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TEMPO-initiated oxidation of 2-aminophenol to 2-aminophenoxazin-3-one

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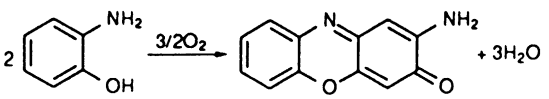
Abstract

The oxidation reaction of 2-aminophenol (OAP) to 2-aminophenoxazin-3-one (APX) initiated by 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) has been investigated in methanol at ambient temperature. The oxidation of OAP was followed by electronic spectroscopy and the rate constants were determined according to the rate law $-\mathrm{d[OAP]}/\mathrm{d}t = k_{\mathrm{obs}}[\mathrm{OAP}][\mathrm{TEMPO}]$. The rate constant, activation enthalpy and entropy at 298 K are as follows: k_{obs} ($\mathrm{dm^3\,mol^{-1}\,s^{-1}}$) $= (1.49 \pm 0.02) \times 10^{-4}$, $E_{\mathrm{a}} = 18 \pm 5\,\mathrm{kJ\,mol^{-1}}$, $\Delta H^{\ddagger} = 15 \pm 4\,\mathrm{kJ\,mol^{-1}}$, $\Delta S^{\ddagger} = -82 \pm 17\,\mathrm{J\,mol^{-1}\,K^{-1}}$. The results of oxidation of OAP show that the formation of 2-aminophenoxyl radical is the key step in the activation process of the substrate.

Keywords: Phenoxazinone synthase; 2-Aminophenol oxidation; Questiomycin A; TEMPO

1. Introduction

The oxidation of aromatic amines in human erythrocytes is very useful for producing phenoxazine compounds via the intervention of human oxyhaemoglobin [1]. One of the main objectives has been the elucidation of the oxidation product of the aromatic amines, which has been shown to be a phenoxazine [2–4] (Eq. (1)). 2-Amino-3*H*-phenoxazin-3-one (APX), also known as questiomycin A, is related to the naturally occurring antineoplastic agent actinomycin D, which acts by inhibiting DNA-directed RNA synthesis [5,6] and is used clinically for the treatment of certain types of cancer [7]. APX has been used as a model for the behaviour of actinomycin D.



(1)

Phenoxazinone synthase catalyses the oxidative coupling of two molecules of substituted 2-aminophenol (OAP) to the phenoxazinone chromophore in the final step at the biosynthesis of actinomycin D. The enzyme phenoxazinone synthase [8,9], a type 2 copper-containing oxidase (subunit molecular mass 88,000, 3.7 Cu per subunit), is naturally found in the bacterium *Streptomyces antibioticus* [10] and has been cloned and overexpressed in *S. lividans* [11].

Actinomycin biosynthesis has generated interest in the conversion of OAP into APX catalysed by

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transition-metal complexes with a view to the possible modelling of phenoxazinone synthase activity. The oxidation of OAP by dioxygen can be catalysed by cobalt(II) salts, cobalt(II)-phthalocyanine derivatives and copper compounds [12–15]. Some tris(oxalato) cobalt complexes are also known to be strong oxidising agents in aqueous solution [16,17]. The kinetics of reduction of tris(oxalato)cobaltate(III) by amines have been investigated [18,19] in aqueous acidic media.

The oxidative condensation mechanism of OAP represents a six-electron oxidative coupling process and appears to take place stoichiometrically in a series of three two-electron oxidations, alternating with conjugate addition of the OAP moiety to *o*-benzoquinone imine type intermediates, followed by tautomerisation steps. Cyclisation occurs with the concomitant reduction of molecular oxygen to water. A sequence of stable intermediates has been established but no free radicals have been detected and no evidence is available to establish whether electron transfer occurs in single electron steps throughout the enzymatic process [20]. Earlier results showed that the cobaloxime-containing biomimic system involves ESR-detectable 2-aminophenoxyl type free radicals as key intermediates, indicating that the enzymatic mechanism proposed earlier may merit reconsideration [15]. In order to disclose a possible radical pathway of the mechanism of these reactions, we carried out studies on free radical-mediated oxidation of OAP.

2. Experimental

2.1. General procedure

All manipulations were carried out under Ar atmosphere by using standard Schlenk technique. Methanol (water content less than 0.1%) and all the materials used in the present work such as OAP and 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) were supplied by the Sigma–Aldrich. Gaseous oxygen from Messergriesheim was 99.6% and passed through P_2O_5 and Blaugel in order to remove traces of water and other impurities. Electronic spectra were measured on a Shimadzu UV-160 spectrometer using quartz cells.

2.2. Synthesis of APX

In a typical reaction, OAP (0.38 g, 3.5 mmol), TEMPO (0.55 g, 3.5 mmol) in methanol were stirred at 50 °C under dioxygen for 10 h. Thereafter diethyl ether was layered on the solution and left at −20 °C for 2 h to afford a red-brown solid material, which was then filtered off and recrystallised from benzene to give APX as red crystals (0.2 g, 54%), m.p. 254–256 °C. This could easily be identified as the same product obtained upon oxidation of OAP by HgO [21].

2.3. Kinetic measurements

Kinetic measurements were carried out at constant partial pressure of dioxygen with vigorous stirring in a thermostated reaction vessel at five temperatures (range (25–50 °C) ± 0.5 °C) equipped with a syringe inlet for taking samples at regular time intervals. The samples were diluted and then submitted to UV–Vis analysis. The concentration of the oxidation product of OAP was monitored spectrophotometrically at 435 nm [12]. Dioxygen uptakes were also measured in a constant pressure gas-volumetric apparatus. The volume of absorbed dioxygen was red periodically using a gas burette. The rate of the oxidation reaction was independent of the stirring rate, excluding eventual diffusion control effects.

3. Results and discussion

We have found that the oxidation of OAP to APX by dioxygen is initiated by TEMPO. The reaction takes place at ambient temperature and 1 bar (105 Pa) O_2 pressure in MeOH. The kinetics of the reaction was followed as a function of time by spectrophotometry at $\lambda_{max} = 435$ nm of APX. OAP has no absorption in the visible range. Experimental conditions are summarised in Table 1. The time sequence of the increase in the absorption band of the oxidation product of OAP initiated by TEMPO at 323 K are shown in Fig. 1 with reaction time of 5 h.

A simple rate law for the oxidation reaction is given in Eq. (2). To determine the rate dependence on the three reactants, oxidation runs were performed at various initial OAP (Table 1; experiments 1–4) and TEMPO concentrations (Table 1; experiments 4–8),

Table 1
Kinetic data for the TEMPO-initiated oxidation of OAP in MeOH

Experiment No.	Temperature (°C)	10^2[OAP] (mol dm^{-3})[a]	10^2[TEMPO] (mol dm^{-3})[a]	$10^6 k'$ (s^{-1})	$10^4 k_{obs}$ (mol^{-1} dm^3 s^{-1})	-10^7 d[OAP]/dt (mol dm^{-3} s^{-1})
1	25	5.00	3.00	4.49	1.49 ± 0.02	2.25
2	25	7.00	3.00	4.32	1.44 ± 0.04	3.03
3	25	9.00	3.00	4.53	1.51 ± 0.05	4.08
4	25	11.00	3.00	4.30	1.43 ± 0.08	4.73
5	25	11.00	0.50	0.89	1.78 ± 0.07	0.98
6	25	11.00	1.00	1.43	1.43 ± 0.04	1.58
7	25	11.00	2.00	2.98	1.49 ± 0.05	3.28
8	25	11.00	4.00	5.78	1.45 ± 0.07	6.36
9	25	11.00	3.00	5.48	1.52 ± 0.06[b] *1.49 ± 0.02*[c]	6.03
10	35	11.00	3.00	5.51	1.83 ± 0.07	
11	40	11.00	3.00	5.99	2.00 ± 0.08	
12	45	11.00	3.00	6.77	2.26 ± 0.11	
13	50	11.00	3.00	7.42	2.47 ± 0.13	

[a] In 40 cm^3 MeOH.
[b] Under air.
[c] Mean value of the kinetic constant k_{obs} and its standard deviations $\sigma(k_{obs})$ were calculated as $k_{obs} = \left(\sum_i w_i k_i / \sum_i w_i\right)$ and $\sigma(k_{obs}) = \left(\sum_i w_i (k_i - k_{obs})^2/(n-1)\sum_i w_i\right)^{1/2}$, where $w_i = 1/\sigma_i^2$.

furthermore at different dioxygen pressures (Table 1; experiments 4,9) under pseudo-first-order conditions.

$$-\frac{\mathrm{d[OAP]}}{\mathrm{d}t} = \frac{\mathrm{d2[APX]}}{\mathrm{d}t} = k[\mathrm{OAP}]^m[\mathrm{TEMPO}]^n[\mathrm{O_2}]^q \quad (2)$$

Eq. (2) can be simplified to Eq. (3) under pseudo-first-order conditions (constant TEMPO and O_2 concentrations), where $k' = k[\mathrm{TEMPO}]^n[\mathrm{O_2}]^q$.

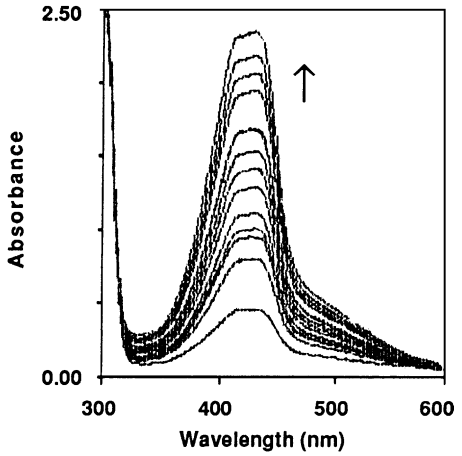


Fig. 1. Time sequence of the increase in the absorption band of APX at the TEMPO-initiated reaction during experiment 13 in Table 1.

$$-\frac{\mathrm{d[OAP]}}{\mathrm{d}t} = \frac{\mathrm{d2[APX]}}{\mathrm{d}t} = k'[\mathrm{OAP}]^m \quad (3)$$

Measuring the time dependence of the change of concentration of APX during the oxidation shows that plots of log(OAP) vs time were linear in experiments 1–4, indicating that the reaction is first-order with respect to substrate concentration. From variations of the reaction rates, plots of $-$d[OAP]/dt vs the initial OAP concentration $[\mathrm{OAP}]_0$ (Fig. 2) were also linear in experiments 1–4 with a correlation coefficient of 99.89%, reinforcing that the reaction is indeed first-order with respect to substrate concentration. This means that $m = 1$.

Kinetic measurements of the reaction rate with respect to TEMPO concentration (Table 1; experiments 4–8) indicated a first-order dependence ($n = 1$). Plots of k' vs $[\mathrm{TEMPO}]_0$ (Fig. 3) for the above five experiments gave a straight line with a correlation coefficient of 99.95%.

Experiments made in an atmosphere of O_2 or air (Table 1; experiments 4, 9) showed that the reaction rate is independent of the dioxygen concentration. Plots of dioxygen uptake vs time (Fig. 4) for the oxidation of OAP was linear, confirming also that

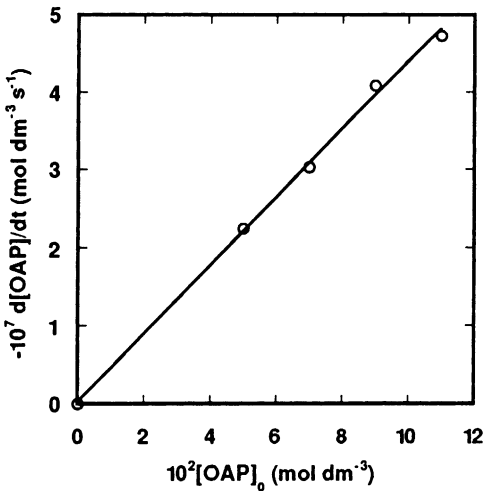


Fig. 2. Plot of oxidation rate of OAP vs. its initial concentration (experiments 1–4, Table 1).

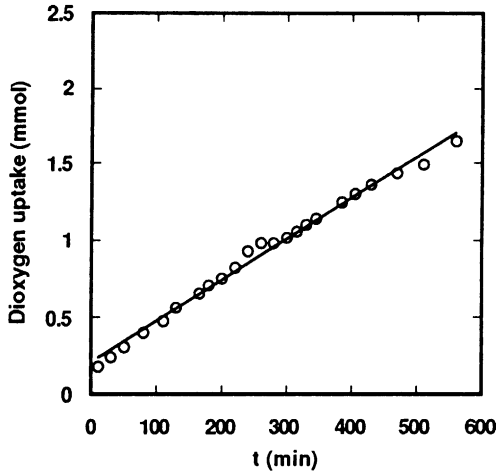


Fig. 4. Typical dioxygen uptake curve for the TEMPO-initiated oxidation of OAP. $[OAP]_0 = [TEMPO]_0 = 3.5 \times 10^{-2}$ mmol; $T = 323$ K; solvent MeOH.

the reaction is indeed zero-order with respect to the dioxygen concentration ($q = 0$).

According to the kinetic data obtained, the TEMPO-initiated oxidation of OAP by dioxygen obeys an overall second-order rate equation with $m = n = 1$, $q = 0$ in Eq. (3), from which the mean value of the kinetic constant k_{obs} of $(1.49 \pm 0.02) \times 10^4\,mol^{-1}\,dm^3\,s^{-1}$ at 298 K was obtained (Table 1). The activation parameters for the oxidation reaction were determined from the temperature dependence of the kinetic constant k_{obs}. The activation energy (E_a) was deduced from the Arrhenius plot and the other activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were calculated from Eyring's equation. The temperature-dependent reaction rate measurements in the range 298–323 K (Table 1; experiments 1–13) resulted in a straight line in the Eyring plot with a correlation coefficient of 99.88% (Fig. 5) and activation parameters $E_a = 18 \pm 5\,kJ\,mol^{-1}$, $\Delta H^{\ddagger} = 15 \pm 4\,kJ\,mol^{-1}$, $\Delta S^{\ddagger} = -82 \pm 17\,J\,mol^{-1}\,K^{-1}$, respectively. Though activation parameters are often not the discriminating factors in recognising the reaction pathway, the negative entropy of activation in this reaction, however, clearly indicates an associative reaction mode in the rate-determining step.

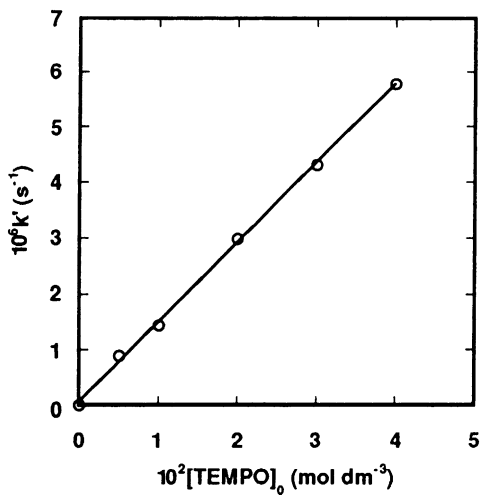


Fig. 3. Plot of pseudo-first-order reaction rate constant (k') vs. the initial TEMPO concentration for the oxidation of OAP (experiments 4–8, Table 1).

A reaction mechanism that fits the chemical, spectroscopic, kinetic and thermodynamic data is shown in Scheme 1. OAP and its derivatives are assumed to be reactive substrates in the enzyme reaction. It is also known that OAP in an atmosphere of air undergoes slow oxidation to APX in acetonitrile or methanol at room temperature. The autoxidation of OAP in acetonitrile under air was studied and the rate low $-d[OAP]/dt = k_{obs}\,[OAP][O_2]$ ($k_{obs} = (1.46 \pm 0.03) \times 10^{-8}\,mol^{-1}\,dm^3\,s^{-1}$ at room temperature) was obtained [12], indicating that the uncatalysed reaction is more slower than the catalytic

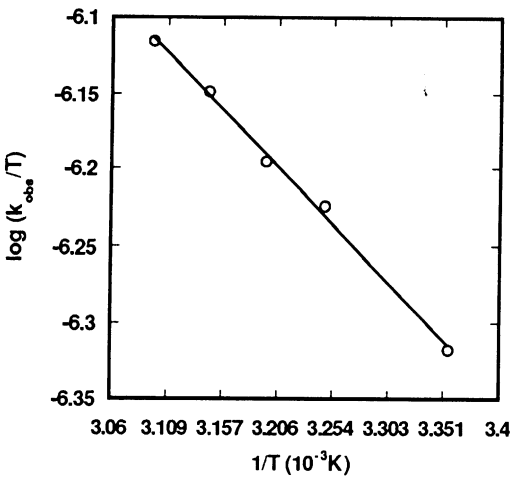
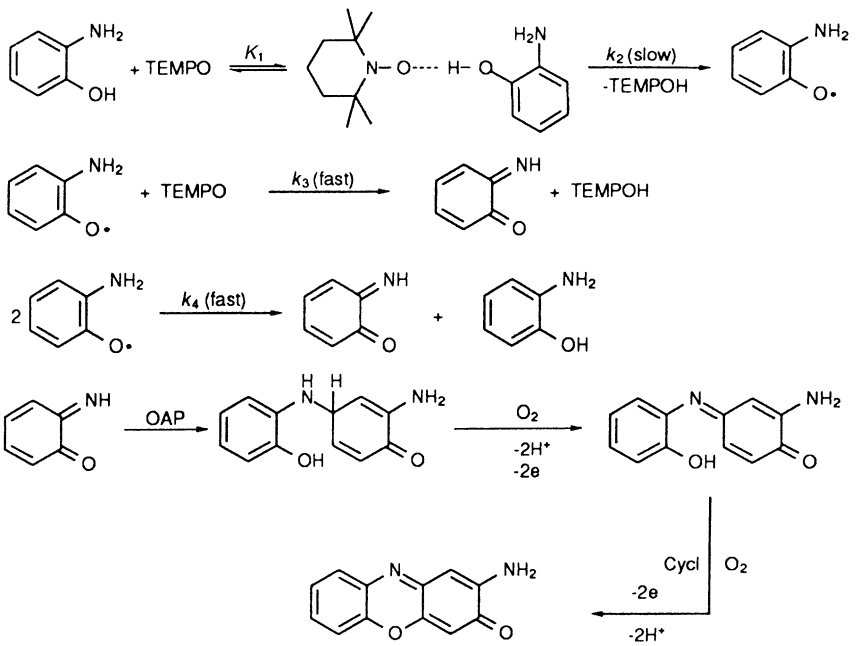


Fig. 5. Eyring plot for the oxidation of OAP (Table 1).

reaction. The low value for the rate constant can be explained by the fact that the substrate itself does not have a sufficiently negative redox potential to react directly with dioxygen at acceptable rates.

We believe that in the presence of the free radical TEMPO, the substrate OAP is hydrogen bonded via its OH group to the nitroxyl oxygen in a rapid pre-equilibrium (k_1/k_{-1} being small). Intramolecular H-atom transfer from the OH group of OAP to TEMPO in the hydrogen-bonded adduct is the rate-determining step (k_2 is slow), generating the 2-aminophenoxyl radical, which is capable of closing the gap in redox potential, thereby sustaining the catalytic cycle. After that the 2-aminophenoxyl radical may react in a radical–radical reaction with subsequent TEMPO (k_3, fast) or disproportionate (k_4, fast) leading to the key intermediate *o*-benzoquinone monoimine. Its further conversion into the product APX can be rationalised by the reactions described in Ref. [17] and showed in Scheme 1. The overall reaction requires several oxidative dehydrogenation steps involving OAP, dioxygen and *o*-benzoquinone monoimine as reactants on the way to APX.

Applying steady-state treatment for this mechanism, $d[OAP–TEMPO]/dt = 0$, the rate equation (4) can be deduced, which after some simplification (k_2/k_{-1} and K_1[TEMPO] being much smaller than 1) is in good agreement with an overall second-order rate



Scheme 1.

expression according to the experimental data obtained in the kinetic measurements.

$$-\frac{\mathrm{d[OAP]}}{\mathrm{d}t} = \frac{K_1 k_2 [\mathrm{OAP}][\mathrm{TEMPO}]}{(k_2/k_{-1}) + 1 + K_1[\mathrm{TEMPO}]} = k_{\mathrm{obs}}[\mathrm{OAP}][\mathrm{TEMPO}] \quad (4)$$

The results outlined above suggest clearly that the oxidation of OAP via a radical reaction pathway is feasible, and cannot be excluded from the list of possible ways of OAP oxidation including the enzymatic reaction as well.

Acknowledgements

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References

[1] A. Tomoda, J. Yamaguchi, H. Kojima, H. Amemiya, Y. Yoneyama, FEBS Lett. 196 (1986) 44.
[2] L.I. Simandi, Int. Rev. Phys. Chem. 8 (1989) 21.
[3] L.I. Simandi, T. Barna, S. Nemeth, J. Chem. Soc., Dalton Trans. (1996) 473.
[4] P.W. Iseminger, M. Gregory, J.R. Timothy, G. Caple, A.G. Sykes, J. Org. Chem. 62 (1997) 2643.
[5] M. Homma, A.F. Graham, Biochim. Biophys. Acta 61 (1962) 642.
[6] H. Nakazawa, F.E. Chou, P.A. Andrews, N.R. Bachur, J. Org. Chem. 46 (1981) 1493.
[7] E. Frei, Cancer Chemother. Rep. 58 (1974) 49.
[8] E. Katz, H. Weissbach, J. Biol. Chem. 237 (1962) 882.
[9] C.E. Barry, P.G. Nayar, T.P. Begley, J. Am. Chem. Soc. 110 (1988) 3333.
[10] C.E. Barry, P.G. Nayar, T.P. Begley, Biochemistry 28 (1988) 6323.
[11] G.H. Jones, D.A. Hopwood, J. Biol. Chem. 259 (1984) 14151.
[12] Z. Szeverenyi, E.R. Milaeva, L.I. Simandi, J. Mol. Catal. 67 (1991) 251.
[13] Z. Szeverenyi, E.R. Milaeva, L.I. Simandi, Stud. Surf. Catal. 99 (1991) 616.
[14] A. Tomoda, J. Yamayuchi, H. Amemiya, Y. Yoneyama, FEBS Lett. 34 (1993) 717.
[15] L.I. Simandi, T.M. Barna, L. Korecz, A. Rockenbauer, Tetrahedron Lett. 34 (1993) 717.
[16] K.V. Krishnamurty, G.M. Harris, Chem. Rev. 61 (1961) 213.
[17] A.B. Zaki, M.Y. El-Sheikh, J. Evans, S.A. El-Safty, Polyhedron 19 (2000) 1317.
[18] A.K. Basak, D. Banerjea, Indian J. Chem. Soc. 54 (1977) 1015.
[19] A.K. Basak, D. Banerjea, Indian J. Chem. 17A (1979) 250.
[20] Y. Yano, M. Ikuta, Y. Amamiya, T. Nabeshima, Chem. Lett. (1991) 461.
[21] G.W.K. Cavill, Tetrahedron 12 (1961) 139.