1.5th order in monomer.[7,12d] Our preliminary data do not establish the order in BH with confidence, partly because of the very large transfer constant of BH which leads to high conversions of BH even at conversions of styrene as low as 2%. In styrene, at 60° the order in BH is about 0.5;[16a] at 98° it is about 0.3.[16b] The order in monomer also is somewhat complex. At 98° and at styrene concentrations above 5 *M*, the order in monomer is the expected 1.5. Below this concentration, the rearrangement of BH apparently competes with eq 6, the concentration of BH is lower than expected, thermal initiation (which is 2.5th order in styrene[8]) makes an appreciable contribution to the total rate of polymerization, and the observed order in styrene is about 2. The apparent activation energy for the BH-initiated polymerization of styrene is 13 kcal/mol. Plots of $1/\bar{P}_n$ versus $[BH]_{av}$ gave 6.8 and 4.6 for C, the transfer constant of BH, at 60 and 98°, respectively, and 10 kcal/mol for the activation energy for transfer, eq 7.[17]

$$BH + M_n\cdot \longrightarrow B\cdot + \text{polymer} \qquad (7)$$

It is of special interest to determine whether BH will initiate the polymerization of a monomer which does not undergo self-initiated polymerization. Experiments with methyl acrylate at 60° show that BH does initiate this monomer, and the order in BH is approximately 0.6.[16d]

Clearly it is important to demonstrate that it is BH itself, rather than an adventitious impurity in it, which is responsible for the initiation we have observed. Although we were not able to perform prepolymerizations and vacuum transfers with BH, several arguments make it very unlikely that anything other than BH itself could explain our observations. First, brief aeration of BH did not change its mass spectrum. Second, even if the hydroperoxide of BH, BOOH, were formed *in situ*, it probably could not give the observed rates. All the hydroperoxides which have been studied decompose in styrene with similar rates.[18a,b] At 70°, 0.02 *M tert*-butyl hydroperoxide is needed to obtain a rate of polymerization, R_P, of 1×10^{-5} *M* sec^{-1};[18a] we observe the same R_P at 60° using only 0.01 *M* BH. Third, and perhaps most convincing, the transfer constant of BH, about 5, is much larger than the transfer constant for any known peroxidic compound.[19] If a minor impurity in BH were the actual transfer agent, it would have to have a transfer constant of 100 or more; such compounds are virtually unknown,[19] and it is very doubtful that one is produced in our system. Thus, we conclude that BH shares with the styrene Diels–Alder adduct AH the remarkable property of being reactive enough to donate a hydrogen atom to an olefin.

We wish to suggest that MAH reactions such as eq 8 may be more common than has been recognized. (In eq 8, QX is any material with a labile atom or group and M is generally, although not necessarily, an olefin.) Systems in which there is evidence for an MAH reaction include *H-atom transfers from* AH,[7] BH,[15] hydroperoxides,[1,18] thiols,[20] dihydropyridine,[21] and even ethane[2] and *halogen-atom transfers from* fluorine,[22] chlorine,[13b] probably bromine,[1,2,13b] iodine,[23] and hypohalites.[24] In fact, we suggest that most compounds which have X atoms or groups which are sufficiently labile so as to transfer in styrene, eq 9, with a transfer constant near unity (*i.e.*, a rate constant near 10^2 M^{-1} sec^{-1} at 60–100°) also undergo eq 8.

$$Q\text{–}X + M \xrightarrow{\text{MAH}} Q\cdot + X\text{–}M\cdot \qquad (8)$$

$$Q\text{–}X + M_n\cdot \xrightarrow{\text{transfer}} Q\cdot + M_n\text{–}X \qquad (9)$$

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(16) (a) 10^{-2} *M* BH for 4 hr produces: 2.2% conversion to polymer; 47% conversion of BH; $\bar{P}_n = 122$; $R_P = 1.3 \times 10^{-5}$ M^{-1} sec^{-1}; (b) 3×10^{-2} *M* BH for 20 min produces: $\bar{P}_n = 55.9$; $R_P = 1.5 \times 10^{-5}$; (c) styrene varied from 8.0 to 2.7 *M* in benzene; (d) 10^{-1} *M* BH for 18 hr produces $R_P = 1.2 \times 10^{-5}$.

(17) These values are similar to those for thiols: W. A. Pryor, "Mechanisms of Sulfur Reactions," McGraw-Hill, New York, N. Y., 1962, p 84.

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(19) J. Brandrup and E. H. Immergut, Ed., "Polymer Handbook," Interscience, New York, N. Y., 1966.

(20) At high RSH/M ratios, Hiatt and Bartlett[9] observe an anomolous acceleration in radical production. They postulate that this is due to homolysis of adventitious disulfide impurities. However, an MAH reaction between thiol and styrene is much more likely.[7]

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Spectroscopic Properties of Protoheme Complexes Undergoing Reversible Oxygenation

Sir:

Recently a number of reports[1–3] have appeared describing the reversible oxygenation of heme complexes as model systems for oxygen-carrying hemeproteins, *i.e.*, myoglobin. In each of these systems, a synthetic heme containing covalently attached axial ligand or sterically hindering groups was used to achieve a minimal structure which would enable reversible oxyheme formation. In addition these models contained an imidazole axial ligand, which has been considered an essential component of the system,[1,2] much as the proximal histidine residue functions in myoglobin. However, the spectroscopic properties of the deoxy- and oxyheme complexes in these reports only partially resemble those properties of the protein. In this com-

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(3) J. P. Collman, R. R. Gagne, T. R. Halbert, J. Marchon, and C. A. Reed, *J. Amer. Chem. Soc.*, **95**, 7868 (1973).

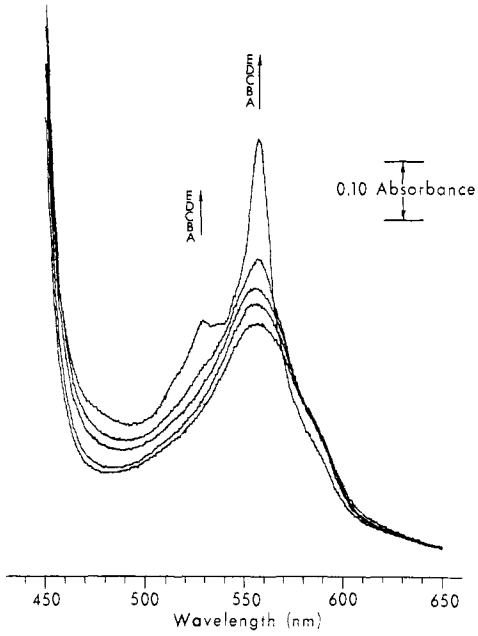

Figure 1. Variable temperature spectra of ferrous protoheme IX 2-methylimidazole system. Protoheme with 0.10 *M* 2-methylimidazole in 1-butanol: temperatures, (A) 25°, (B) −2°, (C) −26°, (D) −53°, (E) −75°.

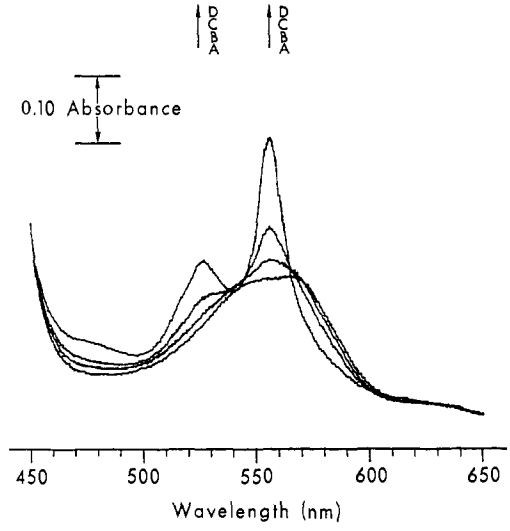

Figure 2. Variable temperature spectra of ferrous protoheme IX *tert*-butylamine system. Protoheme with 1% (v/v) *tert*-butylamine in 1-butanol: temperatures, (A) 25°, (B) −23°, (C) −42°, (D) −65°.

munication we wish to describe spectroscopic properties of complexes of the natural protoheme IX in solution with either an imidazole or a primary amine ligand which also exhibit reversible oxygenation.

During current investigations of the low temperature spectra of low-spin ferrous heme complexes, we observed[4] that ferrous heme in aqueous glycerol solutions of the sterically hindered ligands 2-picoline, 2-methylimidazole, and *tert*-butylamine undergoes an apparent temperature-dependent change in coordination. These complexes show spectra consistent with high-spin pentacoordinate complexes at room temperature and low-spin hexacoordinate complexes at −196°. The corresponding high-spin pentacoordinate structure of the active site of deoxymyoglobin suggested that these model heme complexes may also undergo reversible oxygenation. Conditions were chosen to favor the formation of a stable oxyheme complex.[5-7] Ferrous protoheme IX complexes, *ca.* 1–3 × 10^{-5} *M*, of 2-methylimidazole and *tert*-butylamine in 1-butanol were prepared under an atmosphere of dry argon by reduction of hemin with *ca.* 1% by volume of a concentrated $Na_2S_2O_4$ solution in 0.01 *N* NaOH. The solution was then dried over anhydrous Na_2SO_4 prior to the spectroscopic measurements. Variable temperature spectra of these systems in Figures 1 and 2 indicate the presence of high-spin pentacoordinate complexes near ambient temperatures,[4,8,9] which are similar to the spectra of model heme complexes with one strong field axial ligand.[10-12] Upon cooling to −80°, the spectra show a gradual transition to a characteristic hemochrome spectrum, indicative of a low-spin hexacoordinate complex. In each of these heme systems there exists a temperature range at which the spectrum is closely similar to the high-spin pentacoordinate deoxymyoglobin,[13] with one broad maximum at 556 nm in both the two model complexes and the protein. These spectral assignments provide an interpretation of the temperature dependence of the spectrum of the synthetic deoxyheme complex with covalently attached ligand.[2]

Spectroscopic changes associated with the reversible oxygenation of the deoxyprotoheme complexes of 2-methylimidazole and *tert*-butylamine are shown in Figures 3 and 4, respectively. Equilibration of these solutions with dry oxygen at −80° yielded spectra of oxyheme complexes as shown in Figures 3B and 4B. Spectrum 3B with maxima at 575 and 544 nm is similar to the oxyheme complex observed in one recent report[2] for which the intensity of the α-band was much less than the intensity of the β-band. However, spectrum 4B is characterized by an α/β ratio slightly greater than unity with maxima at 574 and 538 nm, which is similar to oxymyoglobin[13] with an α/β ratio of 1.02 and maxima at 581 and 543 nm. Equilibration of these solutions with CO at −80° resulted in spectra 3C and 4C which changed to 3D and 4D on warming to room temperature. Flushing solutions of the CO–heme complexes with argon, followed by a brief photolysis, yielded spectra 3E and 4E which correspond to the ambient spectra of the deoxyheme complexes shown in Figures 1 and 2, respectively. Thus no apparent oxidation occurred during the overall process. Equilibration of these deoxyheme solutions with oxygen at room temperature resulted in typical oxidized high-spin hemin complexes.

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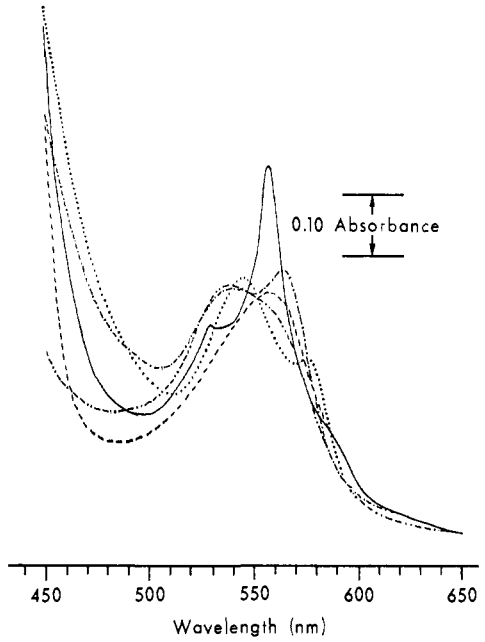

Figure 3. Spectral changes of ferrous protoheme IX 2-methylimidazole system associated with a reversible oxygenation process. Solution conditions as in Figure 1: A (——) deoxyheme at −80°, B(· · · · ·) equilibration of A with O_2 at −80°, C (- - · · - -) equilibration of B with CO at −80°, D (—·—) solution C at 23°, E (- - - -) equilibration of D with Ar followed by photolysis at 23°.

To support the optical assignment of the oxyheme complex, Mössbauer measurements at 4.2°K of the *tert*-butylamine oxyheme, using ^{57}Fe enriched protoheme IX *ca.* 4×10^{-4} *M*, were obtained. The Mössbauer spectrum indicated a sharp doublet, line width *ca.* 0.28 mm/sec, having a quadrupole splitting ΔE_Q of 2.37 ± 0.02 mm/sec and an isomer shift δ of 0.326 ± 0.02 mm/sec, relative to iron metal. These data compare well with ΔE_Q of 2.24 mm/sec and δ of 0.24 mm/sec at 1.2°K, and ΔE_Q of 2.19 mm/sec and δ of 0.26 mm/sec at 77°K for oxyhemoglobin.[14] Allowing this sample to oxidize by warming to ambient temperature produced a paramagnetic Mössbauer spectrum at 4.2°K typical of ferric heme systems, with no indication of the oxyheme doublet.

A number of observations and conclusions can be made from these results. First, the close spectral similarity between the amine oxyheme complex and the native protein is striking when considering the large differences between proximal axial ligands in these heme complexes. Second, the results indicate an imidazolyl-type base is not unique as an axial ligand in its ability to effect the formation of an oxyheme complex. In contrast to imidazole,[15] the *tert*-butylamine is unable to π-bond with the heme and thus indicates that π-bonding between a proximal ligand and oxyheme is not essential. Third, the temperature dependence of the spectra of deoxy and CO complexes in these and other model systems[2] draws awareness to the possible misinterpretation of spectra of heme complexes when observed at below ambient temperatures and to the possible dissimilarities between model complexes and hemeproteins. Thus, deoxymyoglobin does not show

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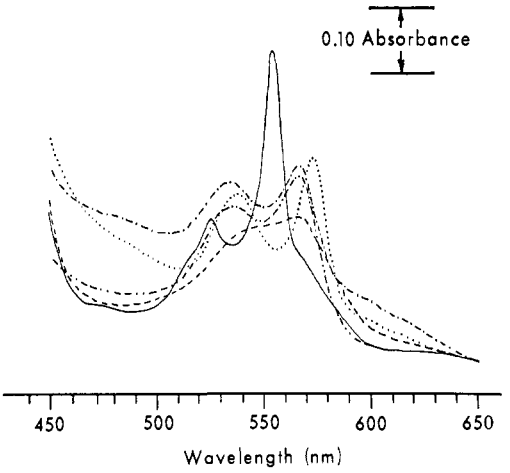

Figure 4. Spectral changes of ferrous protoheme IX *tert*-butylamine system associated with a reversible oxygenation process. Solution conditions as in Figure 2: A (———) deoxyheme at −80°, B (· · · · ·) equilibration of A with O_2 at −80°, C (—·—) equilibration of B with CO at −80°, D (- - · · - -) solution C at 22°, E (- - - -) equilibration of D with Ar followed by photolysis at 22°.

absorption spectra changes indicative of either a change in coordination or spin state even at −196°.[4] Finally, the results demonstrate that the natural heme prosthetic group is able to reversibly bind molecular oxygen in solution in the absence of apoprotein or the covalent linkage of any grouping as long as the pertinent chemical and physical conditions are controlled.

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Simple Dioxygen Heme Complexes Formed in *N,N*-Dimethylformamide

Sir:

Two soluble Fe(II) porphyrins recently have been described[1,2] which form reversible oxyhemochromes.[3] Both are iron porphyrin derivatives possessing structural modifications designed to enhance the formation and stability of a complex with molecular oxygen. That

(1) C. K. Chang and T. G. Traylor, *J. Amer. Chem. Soc.*, **95**, 5810, 8477 (1973).

(2) J. P. Collman, R. R. Gagne, T. R. Halbert, J. C. Marchan, and C. A. Reed, *J. Amer. Chem. Soc.*, **95**, 7868 (1973).

(3) In accord with the term "oxyhemoglobin," the term "oxyhemochrome" is proposed to denote complexes composed of a nitrogenous base, an Fe(II) porphyrin, and O_2. The corresponding CO complexes are called carboxyhemoglobin and carboxyhemochrome. Although the term "oxyhemochrome" appears in the older literature in reference to hemochromes in which oxidation of the porphyrin ring has occurred,[4] the term now seems more appropriate for the reversible dioxygen complexes. The stoichiometry of the O_2 complex of I has been established to be one O_2/heme.[5]

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