Electrochemical Studies of Biologically-significant Pterin Compounds

JAMES LEHNEN, BARBARA M. WHITE and MAUREEN J. KENDRICK*

Department of Chemistry, University of Alabama, Tuscaloosa, AL 35487 (U.S.A.)

(Received June 2, 1989; revised August 18, 1989)

Abstract

Cyclic voltammetry has been used to study a wide variety of pterin derivatives and their redox behavior. The effect of coordinated molybdenum and tungsten on the pterin redox potentials has also been studied. Our results show that the pterins coordinated to metals appear to be good electron sources for metalloenzyme systems that require multielectron redox processes.

The study of redox states of various pterin derivatives is of great importance in preparing synthetic analogues for the active sites of molybdenum-containing metalloenzymes. Recent evidence that xanthine oxidase and sulfite oxidase, two of these enzymes, contain the pterin ligand system in the active site has been provided by Johnson and Rajagapolan [1]. In addition, Taylor *et al.* have completed a total synthesis of a pterin found in nature, urothione, and have provided structural data that agrees excellently with the pterin isolated from the enzymatic system [2]. Although a definitive structure for these active sites has not yet been determined, a proposed structure with substantial supporting evidence is available, and is given in Fig. 1 [2].

In preparing synthetic analogues for these active sites, we have used cyclic voltammetry in the study of a wide range of pterin derivatives and their redox behavior. The capability of these pterin compounds to act as enzyme cofactors depends significantly on their accessible redox states and overall electrochemistry. We have isolated and characterized (^{1}H and ^{13}C NMR, FT-IR, UV–Vis) each molybdopterin complex and the electrochemical processes of the

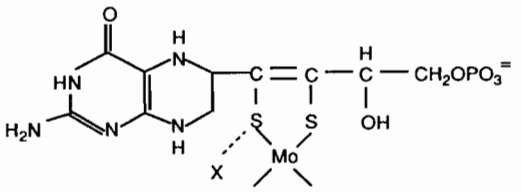

Fig. 1. Proposed structure for the active site environment of some molybdoenzymes.

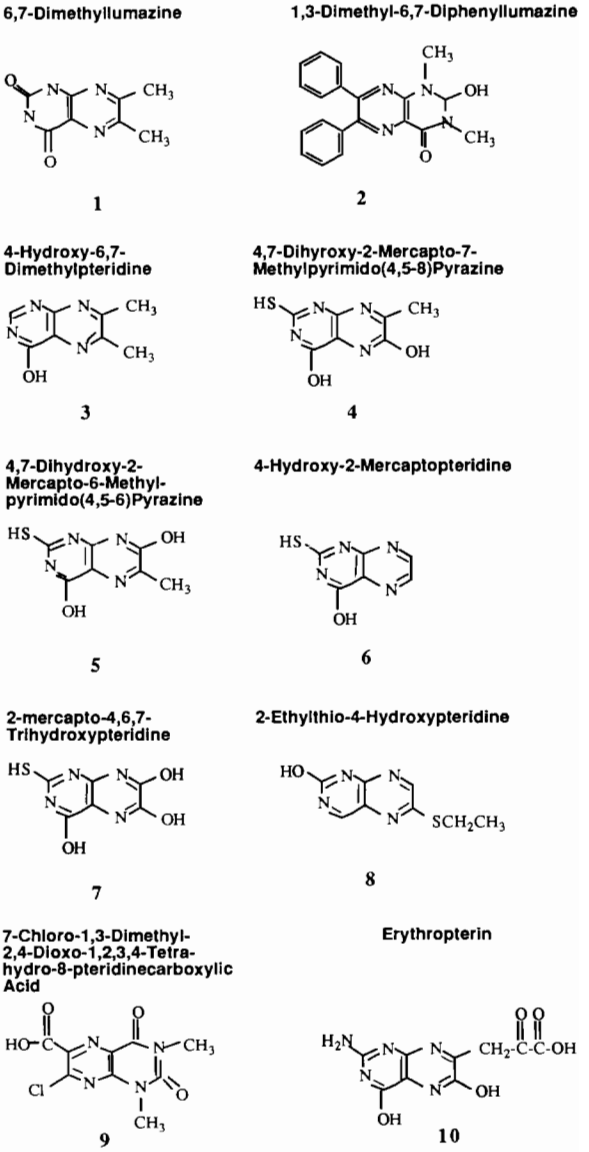

Fig. 2. Structures of pterin complexes studied.

*Current address: Department of Chemistry, Allegheny College, Meadville, PA 16335, U.S.A.

0020-1693/90/$3.50

TABLE 1. Redox potentials and current ratios of pterins[a]

Compound	Solvent	E_{p_a}(V)	E_{p_c}(V)	i_{p_a}/i_{p_c}
1 6,7-Dimethyllumazine	DMF	−0.56	−0.53	0.46
2 1,3-Dimethyl-6,7-diphenyllumazine	DMF	1.19 −0.67	−1.31	0.11
3 4-Hydroxy-6,7-dimethylpteridine	DMF	−0.88 −1.43	−0.58	1.06
4 4,6-Dihydroxy-2-mercapto-7-methylpyrimido-(4,5,8)-pyrazine	DMF	1.14	−0.27	1.65
5 4,7-Dihydroxy-2-mercapto-6-methylpyrimido-(4,5-8)-pyrazine	DMF		−0.27	
6 4-Hydroxy-2-mercaptopteridine	DMF	1.01	−0.42	1.45
7 2-Mercapto-4,6,7-trihydroxypteridine	DMF	0.53 0.86	−0.55	1.10
8 2-Ethylthio-4-hydroxypteridine	DMF	−0.60	−0.30	0.40
9 7-Chloro-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydro-6-pteridine carboxylic acid	DMF	1.17 0.64	1.00 −0.30	16.3 1.20
10 Erythropterin	DMF	0.67	−0.35	2.00

[a]Experimental conditions: DMF solvent, 0.1 M tetra-n-butylammonium hexafluorophosphate as supporting electrolyte. Pt wire/Pt disk/Ag–AgCl electrode system used with a scan rate of 250 mV/s. Compound **11** not listed above is 7-amino-1,3-diethyl-2,4-pteridinedione displayed $E_c = -0.719$ V.

TABLE 2. Effect of molybdenum on redox behavior[a]

Compound	No molybdenum		Plus molybdenum	
	E_{p_a}(V)	E_{p_c}(V)	E_{p_a}(V)	E_{p_c}(V)
Xanthopterin monohydrate	−0.42*	−0.25*	0.74	−0.87
	0.54	−1.01*	1.14	−0.22*
	0.98			−1.29
Lumazine monohydrate	1.08	−0.28	1.22	−0.30*
	−0.44*	−0.81*	0.74	−1.33
			−0.54*	−1.58
7-Amino-1,3-diethyl-2,4-(1H, 3H)-pteridinedione	no redox behavior shown except for that of solvent–electrolyte			
2-Amino-6,7-diphenyl-4-hydroxypyrimido-(4,5-B)-pyrazine	no redox behavior shown except for that of solvent–electrolyte			

[a]Experimental conditions: 0.1 M tetra-n-butylammonium perchlorate; Pt disk, Pt wire and Ag/AgCl as the working, auxiliary and reference electrodes; dimethylformamide solvent. Scan rate 250 mV/s. Starred items: solvent–electrolyte system peaks.

resulting species were studied in a like manner. These synthetic attempts at molybdoenzyme models have just recently begun, and are carried out according to Burgmayer and Steifel [3], Kaul *et al.* [4] and Harlan *et al.* [5]. The structures of the pterins used herein excluding xanthopterin and lumazine are given in Fig. 2.

The cyclic voltammograms for each compound and molybdenum derivatives were recorded using a conventional three electrode system on a Bio-analytical Systems voltammograph, with Pt wire, Pt disk, and Ag/AgCl as the auxiliary, working and reference electrodes, respectively. The solvent system consisted of *N,N*-dimethylformamide (DMF) and 0.10 M tetra-n-butylammonium hexafluorophosphate as the supporting electrolyte. The sample was at least 1 mM in concentration. Table 1 summarizes the redox potentials and current ratios for the pterin derivatives studied. Table 2 describes the effect of the addition of the molybdenum center on the redox behaviors of two selected pterins, xanthopterin and lumazine. Figure 3 depicts a cyclic voltammogram of 1 mM of 2-mercapto-4,6,7-trihydroxypteridine in DMF with 0.10 M tetra-n-butylammonium hexa-

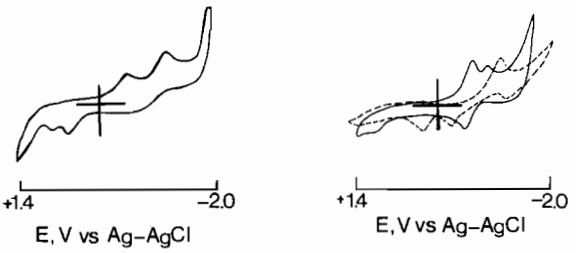

Fig. 3. Cyclic voltammogram of 1 mM 2-mercapto-4,6,7-trihydroxypteridine in dimethylformamide with 0.1 M tetra-n-butylammonium hexafluorophosphate.

Fig. 4. Cyclic voltammograms of 1 mM 1,3-dimethyl-6,7-diphenyllumazine (**2**) (——) and the molybdenum adduct of **2** (- - - -). Experimental conditions are listed in Table 1.

fluorophosphate present, while Fig. 4 shows the effect on the cyclic voltammogram of 1,3-dimethyl-6,7-diphenyllumazine (**2**) when molybdenum is bound to the pterin nucleus. In general, the following points can be summarized based upon the electrochemistry presented herein.

(1) Most of all the pterin derivatives studied display either totally irreversible electrochemical behavior, or quasi-reversible behavior, based upon ΔE and the ratio of the peak currents. Only compounds **3** and **7** showed electrochemical behavior characteristic of reversible behavior.

(2) Even when molybdenum is bound to the pterin nucleus, the electrochemical behavior of the complexes is essentially irreversible.

(3) Coordination of the molybdenum center to a pterin derivative results in more negative ligand redox potentials. Thus pterins coordinated to molybdenum centers appear to be good electron sources for metalloenzyme systems requiring multi-electron redox processes.

(4) We have also isolated the strictly analogous tungsten–pterin complexes for comparison to the molybdo–pterin redox potentials. In every case, the tungsten–pterin complexes exhibit more negative W(VI)/(V)/(IV) redox potentials than the molybdo–pterins, suggesting that tungsten–pterin complexes are too cathodically displaced to perform molybdoenzyme oxo-transferase chemistry [6].

Acknowledgements

We wish to thank the National Science Foundation – Research Opportunities for Women and the Petroleum Research Fund of the American Chemical Society for partial support of this work. James Lehnen was an undergraduate participant in the National Science Foundation Research Experiences for Undergraduates Program.

References

1 J. L. Johnson and K. V. Rajagopalan, *Proc. Natl. Acad. Sci. U.S.A., 79* (1982) 6856.
2 (a) E. C. Taylor and L. A. Reiter, *J. Am. Chem. Soc., 111* (1989) 285; (b) R. C. Wahl and K. V. Rajagopalan, *J. Biol. Chem., 257* (1982) 1354.
3 S. J. N. Burgmayer and E. I. Steifel, *J. Am. Chem. Soc., 108* (1986) 8311.
4 B. B. Kaul, J. H. Enemark, S. L. Merbs and J. T. Spence, *J. Am. Chem. Soc., 107* (1984) 2885.
5 (a) E. W. Harlan, J. M. Berg and R. H. Holm, *J. Am. Chem. Soc., 108* (1986) 6992; (b) J. M. Berg and R. H. Holm, *J. Am. Chem. Soc., 106* (1984) 3035; (c) R. H. Holm and J. M. Berg, *Pure Appl. Chem., 107* (1985) 917, 925.
6 B. W. White and M. J. Kendrick, presented at the *Fourth Int. Conf. Bioinorganic Chemistry, Massachusetts Institute of Technology, Cambridge, MA, U.S.A., July 24–28, 1989.*