bond of the amide group, $\mathrm{C(=O)-N(H)}$, owing to its partial double bond character. In fact, this planarity of the amide group should be expected to suppress steric hindrance to rotation about the other bonds in the protein chain, thereby reducing the change of energy with chain conformation. Furthermore, elastin is known[16] to contain few polar amino acids and the electrical forces should not therefore be expected to contribute appreciably to the conformational character of the chain.

Although the elastin bundle conforms to the condition $(\partial E/\partial L)_{VT} = 0$ for ideal rubber elasticity, the linear stress–strain relation presents a striking contrast to the isotherm reported for rubber. This feature finds qualitative explanation in the fact that elastin consists of separate fibers, curled to different degrees. Consequently some of the fibers do not contribute at all to the force at low extensions. As the extension increases the proportion of the fibers subjected to extension increases; hence more of them contribute to the force. The stress–strain relation which would obtain for a homogeneous polymer is therefore modified to the extent that the initial negative curvature which would otherwise be observed is eliminated. The linearity noted within experimental error over the range of extensions given in Fig. 1 is regarded as fortuitous.

At extensions higher than *ca.* 70% the collagen fibers evidently become taut and hence cause the stress to rise abruptly with further extension. Collagen, being a crystalline polymer where elastic modulus and tensile strength are much higher than those of elastin, prevents rupture of the fiber bundle at higher stresses. In contrast with rubber, the steep rise in stress at elongations near the maximum attainable length is not precipitated by crystallization, but by a permanently crystalline component (collagen) interwoven with the deformable (elastin) component.

The conclusions we have reached depart from the interpretations of Meyer and Ferri[4] and of Wöhlisch and co-workers.[5,6] These investigators concluded from stress–temperature measurements that elastin crystallizes on stretching. Wöhlisch went even so far as to calculate the heat of crystallization of elastin from the stress–temperature relation. The value found was surprisingly low. Failure to take account of de-swelling of elastin in water with elevation of temperature led these investigators to the erroneous inferences. The internal energy changes with length as deduced by them are to be attributed rather to the energy change associated with the aforementioned de-swelling with rise in temperature.

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Hemoglobin Studies. III. The Effect of Pyridine on the Combination of Heme with Carbon Monoxide[1]

BY AKITSUGU NAKAHARA[2] AND JUI H. WANG

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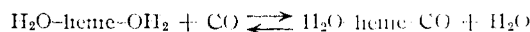
The chemical equilibrium between heme and carbon monoxide was studied in the presence of pyridine. It was shown that small amounts of added pyridine markedly increase the affinity of the heme solution for carbon monoxide in accordance with the ligand-field theory of metal complexes.

It was reported in Paper I of this series that the saturation curve of heme by carbon monoxide corresponds to a simple chemical equilibrium which may be represented by



$$H_2O\text{–heme–}OH_2 + CO \rightleftharpoons H_2O\text{–heme–}CO + H_2O$$

The half-saturation pressure of this reaction was found to be 0.24 cm. which is about 40 times larger than the corresponding value for hemoglobin at room temperature and *p*H 7.4. Since the hemes in hemoglobin are believed to be attached to the imidazole groups of the globin, it seems to be of interest to study the above equilibrium in solutions containing a tertiary amine. Pyridine was chosen in this work instead of imidazole because of the greater solubility of the pyridine–heme complexes.

(1) Paper I, J. H. Wang, A. Nakahara and E. B. Fleischer, THIS JOURNAL, **80**, 1109 (1958); Paper II, J. H. Wang, *ibid.*, 3168 (1958).

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In general when carbon monoxide is equilibrated with an aqueous solution of heme and pyridine, the equilibrium mixture may contain many molecular species such as H_2O–heme–OH_2, H_2O–heme–CO, py–heme–OH_2, py–heme–py, py–heme–CO, etc., where the abbreviation "py" represents pyridine. Thus a quantitative treatment of the equilibrium mixture would include the evaluation of the equilibrium constants for the interconversion of the various pairs of different heme complexes. Fortunately in case of the dilute solutions studied in this work, the treatment of experimental data can be effectively simplified so that the major physical significance of these results can be seen readily without delving in the tedious procedure for evaluating the individual equilibrium constants.

The following solutions at *p*H 11 were prepared and studied: solution A, 0.5×10^{-4} *M* heme/l.; solution B, 0.5×10^{-4} *M* heme + 0.5×10^{-4} *M* pyridine/l.; solution C, 0.5×10^{-4} *M* heme $\times$ 1.0

$\times 10^{-4}$ pyridine/l. The equilibrium spectra of these solutions in the presence and absence of carbon monoxide were examined. These three observations are particularly noteworthy.

(1) In the absence of carbon monoxide, the spectra of the three solutions are, within experimental uncertainties, quantitatively identical.

(2) At P_{CO} = 1 atm., the spectra of the three solutions all show a new pattern, with much more intense absorption in the range 5000–5750 Å. than in the absence of carbon monoxide. But within experimental uncertainties, these three new spectra are again quantitatively identical.

(3) At some intermediate partial pressures of carbon monoxide, *e.g.*, P_{CO} = 0.1 cm., the spectra of these three solutions differ markedly. The spectrum of the solution with the highest pyridine concentration has the most intense absorption band in the 5000–5750 Å. range.

Observation (1) shows that the complex py–heme–py is absent in all of the above solutions, because this complex is known to have an absorption spectrum[3] very different from that of H_2O–heme–OH_2 which is the only species of heme complex in solution A. This observation also shows that the complex py–heme–OH_2 is either absent in the above solutions or that it has, within experimental uncertainties, the same absorption spectrum as that of H_2O–heme–OH_2. Observations (2) and (3) together show that pyridine helps heme to bind CO and that py–heme–CO and H_2O–heme–CO have, within experimental uncertainties, identical spectra.

With these simplifying deductions we may write the degree of saturation, X, of total heme in solution by carbon monoxide as

$$X \equiv \frac{[H_2O\text{-heme-CO}] + [py\text{-heme-CO}]}{[H_2O\text{-heme-}OH_2] + [py\text{-heme-}OH_2] + [H_2O\text{-heme-CO}] + [py\text{-heme-CO}]} = \frac{D - D_1}{D_2 - D_1}$$

where D_1 represents the optical density of one of the above solutions at a given wave length in the absence of carbon monoxide, D and D_2 represent, respectively, the optical densities, at the same wave length, of the same solution when it is partially and completely saturated with carbon monoxide.

The validity of the above expression for the degree of saturation X may be shown as follows. Let

$\epsilon \equiv$ the molar extinction coefficient of H_2O–heme–OH_2 and py–heme–OH_2 (because of observation 1)

$\epsilon' \equiv$ the molar extinction coefficient of H_2O–heme–CO and py–heme–CO (because of observations 2 and 3)

$[H_2O\text{-heme-}OH_2] \equiv a_1$ and $[py\text{-heme-}OH_2] \equiv b_1$ at $p_{CO} = 0$

$[H_2O\text{-heme-CO}] \equiv c_2$ and $[py\text{-heme-CO}] \equiv d_2$ at satn. p_{CO};

$[H_2O\text{-heme-}OH_2] \equiv a$, $[py\text{-heme-}OH_2] \equiv b$, $[H_2O\text{-heme-CO}] \equiv c$, $[py\text{-heme-CO}] \equiv d$ at some intermediate p_{CO}

$l \equiv$ length of the optical path of the absorption cell

$$\therefore D = \epsilon(a + b)l + \epsilon'(c + d)l$$

$$D_1 = \epsilon(a_1 + b_1)l = \epsilon(a + b + c + d)l$$

$$D_2 = \epsilon'(c_2 + d_2)l = \epsilon'(a + b + c + d)l$$

$$\therefore \frac{D - D_1}{D_2 - D_1} = \frac{\epsilon'(c + d) - \epsilon(c + d)}{(\epsilon' - \epsilon)(a + b + c + d)} = \frac{c + d}{a + b + c + d} = X$$

Thus the degree of saturation of the above heme solutions by carbon monoxide may be determined by simple spectrophotometric measurements. The experimental procedure was same as that given in Paper I. These results are summarized in Table I.

TABLE I

THE EFFECT OF PYRIDINE ON THE CHEMICAL EQUILIBRIUM BETWEEN HEME AND CARBON MONOXIDE

Total heme = 0.5 $\times 10^{-4}$ mole l.$^{-1}$; temp. = 21–24°; pH 11.0 ± 0.1

Total pyridine (10^{-4} mole l.$^{-1}$)	P_{CO} (cm.)	Degree of satn. (%)
0.5	0.06	42.6
.5	.19	68.4
.5	.33	79.7
.5	.66	88.0
.5	.79	90.0
.5	1.47	94.7
1.0	0.04	48.3
1.0	.09	66.8
1.0	.10	68.7
1.0	.16	76.9
1.0	.35	88.3
5.0	.05	60.9
5.0	.21	87.4
5.0	.24	90.3
5.0	.50	93.9
5.0	.97	97.3

Table I includes, in addition, data for heme solutions containing 5.0 $\times 10^{-4}$ mole of pyridine per l.

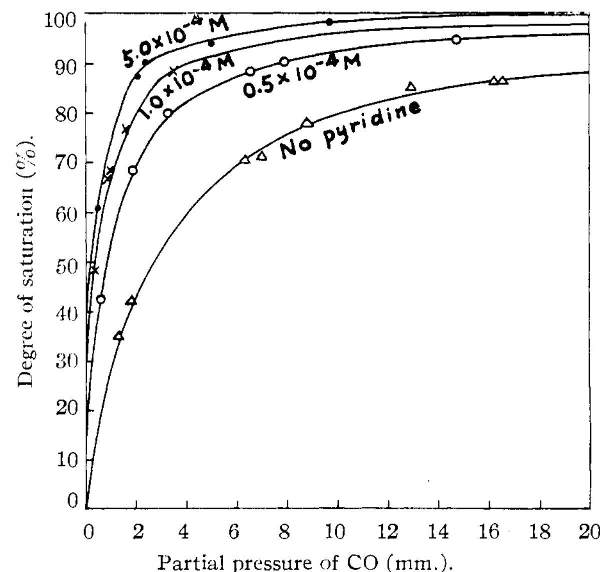

Fig. 1.—Effect of pyridine on the equilibrium between heme and carbon monoxide.

(3) See for example, R. Lemberg and J. W. Legge, "Hematin Compounds and Bile Pigments," Interscience Publishers, Inc., New York, N. Y., 1949, pp. 166, 175.

The spectrum of this solution in the absence of carbon monoxide is no longer the same as that of solutions A, B and C but shows the presence of a minute amount of the complex py–heme–py. However the present procedure should still give the essentially correct value of the degree of saturation, provided that the D_1 value was determined from the corresponding absorption spectrum. The data in Table I, together with similar data in the absence of carbon monoxide reported in Paper I, are plotted in Fig. 1 for comparison.

It is clearly shown in Fig. 1 that the small amounts of added pyridine markedly increase the affinity of the heme solutions for carbon monoxide. Since there is a reciprocal relationship between pyridine and carbon monoxide through their interaction with the same ferrous ion in heme, it may be inferred from above that carbon monoxide also helps the heme to bind pyridine on the opposite side of the heme plane. This is not surprising if one recalls that when heme combines with carbon monoxide, the complex is changed from a high-spin to a low-spin state by the stronger total ligand-field of all six coördinating groups surrounding the ferrous ion in the new complex. The greater thermodynamic stability of py–heme–CO as compared to H_2O–heme–CO is clearly due to the stronger ligand-field of the pyridine as compared to that of the water molecule. These heme complexes are particularly convenient models for ligand-field effect studies because of the absence of direct steric interference between the two coördinating groups on opposite sides of the heme plane.

If the concentration of pyridine in a heme solution is increased continually, the affinity of the system for carbon monoxide should eventually decrease, because then the pyridine tends to combine with heme from both sides and compete with the carbon monoxide.

It may also be of interest to note from Fig. 1 that the half-saturation pressure for carbon monoxide in 0.5×10^{-4} M heme + 5.0×10^{-4} M pyridine solution is 0.029 cm. which is only higher by a factor of 5 than the corresponding value for hemoglobin at pH 7.4.

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