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# OXIDATION POTENTIALS OF NICKEL THIOLATES, AND Ni(III) SITES OF HYDROGENASES IN $H_2$ UPTAKE BACTERIA

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Oxidation potentials of 15 nickel thiolates were surveyed in aprotic media with differential pulse polarography and cyclic voltammetry in terms of the nickel(III) site of hydrogenases of hydrogen uptake bacteria. The potential distribution of the thiolates in relation to the $RS^-$/Ni ratio suggests that the number of the cysteinyl residues coordinating to the Ni(III) in the enzymes is 4 if there are no other kind of anionic residues around the metal.

Ni(III) site of so-called uptake[1-2] hydrogenases, which physiologically take indispensable roles in hydrogen metabolism of archaic bacteria such as methanogenic bacteria,[3-4] purple bacteria,[2] sulfate reducing bacteria,[5-6] or hydrogen oxidizing bacteria,[7] is known to have abnormal $Ni^{3+}/Ni^{2+}$ redox potential.[8] The potential, (-150 mV— -220 mV vs. NHE; This corresponds to ca. -550 mV— -650 mV vs. SCE in polar organic media when corrected for the standard potential shift from NHE to SCE in aqueous solution, and the aqueous-nonaqueous liquid junction potential[9]), is far lower than the redox potentials of of familiar nickel(II) complexes. Recent EXAFS experiments on hydrogenases of M. thermoautotrophicum[10] and D. gigas[11] have clarified that the Ni(III) centers are surrounded by 3 or 4 sulfur atoms, which should originate from the cysteinyl residues of the enzymes. The half wave potentials, $E_{1/2}(Ni^{3+}/Ni^{2+})$, of nickel complexes shift in general to negative side in nickelate because of the dense electron packing at the metal centers. Then, the Ni(III) site will take the nickelate forms, $[Ni^{III}(S\text{-}cys)_nX_m]^{1-}$, in the isolated enzymes. In the preceeding paper, we have demonstrated the syntheses of several thiolato-nickelate compounds and their $Ni^{2+} \rightarrow Ni^{3+}$ oxidation potentials by the anodic peak positions of cyclic voltammograms, which were favorably low but still not enough to be compared with that of Ni-containing hydrogenases.[12]

The purpose of this letter is to add new tetrahedral complexes, $[Ni(SR)_4]^{2-}$, to thiolato-nickelate group, to reveal their oxidation potential ranges in a more inclusive way, and thereby to make a suggestion about the number of cysteinyl $S^-$ anions around Ni(III) in the hydrogenases.

The new compounds, $(NEt_4)_2[Ni(SR)_4]^{2-}$ ($RS^-$=p-$MeC_6H_4S^-$, m-$MeC_6H_4S^-$, p-$ClC_6H_4S^-$

$p\text{-}NO_2C_6H_4S^-$, $Bu^tS^-$), were synthesized from $(NEt_4)_2[NiCl_4]$ and the corresponding sodium thiolates in strictly dried aprotic media according to the way described in the previous paper.[12] The recrystallization conditions, crude yield, and the analytical data are listed in Ref. 13. $[Ni_2(edt)_3]^{2-}$, $[Ni_3(edt)_4]^{2-}$ (edt=1, 2-ethanedithiolate), $[Ni(SEt)_2]_6$, and $[Ni(SPh)_4]^{2-}$ were prepared by the method of Ref. 10, and $(NMe_4)_2[Ni_2(SEt)_6]$ by the method of Ref. 14.

Among the compounds listed above, $[Ni(SR)_4]^{2-}$ undergoes ligand dissociation in polar aprotic solvents (ex. DMSO, $CH_3CN$ ---), as known generally for $[NiX_4]^{2-}$ ($X^-=Cl^-$, $Br^-$, $I^-$, $PhS^-$).[15] Equation of the dissociation equilibrium and the equilibrium constants determined by $^1H$-NMR are as follows; Eq. 1 and Table 1.

$$\underset{\text{(paramagnetic)}}{[Ni(SR)_4]^{2-}} \rightleftharpoons \underset{\text{(diamagnetic)}}{[Ni(SR)_3]^{1-}} + RS^- \qquad (1)$$

Table 1. The dissociation constants[a)]

| $RS^-$ | $p\text{-}MeC_6H_4S^-$ | $m\text{-}MeC_6H_4S^-$ | $PhS^-$ | $p\text{-}ClC_6H_4S^-$ | $p\text{-}NO_2C_6H_4S^-$ | |
|---|---|---|---|---|---|---|
| K[b)] | 20.8 | 12.5 | 4.35 | 2.55 | 9.49 | mM |

a) $K=[Ni(SR)_3^-][RS^-]/[Ni(SR)_4^{2-}]$. b) In DMSO, 301 K, 1 M=1 mol $dm^{-3}$.

Electrochemistry of $[Ni(SR)_4]^{2-}$ was complicated because of this equilibrium. Therefore, cautious experiments were performed to obtain the oxidation potentials of the tetra- and tri-thiolates, controlling the concentrations of both species based on the law of mass action. The oxidations of all the nickel(II) thiolates were irreversible in aprotic media, and the anodic peak positions of the cyclic voltammograms (glassy carbon, vs. SCE, in DMSO) were scan rate dependent (ex. $(\partial Epa/\partial \log v)_{22\,^\circ C}=-0.028$ for $[Ni(SR)_4]^{2-}$; $RS^-=p\text{-}MeC_6H_4S^-$), suggestive of "pure kinetic" case.[16] Hence, to minimize the scanning error, as well as to obtain distinct redox positions, differential pulse polarography (DPP) was applied for the complexes,[17] although the application of this method to a solid electrode should be done carefully to avoid experimental error due to adsorption.[18]

The result is illustrated in Fig. 1, plotting the anodic peak position of DPP,Ea, (glassy carbon, vs. SCE, in DMSO, Mod. Amp. = 10 mV, Scan Rate = 5 mV $s^{-1}$, scanned from negative to positive side) in ordinate, and pKa of the corresponding thiol[19] in abscissa, (Ka, proton dissociation constant of a thiol, is used here as a guide for the electron donation of the thiolate). As is shown in Fig. 1, the potential shifts from the positive to the negative as the electron donation of the thiolate increases, both for $[Ni(SR)_4]^{2-}$ and $RS^-/Ni=3$ series.

The location of tetra-cysteinate, estimated from the pKas of N-acetyl derivatives of penicillamine and 2-mercaptoisoleucine,[19] is shown in the figure by a dotted line. The oxidation potentials of nickel containing hydrogenases vs. SCE in DMSO overlap closely with this area. The potential drops in $RS^-/Ni=3$ series and others with $RS^-/Ni<3$ are insufficient to reach to the hydrogenase region. Therefore, if there is no anionic ligand other than cysteinates at the nickel site in the enzymes, the number of sulfur atoms will be 4 as reported for D. gigas by EXAFS.[11] In case of mixed ligation of cysteinate with other anionic coordinating residues (such as histydinate, tyrosinate, glutamate, ---

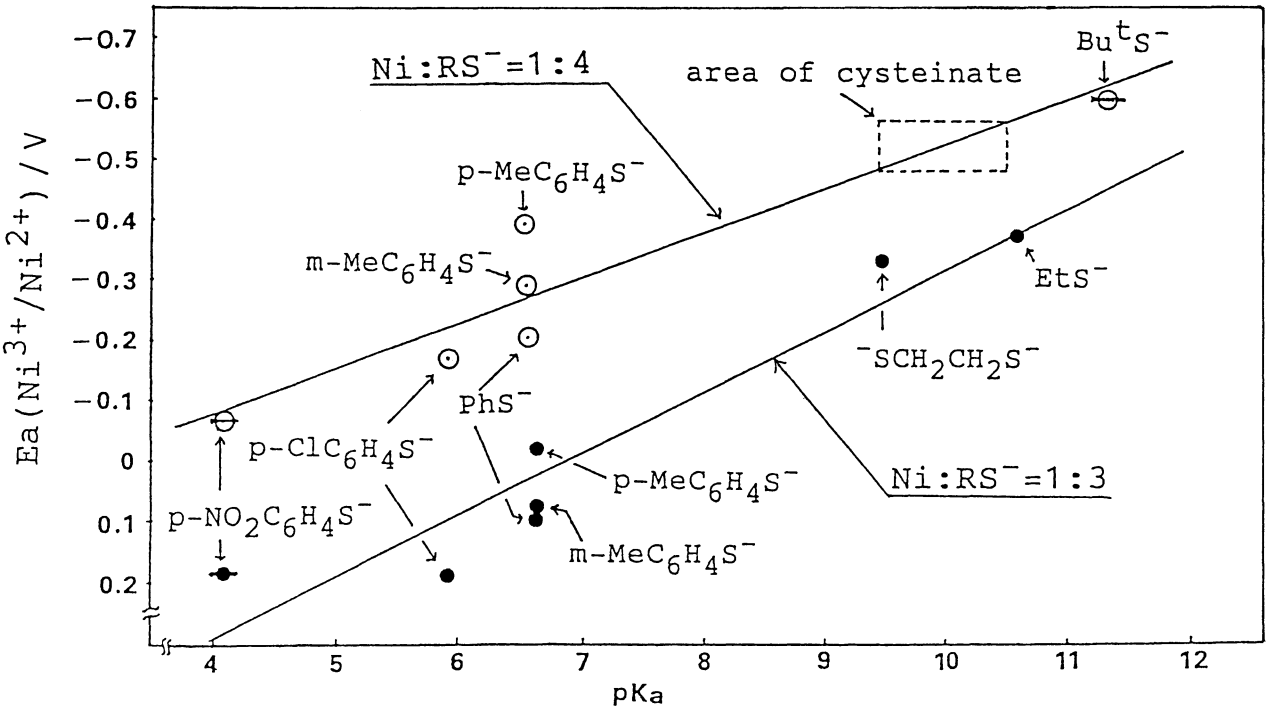


Fig. 1. Anodic peak position, $Ea(Ni^{3+}/Ni^{2+})$ by DPP, of tetra- or tri-thiolate vs. pKa of the corresponding thiol. ---- Ea of $[Ni(SEt)_2]_6$ and $[Ni_3(edt)_4]^{2-}$ were +0.6 and -0.16 V, respectively. pKa of $p\text{-}NO_2C_6H_4SH$ in water was estimated from the reported value in 40% aq. EtOH soln., and the pKas of $Bu^tSH$ and $edtH_2$ from other alkyl thiols'.[19]

and so on) to the center, the number of the sulfur atoms will diminish conserving the total number of the anionic residues about 4. The case of F-420 reducing hydrogenase of M. thermoautotrophicum, which was reported to have 3 sulfur atoms (EXAFS),[10] might conform to this type.

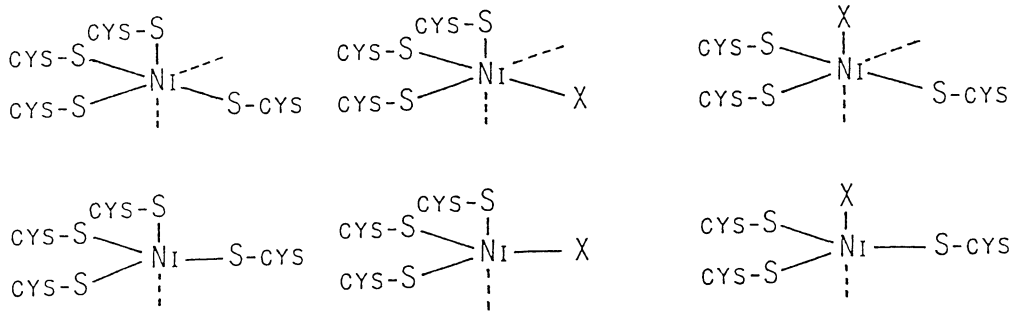


Fig. 2. Proposal for the site.

Finally, the author propose here as the site geometry of Ni(III) in uptake hydrogenases, square pyramidal, distorted octahedral, or trigonal bipyramidal coordinations as drawn in Fig. 2, considering the present result, the EXAFS data,[10-11] and the strong anisotropy of Ni(III) EPR (low spin, S=1/2),[2-7] although the last geometry does not always realize low spin in a crystal field calculation.[20]

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References and Notes

1) R. Cammack, W. V. Lalla-Maharajh, and K.Schneider, "Interaction between Iron and Proteins in Electron Transport and Oxygen Utilization," ed by C. Ho, Elsevier/North-Holland, Amsterdam (1980), Vol. 2, p. 411.
2) S. P. J. Albracht, J. W. VanDer Zwaan, and R. D. Fontijn, Biochim. Biophys. Acta, 766, 245 (1983).
3) J. R. Lancaster, Jr., FEBS Lett., 115, 285 (1980).
4) N. Kojima, J. A. Fox, R. P. Hausinger, L. Daniels, W. H. Orme-Johnson, and C. Walsh, Proc. Natl. Acad. Sci. U.S.A., 80, 378 (1983).
5) J. LeGall, P. O. Ljungdahl, I. Moura, H. D. Peck, Jr., A. V. Xavier, J. J. G. Moura, M. Teixera, B. H. Huynh, and D. V. DerVartanian, Biochem. Biophys. Res. Commun., 106, 610 (1982).
6) W. V. Lalla-Maharajh, D. O. Hall, R. Cammack, and K. Krishna Rao, Biochem. J., 209, 445 (1983).
7) C. G. Friedrich, K. Schneider, and B. Friedrich, J. Bacteriol., 152, 42 (1982).
8) R. Cammack, D. Patil, R. Aquille, and E. C. Hatchkian, FEBS Lett., 142, 289 (1982); M. Teixeira, I. Moura, A. V. Xavier, D. V. Dervartanian, J. Legall, H. Peck,Jr., B. H. Huynh, and J. J. Moura, Eur. J. Biochem., 130, 481 (1983).
9) B. V. Depamphilis, B. A. Averill, T.Herskovitz, L. Que, Jr., and R. H. Holm, J. Am. Chem. Soc., 96, 4159 (1974).
10) P. A. Lindahl, N. Kojima, R. P. Hausinger, J. A. Fox, B. K. Teo, C. T. Walsh, and W. H. Orme-Johnson, J. Am. Chem. Soc., 106, 3062 (1984).
11) R. A. Scott, S. A. Wallin, M. Czechowski, D. V. DerVartanian, J. Legall, H. Peck, Jr., and I. Moura, J. Am. Chem. Soc., 106, 6864 (1984).
12) T. Yamamura, H. Miyamae, Y. Katayama, and Y. Sasaki, Chem. Lett., 1985, 269.
13) p-$MeC_6H_4S^-$;acetonitrile/ether(Y,66.2%),C64.87%(65.02),H8.63(8.44),N3.67(3.45). m-$MeC_6H_4S^-$;acetonitrile/ether(Y,41.0%),C65.02%(65.09,H8.48(8.44),N3.62(3.45). p-$ClC_6H_4S^-$;acetonitrile(Y,63.0%),C53.57%(53.76),H6.42(6.32),N3.45(3.13). p-$NO_2C_6H_4S^-$;acetone after washed with EtOH(Y,46.8%),C50.48%(51.13), H6.35(6.44),N8.78(8.95). $Bu^tS^-$;acetonitrile/ether(Y,47.3%),C55.91%(56.86), H11.45(11.33),N4.14(4.14). Calcd. in parentheses.
14) A. D. Watson, CH. Pulla Rao, J. R. Dorfman, and R. H. Holm, Inorg. Chem., 24, 2820 (1985).
15) D. M. L. Goodgame, M. Goodgame, and F. A. Cotton, J. Am. Chem. Soc., 83, 4161 (1961); D. G. Holah, and D. Coucouvanis, ibid., 97, 6917 (1975).
16) R. S. Nicholson and I. Shain, Anal. Chem., 36, 706 (1964).
17) H. Matsuda, Bull. Chem. Soc. Jpn., 53, 3439 (1980).
18) Dropping mercury electrode was not used because of the practical difficulty. All the measurements were performed under argon using a glove box.
19) J. P. Danehy and K. N. Parameswaran, J. Chem. Eng. Data, 13, 386 (1968).
20) C. A. L. Becker, D. M. Meek, and T. M. Dunn, J. Phys. Chem., 74, 1568 (1970).

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