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Two Types of Molybdenum Sites in Mo Enzymes, Characterized by the New Indicator of ESR Parameters

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ESR and EXAFS studies on the various molybdenum enzymes have aroused wide interest in the structure of Mo active sites [1–3]. These physical methods have directly characterized the Mo sites. Recent EXAFS data have implicated the presence of either two or three thiolate donor atoms in the Mo coordination sphere, and terminal oxygen atoms in sulfite oxidase and xanthine oxidase [3]. Although most of the enzymes exhibit Mo(V) ESR signals during enzyme turnover [1, 2], little information is revealed on the structure and mechanism of action of the Mo centers. The Mo(V) ESR signals of the enzymes vary under the experimental conditions (pH, buffer, and reaction time *etc.*). In addition, there are few ESR data for thiol–Mo(V) complexes of well-defined structures [4]. Herein, we found a new treatment of ESR parameters which classifies the Mo enzymes and the Mo(V) complexes into two types.

The Mo(V)–thiol (selenol) complexes were prepared by addition of the ligands (0.1 mmol) to $MoO_2(acac)_2$ (0.02 mmol) in 2 ml dry DMSO at room temperature [5]. The complexes were reduced by $NaBH_4$ or excess ligands, and the ESR spectra of monomeric Mo(V) complexes were then obtained both at 293 K in solution and at 77 K in the frozen state. X-band ESR measurements were made using a JEOL JES-FE-3X spectrometer equipped with 100 KHz field modulation. The selenol ligands were synthesized according to Klayman's method [6], with some modulations.

Table I summarizes the ESR parameters of the Mo enzymes and the Mo(V) complexes. A good correlation was found between their $(g_3 - g_1)$ and g_3 values (see Fig. 1). It is clear from this figure that the ESR detectable Mo(V) ions can be grouped according to the $(g_3 - g_1)$–g_3 relation. One class (designated type 1) has wider g-anisotropy than the other class (type 2). Of special interest is the fact that most of the native Mo enzymes fall in a class of type 2. On the other hand, many Mo complexes with one or two thiolates and the inactivated Mo enzymes are included in the type 1 and type 2′ respectively.

Table II gives the O=Mo–(*cis*)X angle (°) and the ESR parameters of the oxo Mo(V)–chloride com-

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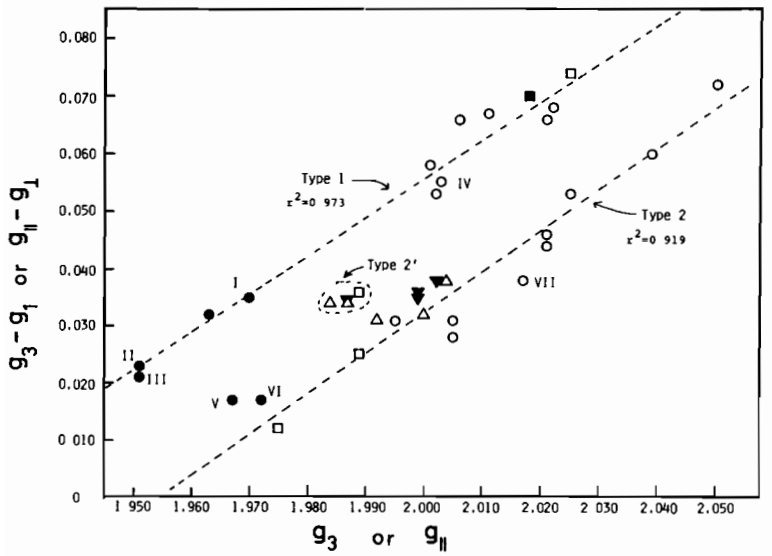

Fig. 1. Plot of ESR parameters for Mo(V) complexes and Mo enzymes. (○) thiol complex, (●) chloride complex. (□) xanthine oxidase, (△) sulfite oxidase (▲) nitrate reductase, (■) NADPH dehydrogenase.

TABLE I. ESR Parameters of Mo(V) Complexes and Mo Enzymes.

Complex	g values (±0.001)					A values (gauss)				$g_3 - g_1$	Type
	g_1	g_2	g_3	g_{av}[a]	g_{rt}[b]	A_1	A_2	A_3	A_{rt}[b]		
Cysteine methylester	1.955	1.955	2.021	1.977	1.976	28	28	59	38	0.066	1
Cysteamine	1 954	1.954	2.022	1.977	1.976	28	28	59	38	0.068	1
N,N-dimethylcysteamine	1.977	1.977	2.021	1.992	1.990	26	26	56	36	0.044	2
Dimercapto ethane	1.978	1.978	2.050	2.002	2.001	22	22	53	32	0.072	2
1,3-Dimercapto propane	1.972	1.972	2.025	1.990	1.989	26	26	56	36	0.053	2
Dithiothreitol	1.964	1.971	1.995	1.977	1.975			66	40	0.031	2
Dimercapto succinic acid	1.979	1.979	2.039	1.999	1.998	22	22	53	32	0.060	2
α-Mercaptopropionyl-glycine	1.943	1.961	2.001	1.968	1.967			64	42	0.058	1
β-Mercaptopropionyl-glycine	1.957	1.975	2.021	1.990				56		0.046	2
Mercaptoacetyl-histidine	1.949	1.949	2.002	1.966	1.966				43	0.053	1
$MoOClL_1$[c]	1.940	1.949	2 006	1.965	1.966	33	25	65	41	0.066	1
$MoOClL_2$[c]	1.944	1.958	2.011	1.971	1.970	25	39	61	41	0.067	1
$[Et_4N][MoOL_3]$[c]	1.974	1.977	2.005	1.985						0.031	2
$[Et_4N][MoOL_4]$[c]	1 977	1.977	2.005	1.986						0.028	2
Selenocysteamine	1.951	1.965	2.104	2.007	2.004			52		0.153	1
β-Methyl selenocysteamine	1.951	1.966	2.105	2.007	2.009			51		0.154	1
N-Methyl selenocysteamine	1.996	2.023	2.042	2.020	2.019					0.046	2
N,N-Dimethyl selenocysteamine	1.999	1.999	2.076	2.025	2.022					0.077	2
K_2MoOF_5 single crystal[d]	1.911	1.911	1.874	1.905		51	51	106	69	−0.037	1
$MoOCl_5^{-2}$ single crystal[d]	1.940	1.940	1.963	1.947		36	36	82	51	0.032	1
$MoOBr_5^{-2}$ single crystal[d]	1.945	1.945	2.090	1 993		30	30	66	42	0.145	1
Xanthien oxidase[e]											
very rapid	1.951	1.956	2.025	1.977		37	24	41	34	0.074	1
rapid complex I	1.964	1.969	1.989	1.974				64		0.025	2
inhibited	1.953	1.977	1.989	1.973		57	25	57	46	0.036	2′
slow	1.957	1 970	1.975	1 967		70				0.012	2
Sulfite oxidase[f]											
pH 7 5 (low pH)	1.968	1.968	2.000	1.979		46	46	63	51	0.032	2
pH 10 (high pH)	1.950	1.961	1.984	1.965				55		0.034	2′
pH 6.5 (low pH)	1.966	1.972	2.004	1 981						0.038	2
pH 10 (high pH)	1.953	1.964	1.987	1.968						0.034	2′
pH 7.3 (phosphate buffer)	1.961	1.969	1.992	1.974						0.031	2
Nitrate reductase[g]											
pH 7.8 (low pH)	1.963	1.986	1 999	1 983						0.036	2
pH 9 7 (high pH)	1 953	1.984	1.987	1.975		35	45	35	38	0.034	2′
nitrate complex	1.964	1.986	2.002	1.984						0.038	2
nitrite complex	1.964	1.985	1.999	1.983						0.035	2
NADPH dehydrogenase[h]	1.948	1.977	2.018	1.981		48	47	52	49	0 070	1

[a] $g_{av} = 1/3(g_1 + {}_2 + g_3)$. [b] Room temperature [c] DMF, see reference 4. [d] See reference 1. [e] Reference 2 [f] Reference 10. [g] Reference 11. [h] Reference 12.

plexes whose single crystal X-ray structures are known [7]. The complexes I–III, with octahedral six coordination structure, belong to the type 1 while the complexes V and VI with five coordination and (distorted) square pyramidal structure fall within the type 2. The O=Mo–X angle (>100°) of the latter complexes is wider than that of the former. The well-characterized thiol–Mo(V) com-

TABLE II. Intratomic Angles (°) and ESR Parameters in $[MoOX_4{*}Y]$ Complexes.

	Complex	Angle (°)								ESR Parameter				Type
		O=Mo–X_1		O=Mo–X_2		O=Mo–X_3		O=Mo–X_4		g_1	g_2	g_3	$g_3 - g_1$	
I	$[AsPh_4][MoOCl_4(H_2O)]$[a]	(Cl)	99.0	(Cl)	99.0	(Cl)	99.0	(Cl)	99.0	1.935	1.935	1.970	0.035	1
II	$[MoOCl_3(P(NMe_2)_3O)_2]$[a]	(Cl)	94.5	(Cl)	96.5	(Cl)	97.0	(O)	95.6	1.928	1.932	1.951	0.023	1
III	$[MoOCl_3(PPh_3O)_2]$[a]	(Cl)	95.4	(Cl)	97.2	(Cl)	93.6	(O)	96.1	1.929	1.935	1.951	0.021	1
IV	$[MoOCl(tox)_2]$[b]	(Cl)	103.0	(S)	106.3	(N)	93.3	(S)	89.2	1.948	1.952	2.003	0.055	1
V	$[AsPh_4][MoOCl_4]$[a]	(Cl)	105.2	(Cl)	105.2	(Cl)	105.2	(Cl)	105.2	1.950	1.950	1.967	0.017	2
VI	$[MoOCl_3(SPPh_3)]$[a]	(Cl)	110.5	(Cl)	102.0	(Cl)	110.9	(S)	100.9	1.955	1.959	1.972	0.017	2
VII	$[MoO(SPh)_4]^-$[c]	(S)	110.2	(S)	108.5	(S)	111.2	(S)	102.5	1.979	1.979	2.017	0.038	2

*X; O=Mo–(*cis*)X, in-plane ligand. [a]Reference 7. [b]References 4 and 8. [c]Reference 9.

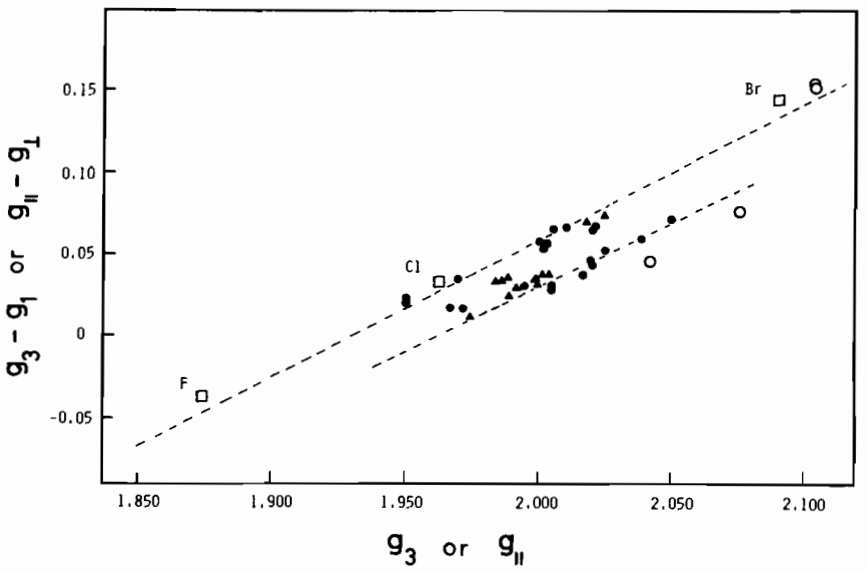

Fig. 2. Donor effect of ESR parameters for Mo(V) complexes. (●) thiol complexes, (▲) Mo enzymes, (○) selenol complexes, (□) $MoOX_5^{-2}$ (X = F, Cl, Br; single crystal).

plexes IV [8] and VII [9] exhibited the same result as the Mo(V)–chloride complexes, though the ESR measurements of the former were performed in solution. It seems likely that the type 1 complexes have octahedral structure, and the type 2 complexes have square pyramidal structure.

Figure 2 shows the effect of coordination donor atoms on the ESR parameters of the Mo(V) complexes. Oxo Mo(V)–halide complexes fit well on the type 1 line. With increasing ionic radius of halide ligands the g values of the halide complexes increase, whereas the A values decrease (see Table I). The result suggests that the unpaired electron in the d_{xy} orbital of Mo atom was strongly delocalized by the $d\pi$ back donation between Mo and in-plane ligands. Indeed, oxo-Mo(V) complexes generally have a $(d_{xy})^1$ ground state. On the other hand, the two types of ESR signals were also observed for the Mo(V)–selenocysteamine and its derivative complexes. It is of interest that the replacement of S by Se gives a distinctice effect between the type 1 and type 2. The result suggests that the type 1 complexes are more strongly affected by the delocalization of the unpaired electron than are the type 2 complexes. Thus the distinction of the donor effect on the two types of complexes can be explained by the difference of the complex structures: since the Mo atoms in the complexes are present in the center of the in-plane ligands for the type 1 complexes with the octahedral structure and upon the in-plane ligands on the type 2 complexes (square pyramidal structure), the overlap between the d_{xy} orbital of the

Mo atom and the π orbital of the in-plane donor atom for the type 1 complexes is larger than in the type 2 complexes. Consequently, the ESR parameters of the type 1 complexes are very strongly affected by the replacement of the in-plane donor atoms.

The present ESR correlation, if confirmed by further structural studies, provides a potential probe for the structure of Mo active sites using ESR data on Mo enzymes.

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References

1 E. I. Stiefel, *Prog. Inorg. Chem., 22,* 1 (1977).
2 R. C. Bray and J. C. Swann, *Structure and Bonding, 11,* 107 (1972).
3 S. P. Cramer, H. B. Gray and K. V. Rajagopalan, *J. Am. Chem. Soc., 101,* 2772 (1979);
J. M. Berg, K. O. Hodgson, S. P. Cramer, J. L. Corbin, A. Elsberry, N. Pariyadath and E. I. Stiefel, *ibid., 101,* 2774 (1979),
T. D. Tullius, D. M. Kurtz, Jr., S. D. Conradson and K. O. Hodgson, *ibid , 101,* 2776 (1979).
4 J. T. Spence, M. Minelli, P. Kroneck, M. I. Scullane and N. D. Chasteen, *J Am. Chem Soc., 100,* 8002 (1978),
M. I. Scullane, R. D. Taylor, M. Minelli, J. T. Spence, K. Yamanouchi, J. H. Enemark and N. D. Chasteen, *Inorg. Chem., 18,* 3213 (1979);
J. T. Spence, M. Minelli and P. Kroneck, *J. Am. Chem. Soc , 102,* 4538 (1980).
5 N. Kim, S. Kim, P. A. Vella and J. Zubieta, *Inorg Nucl. Chem. Letters, 14,* 457 (1978).
6 D. L. Klayman, *J. Org. Chem., 30,* 2454 (1965).
7 C. D. Garner, L. H Hill, F. E. Mabbs, D. L. McFadden and A. T. McPhail, *J. Chem. Soc., Dalton,* 1202 (1977);
C. D. Garner, P. Lambert, F. E. Mabbs and T. J. King, *ibid ,* 1191 (1977),
C. D. Garner, P. Lambert, F. E. Mabbs and T. J. King, *ibid.,* 1191 (1977);
C. D. Garner, L. H. Hill, F. E. Mabbs, D. L. McFadden and A. T. McPhail, *ibid.,* 853 (1977);
C. D. Garner, N. C. Howlader, F. E. Mabbs, P. M. Boorman and T. J. King, *ibid.,* 1350 (1978),
C. D. Garner, N. C. Howlader, F. E. Mabbs, A. T. McPhail and K. D. Onan, *ibid.,* 1848 (1978).
8 K Yamanouchi and J H. Enemark, *Inorg. Chem., 18,* 1626 (1979).
9 J. R. Bradbury, M. F. Mackay and A. G. Wedd, *Aust J. Chem., 31,* 2423 (1978);
J. R. Bradbury, G. R. Hanson, I. W. Boyd, S. F. Gheller, A. G. Wedd, K. S. Murray and A. M. Bond, in 'Proceedings of the 3rd Climax Symposium on Chemistry and Uses of Molybdenum', H. F. Barry and P. C. H. Mitchell, Eds., Climax Molybdenum Co., Michigan, 1979, pp. 300–309.
10 H. J. Cohen, I. Fridovich and K. V. Rajagopalan, *J Biol. Chem.,* 246, 374 (1971),
M. T. Lamy, S. Gutteridge and R. C. Bray, *Biochem J., 185,* 397 (1980).
11 S. P. Vincent and R. C. Bray, *Biochem. J., 171,* 639 (1978).
12 D. V. Dervartanian and R. Bramlett, *Biochim. Biophys. Acta, 220,* 443 (1970).