CHEMISTRY LETTERS, pp. 1181-1186, 1976. Published by the Chemical Society of Japan

ELECTRON PARAMAGNETIC RESONANCE STUDIES OF NITRIC OXIDE-HEME-POLYMER COMPLEX

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The electron paramagnetic resonance (EPR) spectra of nitric oxide-heme-polymer complex were measured under the various conditions. From the analysis of the hyperfine structure by second derivative display, it is suggested that the linkage between NO and heme-iron is altered greatly with the function of the extension of polymer chain.

The technique of the EPR measurements on the nitric oxide complexes has proved its usefulness for elucidating the structure-function relationship of various hemoproteins[1-3], and the model systems[4,5]. Recently, we have shown the novel and convenient method of the second derivative display using high frequency modulation, which is applied to the NO-complex of myoglobin[6] and some other hemoproteins[7]. In the polymer systems, the reactivity of heme-iron towards various ligands have been accepted the much interests and subjected the extensive work[8,9].

In this communication, the NO-heme-poly-4-vinylpyridine (PVP) complex have been subjected to the EPR measurements under the various conditions together with the comparison of the monomeric system. An attempt is made to correlate the alteration of heme-NO linkage and the conformation change of the polymer chain.

PVP was polymerized by the use of azobisisobutylonitrile as an initator in methanol. The small amount of freshly prepared hemin solution was mixed with the N,N'-dimethylformamide (DMF) solution containing the various pyridine derivatives. Final concentrations of hemin and pyridines are varied between $10^{-5}\sim10^{-4}$ M and $1 \times 10^{-2}\sim 5 \times 10^{-1}$ M, respectively. The PVP ($\bar{M}n\approx10^{5}$) was dissolved in the solvent, and then hemin solution was added. After degassed by using conventional vacuum line, reduction of hemin was performed by addition of small amount of sodium dithionite and then NO gas was introduced, according to Kon[4] et al.

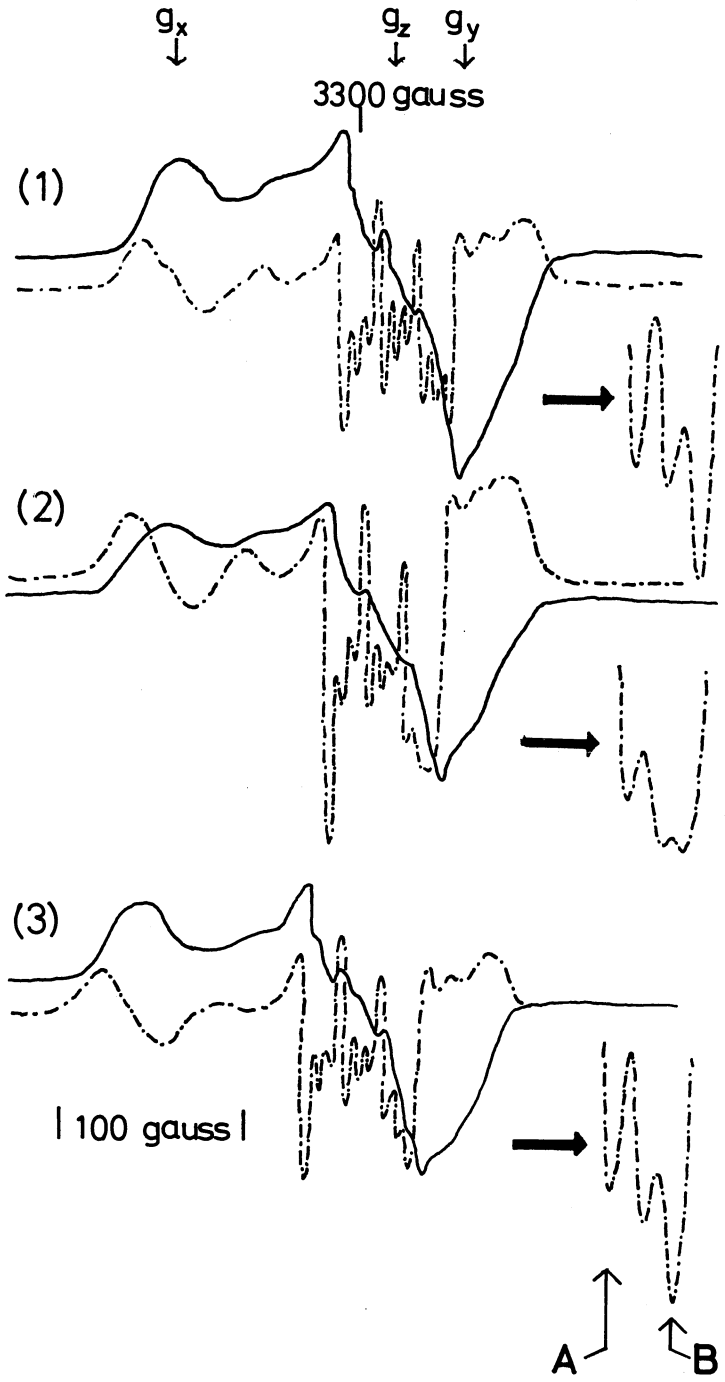

Fig. 1: The EPR absorption spectra of NO-heme-pyridine in DMF (1), NO-heme-PVP in DMF (2) and NO-heme-PVP in MeOH (3).
———— First derivative. —·—·—·— Second derivative.

Varian E line spectrometer was used for the EPR absorption measurements where, in the second derivative display, 1 KHz field modulation was employed, together with the 100 KHz field modulation for first derivative display. Microwave frequency and power were set to be 9.301 GHz and 5mW, respectively. All the measurements were made at liquid nitrogen temperature using dewar which was inserted into the cavity.

Figure 1 compares the EPR spectra of NO-heme-PVP complex in DMF and methanol (MeOH), together with the spectrum of NO-heme pyridine (Fig. 1-(1)), where DMF and MeOH are poor and good solvents for PVP, respectively. The 9 lined hyperfine structure (hfs) is clearly seen in the second derivative display, which is hardly observed in the first derivative spectra. This 9 lined hfs centered at g_z can be attributed to the coupling of both nitrogen atom of NO and coordinated pyridine through heme-iron. In the DMF solution, as is shown in the expanded scale in the figure, the hfs of polymer system (Fig. 1-(2)) is rather unclear than that of heme-pyridine complex, which is, however, turned into distinct one, when the solvent is changed into MeOH (Fig. 1-(3)). EPR spectra of NO-heme-PVP was found to be independent on the concentration of PVP. The alteration of the EPR spectra of Fig. 1-(2) and 1-(3) does not originate from the difference of the solvent itself, since using pyridine or 4-ethylpyridine, the solvent effect on the EPR spectra is very small as compared with results of Fig. 1-(2) and 1-(3).

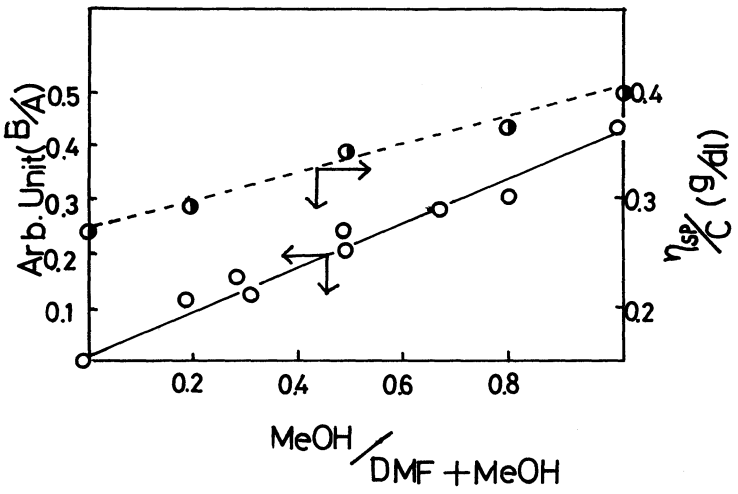

Fig. 2: Solvent effect on the EPR hyperfine structure of NO-heme-PVP.
———— The ratio of the absorption at (A) and (B).
------- The reduced viscosity of PVP.

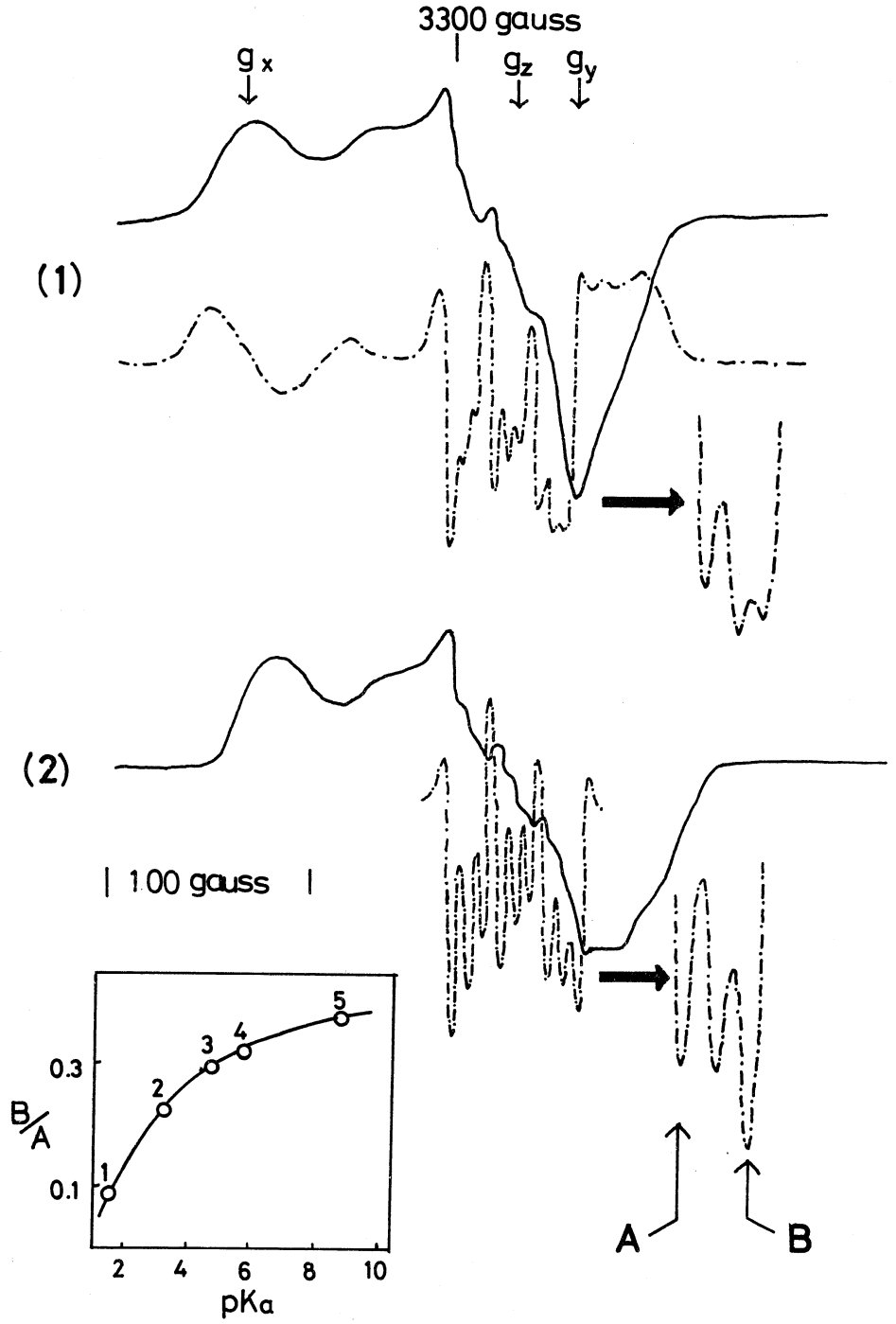

Fig. 3: The EPR absorption spectra of NO-heme-pyridine derivatives.
(1) cyanopyridine. (2) aminopyridine.
The ratio of absorption at (A) and (B) is plotted against the pKa of pyridine derivatives, where (1) cyano-, (2) acetyl-, (3) hydrogen-, (4) methyl-, (5) amino-.

The absorption ratio arrowed as A and B is plotted against the volume fraction of MeOH, where the reduced viscosity is also included in the figure (Figure 2). The hfs (B/A) changes continuously with the changes of the volume fraction of MeOH. From the data of the reduced viscosity, it can be said that when the polymer chain is extended, the hfs (B/A) becomes larger.

Figure 3 illustrates the EPR spectra of NO-heme complexes with various 4 substituted pyridine derivatives. The hfs of aminopyridine (Fig. 3-(2)) is seen more distinctly than that of cyanopyridine (Fig. 3-(1)). Similarly in Fig. 1, the ratio of A to B in the spectra is plotted against the pKa values of those pyridine derivatives. As shown in the inserted figure, the extent of the hfs (B/A) is directly related to the basicity of nitrogen atom of each pyridine derivatives.

Here, the parameter of hfs (B/A) can be regarded as the degree of the delocalization of the unpaired electron on the fifth coordinated nitrogen base, since the linear relationship between the coupling constant and the (B/A) is observed experimentally[10]. Therefore, it can be said that the increase of (B/A) correspond to delocalize the electron on the nitrogen atom of fifth ligand. This shows that, in Fig. 3-(2), the increase of basicity accompanies the increase of the spin-density on the nitrogen atom of pyridine ring.

The remarkable finding of the present study is that the linkage between NO-iron pyridine alters greatly within the polymer-heme complex. For example as is seen in Fig. 2, the hfs (B/A) of PVP in DMF and MeOH are 0.05 and 0.4, respectively. These values may correspond to the extrapolated value of pKa = 0 and pKa = 10 of Fig. 3, which are obtained from relationship between pKa and hfs (B/A). Thus the "apparent" basicity of nitrogen atom of pyridine in PVP seems to be changed more than 10 pKa unit depending on the function of extension of polymer chain. Using pyridine itself, the solvent effect on the EPR spectra is examined, and hfs (B/A) is found to be 0.32 and 0.37 for DMF and MeOH, respectively, which is verysmall as compared with the polymer system (cf. Fig. 2). This suggests that there might exist the other factors affecting the EPR spectra, such as the polarity of heme-environment and the steric hinderance in the polymer chain. For example, the nearly identical values of hfs (B/A) in MeOH with both cases show that the NO-heme-pyridine linkage in PVP behaves as similar manner to monomeric system. With DMF, however, the fact that hfs (B/A) of PVP is much small value compared with that of pyridine itself, may suggest the absence of the interaction between the solvent and heme-moiety enbeded in some peculiar polymer conformation. At present, however, the conclusive explanation is not obtained yet, which is the object of our proceeding works[11].

In summary, the present method can provide the possibility to see directly the "event" occured in the NO-heme-polymer system at the microscopic view point.

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(Received August 26, 1976)