Since neither the ORD results nor the previous $^{13}C$ NMR data[4] nor the molecular model studies[3] support the Hartley concept, the time has arrived to consider favorably an alternative micelle model.[12]

**Acknowledgments.** This work was supported by the National Science Foundation, the National Institutes of Health, and the donors of the Petroleum Research Fund, administered by the American Chemical Society.

(12) Not only do we not take literally the "asterick" micelle attributed to Hartley, neither did Hartley. He states, "It has no physical basis and is drawn for no other reason than that the human mind is an organizing instrument and finds unorganized processes uncongenial." G. S. Hartley, "Aqueous Solutions of Paraffin-chain Salts: A Study in Micelle Formation", Paris, Hermann and Co., London, 1936, p 44.

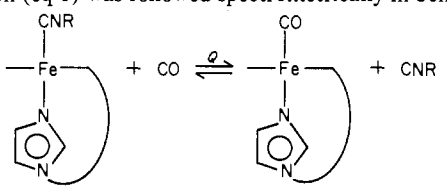
**F. M. Menger,* B. J. Boyer**
*Department of Chemistry, Emory University*
*Atlanta, Georgia 30322*
*Received January 2, 1980*

## Isocyanide Binding to Chelated Protoheme. Kinetic Criteria for Distal Steric[1] Effects in Hemoproteins

*Sir:*

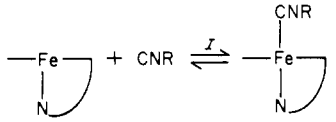
In previous studies, chelated hemes such as protoheme monomethyl ester mono-3-imidazolylpropylamide (**1**) were shown to model the CO and $O_2$ binding to the R state of hemoglobin.[1-3] Herein, we describe studies of isocyanide binding to **1** which quantify the large distal steric effects in hemoglobin (Hb) and myoglobin (Mb), first recognized in the classic work of St. George and Pauling.[4] In the accompanying communication, we report isocyanide binding to a cyclophane heme, designed to model these distal steric effects in hemoproteins.

Early work of Pauling and others[5,6] on simple hemes is complicated by formation of both mono- and diisocyanide complexes. The binding of isocyanides to the five-coordinated chelated heme **1** is more directly comparable to Hb and Mb.[7] The CO/CNR competition (eq 1) was followed spectrometrically in benzene (see




(1)

Figure 1). Equilibrium constants for the direct binding (eq 2) were then calculated from the known CO binding constant for **1** in benzene (Table I).




(2)

Steric effects are unimportant in binding to **1**. The affinities of the isocyanides correlate with the electron-withdrawing properties of R, the electron-withdrawing tosylmethyl group enhancing the $\pi$-acceptor properties of the isocyanide and thus binding more strongly. Solvent effects on isocyanide binding are as small as those on CO binding.[2] The value of $Q$ is two times smaller in aqueous cetyltrimethylammonium bromide suspension than it is in benzene.

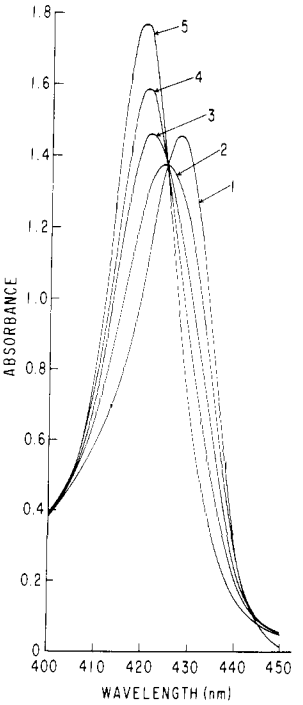



**Figure 1.** Spectral changes accompanying addition of carbon monoxide to **1**-*t*-BuNC in benzene. [*t*-BuNC] = $3 \times 10^{-3}$ M. [CO] $\times 10^4$ = 0, 5.86, 11.7, 23.4, and 65 M in 1 → 5.

**Table I.** Isocyanide Binding to 1 in Benzene

| | $Q^a$ | $I$,[b] $M^{-1}$ |
|---|---|---|
| *n*-BuNC | 0.9 | $4.4 \times 10^8$ |
| *t*-BuNC | 2.4 | $1.7 \times 10^8$ |
| TMIC | 0.06 | $7 \times 10^9$ |

[a] Equation 1. Symbols are those in common usage.[7] [b] Equation 2, calculated from $Q$ by using CO binding constant of $4 \times 10^8$ $M^{-1}$ for 1.[2]

Comparison with isocyanide binding to proteins shows $\sim 10^4$ times poorer binding of *n*-BuNC to Mb compared to **1** and over $10^5$ times for *t*-BuNC or *p*-toluenesulfonylmethyl isocyanide (TMIC). These large differences are due to distal steric effects present in the restricted pocket of Mb which are not present in **1**.

The kinetic studies on **1** were carried out to determine the dynamic differences between **1** and Mb. Flash photolysis studies of **1**-CNR are complicated by the much smaller quantum yield for **1**-CNR compared to **1**-CO and the smaller spectral differences between **1** and **1**-CNR, $\lambda_{max}$ = 428 nm. The rate of BuNC association with **1** can be obtained from flash photolysis of **1**-CO in the presence of BuNC in a technique exactly analogous to that used for determining $O_2$ kinetic data for Hb, Mb,[7] or **1**.[1,3] Photolysis of benzene solutions containing primarily **1**-CO ([Hm] = $5 \times 10^{-6}$ M, [CO] = $6 \times 10^{-5}$ M, [BuNC] = $1 \times 10^{-5}$ to $5 \times 10^{-5}$ M) results in most of the intermediate, **1**, being trapped as the **1**-BuNC even when [CO] is in large excess over [BuNC]. This can only occur if the rate constant for BuNC association with **1** is larger than that for CO.

Absorbance changes at 440 nm proved most convenient for following the rate of isocyanide association. Under conditions where BuNC adds 10 times faster than CO, clean pseudo-first-order kinetics are followed, giving an association rate constant of $2.2 \times 10^8$ $M^{-1}$ $s^{-1}$ for *n*-BuNC.

Attempts to obtain the dissociation rate for **1**-BuNC by following the rate of the slow reaction after photolysis in CO-saturated benzene (eq 3) containing varying [BuNC] ($3 \times 10^{-5}$ to 3

**1**-BuNC + CO → **1**-CO + BuNC (3)

(1) Traylor, T. G.; Chang, C. K.; Geibel, J.; Berzinis, A.; Mincey, T.; Cannon, J. *J. Am. Chem. Soc.* **1979**, *101*, 6716.
(2) White, D. K.; Cannon, J. B.; Traylor, T. G. *J. Am. Chem. Soc.* **1979**, *101*, 2443.
(3) Geibel, J.; Cannon, J.; Campbell, D.; Traylor, T. G. *J. Am. Chem. Soc.* **1978**, *100*, 3575.
(4) St. George, R. C. C.; Pauling, L. *Science* **1951**, *114*, 629.
(5) Keilin, J. *Biochem. J.* **1949**, *45*, 440.
(6) Imai, Y.; Sato, R. *J. Biochem. (Tokyo)* **1968**, *64*, 147.
(7) Antonini, E.; Brunori, M. "Hemoglobin and Myoglobin in Their Reactions with Ligands", North-Holland Publishing Co.: Amsterdam, 1971; p 259.

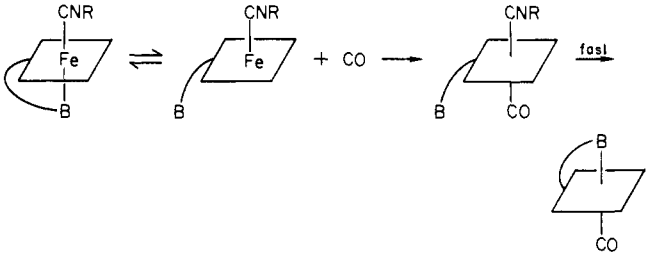
0002-7863/80/1502-5938$01.00/0 

Table II. Comparison of the Equilibrium Constants ($I$ and $L$) and Kinetic Constants for Association ($i'$ and $I'$) and Dissociation ($i$ and $l$) in the Binding of $n$-BuNC and CO to Hemes

| heme | $i'$, $M^{-1}$ $s^{-1}$ | $i$, $s^{-1}$ | $I$, $M^{-1}$ | $I'$, $M^{-1}$ $s^{-1}$ | $l$, $s^{-1}$ | $L$, $M^{-1}$ |
|---|---|---|---|---|---|---|
| chelated protoheme (1)[a] | $2.2 \times 10^8$ | 0.5[b] | $4.4 \times 10^8$ | $1.1 \times 10^7$ | 0.025[c] | $4 \times 10^8$ |
| | $(1.7 \times 10^8)$[d,e] | (0.023)[d] | $(7 \times 10^9)$[d] | | | |
| Mb[f] | $5.8 \times 10^4$ | 1.0 | $5.7 \times 10^4$ | $5 \times 10^5$ | 0.017 | $3 \times 10^7$ |
| | $(2.3 \times 10^2)$[d] | (0.01)[d] | $(2.5 \times 10^4)$[d] | | | |
| Hb(R) $\alpha$[g] | $3.5 \times 10^4$ | 0.32 | $1.1 \times 10^5$ | $6 \times 10^6$ | 0.009 | $7 \times 10^8$ |
| Hb(R) $\beta$[g] | $2.4 \times 10^5$ | 4.0 | $6 \times 10^4$ | | | |

[a] In benzene, 20 °C. [b] Calculated from $I$ and $i'$. [c] Reference 8. [d] For (p-toluenesulfonyl)methyl isocyanide. [e] Calculated from $I$ and $i$. [f] Reference 10. [g] References 2 and 11.

$\times 10^{-3}$ M) gave rates independent of [BuNC], $k = 0.6$ $s^{-1}$. This indicates that CO and BuNC do not compete for **1**, but the system returns to equilibrium via a "base-off" path.[3]




Direct determination of the dissociation rate for TMIC was obtained by mixing **1**-TMIC with chelated mesoheme[8] and following the formation of mesoheme–TMIC at 418 nm or the disappearance of **1**-TMIC at 428 nm. This method prevents reaction via the base-off pathway. From $i_{TMIC} = 0.023$ $s^{-1}$ and $I_{TMIC}$, the on rate for TMIC is calculated $i' = 1.7 \times 10^8$ $M^{-1}$ $s^{-1}$. The electronic differences between TMIC and BuNC appear only in the off rates.

Data for TMIC binding to purified sperm whale myoglobin[9] were obtained in phosphate buffer, pH 7.3. The dissociation rate was obtained by CO displacement. Spectral data for Mb–TMIC [$\lambda_{max}$ = 431, 529, 559 nm ($\epsilon \times 10^3$ = 161, 13.2, 15.3, respectively)] are similar to those reported for other isocyanide derivatives of Mb.[7]

The equilibrium and kinetic differences between **1** and Mb or Hb are summarized in Table II. BuNC binding to Hb shows chain heterogeneity which is sensitive to pH and phosphates.[11] The $10^4$ times difference between **1** and the proteins is seen to arise almost exclusively from association differences. The most remarkable example is the binding of TMIC, which suffers a $10^6$ times decrease in association rate and a twofold decrease in dissociation rate in going from **1** to Mb! Large variations in CNR binding to proteins with increasing steric bulk of R also arise almost exclusively from association-rate differences.[10] These facts are most consistent with a conformationally flexible heme pocket where most of the pocket rearrangement occurs prior to the transition state for ligand addition. The lower association rate for CO addition to Mb suggests some distal steric hindrance is present.[3] The similarity between CO association rates for **1** and Hb(R) suggests little or no steric effects in the R state.

These results and previous kinetic studies afford a kinetic method for distinguishing among the various effects in hemoprotein ligation. Electronic effects in the ligand or proximal base[8] are reflected predominantly in the dissociation rates. Distal side steric effects appear in the association rates.[12] Proximal base steric strain has an approximately equal effect on association and dissociation rates.[2]

**Acknowledgments.** This work was supported by National Institutes of Health Grant HL-13581 (T.G.T.).

**T. G. Traylor ,* D. V. Stynes**[13]
*Department of Chemistry, D-006*
*University of California, San Diego*
*La Jolla, California 92093*

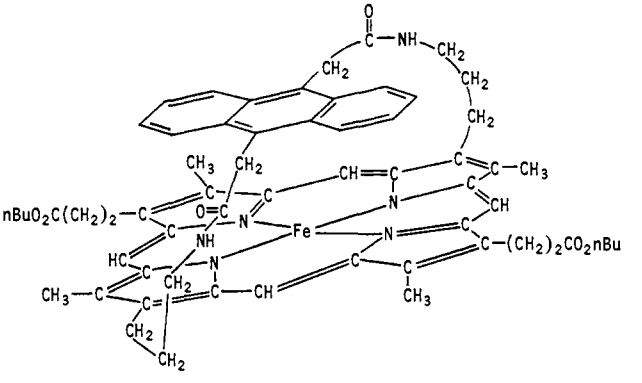
*Received March 24, 1980*

## Cyclophane Hemes. 3. Magnitudes of Distal Side Steric Effects in Hemes and Hemoproteins

*Sir:*

The occurrence and magnitude of the distal side steric effects in hemoproteins remain a subject of great interest.[1-6] On the basis of the similar affinities of simple model compounds and hemoglobin for CO and $O_2$, we proposed[2] that R-state hemoglobin reacts with CO without distal side steric effects whereas myoglobin, having a lower CO affinity, is subject to this effect. Others[3,4] have suggested that all hemoproteins are subject to distal side steric effects on CO, reducing their affinities relative to model compounds.

We report a comparative study of CO and isocyanide binding to the hindered model compound[5] anthracene-6,6-cyclophane, **1**, which establishes the relative steric effects on ligands of various




**1**

Anthracene Heme Cyclophane

(1) Antonini, E.; Brunori, M. "Hemoglobin and Myoglobin in Their Reactions with Ligands"; North-Holland Publishing Co.: Amsterdam, 1971; pp 85–95.
(2) (a) Geibel, J.; Cannon, J.; Campbell, D.; Traylor, T. G. *J. Am. Chem. Soc.* **1978**, *100*, 3575. (b) Traylor, T. G.; Berzinis, A. P. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3171.
(3) Collman, J. P.; Brauman, J. I.; Doxsee, K. M.; Halbert, T. R.; Suslick, K. S. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 564.
(4) Moffatt, K.; Deatherage, J. F.; Seybert, D. W. *Science (Washington, D.C.)* **1979**, *206*, 1035.
(5) Traylor, T. G.; Campbell, D.; Tsuchiya, S. *J. Am. Chem. Soc.* **1979**, *101*, 4748.
(6) Jones, R. D.; Budge, J. R.; Ellis, P., Jr.; Linard, J.; Summerville, P. A.; Basolo, F. *J. Am. Chem. Soc.* **1979**, *101*, 4762.
(8) Traylor, T. G.; Campbell, D.; Sharma, V.; Geibel, J. *J. Am. Chem. Soc.* **1979**, *101*, 5376.
(9) Keyes, M. H.; Falley, M.; Lumry, R. *J. Am. Chem. Soc.* **1971**, *93*, 2035.
(10) Blanck, J.; Ruckpaul, K.; Scheler, W.; Jung, F. *Eur. J. Biochem.* **1972**, *25*, 476.
(11) Olsen, J. S.; Gibson, Q. *J. Biol. Chem.* **1971**, *246*, 5241; **1972**, *247*, 1713.
(12) Traylor, T. G.; Campbell, D.; Tsuchiya, T. *J. Am. Chem. Soc.* **1979**, *101*, 4748.
(13) On sabbatical leave from York University, Downsview, Ontario, Canada.

0002-7863/80/1502-5939$01.00/0 