THE EFFECT OF PRESSURE ON THE OXIDATION STATE OF IRON, III.   HEMIN AND HEMATIN*

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Hemin and hematin are prototype molecules for hemoglobin.   In both cases, ferric iron is inserted at the center of a planar porphyrin ring so that the Fe(III) ion is in a site of essentially square planar symmetry with four nitrogen ligands. In hemin, however, there is a chloride ion attached to the iron, and the iron is displaced 0.475 Å out of the plane of the ring.[1]   In hematin, the Cl^- ion is replaced by OH^-; although the details are not as well determined as for hemin, the symmetry is very similar.

This paper presents the results of high-pressure Mössbauer studies of these two compounds.   The emphasis is on the effect of pressure on the oxidation state of iron, although the unusual behavior of the isomer shift and quadrupole splitting of the ferric ion is also discussed.

In a previous communication,[2] we presented some rather qualitative results on ordinary hemin.   In this work, we synthesized hemin and hematin enriched to 85 per cent in Fe^{57}.   The hemin was made by the method of Moss,[3] whereas hematin was obtained from the hemin by the technique of Fischer *et al.*[4]   The compounds were analyzed chemically, and optical spectra that showed the characteristic visible spectra were taken.[5]

In a series of studies[2, 6–9] we showed that in many compounds, ferric ion reduces to the ferrous state with increasing pressure, that this is a reversible process,
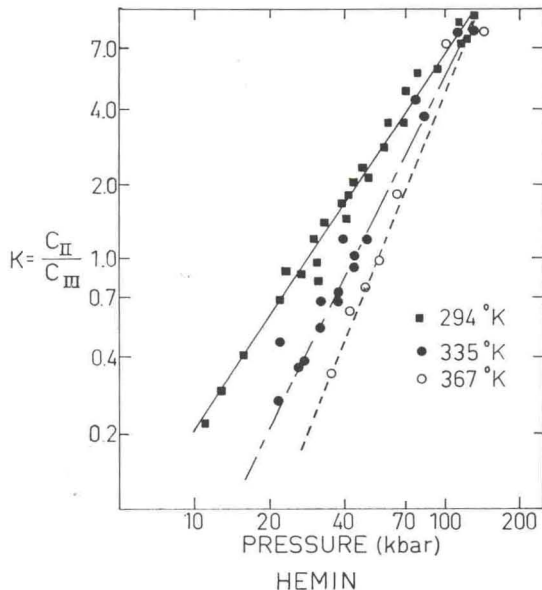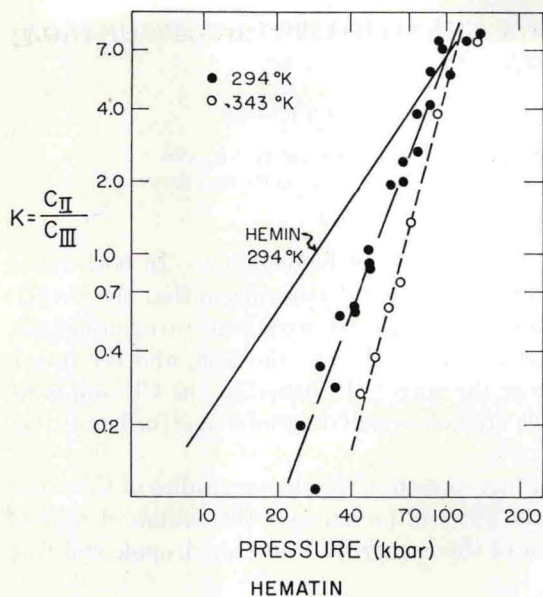

FIG. 1.—Ln K vs. ln P in hemin.

Fig. 2.—Ln K vs. ln P in hematin.

and that the dependence of the equilibrium on pressure is of the form $K = C_{II}/C_{III} = AP^B$. Figures 1 and 2 show that this description also applies to hemin and hematin. The constants A and B are given in Table 1. For more ionic materials such as the halides or phosphate, $B \cong 0.5$. From thermodynamic arguments,

$$\frac{\partial \ln C_{II}}{\partial \ln P} = \frac{P(V^{III} - V^{II})}{RT}(C_{III}) = B(C_{III}), \tag{1}$$

so that B is directly related to the difference in volume between the normal ferric ion with its ligands and the ferrous ion with associated (electron-deficient) ligands. The local distortion accompanying reduction is apparently considerably larger for more covalently bonded iron, as in the compounds under discussion here.

In a previous analysis,[8] we showed that the electron transfer is from a ligand nonbonding level to a predominantly metal antibonding level. This charge transfer can frequently be observed optically and shifts to lower energy with increasing pressure. The high-pressure reduction is a thermal process and thus need not follow the Franck-Condon principle which demands that it occur verti-

TABLE 1. *Constants A and B for* $K = AP^B$.

Compound	A	B
Hemin		
294°K	5.5×10^{-3}	1.53
335°K	4.2×10^{-4}	2.04
367°K	3.5×10^{-5}	2.57
Hematin		
294°K	2.7×10^{-5}	2.67
343°K	1.4×10^{-7}	3.77

cally on a configuration coordinate diagram,[8] so that it involves much less energy than the corresponding optical process.

The optical charge transfer spectra of hemin and hematin are distinctly different.[5] As can be seen from Figures 1 and 2 and Table 1, the pressure at which significant reduction appears, and the values of B (or of $(V^{III} - V^{II})$) are different in the two compounds. There is thus a strong inference that the electron transfer involves the ion (chloride or hydroxide) that is out of the plane of the molecule more than the four nitrogens in the plane. The large value of $(V^{III} - V^{II})$ is more reasonable in this case also. There is, of course, the possibility of interaction between the "external" ion and the ring through the iron.

The pressure-induced reduction of Fe(III) is an endothermic process in all the compounds previously studied. This can reasonably be explained, as one is concerned with the thermally induced transfer of electrons from the ground to the excited electron state, and the usual Boltzmann factor $\exp(-E/kT)$ would give an increased conversion with increasing temperature. Figures 1 and 2 show that the reduction of hemin and of hematin is an exothermic process. An isobar confirmed the fact that the reduction did, indeed, decrease with increasing temperature. Heats of reaction are listed in Table 2; they decreased rapidly with increas-

TABLE 2. *Heats of reaction.*

Pressure (kb)	ΔH (ev)	
	Hemin	Hematin
20	0.22	—
40	0.15	0.23
60	0.11	0.15
80	0.075	0.10
90	0.057	0.052

ing pressure. The apparent explanation is that increased temperature tends to deform the potential wells so that E increases more rapidly than T. As the pressure increases, the distorting effect of higher temperatures becomes much smaller.

The primary purpose of this study was the investigation of the oxidation state of iron in hemin and hematin, as discussed above. In the course of the investigation, interesting data were accumulated on the effect of pressure on the isomer shift (s-electron density of the nucleus) and quadrupole splitting (interaction between the nuclear quadrupole moment and an electric field gradient at the nucleus) of the ferric ion in these compounds. The results are shown in Figures 3 and 4. The isomer shifts are given relative to metallic iron at one atmosphere. Our results for atmospheric isomer shift agree closely with those of Bearden et al.,[10] although our initial quadrupole splitting is smaller than theirs. There are some quantitative differences between hemin and hematin, but the results are so similar that only a single discussion is necessary. The dotted lines represent the qualitative results for hemin obtained on the unenriched compounds.[2]

For most compounds, a monotonic decrease in the isomer shift amounting typically to 0.08–0.12 mm/second in 150 kb is observed with increasing pressure (i.e., an increase of electron density at the nucleus). This can be associated
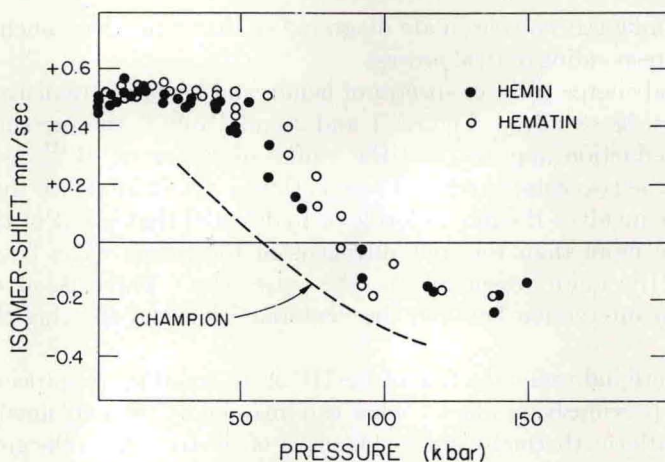
Fig. 3.—Isomer shift vs. pressure for Fe(III) ion in hemin and hematin.

with orbital distortion effects, for example, spreading of the $3d$-orbitals due to interaction with the ligands which reduces the shielding of the $3s$-orbitals, and compression of the s-orbitals.[11]   The ferric quadrupole moment almost always increases with pressure, since it is due to the field imposed by the ligands at the nucleus, which increases essentially as $1/r^3$, where r is a metal-ligand distance. For hemin, Moss[3] has obtained the following expression for the electric field gradient:

$$V_{zz} = \frac{-q_r \left| r_r^2 - 2z_r^2 \right|}{\left| r_r^2 + z_r^2 \right|^{5/2}} + \frac{2q_1}{z_1^3}, \tag{2}$$

where q_1 is the charge on the chloride and q_r the charge on the ring, z_1 the iron-chloride distance, z_r the distance of the iron above the ring, and r_r the effective charge radius of the ring.   As discussed in a previous paper,[2] there are reasons for assuming that the electric field gradient is positive.

From Figure 3, we see that the isomer shift at first increases slightly with pressure (i.e., the electron density at the nucleus decreases); in the region of 20–40 kb a maximum is observed, and at higher pressures there is a very large decrease in isomer shift, which amounts to 0.8 mm/second in 100 kb.   The quadrupole splitting increases considerably in the low-pressure region, passes through a maximum, and then decreases rapidly.   Within our ability to fit the data with Lorentzian peaks, it is zero at the highest pressures.

On the one hand, it must be kept in mind that we are looking at the *ferric* ion isomer shift and quadrupole splitting—these are *unconverted* molecules; on the other hand, the maximum in the isomer shift and quadrupole splitting occur in the same pressure range and at a pressure where a significant fraction of the molecules are reduced.   Furthermore, all these events occur at a measurably higher pressure in hematin than in hemin.   The probability is very high that they are related.
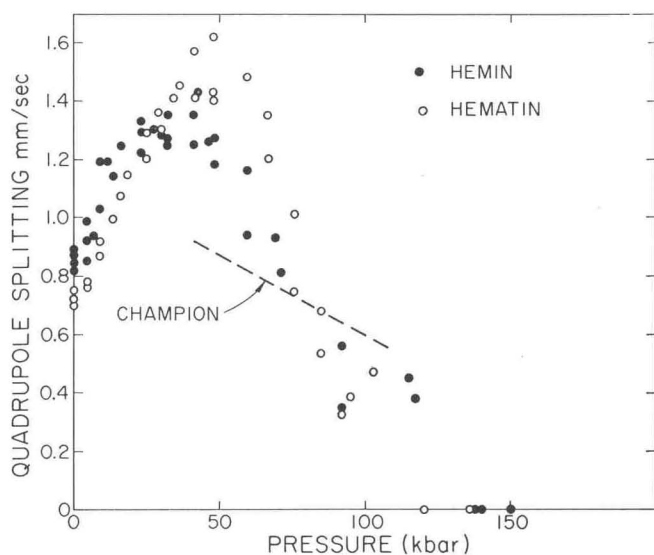
FIG. 4.—Quadrupole splitting vs. pressure for Fe(III) ion in hemin and hematin.

Let us consider first the low-pressure region. From equation (2), we see that a transfer of charge to the ring from the bonding metal orbitals would result in an increase in quadrupole splitting and quite possibly an increase in isomer shift by changing the shielding or the s-character of the bonding orbitals. As conversion increases, the probability that an unconverted molecule would have one or more converted neighbors increases. The reduced ions have a deficiency of electrons on the chlorine and possibly on the ring. These charge deficiencies can interact with the electronic wave functions on an unconverted molecule, making metal-to-ring transfer in the bonding orbitals more difficult and the reverse transfer easier. This could be one factor in the change of direction of the isomer shift and quadrupole splitting. In addition, one would expect a decrease in isomer shift due to the spreading of the $3d$-orbitals, as occurs in most compounds. Interaction of the Cl^- ion with charge-deficient neighbors could spread out its charge, changing z_1 and further affecting the quadrupole splitting. There is not sufficient information for a definitive calculation, but the above factors could reasonably account for the observed results.

The ferrous ion formed from both hemin and hematin has an isomer shift in the neighborhood of 0.45–0.50 relative to metallic iron, which varies little with pressure. This is significantly lower than the isomer shift for normal high-spin Fe(II). Pullman's[12] suggestion that back donation from the metal d-orbitals to the delocalized π-orbitals of the ring is important in metalloporphyrins is probably the explanation. The quadrupole splitting of the Fe(II) hemin spectrum varies from 2.9 mm/second at 20 kb to 2.8 mm/second at 100 kb; that from hematin was about 0.1 mm/second greater. These are typical values for high-spin ferrous ion.

There has been considerable discussion of the effect of spin-spin relaxation on

the peak shapes in hemin,[2, 13] which we will not take up here, but it might be mentioned that the ratio of ferric peak widths was about 2:1 at atmospheric pressure, and less than 1.2:1 just before the quadrupole splitting disappeared.

Summary.—The effect of pressure and temperature has been studied on the Mössbauer spectra of hemin and hematin. Both reduce reversibly to the ferrous state with pressure, with an equilibrium constant given by the relationship $K = AP^B$. The hemin reduces at measurably lower pressures than the hematin. The reaction is exothermic in these compounds, which is in contrast to all previous systems studied. This observation is related to deformation of the potential wells with temperature. Both the isomer shift and quadrupole splitting for the ferric ion increase with pressure in the low-pressure region, go through a maximum at the pressure where the conversion first becomes significant, and then decrease rapidly at higher pressures. The effects of electron transfer between the porphyrin ring and the iron, and the interaction with neighboring molecules containing reduced iron are considered.

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