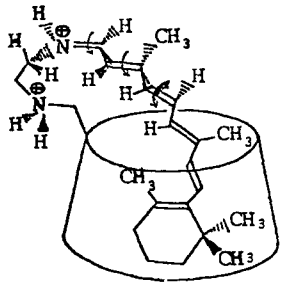

Figure 2. Schematic representation of plausible binding of retinal moiety of $3\cdot(H^+)_2$ based on CPK model.

important to note that in aqueous solution, $3\cdot(H^+)_2$ showed a further red shift from 476 (in ethylene glycol) to 497 nm; the latter wavelength is practically the same as those of native rhodopsins (see Table I). This significant red shift can not be interpreted by the simple binding of the chromophore in a hydrophobic cavity, since, on the contrary, $4\cdot(H^+)_2$ or $5\cdot(H^+)$ showed a substantial *blue shift* in the hydrophobic cavity of unsubstituted $\beta$-cyclodextrin (−26 and −17 nm, respectively). Therefore, this significant and unique red shift (21 + 26 = 47 nm or 53 nm from $4\cdot(H^+)_2\cdot\beta$-CD to $3\cdot(H^+)_2$) caused by covalent combination of the CO recognition site and the hydrophobic binding site is neither due to a solvent effect nor to simple binding in a hydrophobic cavity.

In the literature, there are many mechanisms postulated to interpret the unique and remarkable red shift,[10] which can, in principle, be arranged into the following three categories: (a) the effect of the large polarizability of the microenvironment, most probably of aromatic amino acid residue(s) in opsin's binding site;[11] (b) the abnormally large electrostatic effect of the appropriately located counteranion and/or the additional anion or cation along the surface of the binding site of opsin[12] upon the protonated Schiff base; (c) the possible twist or distortion of the retinal moiety caused by the tight binding by opsin.[2f,13]

Since $3\cdot(H^+)_2$ (Figure 2) is located in a very similar electrostatic and/or microenvironment to $4\cdot(H^+)_2 + \beta$-CD, the remarkable red shift observed for $3\cdot(H^+)_2$ (+53–47 nm) can not be attributable to the electrostatic or microenvironmental effects alone, but rather to a unique combination of these effects, or these effects including the twist mechanism[14] seem to be operating. Apparently, the electrostatic mechanism is operating as is seen from the comparison of $5\cdot H^+$ with $4\cdot(H^+)_2$ (+21 nm). The present model study is a very suggestive one which should lead to further insights into the rhodopsin mechanism, and additional model studies are now underway.

## References and Notes


(1) S. Hecht, *J. Gen. Physiol.*, **2**, 499 (1920).
(2) (a) D. Bownds, *Nature (London)*, **216**, 1178 (1967); (b) M. Akhtar, P. T. Blosse, and P. B. Dewhurst, *Chem. Commun.*, **13**, 631 (1967); (c) G. E. Busch, M. L. Applebury, A. A. Lamola, and P. M. Rentzepis, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 2802 (1972), (d) A. Lews, R. S. Fager, and E. W. Abrahamson, *J. Raman Spectrosc.*, **1**, 465 (1973); (e) A. R. Oseroff and R. H. Callender, *Biochemistry*, **13**, 4243 (1974); (f) B. Honig, A. Warshel, and M. Karplus, *Acc. Chem. Res.*, **8**, 92 (1975); (g) W. H. Waddell, R. Crouch, K. Nakanishi, and N. J. Turro, *J. Am. Chem. Soc.*, **98**, 4189 (1976); (h) M. Marcus and A. Lewis, *Science*, **195**, 1328 (1977); (i) R. R. Birge, J. A. Bennett, B. M. Pierce, and J. M. Thomas, *J. Am. Chem Soc.*, **100**, 1533 (1978); (j) R. Cookingham and A. Lewis, *J. Mol. Biol.*, **119**, 569 (1978); and references cited therein.
(3) (a) E. W. Abrahamson and R. S. Fager, *Curr. Top. Bioenerg.*, **5**, 125 (1973); (b) B. Honig and T. G. Ebrey, *Annu. Rev. Biophys. Bioeng.*, **3**, 151 (1974); (c) T. G. Ebrey and B. Honig, *Q. Rev. Biophys.*, **8**, 129 (1975); and references cited therein.
(4) E. W. Abrahamson and S. E. Ostroy, *Prog. Biophys. Mol. Biol.*, **17**, 179 (1967).
(5) (a) I. Tabushi, K. Shimokawa, N. Shimizu, H. Shirakata, and K. Fujita, *J. Am. Chem. Soc.*, **98**, 7855 (1976); (b) I. Tabushi, K. Fujita, and L. C. Yuan, *Tetrahedron Lett.*, 2503 (1977); (c) I. Tabushi, N. Shimizu, T. Sugimoto, M. Shiozuka, and K. Yamamura, *J. Am. Chem. Soc.*, **99**, 7100 (1977); (d) I. Tabushi, K. Fujita, and H. Kawakubo, *ibid.*, **99**, 6456 (1977); (e) I. Tabushi, Y. Kiyosuke, T. Sugimoto, and K. Yamamura, *ibid.*, **100**, 916 (1978); (f) I. Tabushi, Y. Kuroda, K. Fujita, and H. Kawakubo, *Tetrahedron Lett.*, 2083 (1978).
(6) (a) R. Breslow and Y. Chao, unpublished result. Cf. Y. Chao, Ph.D. Thesis, Columbia University, (1972). (b) I. Tabushi, K. Yamamura, and K. Shimokawa, Abstracts of the 32th Annual Meeting of Chemical Society of Japan, Tokyo, Vol. III, 1975, p 1301. (c) Y. Matsui, T. Yokoi, and K. Mochida, *Chem. Lett.*, 1037 (1976).
(7) Structural identification was successfully made after treatment of the Schiff base with $NaBH_4$. See ref 2a.
(8) C. S. Irving, G. W. Byers, and P. A. Leemakers, *J. Am. Chem. Soc.*, **91**, 2141 (1969).
(9) In ethylene glycol, hydrophobic binding of the chromophore into CD cavity seems to be much weaker.
(10) *all-trans*- and 11-*cis*-retinal show practically the same absorption maximum.
(11) (a) D. S. Kliger, S. J. Milder, and E. A. Dratz, *Photochem. Photobiol.*, **25**, 277 (1977); (b) C. S. Irving, G. W. Byers, and P. A. Leemakers, *Biochemistry*, **9**, 858 (1970).
(12) (a) P. Blatz, J. Mohler, and H. Navangul, *Biochemistry*, **11**, 848 (1972); (b) A. Kropf and R. Hubbard, *Ann. N.Y. Acad. Sci.*, **74**, 266 (1958); (c) B. Honig, A. D. Greenberg, U. Dinur, and T. G. Ebrey, *Biochemistry*, **15**, 4593 (1976); (d) A. Warshell, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 2558 (1978); (e) R. Mathies and L. Stryer, *ibid.*, **73**, 2169 (1976); (f) A. Lewis, A. Lemley, and R. Cookingham, *ibid.*, **73**, 4266 (1976).
(13) P. Blatz and P. Liebman, *Expt. Eye Res.*, **17**, 573 (1973).
(14) The space-filling molecular model also suggests that $3\cdot(H^+)_2$ is appreciably twisted near carbon 11 when bound into its own $\beta$-CD cavity.



**Iwao Tabushi,* Yasuhisa Kuroda, Kazuhiro Shimokawa**

*Department of Synthetic Chemistry*
*Kyoto University, Yoshida, Kyoto 606, Japan*


*Received November 30, 1978*

## The Iron(II) "Homologous Cap" Porphyrin. A Novel Dioxygen Binder

*Sir:*

As part of our current research program in the area of synthetic oxygen carriers, we have been investigating the base and dioxygen binding of the iron and cobalt "cap" and "homologous cap" porphyrins.[1-4] We report here some experimental results concerning Fe(HmCap)(1-MeIm) which show that the complex is capable of *weakly* binding a second molecule of 1-MeIm and that the complex so formed can bind dioxygen reversibly *without* displacing the weakly bound 1-MeIm ligand. In an accompanying communication, comparisons between the Cap and HmCap systems are made.

The $HmCapH_2$ was prepared in a similar manner to that described for the normal cap porphyrin.[1] The reaction of $HmCapH_2$ with anhydrous ferrous chloride in refluxing THF under dry nitrogen gave Fe(HmCap)Cl: UV $\lambda_{max}(CHCl_3)$ 425, 510, 560 (sh), 590 (sh). Benzene or toluene solutions of the iron(III) porphyrin were reduced using aqueous sodium dithionite[5] to give Fe(HmCap), $\lambda_{max}$(toluene) 542 nm. The presence of bands in the infrared spectra of the iron–dioxygen adducts attributable to $\nu_{^{16}O_2}$ and $\nu_{^{18}O_2}$ confirms that oxygenation has occurred.[6]

Figure 1A shows the spectral changes which occurred during the titration of a toluene solution of Fe(HmCap) with a 0.0566 M 1-MeIm solution in toluene. The observed changes are due primarily to the equilibrium

$$\text{Fe(HmCap)} + \text{1-MeIm} \overset{K^B}{\rightleftharpoons} \text{Fe(HmCap)(1-MeIm)} \quad (1)$$

and a value for log $K^B$ (23 °C) of 3.31 ± 0.05 was obtained. If this titration was followed by one using neat 1-MeIm, the spectral changes shown in Figure 1B were observed and these changes are associated with the equilibrium

$$\text{Fe(HmCap)(1-MeIm)} + \text{1-MeIm} \overset{K_B{}^B}{\rightleftharpoons} \text{Fe(HmCap)(1-MeIm)}_2 \quad (2)$$

for which log $K_B{}^B$ (23 °C) = 0.77. Consistent with this inter-

0002-7863/79/1501-4760$01.00/0 

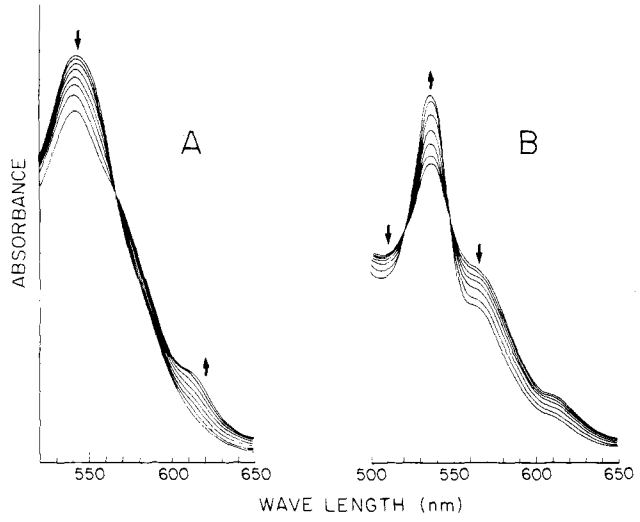


**Figure 1.** A: spectral changes occurring upon titration of an $\sim 10^{-4}$ M toluene solution of Fe(HmCap) with 0.0566 M 1-MeIm in toluene; the final base concentration is $2.8 \times 10^{-3}$ M. B: the titration of a toluene solution of Fe(HmCap)(1-MeIm)$_x$, initially 0.0563 M in 1-MeIm, with neat 1-MeIm; the final base concentration is 0.701 M.

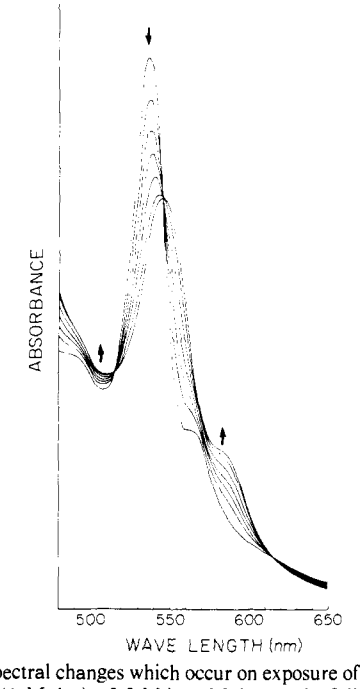


**Figure 2.** The spectral changes which occur on exposure of toluene solution of Fe(HmCap)(1-MeIm)$_2$, 2.2 M in 1-MeIm, to the following pressures of dioxygen at 0 °C; 0, 47.5, 100.2, 173.0, 308.7, 600.3, and 986.6 Torr.

pretation are the following. (a) the $\mu_{eff}$ of toluene solutions of Fe(HmCap)(1-MeIm)$_x$, 0.02 and 1.8 M in 1-MeIm, are, respectively, 4.6 and 3.1 $\mu_B$.[7] That is, as the base concentration is increased, the $\mu_{eff}$ decreases in accord with the coordination of a second base to Fe(HmCap)(1-MeIm) producing a change from high to intermediate spin. (b) Titrations with toluene solutions of 1,2-Me$_2$Im or *t*-BuNH$_2$ showed that only equilibrium 1 was present with log $K^B$ (23°C) equal to 3.61 ± 0.05 and 2.23 ± 0.05, respectively, thus confirming for this system that 1,2-Me$_2$Im, a sterically hindered axial base,[8] and *t*-BuNH$_2$, a bulky axial base, form five- but not six-coordinated Fe(HmCap) complexes.

Figure 2 shows the spectral changes which occur when a toluene solution of Fe(HmCap)(1-MeIm)$_2$, 2.2 M in 1-MeIm, is exposed to various pressures of dioxygen. If dioxygen and 1-MeIm were competing for the sixth coordination site, as indicated in equilibria 2 and

$$\text{Fe(HmCap)(1-MeIm)} + O_2 \underset{}{\overset{K_B^{O_2}}{\rightleftharpoons}} \text{Fe(HmCap)(1-MeIm)}O_2 \quad (3)$$

the apparent $P_{1/2}$ would be a function of base concentation, and the following may be derived:

$$P_{1/2}^{O_2}(\text{apparent}) = (1 + K_B^B[B])P_{1/2}^{O_2}, \quad \text{where } P_{1/2} = (K_B^{O_2})^{-1} \quad (4)$$

The measurement of dioxygen affinities were made at 0 °C where $K_B^B = 8.3 \pm 0.7$. Equation 4 requires that $P_{1/2}^{O_2}$ (apparent) increase by a factor of 3.4 on going from 0.5 to 2 M 1-MeIm. Over this concentration range, however, $P_{1/2}^{O_2}$ (apparent) values are all within the range of 200 ± 10 Torr and, at [1-MeIm] = 0.2 M, $P_{1/2}^{O_2}$ (apparent) = 176 ± 10 Torr. At lower base concentrations increased amounts of baseless Fe(HmCap) cause oxidation to occur. However, the spectral changes for the addition of dioxygen to Fe(HmCap)(1,2-Me$_2$Im) are consistent with its addition to a five-coordinate complex and different from Fe(HmCap)(1-MeIm)$_2$(O$_2$) shown in Figure 2.

The above results indicate that dioxygen and 1-MeIm are *not* competing directly for a sixth coordination site. The inference is that Fe(HmCap)(1-MeIm)$_2$ does reversibly bind dioxygen, and the following equilibria should also be considered:

$$\text{Fe(HmCap)(1-MeIm)}_2 + O_2 \underset{}{\overset{K_{B_2}^{O_2}}{\rightleftharpoons}} \text{Fe(HmCap)(1-MeIm)}_2O_2 \quad (5)$$

$$\text{Fe(HmCap)(1-MeIm)}O_2 + \text{1-MeIm} \underset{}{\overset{K^B_{BO_2}}{\rightleftharpoons}} \text{Fe(HmCap)(1-MeIm)}_2O_2 \quad (6)$$

This reaction scheme permits the derivation of

$$P_{1/2}^{O_2}(\text{apparent}) = [(1 + K_B^B[B])/(1 + K^B_{BO_2}[B])] \times P_{1/2}^{O_2} \quad (7)$$

(where $P_{1/2}^{O_2} = (K_B^{O_2})^{-1}$)

and

$$K_{B_2}^{O_2} = K_B^{O_2} \times (K^B_{BO_2}/K_B^B) \quad (8)$$

Since the observed range of $P_{1/2}^{O_2}$ (apparent) values is so small, it has only been possible to estimate ranges for the dioxygen affinities of Fe(HmCap)(1-MeIm) and Fe(HmCap)(1-MeIm)$_2$, and they are 120–180 and 200 ± 10 Torr, respectively.

The following observations may be made concerning these equilibria: (a) the $K_B^B$ (B = 1-MeIm) found for Fe(HmCap) is approximately three orders of magnitude less than those for other iron porphyrin systems;[9,10] (b) Fe(HmCap)(1-MeIm)$_2$ has intermediate spin ($S = 1$), whereas nonsterically hindered Fe(porphyrin)(1-MeIm)$_2$ complexes are invariably diamagnetic;[7] (c) the dioxygen affinity of Fe(HmCap)(1-MeIm)$_2$ appears to be only slightly lower than that of Fe(HmCap)(1-MeIm). Each of these observations serves to demonstrate the weakness of the second base interaction, but, at present, the manner in which the second base binding occurs is not known.

The above chemistry represents a novel facet of dioxygen binding to metalloporphyrin complexes and is being further investigated.

**Acknowledgment.** This work was supported by grants from the National Institute of Health and National Science Foundation. We thank Dr. T. Szymanski for helpful discussion on the synthesis of the "homologous cap" porphyrin.

## References and Notes


(1) Almog, J.; Baldwin, J. E.; Dyer, R. L.; Peters, M. *J. Am. Chem. Soc.* **1975**, *97*, 226–227.
(2) Almog, J.; Baldwin, J. E.; Huff, *J. J. Am. Chem. Soc.* **1975**, *97*, 227–228.
(3) See the accompanying communication for a schematic representation of the "homologous cap" porphyrin: Budge, J. R.; Ellis, P. E., Jr.; Jones, R. D.; Linard, J. E.; Szmanski, T.; Basolo, F.; Baldwin, J. E.; Dyer, R. L. *J. Am. Chem. Soc.*, following paper in this issue.
(4) Abbreviations: Cap, cap porphyrin; HmCap, homologous cap; $HmCapH_2$, homologous cap porphyrin; 1-MeIm, 1-methylimidazole; 1,2-$Me_2$Im, 1,2-dimethylimidazole; $P_{1/2}{}^{O_2}$, $O_2$ pressure at half saturation.
(5) Brault, D.; Rougee, M. *Biochemistry* **1974**, *22*, 4598–4602.
(6) Jones, R. D.; Budge, J. R.; Ellis, P. E., Jr.; Linard, J. E.; Summerville, D. A.; Basolo, F. *J. Organomet. Chem.*, in press.
(7) Magnetic susceptibilities were determined at 34 °C using the Evans method. At 0.02 M 1-MeIm, Fe(HmCap)(1-MeIm) is the predominant porphyrin species (~90%), whereas at 1.8 M 1-MeIm, it is Fe(HmCap)(1-MeIm)$_2$ (~90%). An error of ±0.2 $\mu_B$ has been assigned to the $\mu_{eff}$ values.
(8) Collman, J. P. *Acc. Chem. Res.* **1977**, *10*, 265–272.
(9) Brault, D.; Rougée, M. *Biochem. Biophys. Res. Commun.* **1974**, *57*, 654–659.
(10) Momenteau, M.; Rougée, M.; Loock, B. *Eur. J. Biochem.* **1976**, *71*, 63–76.
(11) Address correspondence to the Department of Organic Chemistry, The University, Oxford, England.



**John R. Budge, Paul E. Ellis, Jr., Robert D. Jones**
**Jack E. Linard, Fred Basolo***
*Department of Chemistry, Northwestern University*
*Evanston, Illinois 60201*

**Jack E. Baldwin,*[11] Robert L. Dyer**
*Department of Chemistry*
*Massachusetts Institute of Technology*
*Cambridge, Massachusetts 02139*

*Received February 21, 1979*

## The Iron(II) and Cobalt(II) "Cap" and "Homologous Cap" Porphyrins. Base and Oxygenation Equilibria Studies of Relevance to Hemoglobin Cooperativity

*Sir:*

The topic of Hb[1] cooperativity is of considerable interest, and explanations for this complicated phenomenon are being sought through model complex studies.[2,3] Here we report on base and oxygenation equilibria measurements for the iron(II) and cobalt(II) "cap" and "homologous cap" porphyrins (Figure 1).[4,5] The results obtained show that there are significant differences in the base and dioxygen binding of the two

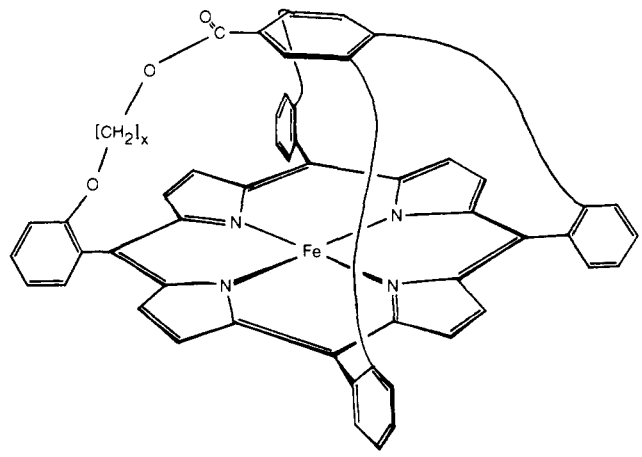


**Figure 1.** Schematic representations of the "cap" ($x$ = 2) and "homologous cap" ($x$ = 3) porphyrin complexes.

porphyrins, and the implications as to the nature of Hb cooperativity are discussed.

A lowering in both the dioxygen and carbon monoxide affinities for Fe(porphyrin)B complexes has been observed when the axial base, B, is changed from 1-MeIm to the sterically hindered 2-MeIm or 1,2-$Me_2$Im; and moreover, these latter complexes are suggested to mimic T-state Hb.[3,6-9] These results have also been used[9] to support the Hoard–Perutz mechanism for cooperativity. This proposes that, in T-state Hb, the protein tertiary structure places greater restraint on the motion of the proximal histidine towards the heme plane as oxygenation occurs. A recent alternative explanation[10] for the R and T states, based on energy minimization calculations, suggests that nonbonding protein–heme interactions in the T state constrain the porphyrin to a "domed" configuration. The additional energy required to "undome" the porphyrin upon oxygenation is reflected in the lower T-state dioxygen affinity. A similar proposal has also been advanced by Hoffman.[11]

Table I summarizes base and dioxygen equilibria data for the "cap" and "homologous cap" systems as well as for some other representative model complexes. Discussing firstly, the "cap" systems, it is apparent that both Fe(Cap)(1-MeIm) and Fe(Cap)(1,2-$Me_2$Im) have considerably lower dioxygen affinities compared with their "picket-fence" porphyrin[3] analogues. However, both the iron "cap" and "picket-fence" porphyrins show comparable reductions in $K_B{}^{O_2}$ on substituting 1,2-$Me_2$Im for 1-MeIm as the axial base. Similar observations may be made for the cobalt complexes although,

**Table I.** Base and Oxygenation Equilibria Data in Toluene

| species | base | log $K^B$ (±0.05)[a] | $P_{1/2}{}^{O_2}$ at 0 °C, Torr | ref |
|---|---|---|---|---|
| Fe(Cap) | 1-MeIm | 2.90 | 4.5 | this work |
| | 1,2-$Me_2$Im | 3.06 | 930 | this work |
| | $t$-$BuNH_2$ | 2.50 | 0.27[d] | this work |
| Fe(HmCap) | 1-MeIm | 3.31 | (120–180) | this work |
| | 1,2-$Me_2$Im | 3.61 | 880[d] | this work |
| | $t$-$BuNH_2$ | 2.23 | 575[d] | this work |
| Co(Cap) | 1-MeIm | 2.32 | $5.9 \times 10^4$, 140[e] | this work |
| | 1,2-$Me_2$Im | 1.84 | (2000–4000)[e] | this work |
| Co(HmCap) | 1-MeIm | 2.28 | (>5000)[e] | this work |
| | 1,2-$Me_2$Im | 1.93 | [f] | this work |
| FeTpivPP(4CIm)PP | | | 0.042 | 9 |
| FeTpivPP | 1,2-$Me_2$Im | 4.5[b] | 4.5 | 9 |
| FeTPP | 2-MeIm | 4.1[c] | | 14 |
| CoTpivPP | 1-MeIm | 4.2[b] | 26.6 | 9, 22 |
| | 1,2-$Me_2$Im | 3.2[b] | 152 | 9, 22 |
| CoT($p$-$OCH_3$)PP | 1-MeIm | 3.37[c] | $3.87 \times 10^3$ | 23, 24 |
| | 1,2-$Me_2$Im | 2.79[c] | | 23 |

[a] At 23.1 ± 0.1 °C unless otherwise stated. [b] At 20 °C. [c] At 25 °C. [d] At −63 °C. [e] At −78 °C. [f] The dioxygen affinity was too small to estimate.

0002-7863/79/1501-4762$01.00/0 