Journal of

The Chemical Society,

Chemical Communications

NUMBER 10/1973 23 MAY

Reaction Between Iron Porphyrins and Carbon Monoxide. A Kinetic Model for Myoglobin

By Dennis V. Stynes and Brian R. James*

(*Department of Chemistry, University of British Columbia, Vancouver 8, British Columbia, Canada*)

Summary Kinetic studies of the reaction between carbon monoxide and (piperidine)$_2$Fe(porphyrin) complexes to give (piperidine)Fe(CO)(porphyrin) show that the mechanism involves predissociation to a five-co-ordinate (piperidine)Fe(porphyrin) complex; the data for the protoporphyrin IX system suggest that the model may resemble closely in kinetic properties the active iron site in deoxy-myoglobin and -hemoglobin.

In studies using models for complex biological protein systems, it is desirable to obtain data on reactions which have an exact analogy in the protein system. In deoxy-myoglobin and -hemoglobin the active site involves a five-co-ordinate high spin iron(II) protoporphyrin IX system.[1] To date, no suitable model system has been reported.† The kinetic studies reported here on the reaction of carbon monoxide with six-co-ordinate Fe(ppIX)pip$_2$ and Fe(tpp)-pip$_2$ complexes[2] (ppIX = protoporphyrin IX, tpp = tetraphenylporphyrin, pip = piperidine) show the importance of five-co-ordinate Fe(porphyrin)pip intermediates, which may serve as such a model. In contrast to the considerable amount of kinetic data concerning reaction of hemoglobin and myoglobin with nitric oxide, carbon monoxide, oxygen, and other ligands,[1] kinetic data for reactions of iron(II) porphyrins have been very limited.[3] In order to understand fully the effect of the globin on the reactivity of hemoglobin and myoglobin, it is essential that kinetic data for the corresponding free hemes be available for comparison with those for the proteins.

The kinetics were studied at 23° in toluene solution which provides a hydrophobic environment reasonably analogous to that found in the protein system. Stop-flow techniques were used to monitor changes in absorbance at 530 nm for Fe(tpp)pip$_2$ and 555 nm for Fe(ppIX)pip$_2$. The carbon monoxide concentration in solution was always in a large excess compared to the porphyrin complexes (*ca.* 5×10^{-5} M) and pseudo-first-order kinetics were observed. The kinetics were studied as a function of carbon monoxide pressure (100—380 Torr) and piperidine concentration (0·5—1·5 M); the minimum half-lives were *ca.* 100 ms. The kinetic data, which show an inverse dependence on piperidine and between first- and zero-order in carbon monoxide, are consistent with the dissociative mechanism shown in equation (1). We have reported elsewhere[4] on the stoicheiometry and thermodynamic data for the overall reaction ($K = k_1k_2/k_{-1}k_{-2}$).

$$(\text{pip})_2\text{Fe(porphyrin)} \underset{+\text{pip},\ k_{-1}}{\overset{-\text{pip},\ k_1}{\rightleftharpoons}} (\text{pip})\text{Fe(porphyrin)} \underset{-\text{CO},\ k_{-2}}{\overset{+\text{CO},\ k_2}{\rightleftharpoons}} (\text{pip})\text{Fe(porphyrin)CO} \quad (1)$$

The rate law, assuming a steady state for the five-co-ordinate intermediate and neglecting k_{-2}, is

$$\text{rate} = \frac{k_1[\text{Fe(porphyrin)pip}_2]}{1 + k_{-1}[\text{pip}]/k_2[\text{CO}]} = k_{\text{obs}}[\text{Fe(porphyrin)pip}_2]$$

Plots of $(k_{\text{obs}})^{-1}$ *vs.* [pip]/[CO] were linear for both the protoporphyrin IX and tetraphenylporphyrin systems, and yielded the k_1 and k_{-1}/k_2 values given in the Table. Values of k_{-2} are readily estimated from a knowledge of the equilibrium constants. The Table also gives some corresponding data for a bispyridinediphenylglyoximate complex reported

† *Added in proof:* Collman and Reed (*J. Amer. Chem. Soc.*, 1970, **95**, 2048) have recently reported on a 2-methylimidazoleFe(tpp) complex.

by Vaska and Yamaji,[5] and some k_{-2} data for hemoglobin and myoglobin systems.

TABLE. *Rate and equilibria data for some* Fe^{II} *complexes (cf. reaction* 1)

	k_1/s^{-1}	k_{-1}/k_2[a]	k_{-2}/s^{-1}	$K/M\ Torr^{-1}$
$(pip)_2Fe(ppIX)$	*ca.* 20	0·002	0·06	2·0[b]
$(pip)_2Fe(tpp)$	*ca.* 11	0·002	0·52	0·13[b]
$(py)_2Fe(dpgh)$[c]	$4·2 \times 10^{-3}$	3·9	$2·5 \times 10^{-5}$	
Hemoglobin[d]			0·029	
Myoglobin[e]			*ca.* 0·02	

[a] Solubility of CO in toluene at 25° taken as $6·5 \times 10^{-3}$ M atm^{-1}; A. Seidell, 'Solubilities of Inorganic and Metal Organic Compounds,' vol. 1, 3rd edn., D. Van Nostrand Co., New York, 1940, p. 218. [b] Ref. 4. Data refer to toluene–piperidine at 23°. [c] Ref. 5. Chlorobenzene solution at 25°. [d] For $Hb_4(CO)_4 \longrightarrow Hb_4(CO)_3 + CO$, in H_2O at pH 9·1, 23°; Q. H. Gibson, *Progr. Biophys. Chem.*, 1959, **9**, 1. [e] Ref. 1, p. 226. In aqueous solution, pH 7, 20°.

The dissociation of carbon monoxide from the low-spin (pip)Fe(porphyrin)CO complexes (k_{-2}) is directly comparable to the corresponding reaction in the myoglobin system where the axial base ligand is an imidazole of the globin. The k_{-2} value for the protoporphyrin IX complex is seen to be within a factor of 2 to 3 for the myoglobin and hemoglobin systems. Barring some fortuitous cancellation of effects, the data suggest that the protein plays little or no role in the dissociation of carbon monoxide from the biological systems. Accepting the hypothesis that the kinetic intermediate is a useful model for deoxymyoglobin, the k_{-1}/k_2 value should also provide information on the effect of the protein on the relative "on rates" (k_2); qualitative agreement between the relative rates of carbon monoxide and nitric oxide binding to $Fe(ppIX)pip_2$, compared to binding to hemoglobin, has been noted.[4]

The data in the Table indicate that the iron glyoxime complexes are not particularly good models for heme protein systems. The porphyrin systems are far more labile than the dpgh system for both ligand and carbon monoxide dissociation; such labilising effects of the porphyrin ligand have been reported for iron(III)[6] and ruthenium(II)[7] systems. Further, the porphyrin systems prefer carbon monoxide to the amine ligands by a kinetic factor of several hundred, while the dpgh system prefers pyridine to carbon monoxide kinetically by a factor of 3·9. The lower ν_{CO} in the porphyrin systems[8] (1970—1980 cm^{-1}) compared with the dpgh complex[5] (1996 cm^{-1}) indicates the greater preference of the porphyrin for carbon monoxide but does not reflect the increased lability.

We thank the National Research Council of Canada for financial support.

(*Received, 26th February* 1973; *Com.* 262.)

[1] E. Antonini and M. Brunori, 'Hemoglobin and Myoglobin in their Reactions with Ligands,' North-Holland, Amsterdam, 1971.
[2] L. J. Radonovich, A. Bloom, and J. L. Hoard, *J. Amer. Chem. Soc.*, 1972, **94**, 2073.
[3] J. O. Alben and W. S. Caughey in 'Heme and Hemoproteins,' eds. B. Chance, R. W. Estabrook, and T. Yonetani, Academic Press, New York, 1966, p. 139; H. W. Kao and J. H. Wang, *Biochemistry*, 1965, **4**, 342.
[4] D. V. Stynes, H. C. Stynes, B. R. James, and J. A. Ibers, *J. Amer. Chem. Soc.*, in the press.
[5] L. Vaska and T. Yamaji, *J. Amer. Chem. Soc.*, 1971, **93**, 6673.
[6] G. N. La Mar and F. A. Walker, *J. Amer. Chem. Soc.*, 1972, **94**, 8607; G. B. Kolski and R. A. Plane, *ibid.*, p. 3740; N. S. Angerman, B. B. Hasinoff, H. B. Dunford, and R. B. Jordan, *Canad. J. Chem.*, 1969, **47**, 3217.
[7] S. S. Eaton, G. R. Eaton, and R. H. Holm, *J. Organometallic Chem.*, 1972, **39**, 179.
[8] J. H. Wang, A. Nakahara, and E. B. Fleischer, *J. Amer. Chem. Soc.*, 1958, **80**, 1109; J. O. Alben and W. S. Caughey, *Biochemistry*, 1968, **7**, 175.