CHEMISTRY LETTERS, pp. 1405-1408, 1981.

THE HIGH SPIN SPECIES IN SOLUTION OF DEUTEROHEMIN WITH TWO IMIDAZOLES COVALENTLY LINKED TO PORPHYRIN RING AND ITS EQUILIBRIUM WITH THE LOW SPIN SPECIES

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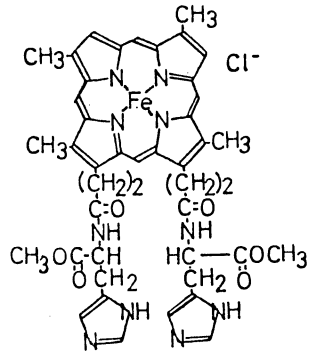
MCD and NMR studies revealed that deuterohemin is, in part, in a five-coordinated high spin form in solution where two imidazoles bound covalently. For the chemical equilibrium between the high spin and the main low spin six-coordinated species, standard enthalpy and entropy changes were -11×10^3 Jmol^{-1} and -52 K^{-1}mol^{-1}, respectively.

Since many hemoproteins have imidazole(s) from histidine residue(s) as axial ligand(s), the nature of ligation of nitrogenous bases to porphyrinatoiron(III) has been subjects of interest.[1-8] Although reactions of porphyrinatoiron(III) and imidazole (or its derivatives) have been studied extensively by mixing the two components,[1-6,8] the hemin with imidazole base(s) covalently bound to porphyrinatoiron(III) will be more appropriate for the direct comparison of ligation of imidazole(s) in model systems with those in native hemoproteins. We have synthesized deuterohemin bis(L-histidinemethylester) (hemin I) with an intention mentioned above and studied its chemical behavior in organic solvents. In the course of the study we have found direct evidence for the existence of a chemical species with high spin which is in equilibrium with a major low spin species. Iron(III)cytochrome b_5 which has two imidazoles as axial ligands exists exclusively as the low spin species at neutral pH values, while metmyoglobin imidazole formed by addition of excess imidazole exists in equilibrium between high and low spin states with the same chemical structure at ambient temperature.[9] Therefore we have tried to identify the high spin species with a different chemical structure and clarify the equilibrium between the high and low spin species.



HEMIN I

Hemin I was synthesized according to the method of Momenteau,[7] and purified by a silicagel column with a methanol-chloroform solution (1:5, v/v).

Magnetic circular dichroism spectra(MCD) were recorded on a JASCO Model J-500 spectrodichrometer with a DP-500 data processor. Nuclear magnetic resonance(NMR) spectra were obtained with a Bruker Model CXP-300 spectrometer with a variable temperature accessory Model B-VT-2000 which has the proton resonance frequency at 300.066 MHz.

The equilibrium reaction was explored through the variation of the magnetic

susceptibility determined by the Evans method[10] by using hexamethyl disiloxane as the standard substance in the temperature range from 260 ∿ 314 K. After correction of the concentrations by estimation of the volume change,[11] the following two equations were used to determine the molar susceptibility (χ_M; emu) and the square of effective magnetic moment (μ_{eff}^2; (B.M.)^2),[12]

$$\chi_M = (3/4\pi)(\Delta\nu/\nu)(1000/c) + \chi_0 M - \chi_D \quad (1)$$

$$\mu_{eff}^2 = (2.83)^2 \chi_M T$$

where $\Delta\nu$ is the difference in the resonance frequencies between the solution and the solvent (in Hz), c is concentration of hemin I (in mole dm^{-3}), χ_0 is the mass susceptibility of the mixed solvent of deuterated methanol and chloroform (2:1, v/v) (-0.015 x 10^{-6} emu), M is the molecular weight of hemin I, χ_D is the diamagnetic susceptibility of hemin I (-507.8 x 10^{-6} emu) and T is the absolute temperature (in K).[13-15]

Fig. 1 demonstrates the temperature dependence of the MCD spectra for hemin I in methanol solution. The peaks from 450 ∿ 550 nm and the trough around 570 nm are attributable to the low spin component, and the trough from 595 ∿ 645 nm is due to the high spin component. The MCD magnitude of the high spin component decreased with lowering temperature, while that of the low spin component increased in concomitant with this change.

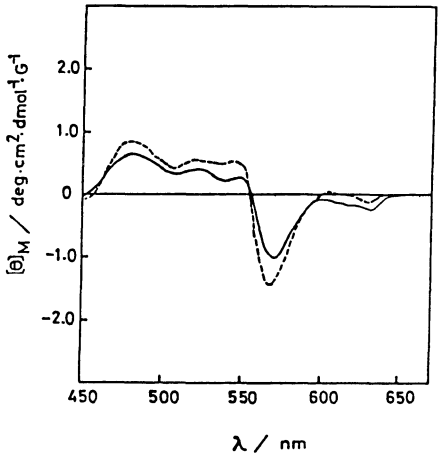


Fig. 1. MCD spectra for hemin I in methanol solution at 317 (——) and 250 (- - - -) K in the hemin concentration of 3.03 x 10^{-4} mol dm^{-3}.

Fig. 2 shows the ^{1}H NMR spectra for hemin I in a mixed solvent of deuterated methanol and chloroform (2:1, v/v) at 297 K. The peaks in the lower field (75 ∿ 35 ppm) is attributable to the high spin component (the peaks from 75 ∿ 65 ppm are possibly assigned to the porphyrin ring methyl proton signals), while the peaks from 25 ∿ 10 ppm is due to the low spin component. High spin peaks decreased by about one third with lowering temperature from 297 to 264 K (not shown).

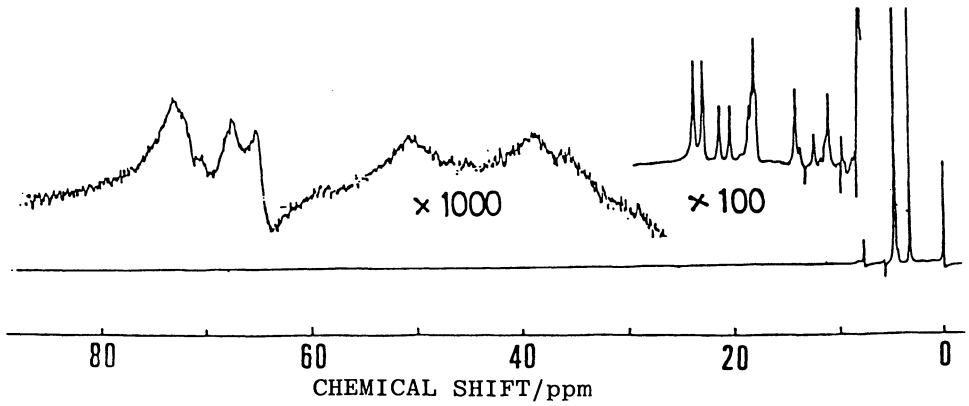


Fig. 2. ^{1}H NMR spectra for hemin I in the mixed solvent of CD_3OD and $CDCl_3$ (2:1,v/v) at 297 K in the hemin concentration of 2.14 x 10^{-3} mol dm^{-3}. The chemical shift is that from TMS.

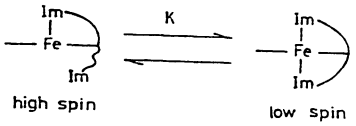
The values of χ_M determined by the Evans method are shown in Fig. 3. They show a minimum (2.52 x 10^{-3} emu) at about 273 K, and increase with both increase

and decrease in temperature. The values of μ_{eff}^2 calculated by the equation (2) approached a constant value in the temperature below 268 K (Fig. 3). The χ_M and μ_{eff}^2 values demonstrate that hemin I exists essentially as the low spin state with very small amount of high spin species in the temperature below 268 K. If we take the values 5 and 35 as the μ_{eff}^2 values, respectively, for pure low and high spin species and α and $1 - \alpha$ for the low and high spin fractions at each temperature, the following equations will hold

$$\mu_{eff}^2 = 5\alpha+35(1 - \alpha) \quad (3)$$

$$K = \alpha/(1 - \alpha) \quad (4)$$

where K is the equilibrium constant for the reaction



(5)

According to the Arrhenius relationship the equilibrium constant K can be written as

$$K = A \exp(\varepsilon/kT) \quad (6)$$

or

$$\ln K = \ln A + \varepsilon/kT \quad (7)$$

where ε is the energy difference between the high spin and the low spin species per molecule. The values K determined from equations 3 and 4 were plotted as a function of the inverse of the absolute temperature in Fig. 4. The linear relation between K and 1/T indicates that the temperature dependence of μ_{eff}^2 is due to the change between two species. The slope and intercept at $1/T = 0$ in the plot yielded the values 3.8×10^{-20} J and 1.9×10^{-3} for ε and A, respectively. Using these values and the relations $\Delta H^\circ = -N \cdot \varepsilon$ and $\Delta S^0 = N \cdot k \cdot \ln A$, one could calculate the standard enthalpy change (ΔH°) and the entropy change (ΔS^0) as -11×10^3 J mol^{-1} and -52 J K^{-1} mol^{-1}.

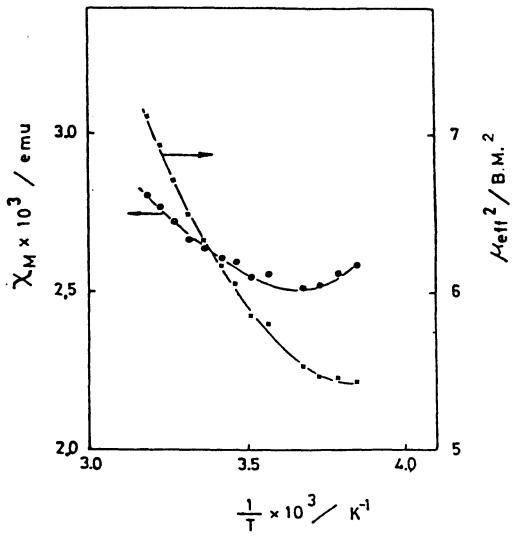


Fig. 3. The temperature dependence of the molar susceptibility (χ_M) and the square of effective magnetic moment (μ_{eff}^2) for hemin I in the mixed solvent of CD_3OD and $CDCl_3$ (2:1,v/v) at the hemin concentration of 2.12×10^{-3} mol dm^{-3}.

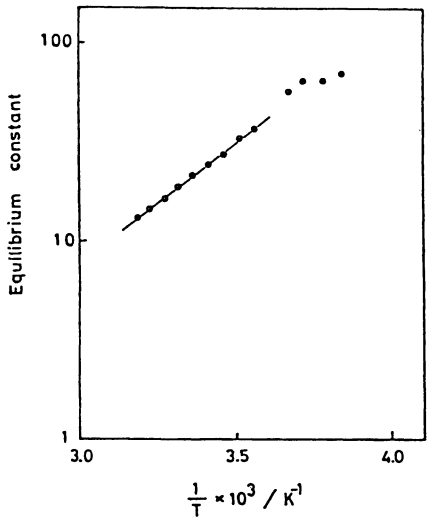


Fig. 4. The temperature dependence of the equilibrium constant (K) for the reaction between the high and the low spin species calculated from the data shown in Fig. 3.

The temperature dependence of the MCD spectra together with that of the NMR spectra indicates that there exists an equilibrium between a high spin component and a low spin component. The NMR spectra for the high spin species in the solution of hemin I have been detected as separate peaks, while the high spin component of metmyoglobin imidazole could not be detected as separate peaks (the NMR peaks appeared at the position of the weighted sum of those of the low and the high spin components).

Therefore the rate of exchange between the high and the low spin species should be slower than the 300 MHz ^{1}H NMR time scale (ca. 10^{-4} s) for the hemin I system, while for the metmyoglobin system it is faster than the NMR time scale. This suggests that the high spin component in the solution of hemin I should be one with a different chemical structure. The spin equilibrium with the same chemical structure such as that found in metmyoglobin imidazole[9] has been excluded also from the temperature independence of the square of effective magnetic moment in the solid state where no chemical equilibrium can be expected.[16] As for the structure of the high spin species in hemin I we tentatively consider a five-coordinated deuterohemin with one imidazole coordinated as shown in equation (5). The other imidazole in hemin I seems to have some interaction with the heme iron, since the deuterohemin with one imidazole covalently bound to the porphyrin ring exhibited a different ^{1}H NMR spectra (the porphyrin ring methyl proton at 40 ppm in the similar solvent system (not shown)) from those for the high spin species in the solution of hemin I. Since the low spin species should correspond to the six-coordinated deuterohemin(bis(imidazolato)deuterohemin), this paper will be the first report on the equilibrium constant (and the thermodynamic parameters) of the reaction between the five-coordinated and six-coordinated hemins with respective one and two imidazole(s) as axial ligand(s).

The standard enthalpy and the standard entropy change for the equilibrium between the high and low spin species show some differences between the reaction in hemin I and that in metmyoglobin imidazole($\Delta H°=-21\times10^{3}$ J mol^{-1}, $\Delta S°=-137$ J K^{-1} mol^{-1}, the values in the reference 9). However the direct comparison can not be made because the contribution from the protein in the latter system may be significant.

In conclusion MCD and NMR spectra for the solution of hemin I revealed the existence of the equilibrium between the high spin (the five-coordinated) and the low spin (the six-coordinated) chemical species. The equilibrium constant and the thermodynamic parameters for the equilibrium will be the first ones determined accurately for the reaction between the five- and the six-coordinated hemin complexes with one and two imidazole(s) as axial ligand(s).

This work was supported in part by Grant-in-Aid for Scientific Research of the Japanese Government.

References

1) B. B. Hasinoff, H. B. Dunford, and G. Horne, Can. J. Chem., 47, 3225 (1969).
2) G. B. Kalski and R. A. Plane, J. Am. Chem. Soc., 94, 3740 (1972).
3) M. Momenteau, Biochim. Biophys. Acta, 304, 814 (1973).
4) T. H. Davies, Biochim. Biophys. Acta, 329, 108 (1973).
5) J. M. Duclos, Bioinorg. Chem., 2, 263 (1973).
6) C. L. Coyle, P. A. Refson, and E. H. Abbott, Inorg. Chem., 12, 2007 (1973).
7) M. Momenteau, M. Rougee, and B. Loock, Eur. J. Biochem., 71, 63 (1976).
8) F. A. Walker, M. W. Lo, and M. T. Ree, J. Am. Chem. Soc., 98, 5552 (1976).
9) T. Iizuka and M. Kotani, Biochim. Biophys. Acta, 181, 275 (1969).
10) D. F. Evans, J. Chem. Soc., 1959, 2003.
11) S. Nagai et al., "Chemistry Handbook (Kagaku Binran)", Maruzen, Tokyo (1966) Basic Part 2, p. 467.
12) 1 B.M. = $eh/4\pi mc = 0.9273\times10^{-23}$ J T^{-1}.
13) D. H. Live and S. I. Chan, Anal. Chem., 42, 791 (1970).
14) D. Brault and M. Rougee, Biochem., 13, 4598 (1974).
15) Y. Huang and R. J. Kassner, J. Am. Chem. Soc., 101, 5807 (1979).
16) K. Okuyama, T. Nozawa, T. Murakami, M. Hatano, and W. Mori, in preparation.

(Received July 27, 1981)