

## Ferrous Porphyrins in Organic Solvents. II. Optical Spectra and Paramagnetic Susceptibilities<sup>†</sup>

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**ABSTRACT:** Spectral data and magnetic susceptibilities of iron(II) deuteroporphyrin dimethyl ester and iron(II) mesotetraphenylporphyrin dissolved in organic solvents are reported. The magnetic moments suggest high spin states with orbital contribution in noncoordinating or weakly coordinating solvents such as benzene, *p*-dioxane, tetrahydrofuran, and *N,N*-dimethylformamide. Ferrous mesotetraphenylporphyrin presents somewhat smaller magnetic moments. The dependences of spectra on the solvent are dis-

cussed along with their correlation with spin states. It appears that two well-defined visible bands are not a sufficient proof for low spin state. On the other hand, a band in the near-infrared region seems related to the high spin state. Besides, a relation is experimentally established between the number of ligands and the spin state. Heme is found to be high or low spin when binding one or two ligand molecules, respectively.

Ferrous and ferric porphyrins may be either in the high or the low spin state, and all these combinations are observed in hemoproteins. For some years, a number of studies have shown that the iron spin state is directly connected to the stereochemistry of the iron porphyrin (for a review, see Hoard, 1971). Thus, the spin state appears to be of great importance with regard to hemoprotein functions (Perutz, 1970). Hemoproteins are conveniently studied by means of visible or near-ultraviolet (uv) spectroscopy. The goal of a number of investigations was therefore to draw close relationships between spin states and optical spectra. Such relationships are well established for ferric hemoproteins or porphyrins (George *et al.*, 1961; Smith and Williams, 1970) and are also currently accepted when iron is in the ferrous state (for instance, see Whitten *et al.*, 1963; Zerner *et al.*, 1966). The deoxyhemoglobin spectra and the carboxyhemoglobin or dipyrindinehemochrome spectra are usually believed typical of high spin and low spin states, respectively.

We present here the paramagnetic susceptibilities and the optical spectra of ferrous deuteroporphyrin dimethyl ester (DeutFe<sup>II</sup>) and ferrous mesotetraphenylporphyrin (Ph<sub>4</sub>PorFe<sup>II</sup>) dissolved in various organic solvents.<sup>1</sup> Our results show that the relationships between spin states and optical spectra must be very cautiously discussed. Spectra of high spin heme may be very different depending on the solvents. Moreover, we draw a parallel between the heme coordination state which has been studied in the accompanying paper and the heme spin state.

### Experimental Section

**Materials.** Sources, preparations, and purifications of porphyrins and chemicals were presented in previous papers (Brault *et al.*, 1971; Brault and Rougee, 1974). Hexamethyldisiloxane of the best available grade was purchased from Spectrometrie Spin Techniques. Optical spectra were recorded using either a Bausch and Lomb Spectronic 505 spectrophotometer calibrated with mercury lines or a Beck-

man DKU spectrophotometer. The nuclear magnetic resonance (nmr) spectra were recorded on a Brücker H.F.X. 90-MHz spectrometer. The electron paramagnetic resonance (epr) spectra were recorded using a Varian 4502 X-band spectrometer.

**Methods.** (1) **PARAMAGNETIC SUSCEPTIBILITY MEASUREMENTS.** The paramagnetic susceptibilities were measured by nuclear magnetic resonance according to the method described by Evans (1959). A reference compound which gives a single resonance line (hexamethyldisiloxane was chosen both for its high boiling point and its unreactivity toward hemes) was added to both the solution of the paramagnetic species and the pure solvent. The solution and the solvent were, respectively, contained in the inner and the outer tubes of a coaxial cell. The outer tube was a standard nmr cell. The inner tube was a capillary tube (1 mm i.d.). Two resonance lines were observed on the nmr spectrum. The shift between them was measured with an accuracy of 0.3 Hz.

The spectrometer was locked on the signal of cyclohexane (or acetic acid) which was added to the outer solvent. The proportion of this added compound does not exceed 1% and does not change the volume susceptibility of the outer solvent. This method was checked with inorganic compounds in aqueous solution (*tert*-butyl alcohol was used as a reference). We obtained the following values: NiCl<sub>2</sub>,  $\chi_M = 4330 \times 10^{-6}$ ; CuSO<sub>4</sub>,  $\chi_M = 1350 \times 10^{-6}$ ; K<sub>3</sub>Fe(CN)<sub>6</sub>,  $\chi_M \approx 2120 \times 10^{-6}$  cgs, which agree with the literature data: NiCl<sub>2</sub>,  $\chi_M = 4433 \times 10^{-6}$  (Seelwood, 1956, p 25); CuSO<sub>4</sub>,  $\chi_M = 1330 \times 10^{-6}$ ; and K<sub>3</sub>Fe(CN)<sub>6</sub>,  $\chi_M = 2290 \times 10^{-6}$  cgs (Handbook of Chemistry and Physics, 1968). Measurements were carried out at 300°K.

The benzene solutions of hemes were prepared by the reduction of chlorohemin according to the "dithionite method" which was described elsewhere (Brault *et al.*, 1971; Brault and Rougee, 1974). A benzene solution of chlorohemin (about  $10^{-3}$  M) was stirred with the aqueous dithionite solution and was concentrated until the heme concentration was about  $3-6 \times 10^{-3}$  M. The heme solution was decanted and transferred in a measuring vessel. Hexamethyldisiloxane was added to the solution (3%). A part of the heme solution was transferred into the capillary tube which was sealed off, while the remaining was diluted with deoxy-

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<sup>1</sup> Abbreviations used are: DeutFe<sup>II</sup>, ferrous deuteroporphyrin dimethyl ester; Ph<sub>4</sub>PorFe<sup>II</sup>, ferrous mesotetraphenylporphyrin.

TABLE 1: Magnetic Moments (in Bohr Magnetons) of Iron(II) Porphyrins Dissolved in Various Solvents (Number of Experiments in Parentheses).

	DeutFe <sup>II</sup>	Ph <sub>4</sub> PorFe <sup>II</sup>
Benzene	5.43 ± 0.05 (11)	4.52 ± 0.28 (8)
<i>p</i> -Dioxane	5.39 ± 0.08 (7)	
Tetrahydrofuran	5.60 ± 0.20 (3)	4.81 ± 0.13 (6)
<i>N,N</i> -Dimethylformamide	5.53 ± 0.09 (6)	5.19 ± 0.18 (3)

genated benzene. Owing to their sensitivity toward air, all the solutions were thoroughly prevented from oxygen contamination and were transferred from one container to another through inox steel tubes by means of excess pressure. The heme concentration and the lack of oxidation were checked by means of the optical spectra of the diluted solution. In order to prepare solutions of heme in other solvents, benzene was evaporated by a nitrogen flow and the heme residue dissolved in the desired solvent.

(2) OPTICAL ABSORPTION MEASUREMENTS. Heme solutions used for optical absorption measurements were prepared in the same way. Solutions were transferred in optical cells (1 mm and 1 cm) sealed to a glass tube stopped by a Teflon cap. Molecular absorbances of hemes dissolved in various solvents were calculated using those obtained in benzene.

For low temperature measurements, hemes were dissolved in toluene-methylcyclohexane (1:1) or 2-propanol-methylcyclohexane (1:1) mixtures which give clear glasses at 77°K. The solutions were contained in sealed quartz tubes (optical path about 2 mm).

## Results

**Paramagnetic Susceptibilities.** The Evans' equation (Evans, 1959) has been transformed in order to get the molar paramagnetic susceptibility. We obtain

$$\chi_M = \frac{3}{2\pi} \frac{\Delta\nu}{\nu} \frac{1000}{C} + \chi_0 M - \chi_D \quad (1)$$

where  $\chi_M$  is the molar paramagnetic susceptibility of heme,  $\Delta\nu$  is the frequency separation between the two resonance lines (hertz),  $\nu$  is the applied field (hertz),  $C$  is the concen-

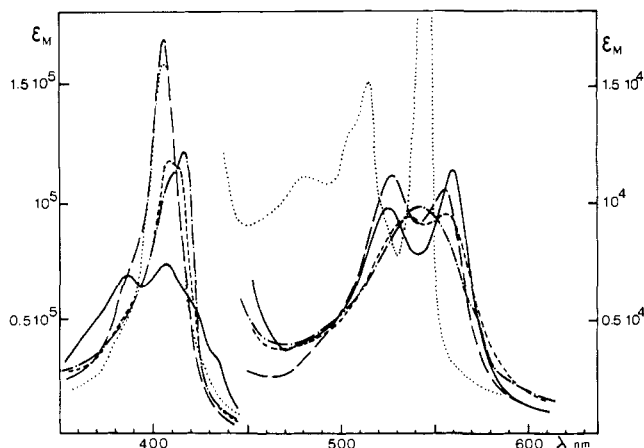


FIGURE 1: Visible and near-uv spectra of iron(II) deuteroporphyrin dimethyl ester dissolved in: (—) benzene; (---) *N,N*-dimethylformamide; (- - -) *p*-dioxane; (- · -) 2-propanol; (···) benzene + pyridine (3%).

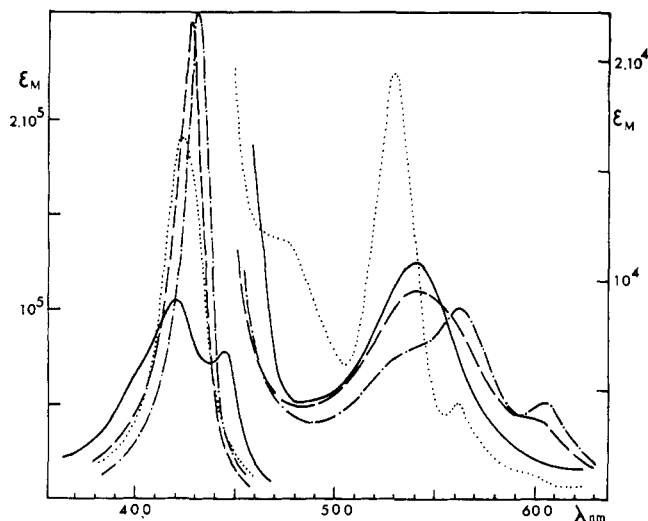


FIGURE 2: Visible and near-uv spectra of iron(II) mesotetraphenylporphyrin dissolved in: (—) benzene; (---) *N,N*-dimethylformamide; (- - -) tetrahydrofuran; (···) benzene + pyridine (3%).

tration expressed in moles per liter,  $\chi_0$  is the mass susceptibility of the solvent,  $M$  is the molecular weight of heme, and  $\chi_D$  is the diamagnetic susceptibility of heme. We express the paramagnetism in terms of the magnetic moment.

$$\mu = 2.84(\chi_M T)^{1/2} \quad (2)$$

The diamagnetic component  $\chi_D$  in eq 1 cannot be checked by means of Evans' method. Indeed, experiments with diamagnetic metalloporphyrins give frequency shifts too low to be measured.  $\chi_D$  was calculated from the diamagnetic susceptibility of mesoporphyrin (Havemann *et al.*, 1961) and the atomic diamagnetic susceptibilities (Seelwood, 1956, p 92). We obtain: DeutFe<sup>II</sup>,  $\chi_D = -561 \times 10^{-6}$ ; Ph<sub>4</sub>PorFe<sup>II</sup>,  $\chi_D = -615 \times 10^{-6}$  cgs. In any case, the diamagnetic component does not exceed 5–10% of the total magnetic susceptibility. In order to check the method and the validity of corrections we have measured the susceptibility of chlorodeuterohemin dimethyl ester and chloromesotetraphenylhemin dissolved in benzene; we obtain  $\mu = 5.95$  and 5.90 BM which agree with the reported value  $\mu = 5.92$  BM (Hambright *et al.*, 1968; Maricondi *et al.*, 1969).

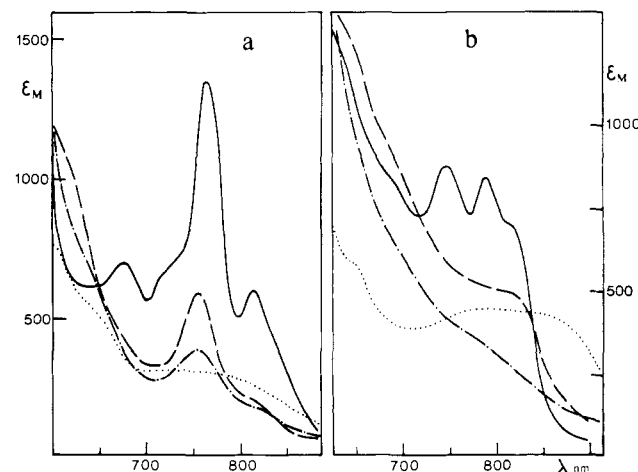


FIGURE 3: Far-red spectra of iron(II) porphyrins. (a) Iron(II) deuteroporphyrin dimethyl ester dissolved in: (—) benzene; (---) *p*-dioxane; (- - -) *N,N*-dimethylformamide; (···) benzene + pyridine (3%). (b) Iron(II) mesotetraphenylporphyrin dissolved in: (—) benzene; (---) tetrahydrofuran; (- - -) *N,N*-dimethylformamide; (···) benzene + pyridine (3%).

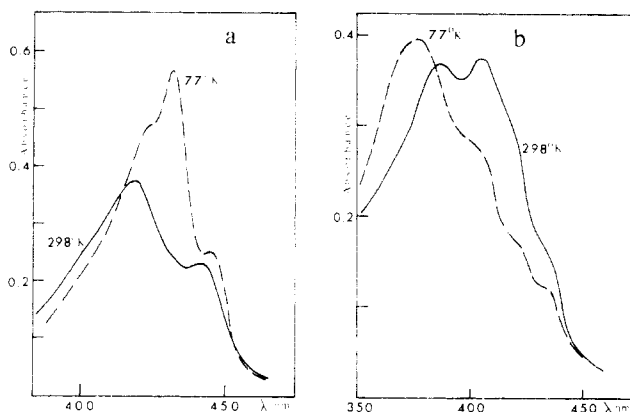


FIGURE 4: Spectra of hemes dissolved in a toluene-methylcyclohexane (1:1) mixture: (a) iron(II) mesotetraphenylporphyrin; (b) iron(II) deuteroporphyrin dimethyl ester; (—) ambient temperature; (---) 77°K.

Several independent measurements of  $\text{DeutFe}^{\text{II}}$  and  $\text{Ph}_4\text{PorFe}^{\text{II}}$  magnetic moments were performed for each solvent. In Table I, we give the mean values with their 95% confidence limits. The number of experiments is given in parentheses.

Hemes oxidize in air leading to oxo-bridged dimers. The magnetic moments of the dimers thus obtained are, respectively,  $3.15 \pm 0.20$  and  $2.30 \pm 0.20$  BM for deuteroporphyrin and mesotetraphenylporphyrin. Oxo-bridged dimers may also be prepared by washing a benzene solution with a basic aqueous solution (Brault *et al.*, 1971). The magnetic moment of the prepared iron(III) deuteroporphyrin dimer is  $3.50 \pm 0.20$  BM.

**Optical Spectra.** The near-ultraviolet, visible, and far-red spectra of  $\text{DeutFe}^{\text{II}}$  and  $\text{Ph}_4\text{PorFe}^{\text{II}}$  dissolved in some organic solvents are given in Figures 1, 2, and 3. Low-temperature spectra are given in Figure 4. Addition of methylcyclohexane (which was required for the obtention of clear glasses) to toluene or 2-propanol solutions of heme does not induce spectral changes. Spectra of heme dissolved in toluene are identical with spectra of heme dissolved in benzene.

## Discussion

**Susceptibility Measurements. METHODS.** The validity of the susceptibility measurements has been ensured by the following experiments. (i) The lack of oxidation of heme solutions was checked by means of optical spectra. (ii) No epr signal was detected at ambient temperature or at 77°K on heme solutions. Thus contamination by paramagnetic impurities is unlikely. The lack of heme signal may be expected for the experiments are not performed at liquid helium temperature. As a matter of fact, Ingram and Bennett (1955) were also unable to detect any signal on ferrous phthalocyanine samples. (iii) The susceptibilities of iron(III) oxo-bridged dimers which were either prepared or obtained as deuteroheme reoxidation products are almost the same. This also argues against any contamination by paramagnetic impurities. It should be noted that our dimer magnetic moments are higher than those reported by Moss *et al.* (1972). However, Cohen (1969) pointed out that contamination of a dimer sample by water may lead to hematin formation accounting for higher magnetic moments. This author has reported  $\mu = 2.68$  BM for the oxo-bridged dimer of mesotetraphenylporphyriniron(III) prepared without extensive drying. In the present experiments, no rigorous drying of the solution was attempted; our data are therefore consistent with Cohen's results. Furthermore, in some ex-

periments, chlorodeuterohemin or chloromesotetraphenylhemin were obtained by bubbling gaseous hydrogen chloride through the solution of reoxidized hemes. Magnetic moments of chlorohemin thus obtained agree within 2% with the literature data (Hambright *et al.*, 1968). (iv) The reliability of both the preparative method and the susceptibility measurements were also proved by the homogeneity of results obtained from various samples. No concentration dependence was found in any solvent; however, precipitation due to high concentration must be avoided. As a consequence, all the measurements were performed in the concentration limits,  $3 \times 10^{-3}$  to  $6 \times 10^{-3}$  M.

**$\text{DeutFe}^{\text{II}}$ .** With regard to the possible pairing of the six electrons of iron(II) porphyrin, three spin states are available;  $S = 0, 1$ , and 2. Their so-called "spin only" moments are  $\mu = 0, 2.83$ , and 4.90 BM, respectively. Our results unambiguously show that the high spin state is obtained when  $\text{DeutFe}^{\text{II}}$  is dissolved in benzene, *p*-dioxane, tetrahydrofuran, and *N,N*-dimethylformamide. As expected, dipyrindenedeuterohemochrome is low spin. The magnetic moments are higher than the "spin only" values. This may be explained in terms of orbital contribution. In cubic symmetry, values as high as 5.7 BM may be expected (Figgis, 1966). Some further quenching of orbital angular momentum may arise as the symmetry is lowered. However, values in the range 5.30–5.40 BM are obtained for tetragonal distorted molecules such as iron(II) complexes of phenanthroline or bipyridyl (Cunningham *et al.*, 1972). The iron(II) derivatives of a tetradentate macrocyclic ligand which have been studied by Dabroviak *et al.* (1972) possess magnetic moments comprised of between 5.27 and 5.95 BM, depending on the supplementary axial ligands. Our results are consistent with all the above.

**$\text{Ph}_2\text{PORFe}^{\text{II}}$ .** Kobayashi and Yanagawa (1972) and Collman and Reed (1973) have measured the magnetic moments of crystalline  $\text{Ph}_4\text{PorFe}^{\text{II}}$  which was prepared from benzene or tetrahydrofuran solutions. They obtained:  $\mu_{\text{C}_6\text{H}_6} = 4.75$  BM,  $\mu_{\text{THF}} = 2.75$  BM (Kobayashi);  $\mu_{\text{C}_6\text{H}_6} = 4.4$  BM,  $\mu_{\text{THF}} = 5.1$  BM (Collman). Their results are similar to ours except the Kobayashi's result on  $\text{Ph}_4\text{PorFe}^{\text{II}}$  crystallized from tetrahydrofuran solution. It appears that  $\text{Ph}_4\text{PorFe}^{\text{II}}$  dissolved in dimethylformamide is essentially high spin.  $\text{Ph}_4\text{PorFe}^{\text{II}}$  dissolved in benzene or crystallized from benzene solution presents smaller magnetic moments. Two facts are suspected for such a lowering: (i) aggregation leading to antiferromagnetic coupling; (ii) contribution from a lower spin state.

Both these phenomena should be temperature dependent. Stacking of heme molecules should produce a broadening and/or a blue shift of the Soret band (Kasha, 1963). This is not observed on the low-temperature spectra of  $\text{Ph}_4\text{PorFe}^{\text{II}}$  dissolved in a toluene-methylcyclohexane mixture (Figure 4a). We feel that the appearance of a new band at low temperature may be concomitant with increasing concentration of a low spin species. At ambient temperature, the two bands in the Soret region are not very well defined and some amount of the low spin species may subsist which might account for the anomalous magnetic moments. As pointed out by Zerner *et al.* (1966) the balance between the spin states of iron in hemes is very subtle, and must drastically depend on the ligand field produced by the chelating molecule. Thus, the ferrous phthalocyanine molecule exists in an intermediate spin state (Lever, 1965). To sum up, the existence of  $\text{Ph}_4\text{PorFe}^{\text{II}}$  as a spin state mixture is not unlikely. However, as aggregation may also occur in the con-

centrated solutions which are required for susceptibility measurements, we cannot attribute the lowering of the magnetic moment to only one of the two phenomena.

**Optical Spectra.** OPTICAL SPECTRA-SPIN STATE RELATIONSHIPS. With regard to both the spectra given in Figures 1 and 2 and the susceptibility measurements, spectra-spin state relationships must be very carefully considered. Visible and Soret spectra of  $\text{DeutFe}^{\text{II}}$  and  $\text{Ph}_4\text{PorFe}^{\text{II}}$  in various solvents are different while the spin state is the same. Besides, spectra of  $\text{DeutFe}^{\text{II}}$  dissolved in *p*-dioxane look like that of carboxyhemoglobin while the spin states are different. Thus, the correlation between a low spin state and two well-defined visible bands which was used by a number of authors (see, for instance, Whitten *et al.*, 1963) is incorrect.

**HEMES IN BENZENE.** It was shown in the accompanying paper that hemes are monomers in benzene solutions so that associations cannot be taken into account for the splitting of the Soret band. On the other hand, this splitting cannot be attributed to an incomplete coordination by water (Brault and Rougee, 1974). In order to check further a possible equilibrium between two states, we have investigated the temperature dependence of the spectra. In the case of  $\text{DeutFe}^{\text{II}}$  (see Figure 4b) no drastic increase of one band to the prejudice of another is observed. We think that the broadening and Soret blue shift which is observed at low temperature could be explained by aggregation (Kasha, 1963). Besides, as inferred from susceptibility measurements, no equilibrium between spin states may be suspected as far as  $\text{DeutFe}^{\text{II}}$  is concerned. The case of  $\text{Ph}_4\text{PorFe}^{\text{II}}$  has been discussed above.

To conclude, the complexity of the Soret band cannot be explained by equilibrium between various states. This complexity may be due to departure from  $D_{4h}$  symmetry. Indeed, iron is expected to lie outside the porphyrin plane in a high spin compound (Hoard, 1971). In addition a complicated situation may arise from the occurrence of equivalent states for the iron d-orbital filling.

**SLIGHTLY COORDINATING SOLVENTS.** Hemes are five coordinated in solvents such as *p*-dioxane, dimethylformamide, and alcohols (Brault and Rougee, 1974). As depicted in Figure 1  $\text{DeutFe}^{\text{II}}$  spectra strongly depend on these solvents. The Soret band is split when  $\text{DeutFe}^{\text{II}}$  is dissolved in 2-propanol. However, the spectra are not temperature dependent, so that equilibrium between states can be ruled out. The broadening of the Soret band observed when  $\text{DeutFe}^{\text{II}}$  is dissolved in 2-propanol and dimethylformamide appears concomitant with the broadening of the visible bands (Figure 1) so that a parallel splitting of the two transitions is likely. We suggest that this behavior may be accounted for by the interaction between groups adjacent to the coordinating atom of the bound solvent molecule and the electrons of the porphyrin ring which removes the degeneracy of the  $e_g$  orbital set (Falk, 1964). As shown by the use of Corey-Pauling-Koltun (CPK) molecular models, such interactions are expected to be greater in the case of 2-propanol than in the case of *p*-dioxane. The splitting of the Soret and visible bands is observed in the same way.

**NEAR-INFRARED SPECTRA.** Transitions centered around 760 and 780 nm are observed when  $\text{DeutFe}^{\text{II}}$  and  $\text{Ph}_4\text{PorFe}^{\text{II}}$  are dissolved in solvents leading to high spin compounds. Although their intensities are very sensitive to the solvent, they may be in most cases distinguished from hemochrome bands (Figure 3). A correlation between these transitions and a high spin state may be supposed. Charge-

transfer transition and d-d transitions may be expected in the near-infrared region. The latter, which are forbidden in square planar compounds, may gain some intensity from vibronic coupling. In both cases, they must be typical of the spin state. We cannot say anything definite on this matter, but it seems that the infrared region may be advantageously considered in relating spectra to spin states.

**Coordination and Spin States.** As pointed out in the accompanying paper (Brault and Rougee, 1974), hemes coordinate only one molecule of solvents such as tetrahydrofuran, *p*-dioxane, *N,N*-dimethylformamide, and alcohols. Moreover, these solvents lead essentially to high spin heme. This may be rationalized in a simple way. High spin heme exists in a square pyramidal conformation (Hoard, 1971) which make unlikely the binding of further ligand. On the other hand, the formation of two strong axial bonds involves an in-plane position of iron and a low spin state. This situation agrees well with theoretical calculations (Zerner *et al.*, 1966).

Collman and Reed (1973) and Kobayashi and Yanagawa (1972) report that crystalline  $\text{Ph}_4\text{PorFe}^{\text{II}}$  is bound to two tetrahydrofuran molecules. This disagrees with our results. However, the crystalline compound may contain solvent molecules which are not directly bound to the iron but clustered in the crystalline framework. This fact emphasizes the difficulty of relating experiments performed in different media. This remark also applies to the  $\text{Ph}_4\text{PorFe}^{\text{II}}$  Mössbauer data reported by Kobayashi *et al.* (1970).

Few other studies have dealt with noncoordinated or weakly coordinated iron(II) porphyrins. Hemes dissolved in mixed solvents have been studied by Whitten *et al.* (1963) but their hypothesis on spin states was only based on optical correlation.

## Conclusion

Spin states and optical spectra of iron(II) porphyrins have been measured using experimental conditions which make valuable a comparison between them. It is concluded that great care must be taken in relating spin states to optical spectra. The near-infrared region must be considered. Besides, as expected from theoretical calculation, five-coordinated iron or six-coordinated iron exists in the high spin or the low spin state, respectively.

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## Equilibrium and Kinetic Aspects of Subunit Association in Immunoglobulin G<sup>†</sup>

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**ABSTRACT:** The noncovalent interaction between light chains and dimeric heavy chains from immunoglobulin G myeloma proteins has been followed by difference spectroscopy in 4 mM sodium acetate buffer (pH 5.4). Red-shifted difference spectra were observed consistent with the transfer of aromatic chromophores from a polar to a nonpolar environment upon subunit association. Considerable variation in the shape and magnitude of the difference spectra was noted from one pair of heavy and light chains to another. Tryptophan perturbations were consistently seen but the involvement of tyrosine and phenylalanine transitions was only apparent for some subunit pairs. Equilibrium binding curves were constructed from spectral and calorimetric data which indicated a 1:1 binding between heavy and light chain. The association of monomeric Fd' with light chain was accompanied by similar changes in molar extinction coefficient, enthalpy, and heat capacity to those seen for reactions involving heavy chain dimer and light chain, thus

confirming that the two binding sites for light chain on heavy chain dimer were independent. The time dependence of the difference spectral changes was used to measure the rate of subunit association. The reactions were second order with rate constants between  $10^2$  and  $10^3$  l. mol<sup>-1</sup> sec<sup>-1</sup> at 25° for several heavy-light chain pairs. The rates showed a marked temperature dependence with Arrhenius activation energies between 16 and 29 kcal mol<sup>-1</sup>. Fd', a fragment corresponding to the amino-terminal half of heavy chain, reacted with light chain at about double the rate observed for heavy chain but showed the same temperature dependence. The kinetic data and information on the dissociation of immunoglobulin G at high dilution have been used to estimate a minimum association constant of  $10^9$  M<sup>-1</sup> governing the interaction of heavy and light chains. At the concentration of subunits used in this study the half-time for noncovalent association is consistent with the known rate of interchain disulfide bond formation *in vivo*.

The immunoglobulin G (IgG<sup>1</sup>) molecule is composed of two identical H chains (mol wt 53,000) and two identical L chains (mol wt 22,500). The four-chain structure is stabi-

lized by strong noncovalent interactions, between the NH<sub>2</sub>-terminal half of an H chain and an L and between the COOH-terminal regions of the two H chains, together with interchain disulfide bonds. During *in vivo* assembly of the molecule noncovalent associations of the subunits must precede interchain disulfide bond formation since sulfhydryl groups must be brought close together before oxidation. The sequence and kinetics of disulfide bond formation have been studied extensively *in vivo* (reviewed by Bevan *et al.*, 1972; Baumal and Scharff, 1973) and, more recently, with an *in vitro* model system (Petersen and Dorrington, 1974). Information on intracellular noncovalent bond formation

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<sup>1</sup> Abbreviations used are: IgG, immunoglobulin G; H, heavy chain; L, light chain; Fd', a fragment corresponding to approximately the NH<sub>2</sub>-terminal half of H chain.