Modeling of the Chemistry of Quinoprotein Methanol Dehydrogenase. Oxidation of Methanol by Calcium Complex of Coenzyme PQQ via Addition−Elimination Mechanism

Shinobu Itoh,* Hirokatsu Kawakami, and Shunichi Fukuzumi*

Department of Applied Chemistry, Faculty of Engineering, Osaka University 2-1 Yamada-oka, Suita, Osaka 565, Japan

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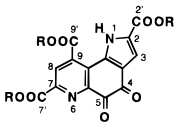
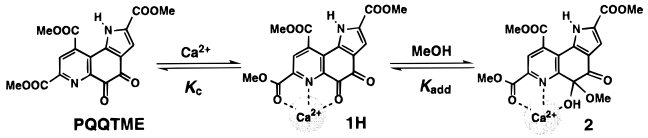
Bacterial methanol dehydrogenase (MDH, E.C. 1.1.99.8) is a quinoprotein that involves a heterocyclic *o*-quinone cofactor PQQ (pyrroloquinolinequinone) as a redox catalyst for the oxidation of methanol to formaldehyde.[1,2] The most curious



R = H; PQQ
R = Me; PQQTME

feature of this enzyme for chemists may be the high reactivity toward methanol, the most inert alcohol.[3] In other words, how does MDH activate PQQ to undergo such a difficult oxidation reaction?[4] Recently, the crystal structure of MDH from methylotrophic bacteria has been determined by two independent research groups to provide full particulars of the enzyme active center. According to the reported X-ray structure, there is one calcium ion strongly bound to PQQ through its C-5 quinone carbonyl oxygen, N-6 pyridine nitrogen, and C-7 carboxylate group in the enzyme active site.[5−7] Davidson and his coworkers have recently suggested that Ca^{2+} plays an important role for the structural stabilization of the enzyme.[8] However, little is known about the catalytic role of Ca^{2+} for the redox reaction in MDH.[9] In this communication, we present the first functional model for MDH, where the calcium complex of a

Scheme 1



PQQ derivative efficiently oxidizes methanol to formaldehyde *via* an addition−elimination mechanism.[10]

When the trimethyl ester of PQQ (PQQTME) was treated with $Ca(ClO_4)_2$ in anhydrous acetonitrile (CH_3CN), the absorption band at 354 nm due to the quinone shifted to 368 nm and the shoulder around 280 nm decreases with clear isosbestic points at 268, 289, 303, 361, and 422 nm.[11] The 1:1 complex formation between PQQTME and Ca^{2+} with the binding constant K_c of 1900 M^{-1} has been determined by analyzing the spectral change.[11] The following ^{1}H- and ^{13}C-NMR data in CD_3CN indicate that the binding position of Ca^{2+} to PQQTME in solution is the same as that to PQQ in the enzymatic system (**1H** in Scheme 1).[12] In the ^{1}H-NMR spectra, the methyl ester protons at the 7-position move toward downfield more than those at the 2- and 9-positions by the complexation with Ca^{2+} ($\Delta\delta$ = +0.14, +0.02, and +0.06, respectively) and the $\Delta\delta$ (downfield shift by the complexation) value of H-8 is also larger than that of H-3 (+0.09 and +0.07, respectively).[11] In the ^{13}C-NMR spectra, C-5 and C-7′ (ester carbonyl carbon at the 7-position) shifted downfield (Δppm = +2.0 and +2.8, respectively), while C-4, C-2′ (ester carbonyl carbon at the 2-position), and C-9′ (ester carbonyl carbon at the 9-position) shifted upfield by the complexation with Ca^{2+} (Δppm = −1.0, −0.2, and −1.0, respectively).[11]

Addition of methanol into a CH_3CN solution of Ca^{2+}-complex **1H** resulted in a spectral change corresponding to the C-5 hemiacetal formation (**2** in Scheme 1).[13] The formation constant K_{add} of the Ca^{2+}-complex with methanol was determined as 3.6 M^{-1} which is six times larger than that measured in the absence of Ca^{2+} (0.63 M^{-1}),[11,13] indicating clearly that the complexation with Ca^{2+} enhances the stability of the C-5 hemiacetal. Coordinative interaction of methanol to Ca^{2+} may also enhance the nucleophilicity of methanol by lowering the pK_a value of the −OH group.

To our surprise, addition of methanol into a deaerated CH_3CN solution of the Ca^{2+}-complex in the presence of a base such as DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) resulted in formation of *reduced PQQTME* (Figure 1). The final spectrum of the reaction mixture is essentially the same as that obtained by the treatment of authentic $PQQTMEH_2$ (quinol form)[14] with $Ca(ClO_4)_2$ and DBU in deaerated CH_3CN, and, more importantly, *it is also very close to the absorption spectrum of fully reduced MDH*.[15] From the reaction mixture in a preparative scale

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(3) Methanol can be used as a solvent for oxidation reactions by highly oxidation-active quinones such as DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) and chloranil (tetrachlorobenzoquinone), see: Becker, H.-D. In *The chemistry of the quinonoid compounds*; Patai, S., Ed.; John Wiley & Sons: New York, 1974; Part 1, pp 335−424.

(4) The two-electron redox potential of free PQQ (−0.175 V vs SCE at pH 7.0) is much lower than that of DDQ and chloranil,[3] see: Kano, K.; Mori, K.; Uno, B.; Kubota, T.; Ikeda, T.; Senda, M. *Bioelectrochem. Bioenerg.* **1990**, *23*, 227.

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(6) (a) Blake, C. C. F.; Ghosh, M.; Harlos, K.; Avezoux, A.; Anthony, C. *Nature Struct. Biol.* **1994**, *1*, 102. (b) Ghosh, M.; Anthony, C.; Harlos, K.; Goodwin, M. G.; Blake, C. *Structure* **1995**, *3*, 177.

(7) The presence of Ca^{2+} in the enzyme active site has been also suggested for other PQQ-dependent enzymes such as ethanol dehydrogenase from *Pseudomonas aeruginosa* and glucose dehydrogenase from *Acinetobacter calcoaceticus*, see: (a) Mutzel, A.; Görisch, H. *Agric. Biol. Chem.* **1991**, *55*, 1721, (b) Geiger, O.; Görisch, H. *Biochem. J.* **1989**, *261*, 415.

(8) Harris, T. K.; Davidson, V. L. *Biochem. J.* **1994**, *303*, 141.

(9) Ca^{2+} in enzymatic systems has been generally believed to act as information mediators, enzyme-structure stabilizers, and enzyme-activity regulators, but little is known about its catalytic role in enzymatic redox reactions, see: Kaim, W.; Schwederski, B. *Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life*; John Wiley & Sons: New York, 1994.

(10) An addition−elimination mechanism through a hemiacetal intermediate and an acid-base catalyzed hydride transfer mechanism have been proposed for the MDH-catalyzed oxidation of methanol,[2] but no direct evidence to support such possibilities has so far been reported.

(11) See Supporting Information.

(12) In spite of our great efforts, a single crystal of the Ca^{2+} complex suitable for the X-ray analysis could not be obtained. However, the binding position of Ca^{2+} to PQQTME has been confirmed by comparing the K_c values and the spectral data of other PQQ model compounds, see: (a) Itoh, S.; Huang, X.; Kawakami, H.; Komatsu, M.; Ohshiro, Y.; Fukuzumi, S. *J. Chem. Soc., Chem. Commun.* **1995**, 2077. (b) Itoh, S.; Kato, J.; Inoue, T.; Kitamura, Y.; Komatsu, M.; Ohshiro, Y. *Synthesis*, **1987**, 1067. (c) Itoh, S.; Fukui, Y.; Haranou, S.; Ogino, M.; Komatsu, M.; Ohshiro, Y. *J. Org. Chem.* **1992**, *57*, 4452. (d) Itoh, S.; Fukui, Y.; Ogino, M.; Haranou, S.; Komatsu, M.; Ohshiro, Y. *J. Org. Chem.* **1992**, *57*, 2788.

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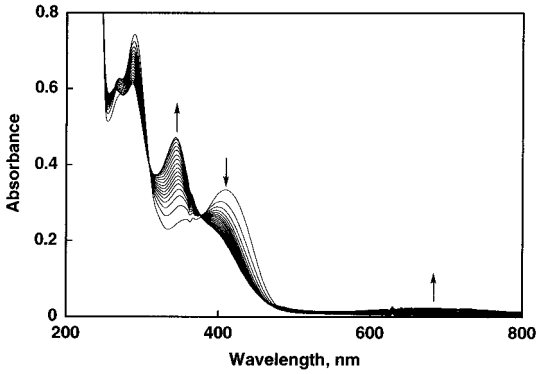


Figure 1. Spectral change observed in the oxidation of CH_3OH (2.2 M) by PQQTME (2.5×10^{-5} M) in the presence of $Ca(ClO_4)_2$ (6.3×10^{-3} M) and DBU (2.4×10^{-3} M) in deaerated CH_3CN at 298 K.

([PQQTME] $= 1.5 \times 10^{-4}$ M, [$Ca(ClO_4)_2$] $= 8.5 \times 10^{-3}$ M, [DBU] $= 2.4 \times 10^{-3}$ M, [CH_3OH] $= 2.2$ M in 100 mL of CH_3CN), a reasonable amount of formaldehyde was isolated as the 2,4-dinitrophenylhydrazone derivative.[16] A similar spectral change (quantitative formation of reduced PQQTME) was observed with ethanol as well as methanol, and the oxidation product, acetaldehyde, was obtained quantitatively as 2,4-dinitrophenylhydrazone derivative. Furthermore, the oxidation of ethanol to acetaldehyde proceeded catalytically (1450% based on PQQTME after 65 h), when the reaction was carried out under aerobic conditions. It should be noted that the presence of both Ca^{2+} and DBU is essential for the redox reaction between PQQTME and the alcohols to occur in our model system.

The pseudo-first-order rate constant (k_{obs}) obtained from the spectral change indicated in Figure 1 shows a Michaelis−Menten type saturation phenomenon when plotted against methanol concentration (Figure 2A).[17] Nonlinear curve fitting using the equation of $k_{obs} = kK_{add}[CH_3OH][DBU]/(1 + K_a[DBU] + K_{add}[CH_3OH])$ derived from the reaction mechanism shown in Scheme 2 provided the kinetic parameters as $K_a = 1.4 \times 10^3$ M^{-1}, $K_{add} = 3.7$ M^{-1}, and $k = 0.42$ M^{-1} s^{-1}, where K_a is the deprotonation equilibrium constant (**1**$^-$ represents deprotonated PQQTME at N-1) and k is the rate constant of the redox reaction from **2** to **3**. Dependence of k_{obs} vs [DBU] (Figure 2B) also afforded a saturation phenomenon as expected from the kinetic equation, from which $K_a = 1.5 \times 10^3$ M^{-1}, $K_{add} = 3.5$ M^{-1}, and $k = 0.42$ M^{-1} s^{-1} were obtained by the computer simulation. The good agreements of those kinetic parameters determined independently from k_{obs} vs [CH_3OH] and k_{obs} vs [DBU] together with the agreement of K_{add} determined by the titration (3.6 M^{-1}) and kinetics (3.5 and 3.7 M^{-1}) support the validity of the proposed mechanism and exclude the intermolecular hydride transfer mechanism from methanol to the quinone. A large kinetic isotope effect of 6.4 on the rate constant k was obtained by using CD_3OD as a substrate, clearly indicating that the base-catalyzed α-proton abstraction from the substrate is rate-determining in the methanol oxidation reaction (from **2** to **3**).

(16) The isolated yield (20%) was just the same as that obtained by the reaction of authentic sample of formaldehyde with 2,4-dinitrophenylhydrazine under the same experimental conditions. This result indicate that formaldehyde is formed quantitatively as in the case of acetaldehyde.

(17) The rate of formation of **3** was monitored after the equilibrium in Scheme 2 was established.

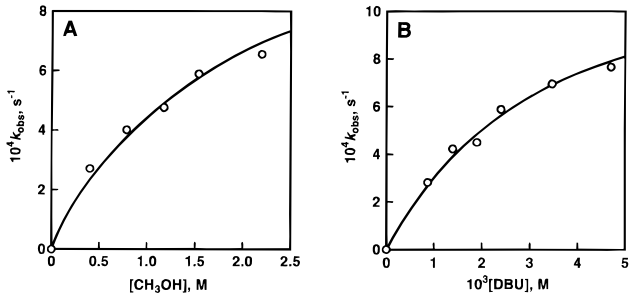
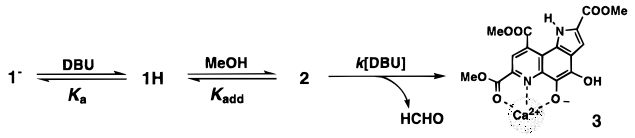


Figure 2. Plot of (A) k_{obs} vs [CH_3OH] (at [DBU] $= 2.4 \times 10^{-3}$ M) and (B) k_{obs} vs [DBU] (at [CH_3OH] $= 1.5$ M) for the oxidation of CH_3OH by PQQTME (2.5×10^{-5} M) in the presence of $Ca(ClO_4)_2$ (6.3×10^{-3} M) and DBU in deaerated CH_3CN at 298 K. The solid lines are drawn based on the computer simulation using the kinetic equation and the parameters indicated in the text.

Scheme 2



In summary, we have demonstrated here that the Ca^{2+}-complex of PQQTME actually facilitates the alcohol-adduct formation at C-5 and also that Ca^{2+} is required for the base-catalyzed oxidative elimination reaction of the adduct. In the enzymatic system, aspartate (Asp) is suggested to be the most likely candidate as the general-base catalyst.[5,6] Alternatively, intramolecular general-base catalysis by the C-4 carbonyl oxygen could be expected to abstract the α-proton of the substrate. Such a possibility will be examined by using other PQQ-model compounds.

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Supporting Information Available: Spectral change of the titration of PQQTME with $Ca(ClO_4)_2$ in anhydrous CH_3CN (S1) and its analytical equations and results (S2), ^{1}H- and ^{13}C-NMR data of PQQTME and its Ca^{2+}-complex in CD_3CN (S3), and spectral change of the titration of Ca^{2+}-PQQTME complex with methanol in CH_3CN (S4) and its analytical results (S5) (5 pages). See any current masthead page for ordering and Internet access instructions.

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