

## Modeling of quinoprotein functions

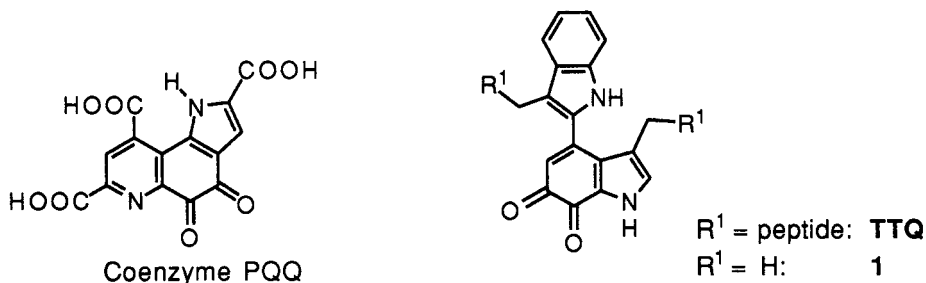
Yoshiki Ohshiro and Shinobu Itoh

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

**Abstract:** The model compound of TTQ (tryptophan tryptophylquinone), the active site cofactor of bacterial methylamine dehydrogenases, was synthesized for the first time.  $^1\text{H}$  NMR analysis and theoretical calculations on the model compound (**1**) indicate that its molecular geometry is close to that of TTQ in the enzyme active site. The redox potential and spectral characteristics (UV-vis and resonance Raman) of **1** were also very similar to those of the native enzymes. Model compound **1** catalyzes the oxidation of benzylamine in  $\text{CH}_3\text{OH}$ , indicating that it possesses the same chemical functions as methylamine dehydrogenases. Product analysis on the reactions of **1** with several amines indicate that the amine oxidation proceeds via a transamination mechanism. In order to study structure-reactivity relationships of TTQ, 4-substituted-6,7-indolequinones were also synthesized and their physicochemical properties and catalytic activity in the amine oxidation were compared to those of model compound **1**.

### INTRODUCTION

A number of redox active enzymes of both bacterial and mammalian origins were thought at one time to contain the cofactor *pyrroloquinolinequinone* (PQQ). This class of enzymes has collectively been called *quinoproteins*. However, recent studies on related enzymes have demonstrated that some quinoproteins possess quinone cofactors other than PQQ.<sup>1</sup> Therefore, much recent attention has been focused on the discovery of new and exciting properties and applications of quinoproteins. However, little has been known about mechanistic details of the redox reactions catalyzed by the quinoproteins. For this reason, we have been carrying out model studies on quinoprotein functions in order to understand the enzymatic mechanisms at a molecular level.<sup>2</sup>



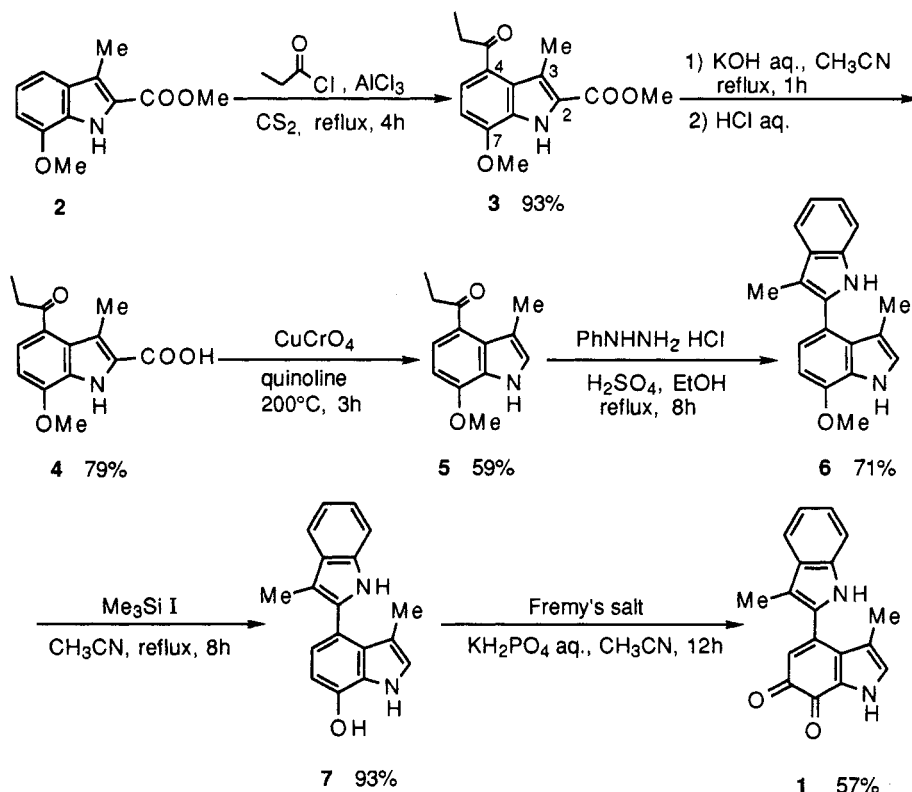
Methylamine dehydrogenase (EC 1.4.99.3, MADH) is a quinoprotein that is isolated from a variety of methylotrophic and autotrophic bacteria. It catalyzes the oxidation of methylamine to formaldehyde and ammonia in a two-electron process.<sup>3</sup> One of the most interesting aspects of MADH is the chemical structure of the active site cofactor. PQQ or a closely related compound used to be regarded as plausible candidates for the organic cofactor,<sup>4-6</sup> and the redox behavior of MADH has been investigated by taking into account the *o*-quinone structure of the cofactor.<sup>7</sup> Recently, however, McIntire and his co-workers

revealed the structure of the cofactor to be that of *tryptophan tryptophylquinone (TTQ)*, not PQQ, by  $^1\text{H}$  NMR and mass spectroscopic investigations on isolated cofactor-bearing peptides.<sup>8</sup> Studies on amino acid sequences and X-ray crystallographic analysis of the native enzymes also supported the proposed structure.<sup>9,10</sup>

The enzymatic mechanism of the amine oxidation by MADH has not been clearly demonstrated yet. A transamination mechanism has been recently proposed for the enzymatic reaction,<sup>8,11</sup> but little was known about the details. Thus, in this study we synthesized and characterized model compounds of TTQ in order to obtain further information on the structure and the reactivity of the active site cofactor.

### SYNTHESIS OF MODEL COMPOUND 1

The synthesis of model compound **1** was accomplished as shown in Scheme 1. Friedel-Crafts acylation on indole derivative **2**<sup>12</sup> with propionyl chloride by the standard method gave the 4-acylated derivative **3**. The position of the acylation in **3** was confirmed by  $^1\text{H}$  NMR; a large NOE, Nuclear Overhauser Effect, (18 %) was detected on 6-H when irradiated at 7-OMe. When the Friedel-Crafts acylation was carried out on 7-methoxyskatole, a propionyl group was introduced predominantly at the 2-position. Indole derivative **3** was then converted into **5** by ester hydrolysis followed by thermal decarboxylation using  $\text{CuCrO}_4$  in quinoline. The second indole ring was constructed by Fischer indolization on **5** with phenylhydrazine hydrochloride with a 71% yield. Deprotection of the methoxy group of **6** by trimethylsilyl iodide gave the 7-hydroxy derivative **7**, which was finally converted into the expected quinone **1** by oxidation with Fremy's salt with a 57% yield. The structures of **1** and **3** - **7** were confirmed by several spectroscopic techniques and elemental analyses.



Scheme 1

## PHYSICOCHEMICAL PROPERTIES OF MODEL COMPOUND 1

X-ray crystallographic investigation of MADHs from *Paracoccus denitrificans* and *Thiobacillus versutus* indicated that the dihedral angle of the two indole rings of TTQ is about  $42^\circ$ .<sup>10</sup> Molecular orbital calculations by the AM1 method indicated that the dihedral angle of the two indole rings of compound 1 is about  $49^\circ$  and the distances between the protons of 3'-Me and those of 3-Me and 5-H are about 4 and 2.5 Å, respectively (Figure 1A).<sup>13</sup> This molecular geometry was also suggested by the observed NOE correlation for 1 (Figure 1B). An NOE approximately 4% was detected between the two methyl groups, but this value is relatively small compared to that between 3'-Me and 5-H (20%). In the case of molecular mechanics calculations on compound 1, minimum steric energy was obtained when the dihedral angle of the two indole rings was about  $55^\circ$ .<sup>14</sup>

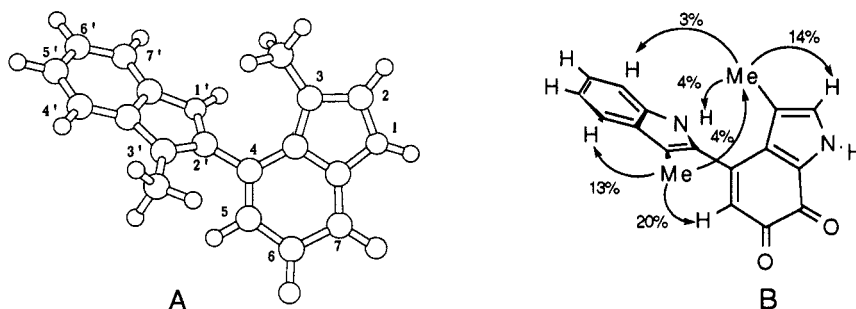


Figure 1. (A) Fully optimized structure of 1 by AM1. (B) Selected NOE correlation for 1.

The two-electron redox potential of 1 was determined by cyclic voltammetry to be  $-188$  mV vs. SCE in a 0.1 M phosphate buffer solution containing 30%  $\text{CH}_3\text{CN}$  (pH 7.4). This value is comparable to that of native MADH from *bacterium* W3A1 ( $E_{1/2} = -148$  mV vs. SCE at pH 7.5).<sup>15</sup> Compound 1 shows a strong absorption at 407 nm ( $\epsilon = 1.07 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ ) with a broad shoulder at 500 - 650 nm in  $\text{CH}_3\text{CN}$  (Figure 2); thus the color of the solution is reddish-brown. Reduction of 1 to  $1\text{H}_2$  (quinol form) by methylhydrazine caused the complete disappearance of the absorptions but gave a new one at 306 nm ( $\epsilon = 1.62 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ ). The spectra of 1 and  $1\text{H}_2$  resemble in shape those of MADH from *Paracoccus denitrificans* ( $\text{MADH}_{\text{ox}}$ ; 440 nm;  $\text{MADH}_{\text{red}}$ ; 326 nm).<sup>7a</sup>

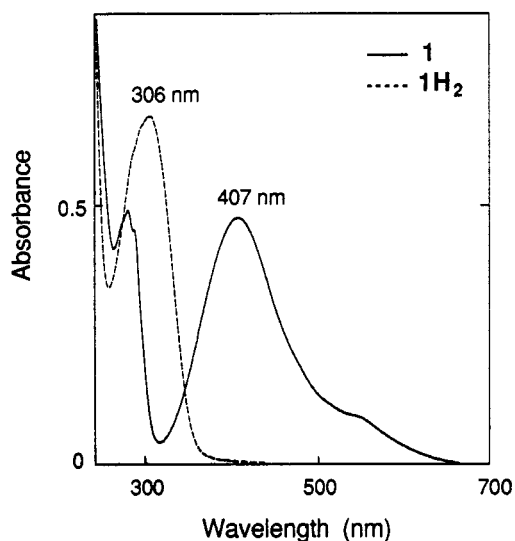
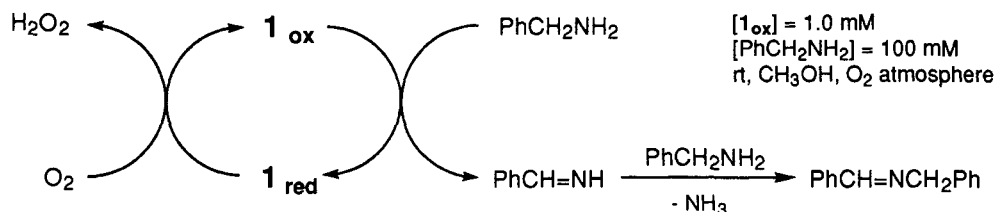


Figure 2. UV-visible spectra of 1 and  $1\text{H}_2$  in  $\text{CH}_3\text{CN}$ .

In the resonance Raman spectrum of **1**, obtained using 457.9 nm excitation (100 mW), strong peaks were detected at 1620 (C=O vibration mode), 1570, 1455, 1170, 1146, 1064, and 955  $\text{cm}^{-1}$ , which are also closely related to those of TTQ in the native enzymes.<sup>11</sup>

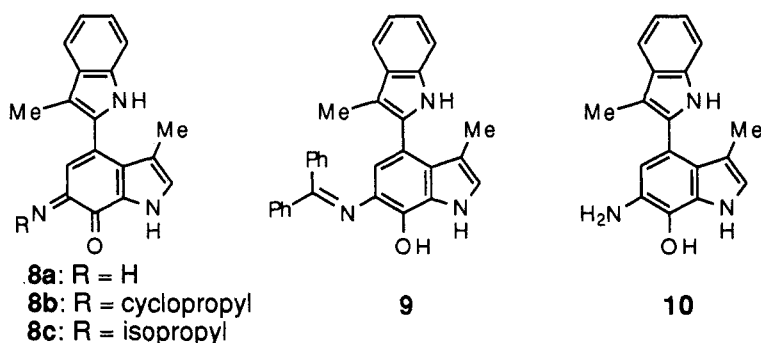
### AMINE OXIDATION BY MODEL COMPOUND **1**

The catalytic efficiency of model compound **1** was examined via the aerobic oxidation of benzylamine *in vitro*.<sup>16</sup> It is noteworthy that **1** acts as a very efficient turnover catalyst in the oxidation reaction. Benzylamine was converted into *N*-benzylidenebenzylamine almost quantitatively in the presence of a catalytic amount of **1** (1 mol%) in  $\text{CH}_3\text{OH}$  under  $\text{O}_2$  atmosphere (Scheme 2). Such efficient amine oxidation does not occur with ordinary quinones.



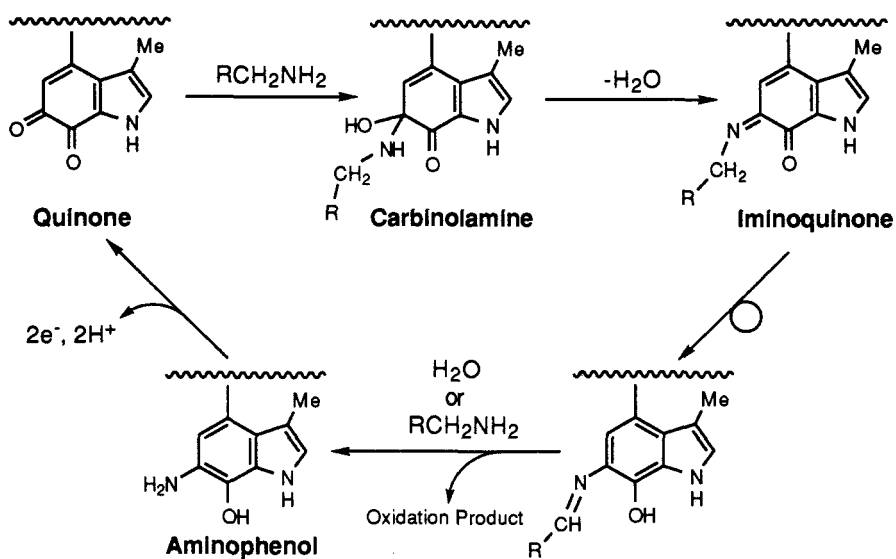
Scheme 2

To learn the oxidation mechanism the reactions of **1** with several amines were investigated under anaerobic conditions. In the reactions with ammonia, cyclopropylamine, and isopropylamine, **1** was converted into the corresponding iminoquinone derivatives **8a** - **8c**, respectively. In the cases of **8b** and **8c**, the products were obtained as a mixture of syn-anti type stereoisomers at the imine function. The addition position of the amine was estimated to be C-6 by a large NOE observed between H-5 and the alkyl group (R) of the amine substrate; in the case of adduct **8b**, 19 % of the NOE was detected between H-5 and the  $\alpha$ -proton of the cyclopropyl group. Theoretical calculations using the MOPAC program on the carbinolamine-type adduct of ammonia suggested that the C-6 adduct is thermodynamically more stable than the C-7 adduct.<sup>13</sup> On the other hand, imine derivative **9** was isolated in the reaction with benzhydrylamine. Furthermore, quantitative formation of aminophenol **10** occurred when benzylamine was used as the substrate.



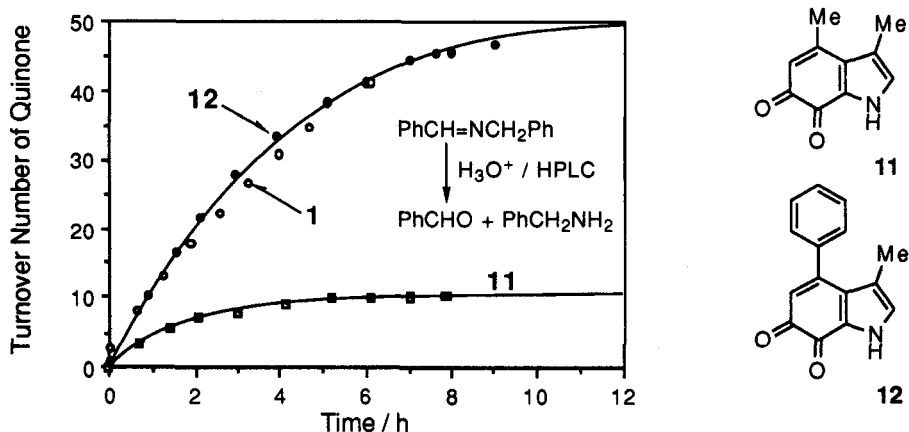
These results clearly indicate that the oxidation of amines by model compound **1** proceeds via the transamination mechanism as shown in Scheme 3. Namely, the amine adds to C-6 of the quinone to form the carbinolamine-type intermediate which is converted into the iminoquinone derivative by dehydration. Rearrangement followed by hydrolysis and/or imine exchange with excess amines affords the aminophenol and the oxidation

product. The aminophenol thus formed is easily reoxidized by molecular oxygen under the basic conditions to yield an efficient catalytic cycle.



### STRUCTURE-REACTIVITY RELATIONSHIPS

TTQ was thought to be formed via post-translational modification of two tryptophan residues of the MADH subunit peptide chains.<sup>9</sup> However, little is known of TTQ biosynthesis, why TTQ has a 6,7-indolequinone skeleton, and the role of the second indole ring at the 4-position. According to the crystal structure of a complex between MADH and its native electron acceptor, amicyanin, the second indole ring is located between the indolequinone moiety of TTQ and the copper site of amicyanin, suggesting its function as an electron-transfer mediator.<sup>17</sup>



**Figure 3.** Time course of the aerobic oxidation of benzylamine (100 mM) catalyzed by the quinone (1 mM) in MeOH at room temperature under O<sub>2</sub>-atmosphere. Formation of PhCHO was followed by HPLC.

In order to answer these questions, we synthesized and characterized several indolequinone derivatives such as 6,7-indolequinones, 4,5-indolequinones, and 4,7-indolequinones. For example, 3,4-dimethyl-6,7-indolequinone (**11**) and 3-methyl-4-phenyl-6,7-indolequinone (**12**) were prepared in similar manners starting from *p*-cresidine and 2-methoxy-5-phenylaniline, respectively (cf. Scheme 1). Interestingly, phenylindolequinone **12** showed high catalytic activity as in the case of compound **1**, while methylindolequinone **11** was inactivated at an early stage of the reaction (Figure 3). Thus, it can at least be said that an aromatic substituent at the 4-position is very important to keep the catalytic activity of the quinone. Further studies on the structure-reactivity relationships are now under investigation.

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