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Supplementary Material Available: Characterization data for 6a-c, 8-10, 12, 14b, and 15 (3 pages). Ordering information is given on any current masthead page.

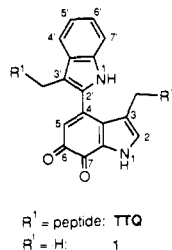
Synthesis and Characterization of the Model Compound of Active Site Cofactor TTQ of Bacterial Methylamine Dehydrogenases

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Received March 16, 1992

Methylamine dehydrogenase (EC 1.4.99.3, MADH) is a quinoprotein (quinone-containing enzyme) which is isolated from a variety of methylotrophic and autotrophic bacteria and catalyzes the oxidation of methylamine to formaldehyde and ammonia in a two-electron step.¹ One of the most interesting aspects of MADH is the chemical structure of the active site cofactor. PQQ (pyrroloquinolinequinone, a novel coenzyme of bacterial alcohol, aldehyde, and glucose dehydrogenases) or a closely related compound used to be regarded as a plausible candidate for the cofactor,²⁻⁴ and the redox behavior of MADH has been investigated by taking account of the *o*-quinone structure of the cofactor.⁵ Recently, however, McIntire and his co-workers revealed the structure of the cofactor to be that of tryptophan tryptophylquinone (TTQ), not PQQ, by ¹H NMR and mass spectroscopic investigations on isolated cofactor-bearing peptides.⁶ Studies on amino acid sequences and X-ray crystallographic analysis of the native enzymes also supported the proposed structure.^{7,8}



The enzymatic mechanism of amine oxidation by MADH has not been clearly demonstrated yet. A transamination mechanism has been recently proposed for the enzymatic reaction,^{6,9} but little was known about the details. Thus in this study, we synthesized

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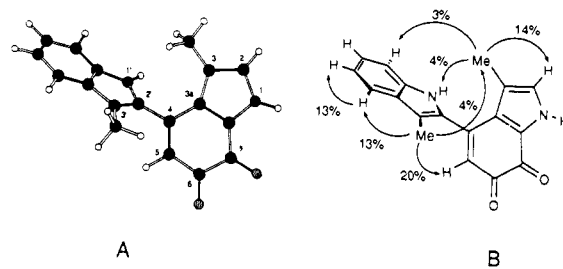
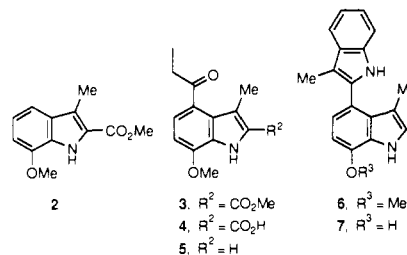


Figure 1. (A) Fully optimized structure of 1 by AM1. The dihedral angle of the two indole rings defined by C(3a)-C(4)-C(2')-N(1') is 54.7°. (B) Selected NOE correlations for 1.

and characterized a model compound of TTQ in order to obtain further information on the structure and the reactivity of the active site cofactor.

The synthesis of model compound 1 was accomplished as follows. Friedel-Crafts acylation on indole derivative 2¹⁰ with propionyl chloride by the standard method gave the 4-acetylated compound 3 (93%), which was converted into 5 by ester hydrolysis (79%) followed by thermal decarboxylation using CuCrO₄ in quinoline (59%). The second indole ring was constructed by Fischer indolization on 5 with phenylhydrazine hydrochloride in 71% yield. Deprotection of the methoxy group of 6 by trimethylsilyl iodide gave the 7-hydroxy derivative 7 (93%), which was finally converted into the expected quinone 1 by oxidation with Fremy's salt in 57% yield.¹¹



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°.⁸ AM1 calculations indicated that the dihedral angle of the two indole rings of compound 1 defined by C(3a)-C(4)-C(2')-N(1') is 54.7° 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).¹² Such a molecular geometry was also suggested by the observed NOE correlations for 1 (Figure 1B). An approximately 4% NOE was detected between the two methyl groups, but this value seems to be relatively small compared to that between 3'-Me and 5-H (20%). In the case of MM2 calculations on a structurally related compound, 4-(3'-methylindol-2'-yl)skatole, minimum steric energy was obtained when the dihedral angle of the two indole rings was 45.8°. ¹³

The two-electron redox potential of 1 was determined to be -188 mV vs SCE by cyclic voltammetry at pH 7.4.¹⁴ This value is comparable to that of native MADH from bacterium W3A1 (*E*_{1/2} = -148 mV vs SCE at pH 7.5).¹⁵ Compound 1 shows a strong absorption at 407 nm ($\epsilon = 1.07 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) with a broad

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(11) Experimental details and analytical data of 1 and 3-7 are provided as supplementary materials.

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(14) The electrochemical measurement was carried out in a 0.1 M phosphate buffer solution containing 30% CH₃CN (pH 7.4) using glassy carbon as a working electrode.

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shoulder at 500–650 nm in CH₃CN; thus, the color of the solution is reddish-brown. Reduction of **1** to 1H₂ (quinol form) by methylhydrazine caused the complete disappearance of the absorptions but gave a new one at 306 nm ($\epsilon = 1.62 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$).¹⁶ These spectra of **1** and 1H₂ resemble in shape those of MADH from *Paracoccus denitrificans* (MADH_{ox}, 440 nm; MADH_{red}, 326 nm).^{5a} In the resonance Raman spectrum of **1**,¹⁷ strong peaks were detected at 1620 (C=O vibration mode), 1570, 1455, 1170, 1146, 1064, and 955 cm⁻¹, which are also closely related to those of TTQ in the native enzymes.⁹

It should be emphasized that model compound **1** acts as a very efficient turnover catalyst in the oxidation of benzylamine under aerobic conditions. *N*-Benzylidenebenzylamine was obtained in the reaction of **1** (1.0 mM) and benzylamine (100 mM) under O₂ atmosphere: 1500% (based on **1**) in CH₃CN after 8 h and 5000% in CH₃OH after 10 h.¹⁸ Mechanistic details and structure–reactivity relationships of the amine oxidation reaction are now under investigation.

Acknowledgment. The present study was financially supported in part by a Grant-in-Aid for Scientific Research (03403016) from the Ministry of Education, Science, and Culture of Japan and also by the Watanabe Foundation. We thank Dr. Akira Nakamura and Dr. Norikazu Ueyama of Osaka University for assistance in obtaining the resonance Raman spectrum, Dr. Yoshihito Tobe of Osaka University for helpful discussions on the AM1 and MM2 calculations, and the Instrumental Analysis Center, Faculty of Engineering, Osaka University, for the measurement of mass spectra on a JEOL JMS-DX303 spectrometer.

Supplementary Material Available: Listings of experimental details of the synthetic procedures and copies of ¹H NMR, ¹³C NMR, IR, mass, UV–vis, and resonance Raman spectra and a cyclic voltammogram of **1** (13 pages). Ordering information is given on any current masthead page.

(16) Quinol 1H₂ was easily isolated from the reaction of **1** and methylhydrazine; see the supplementary material.

(17) The resonance Raman spectrum of **1** was obtained by using 457.9-nm excitation (100 mW) (KBr disk sample).

(18) The aerobic oxidation of benzylamine was performed by the same method reported earlier: Itoh, S.; Mure, M.; Ogino, M.; Ohshiro, Y. *J. Org. Chem.* **1991**, *56*, 6857.

Synthesis of Single Silver Nanoclusters within Spherical Microdomains in Block Copolymer Films

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Recently we have been able to synthesize silver,¹ gold,¹ palladium,² and platinum² nanoclusters in lamellar or cylindrical microphase-separated precursor diblock copolymer films in which metal complexes initially were attached to the monomer comprising one block of the block copolymer. However, the greatest control over the number of atoms or molecules in a cluster should be possible in spherical microdomains. Synthesis of metal clusters in spherical microdomains is related to synthesis of metal and metal sulfide clusters in solution within micelles^{3–6} and vesicles.⁷

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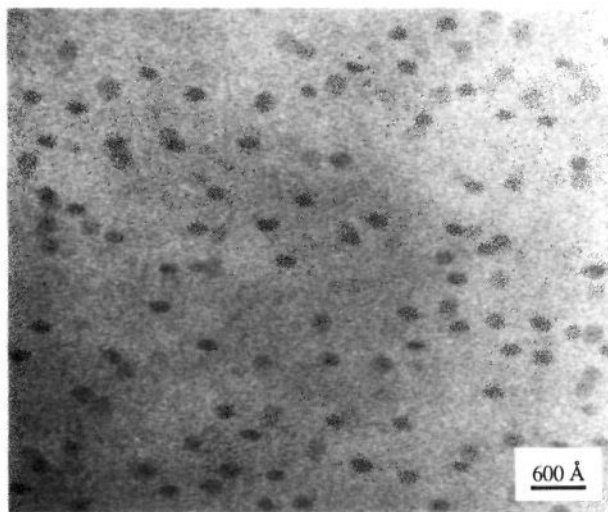
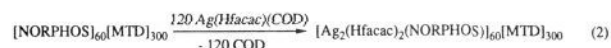
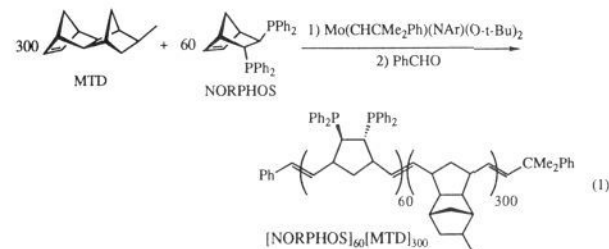


Figure 1. Electron micrograph of Ag-1 (JEOL 200 CX at 200 kV).

We report here that polymer films containing evenly-dispersed silver-containing spherical microdomains can be prepared, and that upon heating these films a single silver cluster (mean diameter <100 Å) forms within each microdomain.

A functionalized phosphine-containing diblock copolymer having the composition [NORPHOS]₆₀[MTD]₃₀₀⁸ (NORPHOS⁹ = racemic 2-*exo*-3-*endo*-bis(diphenylphosphino)bicyclo[2.2.1]heptene; MTD = methyltetracyclododecene; eq 1) was prepared by sequential ring-opening metathesis polymerization of MTD and NORPHOS using Mo(CHCMe₂Ph)(NAr)(O-*t*-Bu)₂^{10,11} (Ar = 2,6-C₆H₃-*i*-Pr₂) as the initiator. (The procedure has been de-



scribed elsewhere.²) The diblock was dissolved in benzene, and 2 equiv of Ag(Hf(acac))(COD)¹² (COD = 1,5-cyclooctadiene; Hf(acac) = [CF₃C(O)CHC(O)CF₃]⁻) per NORPHOS was added. [MTD]₂₀₀ homopolymer (PDI (polydispersity index) = 1.03) was then added in order to yield a polymer mixture containing 3 wt % of the silver-containing block. Films (ca. 0.4 mm thick) were cast by slowly evaporating the solvent under dinitrogen in the dark over a period of 5–8 days.

Thin sections (ca. 400 Å microtomed from the bulk) of the colorless polymer films were analyzed by transmission electron microscopy (TEM). A micrograph of [Ag₂(Hf(acac))₂(NORPHOS)]₆₀[MTD]₃₀₀/[MTD]₂₀₀ (Ag-1; Figure 1) shows silver-

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(8) The polymers have been isolated quantitatively and purified by double precipitation in pentane. GPC analysis of [NORPHOS]₆₀[MTD]₃₀₀ gave *M_n* = 70 000 (calcd 80 030) vs polystyrene with PDI = 1.07. DSC analysis revealed a *T_g* at ca. 177 °C (for poly(NORPHOS)) and 215 °C (for poly(MTD)).

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