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(10) The scheme is consistent with the observed second-order disappearance of **1**. If one applies the steady state approximation, one obtains a term for initiation and dimerization which is second order in **1** and a term for polymerization which has a higher order dependence on **1** and is determined by the nature of the chain termination step. In the preparative experiments the ratio of dimerization of polymerization was 1:1. The kinetic experiments were carried out at a lower concentration of **1** which should then give close to second-order kinetics.
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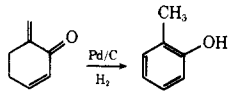

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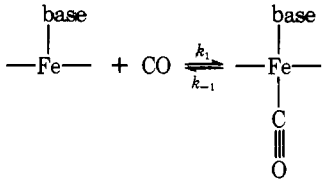
Dual Pathways of Heme Protein Model Compound Reactions with Carbon Monoxide

Sir:

The reactions of carbon monoxide with heme proteins[1] or their five-coordinate model compounds[2-4] are usually written as the simple association-dissociation process shown below (direct association mechanism).

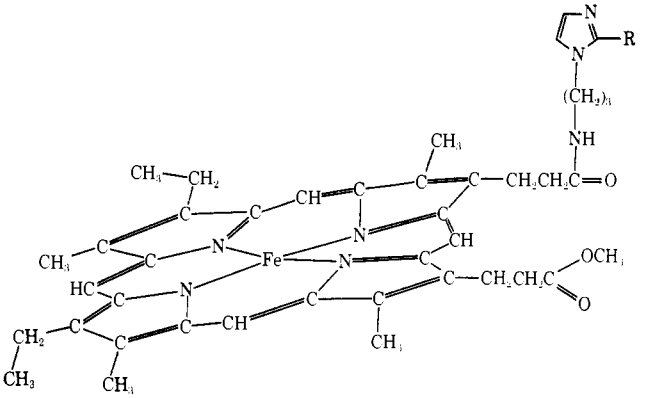


(1)

We have recently reported[4] definitive kinetic evidence for this pathway for compound **1** (see Table I) in water at pH >7. We now report evidence for a different base-elimination mechanism (eq 2-4) in reactions of heme-base compounds with carbon monoxide.

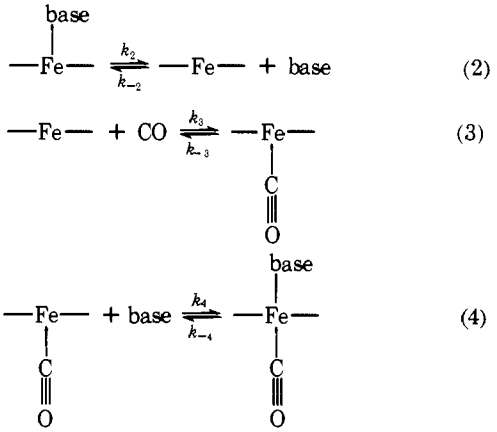
When compound **1** in aqueous CTAB is titrated with acid its Soret band shows an isosbestic change from that of five-coordinate heme (416 nm at pH 9) to that of four-coordinate heme (408 nm, broad, at pH 2)[5] and indicates an apparent pK_a of 3.5.[6] Over the range pH 2-9 the visible spectrum of the corresponding carbon monoxide complex, **1**-CO, is unchanged. This means that at pH ≤2.5, the proximal imidazole in **1** remains complexed with iron only if carbon monoxide is also complexed.[5] It also implies that four-coordinate heme, **1c**, produced in acidic media must complex carbon monoxide before the imidazole can coordinate (base-elimination mechanism).

Table I

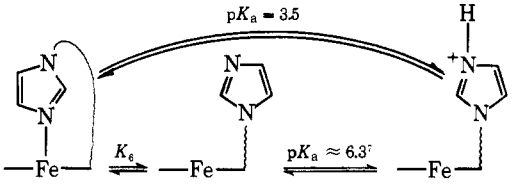


Compound	R	$(l'_{obsd})^{a,b}$ l. mol^{-1} s^{-1}	pK_a[c]
1	H	1.0×10^7	3.6
2	CH_3	1.3×10^8	6.5
Mesoheme dimethyl ester		3.5×10^8	

[a] The observed second-order rate constant for heme–carbon monoxide reaction was measured by the flash photolysis method as a pseudo-first-order reaction in varying concentrations of excess carbon monoxide. [b] Reactions were observed at pH 7.3 in water containing 2% cetyltrimethylammonium bromide (CTAB) and about 10^{-4} M sodium dithionite. [c] pK_a = pH at which the proximal base is half coordinated to iron(II) and the other half protonated.



The kinetic data for **1** strengthen this implication. At a carbon monoxide concentration of 2×10^{-5} M the rate constant for combination with carbon monoxide (l'_{obsd}) increases from 1×10^7 l. mol^{-1} s^{-1} at pH 7 to 3.5×10^8 l. mol^{-1} s^{-1} at pH 2.5.[4] Since the rate constant obtained at pH 2.5 is identical with that obtained for mesoheme dimethyl ester, the reaction of **1** at pH 2.5 with carbon monoxide presumably proceeds via a reaction of **1c** with carbon monoxide ($l'_{obsd} = k_8$), yielding, as the final product, **1a**-CO, and not **1c**-CO.



$$\mathbf{1a}\text{-CO} \xrightarrow{\text{pH 2.5, } h\nu} \mathbf{1a} \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} \mathbf{1b} \underset{k_{-7}}{\overset{k_7\,(H^+)}{\rightleftharpoons}} \mathbf{1c} \underset{k_{-8}}{\overset{k_8\,(CO)}{\rightleftharpoons}} \mathbf{1c}\text{-CO}$$

$$\mathbf{1c}\text{-CO} \underset{k_{-9}\,(H^+)}{\overset{k_9}{\rightleftharpoons}} \mathbf{1b}\text{-CO} \underset{k_{-10}}{\overset{k_{10}}{\rightleftharpoons}} \mathbf{1a}\text{-CO}$$

At pH 7.3 the dominant reaction is direct association of carbon monoxide with **1a**,[4]

$$\mathbf{1a}\text{-CO} \xrightarrow{h\nu} \mathbf{1a} \underset{k_{-11}}{\overset{+CO\ k_{11}}{\rightleftharpoons}} \mathbf{1a}\text{-CO}$$

Following the flash photolysis of **1a**-CO at pH 3 the observed first-order return to **1a**-CO is found to be first order in CO concentration up to about 8×10^{-6} M CO. Above this CO concentration the rate is independent of CO and inversely proportional to hydrogen ion concentration (eq 12) over the range of pH 2-3.

$$\log (k_{obsd}) = 1.24\ \text{pH} - 0.29 \qquad (12)$$

This suggests that at low CO concentration the rate-limiting step is reaction 8, whereas at high CO concentration it changes to the closure steps 9 and 10 (i.e., $l'_{obsd} = K_9 k_{10}$).

The latter mechanism was confirmed by flash spectroscopy. At pH 2.5 and 3.8×10^{-4} M carbon monoxide the Soret band of the intermediate formed about 100 μs after a flash of 200 μs duration[8] was identical with that of mesoheme dimethyl ester-CO (403 nm). This spectrum changed, with an isosbestic point, to that of **1a**-CO (409 nm)[9] at a rate described by eq 12.[10] Alternatively, at pH 7.3 the intermediate formed immediately following photolysis had a Soret maximum at 416 nm[9c] clearly indicating a direct association mechanism at this pH.

The change from the direct association mechanism to the base-elimination mechanism can be achieved at pH 7.3 by introducing steric hindrance into the proximal base as in compound **2**.[11] This has the effect of shifting equilibrium 2 to the right ($k_{-2}/k_2 \cong 5$ for **2** vs. 500 for **1**), although the compound **2** still appears predominantly five-coordinate according to its visible spectrum (λ_{max} 415, 550 nm). This makes $k_3 k_2/k_{-2} > k_1$ for **2** and changes the pathway to reactions 2-4. Again, the product of the reaction is **2a**-CO, a hexacoordinate complex, as indicated by its spectrum.[5b]

Because the hindered 2-methylimidazole forms only five-coordinate complexes with hemes[5a,12,13] and shows no formation of heme(base)$_2$ complexes even at base concentrations as high as 2 M in water or toluene,[12,13b,14] this mixture would seem to constitute a good myoglobin model. The association mechanism (1) would require that the reaction of this mixture with carbon monoxide become independent of the imidazole concentration above the concentration at which five-coordinate heme formation is >99% complete. This is because no hexacoordinate heme is formed which would interfere with the carbon monoxide association. However, we find that in a pH 9 phosphate buffer containing CTAB this mixture reacts with carbon monoxide with second-order rate constants given by eq 13, where B = concentration of 2-methylimidazole.

$$\frac{3.5 \times 10^8}{l'_{obsd}} = K_{13}B + 1 \qquad (13)$$

Although the slope of the $1/l'_{obsd}$ vs. B plot shows a slight decrease at about 0.3 M base (K_{13} = 196 below 0.3 M and K_{13} = 145 from 0.3 to 3 M), there is no indication that the rate becomes independent of base concentration even at 1.6 M base where $l'_{obsd} = 1.4 \times 10^6$ l. mol^{-1} s^{-1}. Similar results are obtained in toluene. We conclude that k_1 would be less than 1.4×10^6 l. mol^{-1} s^{-1}[15] for this mixture, that the reaction proceeds by the base-elimination pathway (eq 2-4) at all concentrations of 2-methylimidazole, and that $K_{13} = k_{-2}/k_2$.

Even with the unhindered base 1-methylimidazole and mesoheme dimethyl ester in pH 7.3 buffer, the base-elimination mechanism obtains at the low concentration of base usually employed in studies of such model systems.[2,3] The l'_{obsd} of such systems is faster than the l'_{obsd} of five-coordinate hemes even at 0.1 M base and the rate constant accurately follows eq 13 up to 0.08 M 1-methylimidazole.[17] This is explicable only if the base-elimination mechanism is followed below this concentration. At 10^{-4} M CO, 5×10^{-6} M heme, and 10^{-4} M 1-methylimidazole and pH 8.5, flash spectroscopy revealed heme-CO and *not* heme-methylimidazole as the intermediate.

Although we have not demonstrated the base-elimination mechanism for reactions of heme proteins, nor are we suggesting it for reactions of myoglobin or hemoglobin, the steric pull on the proximal imidazole which is presumably responsible for altering oxygen binding[16] is similar to the steric effects which tend to remove the proximal imidazole and change the reaction mechanism. Such a change from the direct association to the base-elimination mechanism would have large effects on the observed *on* and *off* rates and could represent an additional mode of control for heme protein ligand binding and oxidation-reduction properties.[18,20]

Acknowledgment. We wish to thank the National Institutes of Health (Grant HL-13581) for financial support of this research and (Grant RR00708) for support of the NMR facilities which were used.

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