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Spectroscopic Evidence for the Redox Reaction between Copper(II) and a Pterin Cofactor Model, 6,7-Dimethyl-5,6,7,8-tetrahydropterin

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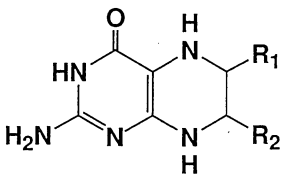
The reactions of Cu(II) with 6,7-dimethyl-5,6,7,8-tetrahydropterin dihydrochloride ($H_4DMP \cdot 2HCl$) in CH_3OH-CH_3CN have been investigated by spectroscopic methods. The redox reaction in the 1:1 Cu(II)–$H_4DMP \cdot 2HCl$ system has been established by detection of the protonated trihydropterin radical ($H_4DMP^{\bullet+}$) and its disproportionation products.

5,6,7,8-Tetrahydrobiopterin (**1**) is a natural pterin derivative required as the cofactor for the catalytic activities of phenylalanine hydroxylase (PAH) and other aromatic amino acid hydroxylases.[1-3] In the presence of the cofactor PAH introduces a hydroxyl group into the benzene ring of phenylalanine by activating O_2, converting phenylalanine to tyrosine in the biosynthesis of neurotransmitters, dopamine, norepinephrine, and epinephrine.[1-3] The tetrameric mammalian PAH contains one non-heme iron per monomer,[1,2,4] whereas the monomeric PAH isolated from *Chromobacterium violaceum* [5] contains a single type II copper[6] with two coordinated histidyl imidazoles in the Cu(II) and Cu(I) states.[7,8]

In the above hydroxylation reaction, **1** is oxidized to 7,8-dihydrobiopterin (quinonoid form).[1,2] Although the oxidation may take place by a one-electron redox process via the trihydropterin radical, no evidence seems to have been reported on formation of the pterin radical species in the course of the enzymatic reaction. 6,7-Dimethyl-5,6,7,8-tetrahydropterin (H_4DMP, **2**), which also has the cofactor activity, has been shown to bind with the Cu(II) center of the bacterial PAH at the N(5) atom.[9] A Cu(II) complex of tris(3-phenylpyrazolyl)hydroborate was observed to undergo a redox reaction with 5,6,7,8-tetrahydropterin (**3**) to give a Cu(I) complex in a preliminary experiment,[10] but no radical species was reported. We have been studying the complex formation and redox reactions of pterin derivatives with Cu(II) complexes such as $Cu(bpy)^{2+}$ (bpy = 2,2'-bipyridine)[11] and established the structure of the first Cu(II) complex isolated.[11b] We here report the spectroscopic evidence for the redox reaction between Cu(II) and $H_4DMP \cdot 2HCl$ in CH_3OH-CH_3CN which gives Cu(I) and equal amounts of H_4DMP and H_2DMP (**4**) via a transient protonated trihydropterin radical $H_4DMP^{\bullet+}$.

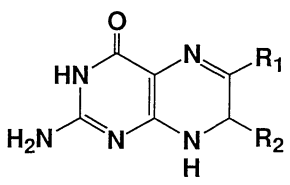
Reduced pterins $H_2DMP \cdot HCl \cdot H_2O$ and $H_4DMP \cdot 2HCl \cdot H_2O$ were prepared from 6,7-dimethylpterin[12]



$R_1 = -CH(OH)-CH(OH)-CH_3$, $R_2 = -H$ (1)

$R_1 = R_2 = CH_3$ (2)

$R_1 = R_2 = H$ (3)



$R_1 = R_2 = -CH_3$ (4)

by reduction with Zn in 0.5 M KOH[13] and catalytic hydrogenation with PtO_2 in 1M HCl (1 M = 1 mol dm^{-3}), respectively. Rapid scan absorption spectra for the 1:1 $[(C_2H_5)_4N]_2[CuCl_4]$–H_4DMP·2HCl system in 50 v/v% CH_3OH-CH_3CN were measured at room temperature with a UNISOKU stopped flow–rapid scan spectrophotometer, the Cu(II) concentration being 0.5 mM. ESR spectra for the 1:1 $Cu(NO_3)_2$–H_4DMP·2HCl system (2 mM) in 50 v/v% CH_3OH-CH_3CN or in 50 v/v% CD_3OD-CD_3CN with and without deuteration were obtained with a JEOL RE-1X ESR spectrometer at room temperature. The spectrum for the non-deuterated system was also measured at 77K by the rapid-mixing-freezing technique. ^{1}H NMR spectra (300 MHz) for the reaction mixture (10 mM) were measured in 50 v/v% CD_3OD-CD_3CN, after the ESR signals for $H_4DMP^{\bullet+}$ had disappeared, with CD_2HCN as the internal reference (δ 1.977 relative to TMS).

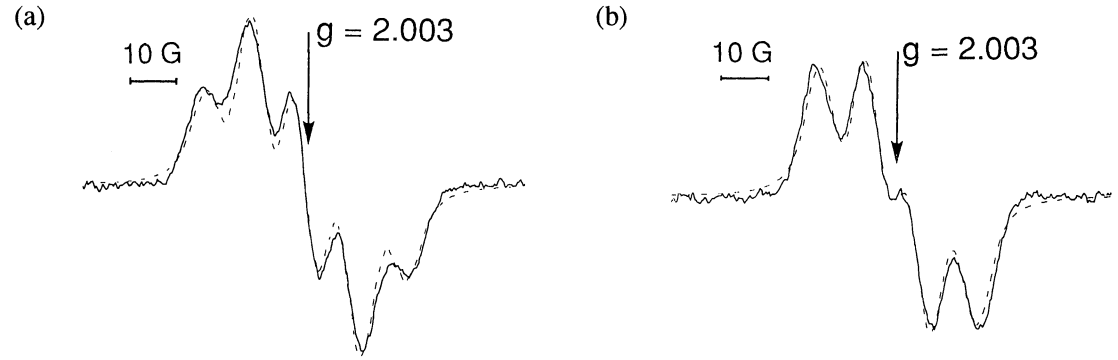


Fig. 1. Observed and simulated ESR spectra of 1:1 $Cu(NO_3)_2$–H_4DMP·2HCl in 50 v/v% CH_3OH–CH_3CN (2 mM)[14] (a) and of the deuterated system in 50 v/v% CD_3OD-CD_3CN (b) under N_2 at room temperature. Observed, ——; calcd., -----. Conditions: microwave frequency, 9.450 GHz; microwave power, 10 mW; modulation, 1G (1G = 0.1 mT).

Addition of $Cu(NO_3)_2$ in CH_3CN (2 mM) to an equimolar amount of H_4DMP·2HCl in CH_3OH under N_2 gave a colorless solution exhibiting the ESR spectrum due to a radical species at g = 2.003 at 298K (Fig. 1(a)),[14] and no Cu(II) signals were observed. The spectrum is very similar to that reported for the pterin radical obtained by oxidation of **3** with H_2O_2 in CH_3OH-CF_3COOH.[15,16] Deuteration of the exchangeable protons of H_4DMP·2HCl prior to the reaction with $Cu(NO_3)_2$ gave the ESR spectrum of the pterin radical (Fig. 1(b)) with a hyperfine pattern different from that shown in Fig. 1(a). The spectra were reasonably simulated by the parameters[17] analogous to those reported[16b] and by considering the highest spin density on N(5) (0.358) with a lower density on N(8) (0.107) as indicated by EHMO calculations. The density on N(5) is supported by the previous calculations[16b,18] and the transmethylation reactions of N-methylated tetrahydropterins.[19] The ESR spectrum obtained for 1:1 $Cu(NO_3)_2$–H_4DMP·2HCl by the rapid-mixing-freezing method showed both the unbound Cu(II) and radical signals, from which 40-50% of Cu(II) used was estimated to be reduced to Cu(I). The ^{1}H NMR spectrum of the final reaction mixture exhibited sharp, well-resolved methyl resonances of protonated H_4DMP (6-CH_3, δ 1.24 (d, J = 6.9Hz, 3H) ; 7-CH_3, δ 1.29 (d, J = 6.6Hz, 3H)) and H_2DMP (6-CH_3, δ 2.38 (s, 3H); 7-CH_3, δ 1.44 (d, J = 6.9Hz, 3H)), indicating that the tetra- and dihydro species were formed in approximately equal amounts and that there was no Cu(II) left unreacted.

Trihydropterins $H_3P^{\bullet}$ are known to disproportionate to tetra- and dihydropterins, H_4P and H_2P, respectively, and the constant K_{com} for the following equilibrium (comproportionation, Eq. 1)

$$H_4P + H_2P \underset{}{\overset{K_{com}}{\rightleftharpoons}} 2H_3P^{\bullet} \qquad (1)$$

has been reported to be 7.1 x 10^{-7} M^{-1} for 6,6,7,7-tetramethyl-5,6,7,8-tetrahydropterin and its dihydro form in CF_3COOH,[20] which shows that the equilibrium lies far to the left, so that the amount of the radical species is very small after the equilibrium has been attained. The NMR spectrum is then interpreted as exhibiting the result of the disproportionation of $H_4DMP^{\bullet+}$. On the basis of the ESR signal intensity calibrated by that of 4-hydroxy-TEMPO (TEMPO = 2,2,6,6-tetramethylpiperidine-N-oxyl radical), the amount of the radical species in the present case was estimated to be 1.8% of $H_4DMP{\cdot}2HCl$ used, and the K_{com} value was then calculated to be 1.3 x 10^{-3} according to Eq. 1.

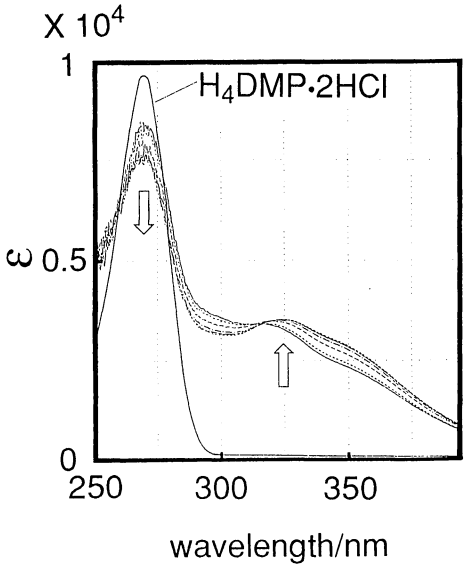


Fig. 2. UV spectral changes for the 1:1 $[(C_2H_5)_4N]_2[CuCl_4]$–$H_4DMP{\cdot}2HCl$ system in the first 20 msec in 50 v/v% CH_3OH-CH_3CN.

Information on the transient species formed during the redox reactions was obtained from the absorption spectral changes in the UV and visible regions measured at room temperature with the stopped flow–rapid scan method. While the Cu(II) d-d band at 920 nm in the 1:1 $[(C_2H_5)_4N]_2[CuCl_4]$– $H_4DMP{\cdot}2HCl$ system in 50 v/v% CH_3OH-CH_3CN disappeared within 20 msec, the spectrum corresponding to the pterin moiety exhibited changes indicating that the rapid oxidation of the tetrahydropterin in the first 5 sec proceeds in two steps, giving first an intermediate spectrum having a maximum at 270 nm and a broad peak at 325 nm with a shoulder at ~350 nm (Fig. 2) and then a spectrum with peaks at 270 and ~335 nm. Because $H_4DMP{\cdot}2HCl$ has an absorption maximum at 268 nm and $H_2DMP{\cdot}HCl$ has peaks at 254 and 361 nm in 50 v/v% CH_3OH-CH_3CN,[21] the observed initial spectral changes may be interpreted as due to formation of the trihydropterin radical and its subsequent disproportionation.

The radical species of H_4DMP obtained by oxidation with $N_3^{\bullet}$ has recently been reported to have a maximum at ~320 nm at pH 7,[22] which corresponds well with the broad peak at 325 nm of the above intermediate spectrum. It is interesting to note in this connection that the oxidations of H_4DMP by PAH,[6] O_2,[18] and by the electrochemical method[23] have been interpreted to indicate that the oxidation process involves one-electron steps with transient formation of pterin radicals. These and the present findings strongly suggest that in 1:1 Cu(II)-$H_4DMP{\cdot}2HCl$ systems H_4DMP undergoes a redox reaction with Cu(II) by a one-electron reaction step via formation of the trihydropterin radical, which then disproportionates to tetra- and dihydropterins.

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