± 0.8 mM.

Conclusion

The cobalt(II) complex of a simple pyridine-and amide-containing copolymer (**P₉**) was prepared and characterized by means of IR and ^{1}H-NMR. The Co^{II}-**P₉** complex shows a significant oxidation activity towards catechol oxidation. The oxidation of catechol by Co^{II}-**P₉** in the presence of H_2O_2 exhibits enzyme-like kinetics, yielding a significant first-order rate constant k_{cat} = 0.026 ± 0.30 s^{-1}, dissociation constant K′ = 1.9 ± 0.6 mM and k_{cat} /K′ = 13.7 $M^{-1}s^{-1}$. The much less activated catechol compound 4,5-dichlorocatechol can also be oxidized by Co^{II}-**P₉** in absence and presence of H_2O_2 to afford rate constants k_{cat} = 3.1 $\times 10^{-4}$

and $1.03 \times 10^{-2} \, s^{-1}$ respectively. The study presented herein has introduced a simple functional model system of catechol oxidase-like enzyme to explore more information about the mechanism of the oxidation chemistry of catechol and analogues.

$$\frac{V_o}{V_{max}} = \frac{[Co - P_9]^\theta}{K_x + [Co - P_9]^\theta} \qquad \text{Eq. 1}$$

$$Co^{II}\text{-}\mathbf{P9} + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} (Co^{II}\text{-}\mathbf{P9})\text{-}S \xrightarrow{K_{cat}} Co^{II}\text{-}\mathbf{P9} + Prod. \qquad \text{Eq. 2}$$

$$rate = \frac{K_{cat}\,[Co^{II}\text{-}\mathbf{P9}]}{K' + [S]} \qquad \text{Eq. 3}$$

$$\frac{[Cat]}{V_o} = \frac{(1 + (K'_{(H_2O_2)}/[H_2O_2])}{V_{max}}[Cat] + \frac{K'_{cat}}{V_{max}}(1 + \frac{K_{i(H_2O_2)}}{[H_2O_2]}) \qquad \text{Eq. 4}$$
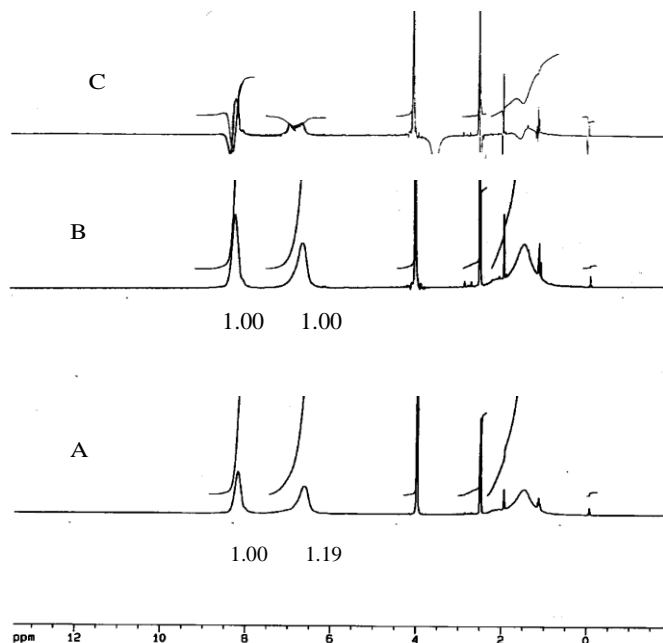

Fig. 1 (A); The ^{1}H-NMR signals of the pyridine ring and the amide NH$_2$ protons of the copolymer in DMSO. (B)The copolymer dissolved in DMSO in addition to a few drops of D$_2$O, where the NH$_2$ signals are disappeared. (C) The change can be clearly seen in the difference spectrum which reveals the NH$_2$ signals.
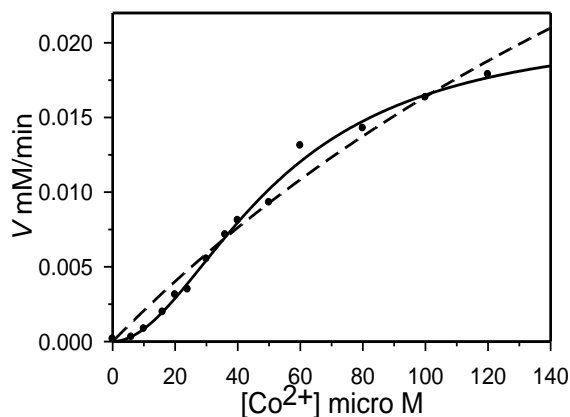
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Fig. 2; Co(II) titration to 40 μM P$_9$ and monitored with the initial rate of the oxidation of 1.0 mM catechol in 50% MeOH/buffer solution (HEPES at pH 8.0) at 25 °C. The solid trace is the best fitting to the Hill equation and dashed trace is the best fit to a quadratic equation.
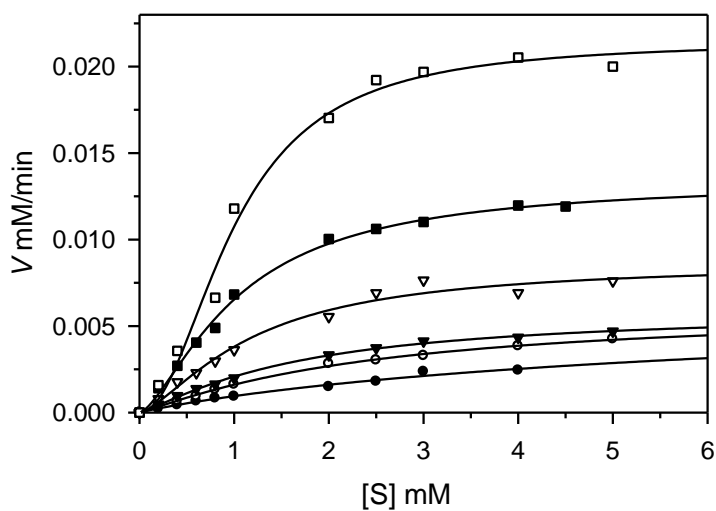

Fig: 3; Initial rate of catechol oxidation by 20 μM Co-P$_9$ in 50% methanol/HEPES buffer solution at pH 8.0 and 25 °C in the presence of zero (●), 0.5 (○), 1.0 (▼), 2.5(▽), 5.0 (■), and 7.5 (□) mM H$_2$O$_2$. The data were fitted to the pre-equilibrium rate law. There seems to have slight cooperativity in the presence of a high concentration of H$_2$O$_2$, which can be fitted to the Hill equation as well.
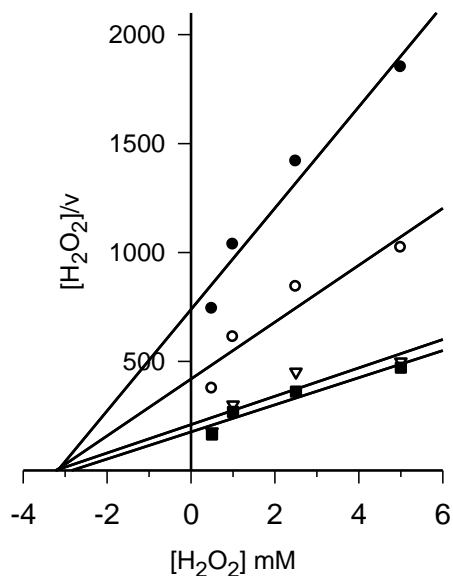
Fig. 4; Hanes plots of the data from Fig. 2 in 50% methanol/ HEPES buffer solution at pH 8.0 and 25 °C using 20 μM of Co^{II}-P9.
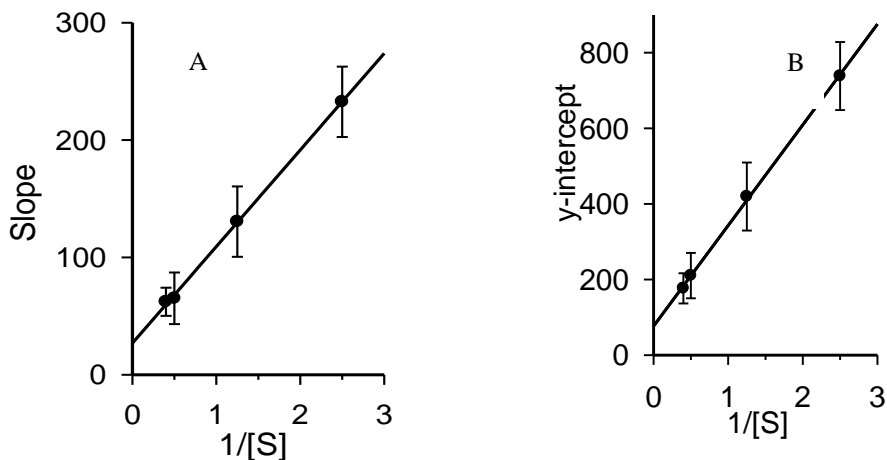

Fig. 5 (A) Replotting the slope obtained from Fig 4 versus 1/[H$_2$O$_2$] reveals true dissociation constants for catechol and H$_2$O$_2$ in a bi-substrate reaction. (B) Repotting the y-intercept obtained from Fig 4 versus 1/[H$_2$O$_2$] reveals true dissociation constants for catechol and H$_2$O$_2$ in a bi-substrate reaction.
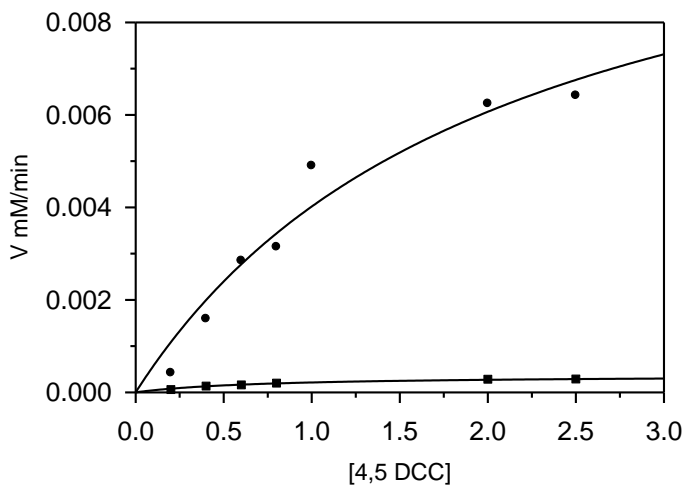
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Fig. 6; initial rate of oxidation of 4,5 dichlorocatechol using 20 μM Co-P9 without H$_2$O$_2$ (■), and with 7.5 mM H$_2$O$_2$ (●). All data were fitted to the pre-equilibrium rate law.

Reference

1. (a) J. HAGGIN, Chem. Eng. News 71 (1993) 23.

    (b) L.I. SIMA´NDI, Catalytic Activation of Dioxygen by Metal Complexes, Kluwer, Dordrecht, 1992.

2. M. TRE´MOLIE`RES, J.B. BIETH, Photochemistry, 23 (1984) 501.

3. D. MEIWES, B. ROSS, M. KIESSHAUER, K. CAMMANN, H. WITZEL, M. KNOLL, M. BORCHARDT, C. Sandermaier, Lab. Med. 15 (1992) 24.

4. F. ZIPPEL, F. AHLERS, R. WERNER, W. HAASE, H.-F. NOLTING, B. KREBS, Inorg. Chem., 35 (1996) 3409.

5. J. REIM, R. WERNER, W. HAASE, B. KREBS, Chem. Eur. J. 4 (1998) 289.

6. J. REIM, B. KREBS, J. Chem. Soc., Dalton Trans. (1997) 3793.

7. M. R. MALACHOWSKI, M.G. DAVIDSON, Inorg. Chim. Acta 162 (1989) 199.

8. K. D. KARLIN, Y. GULTNEH, T. NICHOLSON, J. Zubieta, Inorg. Chem. 24 (1985) 3727.

9. M. R. MALACHOWSKI, M. G. DAVIDSON, J. N. HOFFMAN, Inorg. Chim. Acta 157 (1989) 91.

10. E. V. RYBAK-AKIMOVA, D. H. BUSCH, P. K. KAHOL, N. PINTO, N. W. ALCOCK, H. J. Clase, Inorg. Chem. 36 (1997) 510–520.

11. J. F. LARROW, E. N. JACOBSEN, Y. GAO, Y. P. Hong, X. Y. Nie, C. M. Zepp, J. Org. Chem. 59 (1994) 1939–1942.

12. T. PUNNIYAMURTH, S. VELUSAMY, J. Igbal, Chem. Rev. 105 (2005) 2329–2364.

13. H. OKAWA, H. SAKIYAMA, Pure Appl. Chem. 67 (1995) 273.

14. R. BECHARA, D. BALLOY, J.Y. DAUPHIN, J. Grimblot, Chem. Mater. 11 1999) 1703.

15. L. JI, J. LIN, H. C. ZENG, J. Phys. Chem. B 104 (2000) 1783.

16. T. TSUMAKI, Bull. Chem. Soc. Jpn. 13 (1938) 252–260.

17. D. H. BUSCH, N. W. ALCOCK, Chem. Rev. 94 (1994) 585–623.

18. R. D. JONES, D. A. SUMMERVILLE, F. BASOLO, Chem. Rev. 79 (1979) 139–179.

19. D. CHEN, A. E. Martell, Inorg. Chem. 26 (1987) 1026–1030.

20. D. CHEN, A. E. MARTELL, Y. SUN, Inorg. Chem. 28 (1989) 2647–2652.

21. B. SUN, J. R. CHEN, J. Y. HU, X. J. LI, Chin. Chem. Lett. 12 (2001) 1043–1046.

22. A. I. HANAFY, V. LYKOURINOU, K. S. BISHT, L. J. Ming, Inorg. Chim. Acta 358 (2005) 1247-1252.

23. V. LYKOURINOU, A. I. HANAFY, G. F. Z. DA SILVA, K. S. BISHT, R. W. LARSEN, B. T. LIVINGSTON, A. ANGERHOFER, L. J. MING, Eur. J. Inorg. Chem. (2008), 2584–2592.

24. K. E. GECKLER, N. ARSALANI, J. MACROMOL. Sci., Pure Appli. Chem. A33 (1996) 1165–1179.

25. (a) G. F. Z. da SILVA, W. M. TAY, L. J. MING, J. Bilo. Chem, 280 (2005) 16601-16609.

    (b) J. C. ESPIN, J. TUDELA, F. Garcia-Canovas, Anal. Biochem., 259 (1998) 118

26. K. E. GECKLER, N. ARSALANI, J. MACROMOL. Sci., Pure Appli. Chem. A33 (1996) 1165–1179.

27. L. J. BELLAMY, The Infrared spectra of Complex Molecules, John Wiley, London, (1966).

28. R. M. SILVERSTEIN, F. X. WEBSTER, Spectrometric Identification of Organic Compounds, John Wiley & Sons, Inc., New York, (1996).

29. W. KEMP, Organic Spectroscopy, The Macmillan Press, London and Basingstoke, (1975).

30. R. BLINCK, D. HADZI, *J. Chem. Soc.,* (1959), 453.

31. K. BURGER, I. RUFF, J. Inorg. Nucl. Chem. 27 (1965) 197.

32. J. I. BULLOCK , H. N. TAJIMIR-RIAHI, J. Chem. Soc. Dalton. Trans. (1978) 34.

33. A.B.P. Lever, Inorganic Electronic Spectroscopy, second ed., Elsevier, Amsterdam, 1984, pp. 496–500.

34. S. G. SRIVATSAN, P. NIGAMB , M. S. RAOB , S. Verma, Appl. Catal. A 209 (2001) 327 – 334.

35. G. F. Z. Da Silva and L. J. Ming, Angew. Chem. Int. Ed. 2005, 44, 5501 –5504

36. Leskovac, Comperhensive Enzyme Kinetics, Kluwer/Plenum, Boston, (2002), p. 119.

37. J. R. FLORINI, C. S. Vestling, Biochem. Biophys. Acta. 25 (1957) 575-578.