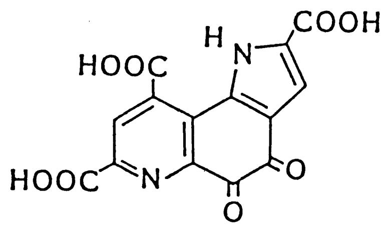
CHEMISTRY LETTERS, pp. 1491-1494, 1989.

Chemical Behavior of Coenzyme PQQ toward Diamines

Minae MURE, Shinobu ITOH, and Yoshiki OHSHIRO*
Department of Applied Chemistry, Faculty of Engineering,
Osaka University, Yamadaoka 2-1, Suita, Osaka 565

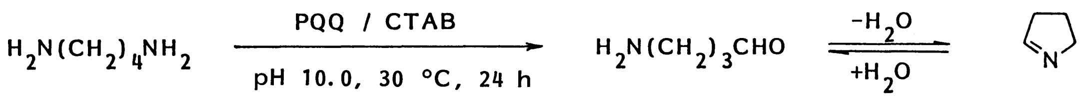
Reaction of the coenzyme PQQ with several α,ω-diaminoalkanes was examined in the CTAB micellar system. α,ω-Diaminoalkanes having 4 to 10 methylene carbons were oxidized to the corresponding aminoaldehydes by PQQ efficiently. On the other hand, ethylenediamine showed competitive inactivation by forming the cyclic adduct, a pyrazine derivative. The reaction mechanism is also discussed.

α,ω-Diaminoalkanes are well known to be specific substrates and/or irreversible inhibitors of quinoprotein (PQQ-containing enzymes) diamine oxidase and lysyl oxidase depending upon the alkylene chain length.[1,2] While the oxidative deamination of monoamines by PQQ has been studied,[3,4] details of the reaction between diamines and PQQ have not been investigated yet. Here we report on the chemical behavior of the coenzyme PQQ toward α,ω-diaminoalkanes to clarify the oxidation and enzymatic inhibition mechanism.



PQQ: 4,5-dihydro-4,5-dioxo-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid

The reaction of PQQ and several α,ω-diaminoalkanes having 4 to 10 methylene carbons was examined spectrophotometrically under <u>anaerobic</u> pseudo-first order conditions ([PQQ] = 4.0×10^{-5}M, [amine] = 4.0×10^{-3}M). In every case, only the reduction of PQQ was observed, indicating that these diamines were good substrates for PQQ-oxidation. The reaction rate increased significantly with increasing the chain length, and the plots of the pseudo-first-order rate constant (k_{obsd}) versus concentration of the diamine showed Michaelis-Menten type saturation phenomena. These results indicate that the oxidation proceeded mainly on the micelle as in the case of monoamines.[5]



(1)

When 1,4-diaminobutane (4.0×10^{-2}M) was treated with a catalytic amount of PQQ (1 mol%) in the presence of CTAB (4.0×10^{-3}M) under <u>aerobic</u> conditions at pH 10.0 and 30 °C for 24 h, the autorecycling oxidative deamination proceeded effi-

ciently as in the case of monoamines to give 4-aminobutylaldehyde in 1028% yield (based on PQQ, Eq. 1).[6)]

The reaction of PQQ with ethylenediamine was examined in detail because ethylenediamine is known to be an irreversible inhibitor of lysyl oxidase.[2)] In the reaction at pH 4.7, a slow increase (k_{obsd}= 1.3 x $10^{-4}s^{-1}$) in A_{286} was observed and the final spectrum was quite different from that of reduced PQQ (Fig. 1a), and the spectrum was hardly affected by aeration. At pH 6.9, the spectrum of PQQ (λ_{max} 250 and 347 nm, in the CTAB micellar system) rapidly changed within 5 min to that having λ_{max}261 and 347 nm with an isosbestic point at 254 nm (Fig. 1b) at initial stage, and then λ_{max} were gradually changed to λ_{max} 286, 302, and 320 nm with new isosbestic points at 243, 274, and 343 nm (Fig. 1c: k_{obsd} = 6.0 x 10^{-4} s^{-1}). Upon introduction of air into the final reaction mixture, absorption at around 302 and 320 nm, which correspond to reduced PQQ (aminophenol and quinol, respectively, see Scheme 1), disappeared and the species absorbing at 286 nm remained. This phenomenon shows that the formation of the species having λ_{max} at 286 nm and the reduction of PQQ occurred competitively at pH 6.9. The similar phenomena were also observed at pH 9.9, but the latter spectral change (formation of pyrazine adduct and reduced PQQ) proceeded very slowly (ca. 5 days).

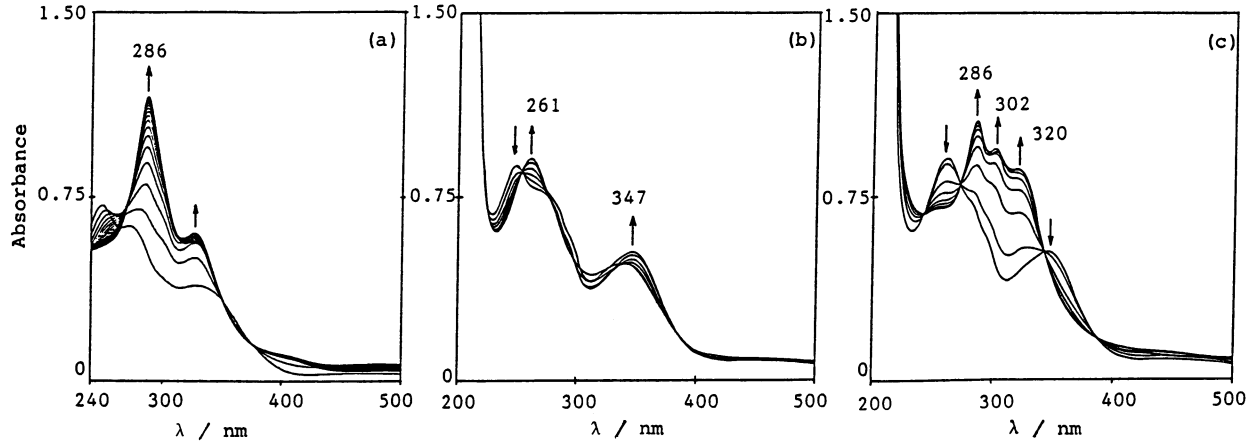
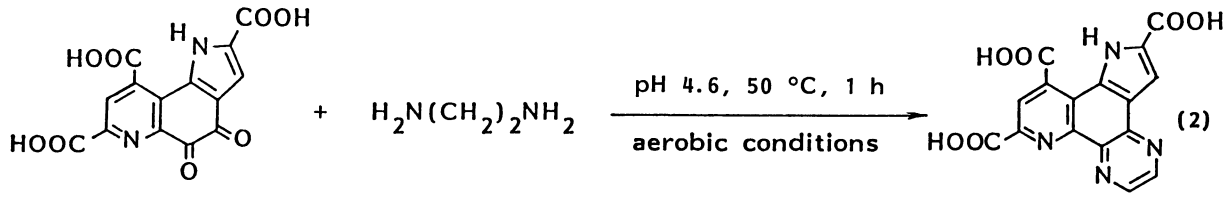


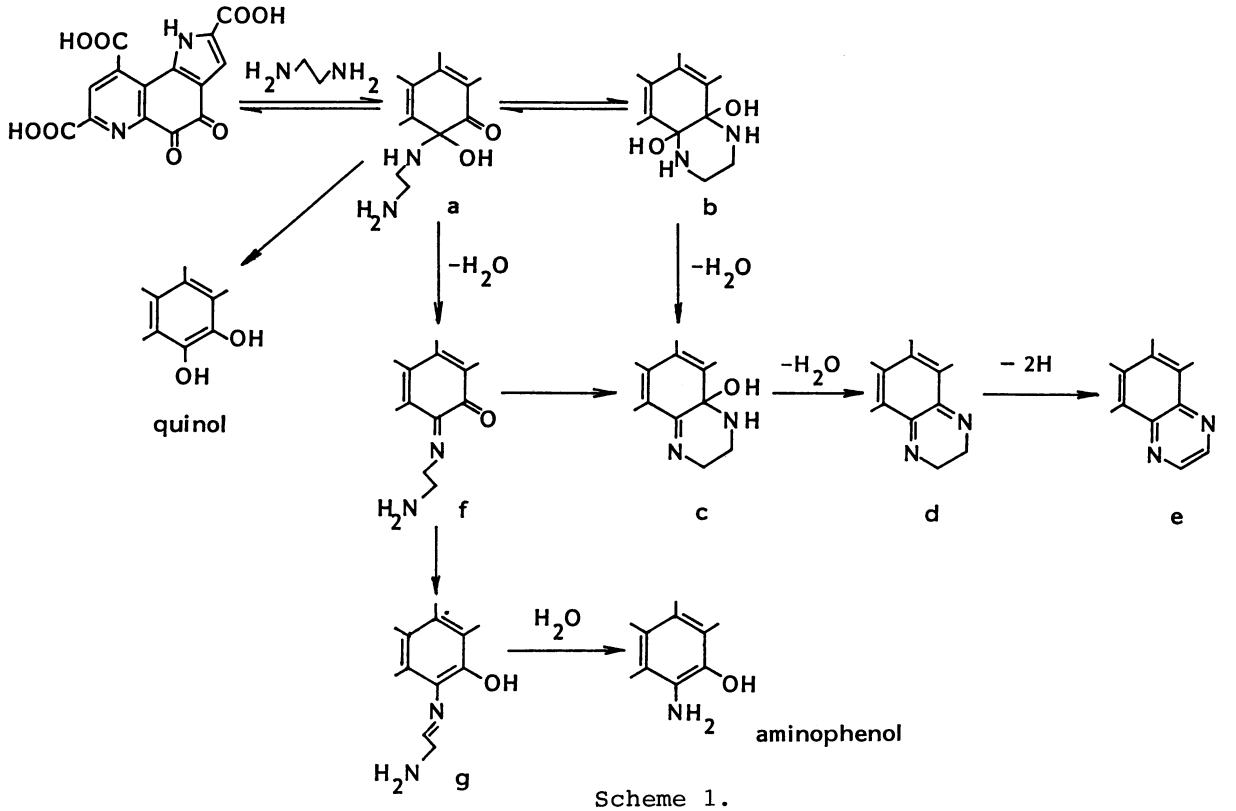
Fig. 1. Spectral changes along the progress of the reaction of PQQ (4.0 x 10^{-5}M) with ethylenediamine in the presence of CTAB (2.0 x 10^{-3}M) under anaerobic conditions at 35 °C. a) at pH 4.7 in 0.05 M acetate buffer, [ethylenediamine] = 4.0 x 10^{-2}M, time: from 3 min to 200 min, b) at pH 6.9 in 0.05 M phosphate buffer, [ethylenediamine] = 4.0 x 10^{-3}M, time: from 30 s to 5 min, c) at pH 6.9, time: from 8 min to 90 min.

In order to identify the product having λ_{max} at 286nm, PQQ (10.8 mg, 3.3 x 10^{-5} mol) was treated with 10-fold excess of ethylenediamine in 3 ml of 0.05 M acetate buffer (pH 4.6) at 50 °C for 60 min. A pyrazine derivative was isolated as a yellow solid in 87% yield (Eq. 2). The structure of the derivative was well confirmed by spectroscopic analyses.[7a)] The similar pyrazine derivatives were also obtained from the reaction of PQQTME (trimethylester of PQQ) with ethylenediamine and PQQ with 1,2-diaminocyclohexane, respectively.[7b,7c)]



We have already proposed that the oxidative deamination of amines by PQQ proceeds via nucleophilic addition of the amine to the carbonyl group of 5-position of PQQ to form a key intermediate of carbinolamine type.[4] The present result, the pyrazine derivative formation, clearly supports our proposed mechanism. Namely, in the reaction with ethylenediamine under neutral conditions, the pyrazine derivative could be formed by cyclization of the carbinolamine intermediate a or of the imine intermediate f followed by aromatization along with the reduction of PQQ (a→quinol, f→g→aminophenol) as shown in Scheme 1. Although details of the aromatization should be further investigated, the reaction seems to be promoted by stabilization of being 18 π aromatic system.

The initial spectral change (Fig. 1b) may correspond to the pre-equilibrium of the formation of intermediate b which is predominantly formed and relatively stable under basic conditions (at pH 9.9). This intermediate gave the pyrazine adduct immediately when the solution was acidified at this stage, which could be attributed to the enhancement of the acid-catalyzed dehydration (b→c→d). Under acidic conditions, the pyrazine formation mainly occurred because the base catalyzed α-proton removal from the intermediates a or f (reduction of PQQ) may be suppressed and acid-catalyzed dehydration reaction is enhanced.



Scheme 1.

In conclusion, we could construct a model system of diamine oxidases and reveal the inhibitory action of ethylenediamine toward PQQ. It should be noted that ethylenediamine acts both as a substrate and as an inhibitor in the catalytic process under neutral conditions.[8] Trackman and Kagan have reported that diamines are oxidized by lysyl oxidase in the course of time- and temperature dependent inhibition.[2] The present results are in good accordance with such a finding in the enzymatic system.

The present work was partially supported by a Grant-in-Aid for Special Project Research from the Ministry of Education, Science, and Culture of Japan (No. 62216013).

References

1) W. G. Bardsley, C. M. Hill, and R. W. Lobley, Biochem. J., 117, 169 (1970).
2) P. C. Trackman and H. M. Kagan, J. Biol. Chem., 254, 7831 (1979).
3) C. Hartmann and J. P. Klinman, Biofactors, 1(1), 41 (1988).
4) S. Itoh, Y. Kitamura, Y. Ohshiro, and T. Agawa, Bull. Chem. Soc. Jpn., 59, 1907 (1986).
5) S. Itoh, M. Mure, and Y. Ohshiro, Yukagaku, 36, 882 (1987).
6) The yield was determined by well known method using o-aminobenzaldehyde; B. Holmstedt, L. Larsson, and R. Tham, Biochim. Biophys. Acta., 48, 182 (1961).
7) a) Pyrazine derivative from PQQ and ethylenediamine: mp > 300 °C (dec.); IR (KBr) 1710, 1560, 1350, and 1244 cm^{-1}; UV-vis (pH 9.9, 0.05 M carbonate buffer) λ_{max} 286 and 326 nm, ^{1}H-NMR (0.1 M K_2CO_3/D_2O, internal reference: $Me_3Si(CH_2)_3SO_3Na$) δ 6.90 (s, 1H, a proton on the pyrrole ring of PQQ), 8.10 (s, 1H, a proton on the pyridine ring of PQQ), 8.36 and 8.42 ppm (two br s, 1H x 2, -CH=CH-); b) Pyrazine derivative from PQQTME and ethylenediamine: mp > 300 °C (dec.); ^{1}H-NMR ($CDCl_3$, TMS) δ 4.02, 4.17, and 4.24 (three s, 3H x 3, $-COOCH_3$), 8.06 (d, J = 2.12 Hz, 1H, a proton on the pyrrole ring of PQQ), 9.00 (s,1H, a proton on the pyridine ring of PQQ), 9.09 (s, 2H, -CH=CH-), 12.75 ppm (br s, 1H, NH). Ms; m/e 394 (M^+), 363 ($M^+ - CH_3O$), 336 (363 - CH_3N); c) Pyrazine adduct from PQQ and 1,2-cyclohexanediamine: mp > 300 °C (dec.); IR (KBr) 3444, 2940, 1714, 1330, and 1250 cm^{-1}; UV-vis (pH 9.9, 0.05 M carbonate buffer) λ_{max} 286 and 332 nm, ^{1}H-NMR (d^6-DMSO, TMS) δ 2.14 and 3.30 (two br s, 2H x 2, $-(CH_2)_2-$), 7.79 (br s, 1H, a proton on the pyrrole ring of PQQ), 8.89 (s, 1H, a proton on the pyridine ring of PQQ), 14.05 ppm (br s, 1H, NH).
8) 1,3-Diaminopropane also showed inhibitory effect on PQQ. However, the reaction was complicated as in the case of the reaction of phenantherenequinone with 1,3-diaminopropane: R. H. McDougall and S. H. Malik, J. Chem. Soc., C, 1969, 2044.

(Received May 1, 1989)