Kinetic Studies on the Oxidation of Thiols by Coenzyme PQQ

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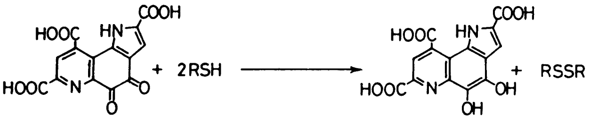
Synopsis. Kinetic studies on the oxidation of thiols by coenzyme PQQ are carried out under anaerobic conditions. A bell shaped pH-rate profile having a maximum rate at around pK_a of the thiol is observed. The rate-determining step changes between acidic and basic sides of the profile indicating the existence of at least one intermediate in the course of the reaction.

Mercapto groups are important in biological systems due to the widespread occurrence of this functional group in many proteins and in natural compounds such as glutathione, cysteine, coenzyme A, and lipoic acid. In particular, reversible oxidation-reduction to the disulfide stage is an essential process in such systems, and thus mechanistic details on the oxidation of thiols by flavins have been demonstrated.[1,2] On the other hand, we have found that coenzyme PQQ (4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]-quinoline-2,7,9-tricarboxylic acid) is an efficient catalyst in air oxidation of thiols.[3] In this paper, we wish to report the kinetic studies on the reaction between PQQ and thiols.[4]

Results and Discussion

Treatment of PQQ (3.4×10^{-2} mmol) and 10-fold excess of benzenethiol in 20% CH_3CN neutral aqueous solution under anaerobic conditions gave $PQQH_2$ (4,5-dihydroxy-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid, 84%) and diphenyl disulfide (99% based on PQQ) as shown in Eq. 1. This reaction was followed spectrophotometrically under pseudo-first-order conditions ([PQQ] ≪ [PhSH], Fig. 1). PQQ was reduced rapidly to $PQQH_2$ with isosbestic points at 274, 336, and 399 nm at pH* 4.65 (see Experimental), and the reaction followed pseudo-first-order rate law up to 90% conversion (Fig. 2). When the concentration of the thiol was kept constant and the pH* was varied using 0.05 M acetate, phosphate, and carbonate (containing 20% CH_3CN, $\mu=0.2$ with KCl), it was found that a plot of log k_{obsd} vs. pH* exhibited a "bell-shaped curve" (Fig. 3). Interestingly, the reaction was found to be first-order in benzenethiol concentration at the acidic side of the bell-shaped curve (pH* 6.3), while it was second-order in benzenethiol concentration at the basic conditions (pH* 9.9) as shown in Fig. 4. This result shows an evidence for a change in rate-determining step between the acidic and the basic conditions. This implies the existence of at least one intermediate, and all one-step mechanisms such as a net hydride transfer may be ruled out.



PQQ PQQH$_2$

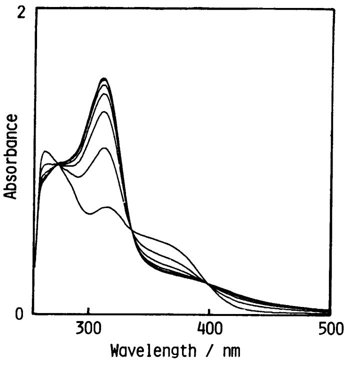


Fig. 1. Sepctral change along the progress of the oxidation of PhSH (4.0×10^{-3} M) by PQQ (4.0×10^{-5} M) in 0.05 M acetate buffer (pH* 4.65, $\mu=0.2$ with KCl, 20% CH_3CN) at 30 °C under anaerobic conditions.

From these results, we assume the following reaction path (Eq. 2).

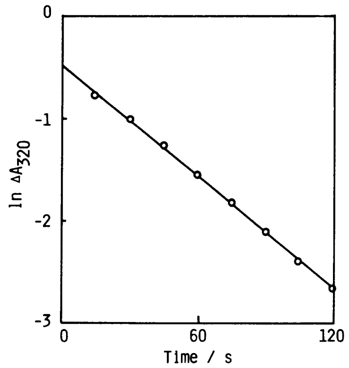


Fig. 2. Pseudo-first-order plot for the oxidation of PhSH by PQQ at pH* 4.65.

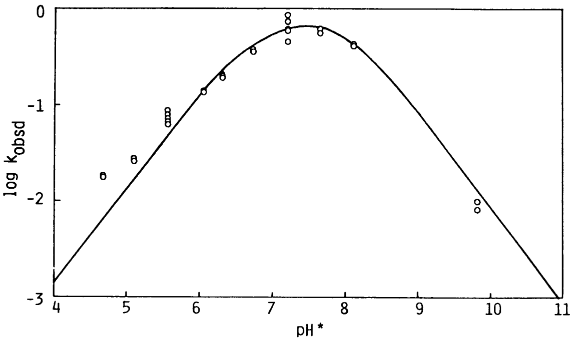


Fig. 3. The pH-rate profile for the oxidation of PhSH by PQQ.

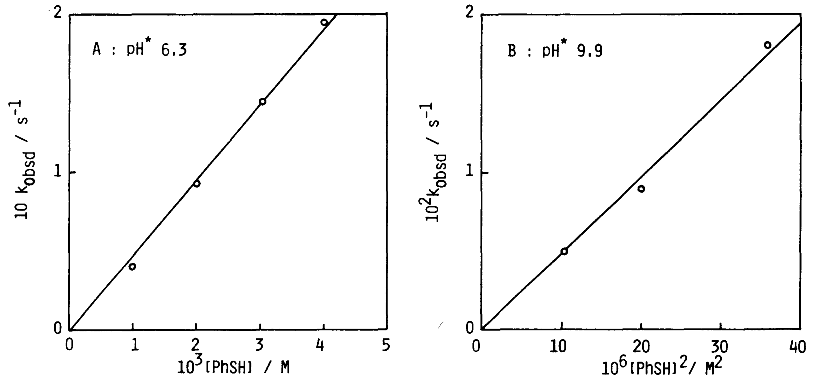


Fig. 4. Dependence of k_{obsd} on benzenethiol concentration at pH* 6.3 (A) and 9.9 (B).

$$\mathrm{PQQ} \underset{k_{-1}}{\overset{k_1[\mathrm{RS}^-]}{\rightleftharpoons}} \mathrm{I}^- \underset{k_{-\mathrm{H}}}{\overset{k_\mathrm{H}[\mathrm{H}^+]}{\rightleftharpoons}} \mathrm{IH} \xrightarrow{k_2[\mathrm{RS}^-]} \mathrm{PQQH}^- + \mathrm{RSSR} \tag{2}$$

(I: intermediate)

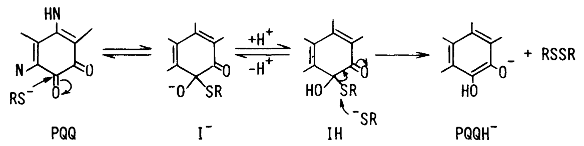
If it is assumed that protonation of I^- occurs rapidly relative to the establishment of the PQQ-I^- equilibrium ($k_H[H^+] \gg k_{-1}$), the rate law can be given as

$$k_{obsd} = \frac{k_1 k_2 K_a{}^2 a_H [RSH]_T{}^2}{k_{-1} K_H (K_a + a_H)^2 + k_2 K_a a_H (K_a + a_H)[RSH]_T} \tag{3}$$

where K_a is the apparent dissociation constant of benzenethiol and K_H is k_{-H}/k_H. Curve-fitting of Eq. 3 (solid line in Fig. 3) by using pK_a value (6.9) determined by spectrophotometric titration under the kinetic conditions provides the following values; $k_1 = 325\ M^{-1}\ s^{-1}$, $k_2/k_{-1}K_H = 1.6\times10^{10}\ M^{-2}$. Using these values, kinetic behaviors mentioned above can be explained with this equation. Namely, under acidic conditions ($K_a \ll a_H$), Eq. 3 can be simplified to Eq. 4 in which k_{obsd} is proportional to $[RSH]_T$ and $a_H{}^{-1}$, respectively. On the other hand, under basic conditions ($K_a \gg a_H$), Eq. 5 is an approximated form where k_{obsd} is proportional to $[RSH]_T^2$ and a_H, respectively. Aliphatic thiols such as 2-mercaptoethanol, cysteine, and 1,4-butanedithiol were also oxidized rapidly at around their pK_as as shown in Table.

$$k_{obsd} = k_1 a_H{}^{-1} K_a [RSH]_T \tag{4}$$

$$k_{obsd} = k_1 k_2 k_{-1}{}^{-1} K_H{}^{-1} a_H [RSH]_T{}^2 \tag{5}$$



Scheme

From buffer dilution studies (0.1—0.5 M at pH* 6.3 and 9.9), it could be concluded that the oxidation of benzenethiol by PQQ is not subject to buffer catalysis. One of the possible mechanism is that involving C-5 attack of the thiolate ion followed by breakdown to reduced PQQ and the corresponding disulfide as shown in Scheme. In this mechanism, the first thiolate ion attack to the quinone is the rate-determining step under the acidic conditions, while the breakdown step is rate-determining under the basic conditions. It is well-known that C-5 quinone carbonyl of PQQ is easily attacked by nucleophiles.[5] Jencks and his co-worker have demonstrated that the addition of a thiolate ion to carbonyl group shows no general acid catalysis.[6] So there may be no general acid catalysis in the first thiolate ion attack to the quinone carbonyl. Further, the acid-base dissociation constant of the quinol group of $PQQH_2$ is determined to be 7.08×10^{-10} M (pK_a 9.15) by spectrophotometric titration. Thus, the breakdown step would not also be subject to general acid catalysis.

Table 1. Pseudo-First-Order Rate Constants for the Oxidation of Thiols by Coenzyme PQQ[a)]

Thiol	pK_a	pH*	k_{obsd}/s^{-1} [b)]
PhSH	6.9	7.2	6.5×10^{-1}
$HOCH_2CH_2SH$	9.7	9.8	8.1×10^{-2}
$HS(CH_2)_4SH$[c)]	10.3	9.1	3.3×10^{-1}
$HOOCCH(NH_2)CH_2SH$	8.5	8.1	3.4×10^{-2}

a) [PQQ]$=4.0\times10^{-5}$ M, [thiol]$=4.0\times10^{-3}$ M, 0.05 M buffer solution ($\mu=0.2$ with KCl, containing 20% CH_3CN) at 30 °C under anaerobic conditions. b) Average values of 3—5 kinetic runs. c) In 40% CH_3CN buffer solution.

Alternatively, free radical mechanism (one-electron transfer; $I^- = PQQ^{\overline{\cdot}}$) may be applicable since stepwise one electron oxidation of thiols is well documented.[7] In that case, however, the reactions at all pH region investigated should be first-order in benzenethiol concentration, since the PQQ semiquinone radical is well-known to be unstable to disproportionate rapidly to PQQ and $PQQH_2$ under these aqueous conditions.[8] If the PQQ semiquinone radical should be comparably stable, kinetics indicative of autocatalytic process would be observed. From these considerations, the ionic mechanism (Scheme) is preferable for the present oxidation.

Experimental

Kinetics. Kinetic measurements for the oxidation of thiols by PQQ were carried out under anaerobic pseudo-first-order conditions. An anaerobic 20% acetonitrile aqueous solution (1.5 ml) containing a thiol (8.0×10^{-3} M) was mixed with 1.5 ml of a 20% acetonitrile buffer solution (0.05 M acetate, phosphate, and carbonate, μ=0.2 with KCl) containing PQQ (8.0×10^{-5} M) under anaerobic conditions in a Thunberg cuvette. The reaction was followed by monitoring the appearance of $PQQH_2$ at 320 nm at 30 °C (Shimadzu UV-240 spectrophotometer equipped with a temperature controlled cell holder, Shimadzu TCC-240). The pH* is a corrected pH value by adding 0.11 to measured pH value in an aqueous buffer solution containing 20% CH_3CN.[9]

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References

1) I. Yokoe and T. C. Bruice, *J. Am. Chem. Soc.*, **97**, 450 (1975).

2) E. L. Loechler and T. C. Hollocher, *J. Am. Chem. Soc.*, **102**, 7312, 7322, 7328 (1980).

3) S. Itoh, N. Kato, Y. Ohshiro, and T. Agawa, *Chem. Lett.* **1985**, 135.

4) Reoxidation of reduced PQQ by molecular oxygen has already been investigated; S. Itoh, Y. Ohshiro, and T. Agawa, *Bull. Chem. Soc., Jpn*, **59**, 1911 (1986).

5) R. H. Dekker, J. A. Duine, J. Frank, P. E. J. Verwiel, and J. Westerling, *Eur. J. Biochem.*, **125**, 69 (1982).

6) G. E. Liehard and W. P. Jencks, *J. Am. Chem. Soc.*, **88**, 3982 (1966).

7) Patai, S. "The Chemistry of the Thiol Group," Wiley; New York (1974).

8) Mixing of PQQ and $PQQH_2$ under appropriate anaerobic conditions did not give the semiquinone radical. Only under strongly alkaline conditions (pH 13), the semiquinone radical can be detected by UV-visible absorption spectroscopy: J. A. Duine, J. Frank, and P. E. J. Verwiel, *Eur. J. Biochem.*, **118**, 395 (1981).

9) G. Douheret, *Bull. Soc. Chim., Fr*, **1968**, 3122.