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L-BAND ESR SPECTRA OF COPPER(II) COMPLEXES WITH CuN_4 CONFIGURATIONS

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SUMMARY: L-band ESR spectrometry has been developed for detecting free radicals or paramagnetic metal ions in intact animals or biological samples which contain much water. Several studies by L-band ESR on free radicals have been reported, but few on paramagnetic elements such as copper(II) or iron(III). We measured L-band ESR spectra of Cu(II) for the first time to investigate the coordination structure around the metal ion in aqueous solution. In X- and L-band ESR, both spectral patterns and parameters have been found to reflect the coordination structure of Cu(II)-polyamine complexes depending on the structures such as square-planar, axially coordinate square-planar, tetrahedral and distorted square-planar (Cu(II) ion is displaced from CuN_4 plane). In addition, in L-band ESR, the coordination structures of Cu(II) complexes relate with spectral pattern, ESR parameter (^{L}A-value), stability constants and halfwave potentials in terms of the electron density on Cu(II). Thus, L-band ESR spectrometry was indicated to be useful to know the coordination structure of Cu(II) proteins or enzymes in aqueous solution.

ESR is a method for detecting unpaired electrons or radicals (1, 2). If informations on pharmacodynamics and distribution of a paramagnetic ion inside living organisms are obtained from outside of the body by measuring ESR, a new direction of diagnosis, treatment of sickness and development of medicines will be established (3, 4). In general, conventional X-

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Abbreviations: ESR, electron spin resonance; en, ethylenediamine; N-Me-en, N-methylethylenediamine; N,N-Me_2-en, N,N-dimethylethylenediamine; N,N'-Me_2-en, N,N'-dimethylethylenediamine; N-Et-en, N-ethylethylenediamine; N,N-Et_2-en, N,N-diethylethylenediamine; N,N'-Et_2-en, N,N'-diethylethylenediamine; N-Pr-en, N-propylethylendiamine; tn, 1,3-trimethylenediamine; chxn, trans-1,2-cyclohexanediamine; py, pyridine; dien, diethylenetriamine; trien, triethylenetetramine; tetren, tetraethylenepentamine; hexen, pentaethylenehexamine; phen, 1,10-phenanthroline; bipy, 2,2'-bipyridine; cyclen, 1,4,7,10-tetraazacyclododecane; cyclam, 1,4,8,11-tetraazacyclotetradecane; Li-TCNQ, tetracyano quinodimethane lithium salt; E_{pa}, oxidation potential; E_{pc}, reduction potential; E, redox potential; $E_{1/2}$, halfwave potential; NHE, normal hydrogen electrode; χ_g, mass susceptibility; log K_β, overall stability constant; g- and A-values, parameters from X-band ESR; ^{L}g- and ^{L}A-values, parameters from L-band ESR.

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band ESR method is difficult to detect paramagnetic metal ions in aqueous solution at room temperature, since X-band microwave (around 9 GHz) is absorbed by water molecule which has a high dielectric constant. On the other hand, L-band microwave (around 1 GHz) has little dielectric loss by water. Therefore, L-band ESR method is expected to be very useful for measuring paramagnetic metal ions in biological samples at room temperature (5-12).

Recently, we found that both X-band ESR parameters and spectral patterns depend on the coordination structure, stability constant and redox property of Cu(II) complex (14). Thus, it has been proposed that unknown coordination structure, stability constants and redox potentials of Cu(II) complexes or proteins can be presumed by measuring X-band ESR spectra (14). On the bases of these results, we have applied L-band ESR method to know the coordination structure of Cu(II) complexes with CuN_4 coordination modes such as square-planar, axially-coordinate square-planar and distorted square-planar as models of Cu(II) proteins or enzymes. The coordination structure of Cu(II) complexes in aqueous solution has been found to be estimated by L-band ESR method similarly to X-band ESR. This paper reports the first measurement and analysis of Cu(II) complexes by L-band ESR spectrometry.

MATERIALS AND METHODS

Ligands used were purchased from Aldrich Chemical, Nacalai Tesque, Tokyo Kasei and Wako-Pure Chemical Industries. X- and L-band ESR spectra were recorded with X- and L-band ESR spectrometers, JES-RE3X and JES-RE3L, JEOL (Japan), respectively, at room (22 ℃) and liquid nitrogen (77K) temperatures. Concentrations of Cu(II) for X- and L-band ESR measurements were 5×10^{-4} M and 0.1 M, respectively, at the molar ratio of Cu:ligand=1:0.5 ~ 1:8. pH-Value of sample solutions for X-band ESR measurement was adjusted to 7.4 with 10^{-2} M 2,6-lutidine - 0.1 M HCl buffer and those for L-band ESR were 5.0, 7.4 and 10.0 with 0.1 M HCl and 0.1 M NaOH. Simulated ESR spectra were obtained with ESPRIT ESR Data System, JEOL (Japan). Visible absorption spectra in aqueous solution were recorded at room temperature on a spectrometer, Ubest-55, JASCO (Japan), at the molar ratio of Cu:ligand=1:4 ($Cu(II)=10^{-2}$) and pH=4~11, adjusted with 0.1 M HCl and 0.1 M NaOH. Cyclic voltammograms were obtained for the solution of acetonitrile-water (1:1) containing 0.1 M tetra-*n*-butylammonium perchlorate with an electrochemical workstation, 100B/W, BAS (U. S. A.) at the molar ratio of Cu:ligand=1:2 ($Cu(II)=10^{-3}$ M). Cyclic voltammetry was performed with a three-electrode system containing glassy-carbon working electrode, platinum counter electrode and Ag/AgCl (saturated NaCl) reference electrode, recorded at a scan rate of 100 mV^{-1}. Halfwave potentials ($E_{1/2}$-value) were calculated as the midpoint between the peek potentials, $E_{1/2} = (E_{pc} + E_{pa})/2$. Magnetic susceptibilities for aqueous solution or powder were measured at room temperature (25℃) with a magnetic susceptibility balance, MK-I, Sherwood Scientific Limited (U. K.).

RESULTS AND DISCUSSION

L-band ESR spectra were obtained at both room and liquid nitrogen temperatures. Observed and simulated spectra of $Cu(II)$-$(en)_2$ complex are shown in Fig. 1. L-band ESR spectral simulation was performed with both X-band ESR parameters (g- and A-values) and microwave

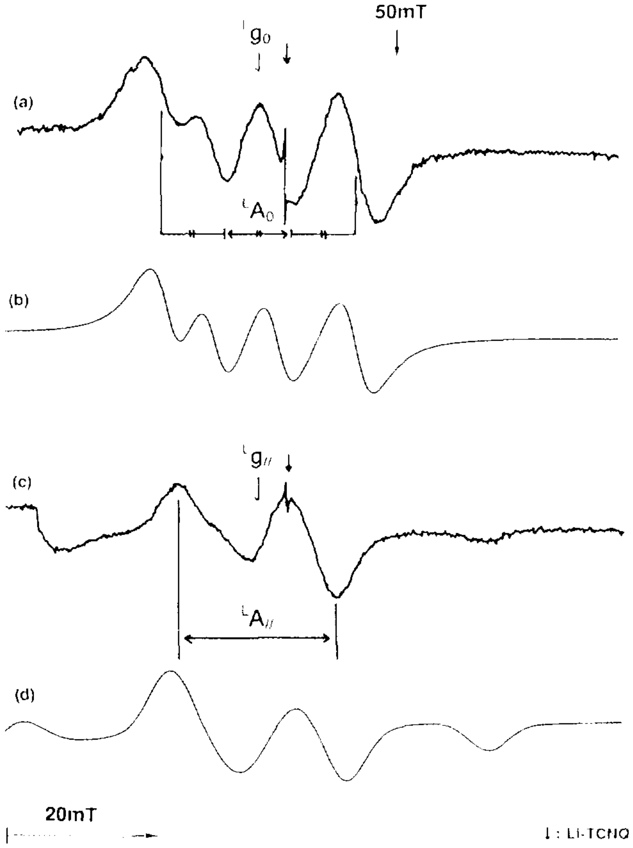

Fig. 1. L-band ESR spectra of Cu(II)-$(en)_2$ complex in aqueous solution. Complexes were prepared at the molar ratio of Cu(II):ligand = 1:4 (Cu(II) = 100mM). (a) Observed spectrum at room temperature, (b) simulated spectrum at room temperature, (c) Observed spectrum at liquid nitrogen temperature and (d) simulated spectrum at liquid nitrogen temperature.

frequency transformed from X-band (around 9 GHz) to L-band (around 1 GHz). The simulated spectrum was fully identical with the observed spectrum, indicating that the parameters of L-band ESR are essentially identical to those of X-band ESR. ESR parameters involving both X- and L-band spectra and other physicochemical parameters such as visible absorption parameters, stability constants, halfwave potentials and magnetic susceptibilities for Cu(II) polyamine complexes are summarized in Table 1.

L-band ESR spectra of Cu(II)-en complex at the molar ratio of Cu(II):ligand =1:1, 1:2 and 1:4 at room and liquid nitrogen temperatures are shown in Fig. 2. At 1:1 molar ratio, Cu(II)-en complex with CuN_2 configuration was formed, as evaluated by the spectral pattern and ESR parameters. But at 1:2 and 1:4 molar ratios, a CuN_4 type complex was formed. When CuN_4 plane was formed and the stability of in-plane bonding was strengthened, ^{L}A-values increased

Table 1. ESR and optical and related parameters for polyamine-Cu(II) complexes at pH 7.4 with CuN_4 coordination mode

Ligand	g-value				A-value ($10^{-4}cm^{-1}$)				λ_{max}	ε		$E_{1/2}$ (mV)	χ_g
	g_0	$^Lg_0$	$g_{//}$	$^Lg_{//}$	A_0	$^LA_0$	$A_{//}$	$^LA_{//}$	(nm)	($M^{-1}cm^{-1}$)	log K_β[a]	(*vs.* Ag/AgCl)	(10^{-6} c. g. s.)
square-planar													
$(en)_2$	2.101	2.169	2.204	2.234	82.9	89.9	209.5	219.4	547	128	19.6	-413	3.954
$(N\text{-}Me\text{-}en)_2$	2.104	2.176	2.208	2.233	83.2	89.3	203.2	221.0	551	84	18.9	-378	3.539
$(N,N\text{-}Me_2\text{-}en)_2$	2.104	2.176	2.203	2.224	80.3	86.1	200.0	213.2	564	156	16.4	-201	3.038
$(N,N'\text{-}Me_2\text{-}en)_2$	2.106	2.182	2.214	2.242	82.8	87.7	200.3	215.6	565	107	17.1	-260	2.992
$(N\text{-}Et\text{-}en)_2$	2.104	2.179	2.208	2.239	82.4	89.0	202.1	219.2	558	112	18.6	-277	3.153
$(N,N\text{-}Et_2\text{-}en)_2$	2.114	2.171	2.247	2.228	79.7	78.4	186.3	198.0	585	210	13.7	-43	2.702
$(N,N'\text{-}Et_2\text{-}en)_2$	2.118	2.203	2.248	2.237	74.5	86.7	185.8	210.3	583	108	15.6	-76	2.520
$(N\text{-}Pr\text{-}en)_2$	2.104	2.187	2.207	2.238	82.2	88.3	200.3	215.3	559	104	18.1	-249	3.071
$(tn)_2$	2.109	2.151	2.220	2.224	78.6	80.6	202.6	210.4	568	106	16.9	-290	3.427
$(chxn)_2$	2.103	—	2.205	—	84.0	—	206.7	—	542	76	20.4	-498	—
cyclam	2.093	—	2.185	—	89.9	—	216.1	—	583	108	27.2	-954	—
$(NH_3)_4$[b]	2.122	—	2.248	—	76.2	—	188.6	—	596	52	13.0	-72	4.744
$(py)_4$[c]	2.133	—	2.304	—	72.1	—	185.8	—	559	104	6.5	+284	2.331
axially-coordinate square-planar													
$(dien)_2$ (6-coordinate)	2.113	2.158	2.212	2.244	65.4	73.2	181.7	203.9	630	110	20.9	-459	2.971
hexen (5-coordinate)	2.110	2.165	2.203	2.255	66.7	71.9	167.6	200.7	627	236	22.1	-617	2.410
tetren (5-coordinate)	2.110	2.176	2.205	2.242	66.7	75.6	163.2	210.0	645	166	22.8	-657	3.050
tetrahedral													
$(phen)_2$	2.145	—	2.271	—	57.3	—	155.8	—	690	42	11.1	+80	3.380
$(bipy)_2$	2.145	—	2.259	—	59.5	—	156.1	—	700	72	12.7	+55	3.480
distorted square-planar													
trien	2.102	2.182	2.200	2.233	80.8	87.6	200.2	214.1	587	144	20.1	-517	
cyclen	2.103	2.145	2.200	2.221	76.0	77.6	191.3	201.2	605	202	24.8	-617	

a: A.E.Martell, Critical Stability Constants, vol. 2 Amines, vol. 5 First Supplement (1982)

b: in the presence of excess ammonia water

c: in the presence of excess pyridine (Cu:py=1:100)

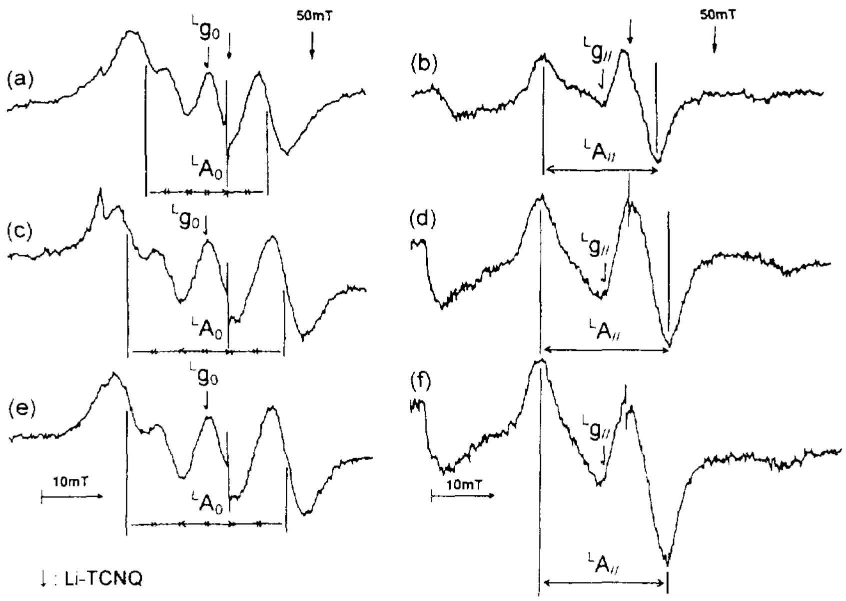

Fig. 2. L-band ESR spectra of Cu(II)-(en)$_2$ complex in aqueous solution at room and liquid nitrogen temperatures. (a) and (b) Cu:en = 1:1, (c) and (d) Cu:en = 1:2, (e) and (f) Cu:en = 1:4. (a), (c) and (e) were measured at room temperature and (b), (d) and (f) were at liquid nitrogen temperature.

($^{L}A_0 = 7.1$, $^{L}A_{//} = 19.1$ and $^{L}A_{\perp} = 1.1$ (mT) to $^{L}A_0 = 8.8$, $^{L}A_{//} = 21.3$ and $^{L}A_{\perp} = 2.6$ (mT)). As observed by the visible absorption spectra, the Cu(II):en = 1:2 complex was fully formed with an increase of pH 5.0 to 10.0.

X-band ESR parameters (g_0- and A_0-values) reflect the coordination structure of Cu(II) polyamine complexes (Fig. 3-(a)). L-band ESR parameter ($^{L}A_0$-value) has also been found to depend on the coordination structure of Cu(II) complexes (Fig. 3-(b)). Axial-coordination or tetrahedral distortion caused a decrease of electron density of unpaired electrons on Cu(II) of N_4-plane (15). Therefore, the axially-coordinate square-planar and tetrahedral complexes have small A_0-value.

The stability constant of the complex is thought to relate with the electron donating ability from ligand to metal (16). Then, we examined the relationship between the over-all stability constants (log K_β, $K_\beta = [ML_n] / [M][L]^n$) and X- or L-band ESR parameters of Cu(II) complexes. Linear relationships between g_0- or $^{L}g_0$-values and log K_β of Cu(II) complexes were found, in which the correlation coefficients were r = 0.775 (X-band) and 0.483 (L-band) for a

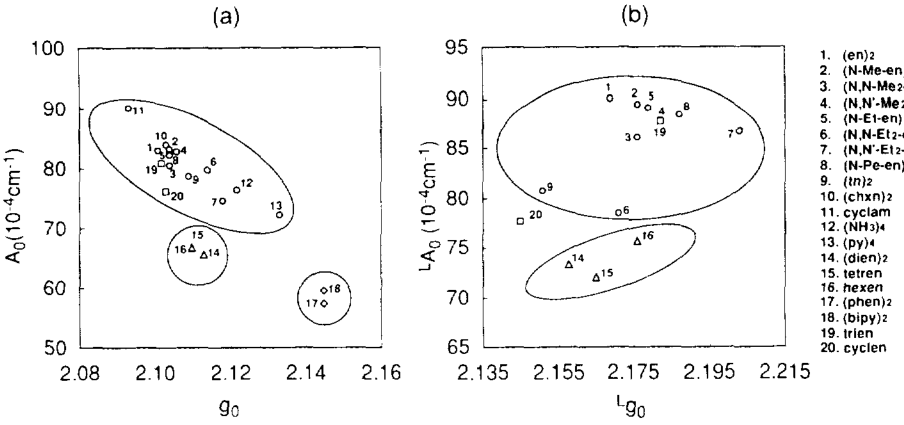

Fig. 3. Plots for (a) g_0-value vs A_0-value and (b) $^{L}g_0$-value vs $^{L}A_0$-value for CuN_4 type complexes. : square-planar, △: axially coordinate square-planar, ◇: tetrahedral and ☐: distorted square-planar.

total 20 and 14 points, respectively (Data not shown). A_0- or $^{L}A_0$-value correlated well linearly with log K_β for the square-planar type complexes, in which the correlation coefficients were r = 0.921 (X-band) and 0.779 (L-band) for a total of 13 and 9 points, respectively (Fig. 4). These results indicate that A- or ^{L}A-value strongly reflects the coordination structure of Cu(II) polyamine complexes. Thus, the complexes with high over-all stability constants give large A_0- or $^{L}A_0$-value and small g_0- or $^{L}g_0$-value. While, in axially-coordinate square-planar or tetrahedral complexes the electron density of CuN_4 plane decreases and shows small A- or ^{L}A-value. These results suggest that g_0- or $^{L}g_0$-value correlates mainly with the stability constant of the complex, while A_0- or $^{L}A_0$-value principally relates to the stability of CuN_4 plane.

Since halfwave potentials of the complexes have a close relation to the electron density on Cu(II) atom, Cu(II) complexes with more negative halfwave potentials are difficult to be reduced to Cu(I) than the complexes with more positive halfwave potentials. Plotting $E_{1/2}$-value *vs*. log K_β of the complexes indicated a good linear relationship with the correlation coefficient r = 0.976 for a total of 20 points. Moreover, $E_{1/2}$-values correlated curvilinearly with g_0-values (the correlation coefficient r = 0.808 for a total 20 points) (Data not shown), indicating that the electron density of the complexes relates to the unpaired electron density of Cu(II) center. From these results, ESR parameters, over-all stability constant and halfwave potential of the Cu(II) complexes have been found to relate each other in terms of the unpaired electron density of Cu(II) center.

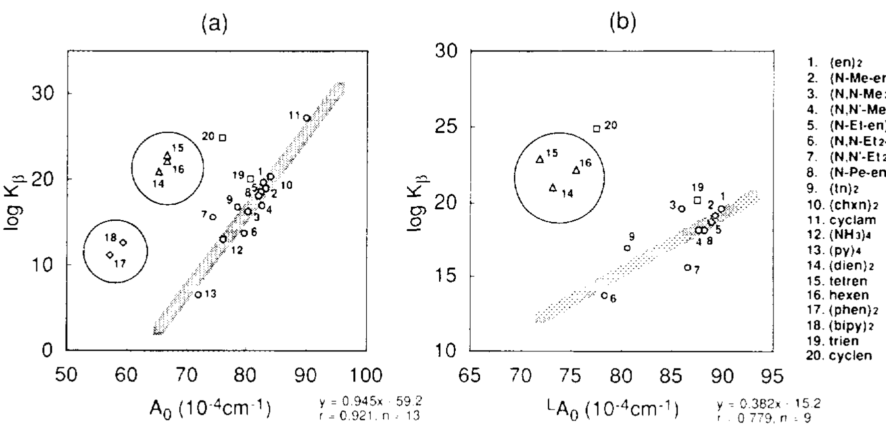

Fig. 4. Plots for (a) A_0-value *vs* log K_β and (b) $^LA_0$-value *vs* log K_β for CuN_4 type complexes. : square-planar, △: axially coordinate square-planar, ◇: tetrahedral and □: distorted square-planar.

In conclusion, L-band ESR spectra were measured for Cu(II) complexes at room and liquid nitrogen temperatures for the first time. The L-band ESR parameters, especially $^Lg_0$- and $^LA_0$-values, depend on the coordination structures of the Cu(II) complexes, similarly to the findings in X-band ESR analyses. Consequently, the coordination structures of the Cu(II) complexes can be presumed by plotting the ^Lg- *vs.* ^LA-values, relating to their stability constants and halfwave potentials of the complex. These results will be useful to assign the coordination structure of Cu(II)-proteins and -enzymes whose structures are not established, by measuring L-band ESR in aqueous solution.

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