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Dating of Human Blood by Electron Spin Resonance Spectroscopy

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A method dating samples by electron spin resonance (ESR) spectroscopy has been developed in the last 10 years and applied to a wide variety of materials of historical, archeological, and geological interest [1]. This method involves ESR measurements of accumulated radicals on lattice defects formed by natural radiation. ESR spectroscopy has been used to investigate the denaturation of hemoproteins such as hemoglobin (Hb) and myoglobin (Mb) [2], although details of the process of degradation of human blood are not yet fully understood. Furthermore, although determination of the age of human bloodstains is important in forensic medicine, no standard method for this purpose is yet available, as far as we know.

During investigations on degradation of hemoproteins in some chemical models [3], we found that ESR signals due to paramagnetic species in human blood can be correlated with the time after bleeding. This paper reports an ESR method dating for human blood by estimation of the signal intensities due to paramagnetic species detected at 77 K. This method for estimating the age of bloodstains by ESR spectroscopy should prove to be not only one of the new fields of bioinorganic chemistry but also of practical use in forensic medicine.

Human blood from three healthy men was soaked into filter paper soon after its extraction without an added anticoagulant, and dried at room temperature. Three small pieces (ca. 2 mm x 15 mm each) of the filter paper containing dried blood were put into a quartz sample tube (5 mm ∅) for ESR measurement. ESR spectra were recorded at liquid nitrogen temperature (77 K) in a JES FE-1XG (X-band) spectrometer (9.1 GHz) with 100 kHz field modulation (modulation amplitute 6.3 G) and 5 mW microwave power, which was calibrated with a Takeda Riken frequency counter, TR 5212. Li-TCNQ and Mn(II) doped in MgO were used as standards. The crystal field parameters of tetragonality (μ/λ) and rhombicity (R/μ), which were originally proposed by Blumberg and Peisach [4], were computed from three ESR g-values by Bohan's method [5].

Figure 1 shows ESR spectra (77 K) of dried human blood on the first day and 4 months after its extraction. Both spectra showed signals due to ferric species and free radicals: those of high-spin ferric heme species at $g = 6.2$, ferric nonheme iron species at $g = 4.3$, two low-spin ferric heme species at $g_1 = 2.92$, $g_2 = 2.26$, and $g_3 = 1.53$ (A) and $g'_1 = 2.45$, $g'_2 = 2.26$, and $g'_3 = 1.90$ (B), and free radicals at around $g = 2.005$. The low-spin ferric heme species were compared with those of several types of low-spin ferric heme complexes, as shown in Table 1. The ESR g-values and the crystal field parameters of the highly anisotropic species (A) were similar to those of complexes having the dinitrogen-heme coordination mode, indicating the formation of a bishistidinato-ferric heme complex in blood. ESR parameters of the small anisotropic species (B) were almost the same as those of complexes with the thiolate-ferric heme-nitrogen axial ligation mode, in which one ligand is a mercaptide, presumably from a cysteine residue, and the other is an imidazole from a histidine residue. On the basis of these results, we speculate that on denaturation of dried blood, the distal position of the apoprotein of the heme in Hb becomes occupied by either the imidazole-nitrogen of histidine or the sulfur of a cysteinate residue. A similar conclusion has been reached by ESR examination of ghosts prepared from acetylhydrazine-treated red cells [6].

The ESR spectra at 77 K were recorded for about 270 days. The four signals at

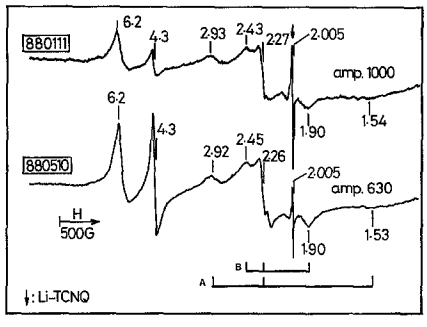

Fig. 1. ESR spectra at 77 K of dried human blood on filter paper of the first day (880111) and 4 months (880510) after its extraction. The recording amplitudes for 880111 and 880510 samples were 1000 and 630, respectively. The g-value at 2.005, due to free radicals, was obtained by expanding the magnetic field and amplifying both the 880111 and 880510 samples. The observed peak-to-trough width was 13.2 G in both samples

g = 6.2 (g6), 4.3 (g4), 2.2 (H), and 2.005 (R) changed with time. The intensities of these four signals were not linearly correlated with the time after blood extraction. However, in logarithmic plots of the ratios of the mean values of two signals for three individuals as functions of time, all six combinations of ratios of two of the four main signals showed good linear correlations: the relations of the ratios of R/g4, g4/g6 and H/g4 with time showed the correlation coefficients of 0.990, 0.984, and 0.980, respectively. The correlation coefficients for other combinations were 0.958 (R/g6), 0.906 (H/g6), and 0.314 (R/H). Thus, the correlations of R/g4, g4/g6, and H/g4 were concluded to be useful for determining the age of human bloodstains. Figure 2 shows a typical example for the logarithmic relationship of the ratio of R/g4 with time. The correlation of the day after blood extraction and the calculated ESR day estimated by the ESR intensity ratio of R/g4 showed a good correlation (correlation coefficient 0.974) for about 270 days. Furthermore, a better correlation (correlation coefficient 0.987) was found for about 120 days, as depicted in Fig. 3, suggesting the practical use of this method for actual samples at least for approximately 100 days. Recently, similar studies were reported by Miki et al. [7], who recorded ESR spectra at room temperature. We think that our method for ESR measurement of blood is more practical for providing information on degradation of blood than measurements at room temperature.

We are now investigating the practical application of this ESR method for dating human blood on various materials under various conditions, and full details will be published.

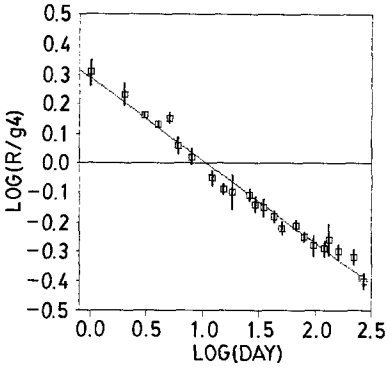

Fig. 2. Logarithmic plot of the intensity ratio (R/g4) of the two different ESR signals as a function of the age of dried human blood. Data are mean values ± S.D. for three individual blood samples

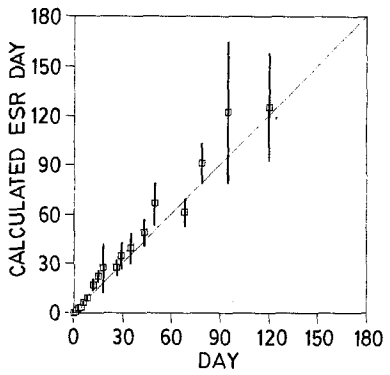

Fig. 3. Correlation of the ESR day as a function of the actual day of blood samples after extractions. ESR day was estimated from the intensity ratio (R/g4). Data are mean ± S.D. for three individual blood samples

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Table 1. ESR and crystal field parameters of low-spin ferric species in blood and model complexes

Species or complex	ESR g-values			Crystal field parameters		Coordination mode	Ref.
	g_1	g_2	g_3	$\lvert\mu/\lambda\rvert$	$\lvert R/\mu\rvert$		
A (880111)	2.93	2.27	1.54	3.35	0.58	N-Fe-N	
A (880510)	2.92	2.26	1.53	3.33	0.58	N-Fe-N	
B (880111)	2.43	2.27	1.90	5.83	0.39	S^--Fe-N	
B (880510)	2.45	2.26	1.90	5.86	0.46	S^--Fe-N	
FePPIXDME$(Im)_2$	2.90	2.25	1.54	3.41	0.57	N-Fe-N	[8]
FePPIXDME $(NMeIm)_2$	2.91	2.25	1.55	3.47	0.56	N-Fe-N	[8]
FePPIXDME(4-NO_2-∅-S^-)(NMeIm)	2.42	2.26	1.91	6.08	0.41	S^--Fe-N	[9]
FePPIXDME(4-NO_2-∅-S^-)(Im)	2.47	2.28	1.89	5.52	0.42	S^--Fe-N	[9]
FePPIXDME(TGE-S^-)(MeOH)	2.35	2.25	1.94	7.00	0.30	S^--Fe-O	[8]
FePPIX(TGE-S^-)(H_2O)	2.31	2.34	1.95	7.47	0.26	S^--Fe-O	[8]
FePPIX(TGE-S^-)$_2$	2.29	2.22	1.96	8.17	0.23	S^--Fe-S^-	[8]
FePPIXDME(TGE-S^-)$_2$	2.29	2.23	1.96	8.01	0.22	S^--Fe-S^-	[8]

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