used with the intact globin sample to determine the site of adduction. In agreement with previous speculation,[12] no diminution of the ester signals was noted, suggesting that the adduct is protected in a hydrophobic portion of the Hb. Simple mixing of the BaP tetrahydrotetrols with globin followed by the acrylamide treatment resulted in a complete loss of tetrol fluorescence, suggesting a not so surprising hypothesis that the interaction of BaP tetrahydrotetrols with hemoglobin differs from that of BPDE with the protein.

Discussion

A particularly important aspect of this research has been to determine whether human Hb adducts formed in vivo could be attributed to a specific structural type. Previous mechanism-based isotope incorporation experiments have suggested that carboxylic esters are the major adducts formed by BPDE both in vitro and in vivo.[12] The remarkably straightforward results from the present study confirm the previous findings. Despite the low adduction level present in the globin sample, its FLN spectrum exhibits adequate signal to noise for confident structural assignments. As expected for individuals environmentally exposed to BaP, the major adduct analyzed by our system is derived from BPDE. This is confirmed by comparison of the FLN spectrum of the intact globin adduct to that of a synthetic ester adduct of *anti*-BPDE. The two spectra are essentially the same, demonstrating that the esters are likely candidates for globin adduct formation in vivo. Additional support for these assignments is obtained by noting the similarities that occur at various excitation wavelengths. The spectral evidence clearly is in support of BPDE esterification taking place at one or more carboxylate sites in human hemoglobin.

Experimental data collected on adducted globin demonstrated the utility of acrylamide in quenching pyrene moieties that adopt a conformation external to the protein where water is available. This is similar to DNA where BPDE and the BaP tetrols form external complexes and quasi-intercalated complexes.[19,20] Acrylamide, when dissolved in water and mixed with adducted globin, does not quench the fluorescence from oxygen or nitrogen type adducts formed in vivo with human Hb. On the other hand, free tetrols mixed with the globin samples are quenched, demonstrating that the BaP tetrols are adopting external conformations. Similar fluorescence quenching has been used to distinguish stereoisomers of a given metabolite bound to a particular nucleic acid base.[16] In future studies we hope to use FLN spectroscopy to determine the stereochemistry of the active metabolite of BaP that alkylates hemoglobin in vivo. Finally, we would like to add that after all stable adducts are decomposed and/or hydrolyzed to BaP tetrols the expected FLN spectrum would appear as the one presented in Figures 3C and 4C: the BaP tetrahydrotetrols + globin mixture, spectra that are very different from those of BPDE adducts.

These studies have shown again that FLN spectroscopy is a viable technique for structural identification. It should be a powerful tool for future structural analysis in the characterization of the origin and nature of different human macromolecular adducts. Whether BPDE-DNA adducts are present after in vivo exposure depends on many additional factors, like DNA repair and the rate of cell proliferation.[21] BPDE-protein adducts are not considered to be pathological lesions, but they provide a useful resource in molecular dosimetry of chemical carcinogens. Our spectroscopic studies show that the major globin adduct is an ester, indicating that the adduct formation occurs either at the C termini of the α or β chains or the side chain of aspartate or glutamate in hemoglobin.

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Communications to the Editor

On the Composition of Yamamoto's Reagent: "RCu·BF_3"

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In 1978, a new class of organocopper reagents derived from treatment of CuI with an organolithium (1 equiv) followed by BF_3·Et_2O (1 equiv) was introduced as a means of effectively displacing allylic leaving groups.[2] More recently, these species have found favor as valued Michael donors in couplings with α,β-unsaturated ketones, esters, and even acids.[3] Their composition, originally scribed as $RBF_3^-Cu^+$,[2] is now more commonly written as "RCu·BF_3" (**1**) to reflect the metathesis between an RLi and CuI (eq 1).[3] The role of the byproduct LiI in the subsequent chemistry of **1** has always been ignored. We now report, by virtue of both spectroscopic and chemical experiments, that the success of Yamamoto's reagent is intimately tied to the presence of lithium iodide in the medium and that "RCu·BF_3" is in fact *not* the species that effects C-C bond formation.

$$\text{RLi} + \text{CuI} \longrightarrow \text{RCu} + \text{LiI} \xrightarrow{BF_3} \text{"RCu·}BF_3\text{"} + \text{LiI} \qquad (1)$$

The ^{1}H NMR spectrum of MeCu·LiI + BF_3 (from CuI + MeLi/Et_2O, 0.15 M in THF at -80 °C, then BF_3·Et_2O) shows, in addition to a major broad singlet at δ -1.25, another minor peak at δ 0.15. Upon warming to -40 °C, the peak at δ -1.25 is rapidly replaced by a new signal at δ -0.77. All three signals are observable at -80 °C if Me_2S (3 equiv) is present initially to maximize solution homogeneity (Figure 1).[4] As more LiI is added to the Yamamoto recipe (eq 1), the peak at δ 0.15 grows in still further. Once an additional 2 equiv of LiI have been added to MeCu·LiI + BF_3, the downfield signal is all that remains at -80

(1) Proctor & Gamble Predoctoral Fellow, 1989-1990, awarded by the organic division of the American Chemical Society.

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(4) MeCu·LiI itself, 0.15 M in 10% HMPA/THF at -80 °C, gives only the signal at δ -1.25.

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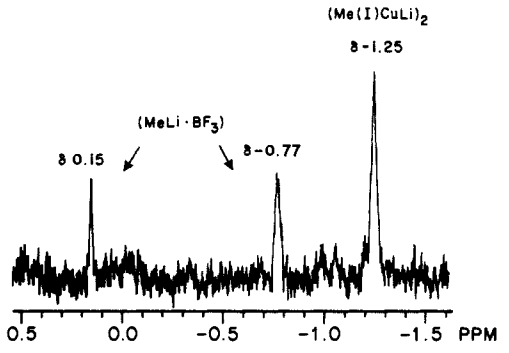
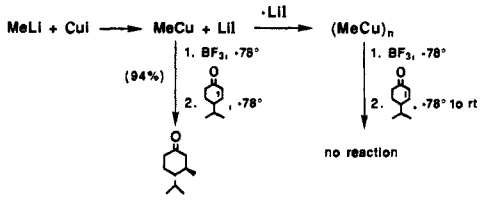
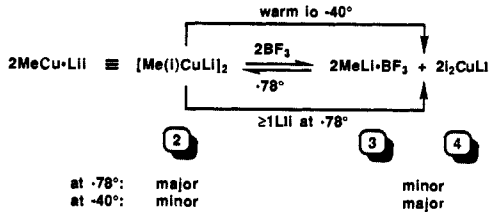


Figure 1. ^{1}H NMR spectrum at −80 °C of MeLi + CuI in THF containing Me_2S (3 equiv) to which has been added BF_3·Et_2O (1 equiv).

Scheme I



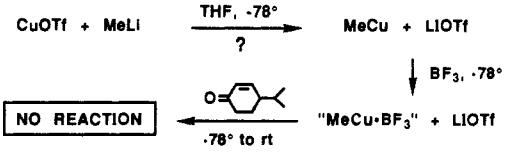
Scheme II



°C in the ^{1}H NMR spectrum![5a] The peaks at δ 0.15 and −0.77 were identified with MeLi·BF_3,[5b] since addition of this additive[6] to low-halide MeLi/Et_2O in THF alone at −80 °C gives rise to these singlets with the complete loss of the original MeLi peak at δ −2.05. Removal of the LiI from the MeCu followed by addition of BF_3 does not afford much, if any, solubilized organocopper (Scheme I), and in fact, these heterogeneous mixtures are completely inert (from −78 °C to room temperature) toward, e.g., cyclohexenone. External introduction of LiI/THF (1 equiv), however, restores a significant degree of solubility and reactivity to this otherwise inert species.[7]

The ability of BF_3 and LiI to sequester MeLi from MeCu·LiI is remarkable, given the >3-V differential between Li^+ and Cu^+ in the electromotive series.[8] Whatever stabilization this form of MeLi enjoys in the company of Lewis acids,[9] its detection to various extents as a function of temperature allows formulation

Scheme III



of a scenerio that describes the Yamamoto protocol (Scheme II). That is, the initially formed MeCu·LiI, solubilized to a large degree by BF_3, is likely to be better thought of as dimeric Me(I)CuLi (i.e., **2**).[10] The BF_3 withdraws fully the elements of MeLi from **2**, leading to MeLi·BF_3 **3** and solubilized CuI (i.e., I_2CuLi, **4**).[14] Such a mode of action by BF_3 is very much related to that which is observed upon its introduction to both higher order cuprate $Me_2Cu(CN)Li_2$ [giving MeCu(CN)Li + MeLi·BF_3][11] and Gilman's reagent $[Me_2CuLi]_2$ (giving Me_3Cu_2Li + MeLi·BF_3).[12]

Additional support for the propensity of BF_3 to remove RLi from R(I)CuLi was derived from an acetylenic system. Treatment of $MeOC(Me_2)C{\equiv}CLi$ with CuI (1 equiv) in THF [to form $MeOC(Me_2)C{\equiv}CCu(I)Li$][15] followed by BF_3 at −80 °C leads to the appearance of $MeOC(Me_2)C{\equiv}CLi{\cdot}BF_3$, as seen in the ^{11}B NMR spectrum (δ −0.62 vs BF_3), identical with that of an authentic sample.[16] Thus, notwithstanding the "dummy" ligand nature of acetylene groups bound to copper,[17] they too will be surrendered under the influence of BF_3.[18]

When only halogen is present, as with CuI·LiI (i.e., $[I_2CuLi]_2$) in THF, then BF_3 removes LiI to form LiI·BF_3 and (presumably) I_3Cu_2Li. The presence of the former species was confirmed by ^{19}F NMR (δ 5.1 vs BF_3).[19] Examination of Yamamoto's reagent by ^{19}F NMR, however, shows none of this complex (LiI·BF_3). Thus, it appears that BF_3 prefers to sequester MeLi, rather than LiI, from **2**.

Finally, to unequivocally corroborate the involvement of iodide ion in Yamamoto's reagent, cuprous triflate (CuOTf)[20] was chosen as an alternative Cu(I) source en route to "RCu·BF_3". Treatment of this salt with MeLi/Et_2O and then BF_3·Et_2O prior to enone addition, as done with CuI (vide supra), *gave no trace of the 1,4-product ketone* (Scheme III).[21]

In summary, the events that occur upon generation of Yamamoto's reagent have been brought to light. It can now be appreciated that *both* BF_3 and LiI[22] are essential ingredients, which

(5) (a) Notwithstanding the presence of only this peak in the spectrum, subsequent 1,4-additions do take place in good yields. (b) Control experiments [i.e., ^{1}H NMR of MeLi + LiI (1 equiv) and even $(MeO)_3B$ (1 equiv) in THF at −80 °C] confirmed that none of the original signal due to "free" MeLi (δ −2.05) remains in the presence of these additives.

(6) (a) Other species identified (^{11}B NMR) in the medium are lithium alkoxides, presumably derived from the opening of THF.[6b] Their effect is minimal since these reactions can be carried out successfully in toluene where such intermediates cannot be present. (b) Eis, M. J.; Wrobel, J. E.; Ganem, B. *J. Am. Chem. Soc.* **1984**, *106*, 3693.

(7) (a) Reintroduction of LiI (1 equiv) afforded yields of 1,4-adduct in the range of 46–55%. Full recovery of activity is not to be expected; cf.: Ouannes, C.; Dressaire, G.; Langlois, Y. *Tetrahedron Lett.* **1977**, 815. The importance of LiI in cuprate chemistry has previously been noted; cf.: Krause, N. *Tetrahedron Lett.* **1989**, *30*, 5219. Bertz, S. H.; Dabbagh, G. *J. Am. Chem. Soc.* **1988**, *110*, 3668. See also: Yatagai, H.; Yamamoto, Y.; Maruyama, K. *Chem. Lett.* **1980**, 669. (b) Addition of either n-$C_5H_{11}O^-Li^+$ or $LiClO_4$ led to, at most, a trace of the 1,4-product. Attempts to fully solubilize $(MeCu)_n$ with HMPA were fruitless.

(8) Latimer, W. M. *The Oxidation States of the Elements and Their Potentials in Aqueous Solutions*, 2nd ed.; Prentice-Hall: New York, 1952.

(9) MeLi is also altered upon addition of $LiClO_4$, although it is intriguing to note that LiBr (2 equiv), unlike LiI, has no effect.

(10) Even in the absence of BF_3, fully soluble organocopper reagents RCu, containing supposedly free LiI from an initial metathesis, show resonances in their ^{7}Li NMR spectra at −75 °C which are *not* characteristic of LiI itself. Rather, the chemical shifts observed are hybridization dependent [n-BuCu·LiI, δ 0.18; a vinylcopper·LiI, δ 0.04; $MeOC(Me_2)C{\equiv}CCu{\cdot}LiI$, δ 0.43; vs free LiI, δ 0.28]. The importance of iodide ion, or other anionic species in solutions of RCu, was first observed and insightfully discussed by House over 20 years ago; cf.: House, H. O.; Fischer, W. F. *J. Org. Chem.* **1968**, *33*, 949.

(11) Lipshutz, B. H.; Ellsworth, E. L.; Siahaan, T. J. *J. Am. Chem. Soc.* **1988**, *110*, 4834.

(12) Lipshutz, B. H.; Ellsworth, E. L.; Siahaan, T. J. *J. Am. Chem. Soc.* **1989**, *111*, 1351.

(13) Reference deleted in press.

(14) The I_2CuLi so formed is undoubtedly maintained in solution as I_2CuLi by the LiI present. It could be easily detected by addition of fresh MeLi/Et_2O, which led to a very short-lived starburst of yellow color (MeCu).

(15) Corey, E. J.; Floyd, D. M.; Lipshutz, B. H. *J. Org. Chem.* **1978**, *43*, 3148.

(16) Formed (at 0.26 M in THF at −78 °C) by addition of BF_3·Et_2O (1 equiv) to the lithiated acetylene. It was necessary to switch to a system of this type since MeLi·BF_3 is not distinguishable from BF_3 alone by ^{11}B NMR.[12]

(17) For a discussion of the various acetylenic ligands that tend to remain on copper, see: Lipshutz, B. H.; Sengupta, S. *Org. React.* (*N.Y.*), in press.

(18) Preferential coupling with an acetylenic ligand from a cuprate by Me_3SiCl has been noted previously; cf.: Lipshutz, B. H.; Ellsworth, E. L.; Siahaan, T. J.; Shirazi, A. *Tetrahedron Lett.* **1988**, *29*, 6677.

(19) Recorded in THF at −75 °C by using a 0.17 M solution of LiI + BF_3 (1:1).

(20) Bertz, S. H.; Gibson, C. P.; Dabbagh, G. *Tetrahedron Lett.* **1987**, *28*, 4251.

(21) When the procedure was carried out in the presence of LiI (1 equiv), however, a 41% yield of the conjugate adduct was realized.[7a]

(22) LiBr, generated from treating CuBr·Me_2S with MeLi/Et_2O, apparently plays a related role, judging from similar chemical results obtained with 4-isopropylcyclohexenone (Scheme I; 97% GC yield).

together impact on the RCu initially formed from metathesis. The precise nature of the reagent, which clearly must involve halide ion (as in, e.g., **2**) along with an assist from the Lewis acid, cannot, however, be identified with certainty at this time.

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Registry No. MeLi, 917-54-4; CuI, 7681-65-4; LiI, 10377-51-2; BF_3, 7637-07-2.

Electronic Structure of Peroxide-Bridged Copper Dimers of Relevance to Oxyhemocyanin

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The active site of oxyhemocyanin (oxyHc) consists of two tetragonal Cu(II) ions separated by ~3.6 Å and exhibits a unique absorption spectrum with an intense band at 580 nm ($\epsilon \sim 1000$ M^{-1} cm^{-1}) and an extremely intense band at 345 nm ($\epsilon \sim 20\,000$ M^{-1} cm^{-1}).[1] These bands have been interpreted as peroxide to Cu(II) charge-transfer transitions from the out-of-plane peroxide Π^*_v and in-plane Π^*_σ orbitals, respectively,[2] where the 12 000-cm^{-1} Π^*_v-Π^*_σ splitting is much larger than the 4000-cm^{-1} splitting observed for peroxide bound to a single Cu(II).[3] On the basis of studies of oxyHc and the met azide derivative,[4] a spectroscopically effective model[2,5] of oxyHc has been developed in which the two coppers are bridged by peroxide in an end-on cis-μ-1,2 fashion and by a second endogenous ligand, likely hydroxide, **1** in Figure 1. However, the recent structural characterization of the first side-on μ-η^2:η^2 peroxide-bridged transition-metal dimer,[6] **2**, provides an alternative possibility for the active site of oxyHc. We report here the results of electronic structure calculations on peroxide-bridged copper dimers with geometries **1** and **2**. We have found that the primary bonding interaction in both dimer systems involves the peroxide Π^*_σ with the antisymmetric combination of Cu d_{xy} orbitals which form the LUMO. In addition, the unoccupied peroxide σ^* orbital of **2** acts as a π acceptor influencing the bonding properties of this complex.

Broken-symmetry, spin-unrestricted SCF-Xα-Scattered Wave (SCF-Xα-SW) calculations[7] were performed on **1** with an end-on cis-μ-1,2 bridging geometry[8] and an effective C_{2v} symmetry and **2** with a side-on μ-η^2:η^2 bridging geometry[9] and an effective D_{2h} symmetry. The symmetries were lowered to C_s and C_{2v} by using

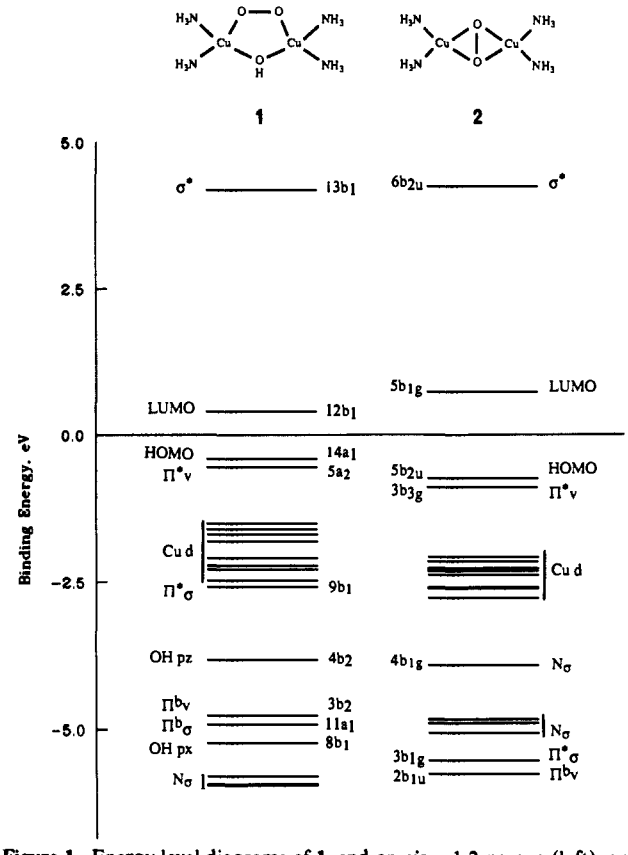

Figure 1. Energy level diagrams of **1**, end-on *cis*-μ-1,2-peroxo (left), and **2**, side-on μ-η^2:η^2-peroxo (right). The energy scale has been linearly shifted such that 0 eV is centered between the HOMO and LUMO and energy levels have been labeled by using the full symmetry designations.

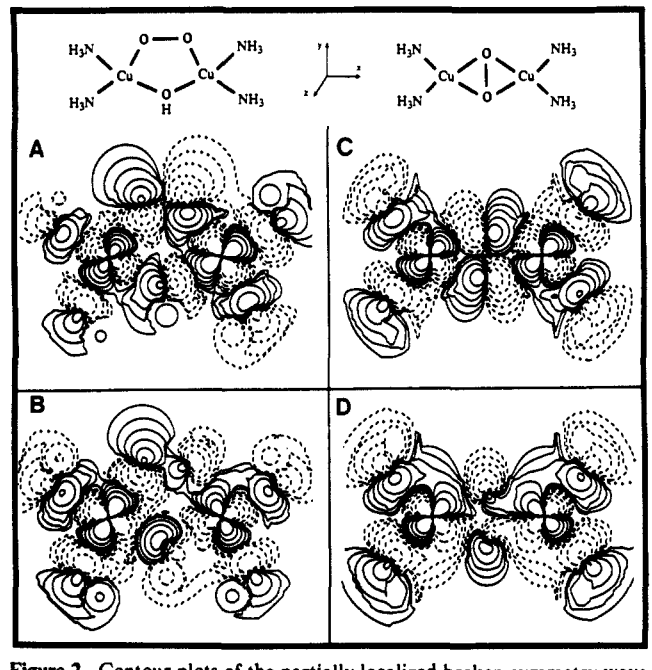

Figure 2. Contour plots of the partially localized broken-symmetry wave functions in the *xy* plane for (A) spin-up level $12b_1$ (LUMO) of **1**, (B) spin-up level $14a_1$ (HOMO) of **1**, (C) spin-up level $5b_{1g}$ (LUMO) of **2**, and (D) spin-up level $5b_{2u}$ (HOMO) of **2**. Contours located at ±0.005, ±0.01, ±0.02, ±0.04, ±0.08, and ±0.16 $(e/bohr^3)^{1/2}$.

the broken-symmetry formalism developed by Noodleman[10] which imposes a mirror up-down spin symmetry on opposite halves of the dimer. As with previous calculations,[11] the radii of the spheres

* Author to whom all inquiries should be addressed.

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(3) Pate, J. E.; Cruse, R. W.; Karlin, K. D.; Solomon, E. I. *J. Am. Chem. Soc.* **1987**, *109*, 2624-2630.

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(6) Kitajima, N.; Fujisama, K.; Moro-oka, Y.; Toriumi, K. *J. Am. Chem. Soc.* **1989**, *111*, 8975-8976.

(7) (a) Slater, J. C. *Adv. Quantum Chem.* **1972**, *6*, 1-92. (b) Johnson, K. H. *Ibid.* **1973**, *7*, 143-185. (c) Case, D. A. *Annu. Rev. Phys. Chem.* **1982**, *33*, 151-171.

(8) Geometric parameters: Cu-Cu = 3.60 Å, Cu-O = 2.00 Å, O-O = 1.44 Å, Cu-N = 2.00 Å, N-H = 1.00 Å, and ∠Cu-O-O = 122.7°. Sphere radii: r(Cu) = 2.95 bohr, r(O) = 1.84 bohr, r(N) = 1.70 bohr, and r(H) = 1.17 bohr.

(9) Geometric parameters: Cu-Cu = 3.56 Å, Cu-O = 1.90 Å, O-O = 1.42 Å, Cu-N = 2.00 Å, and N-H = 1.00 Å. Sphere radii: r(Cu) = 2.70 bohr, r(O) = 1.75 bohr, r(N) = 1.70 bohr, and r(H) = 1.17 bohr.

(10) (a) Noodleman, L.; Norman, J. G., Jr. *J. Chem. Phys.* **1979**, *70*, 4903-4906. (b) Noodleman, L. *Ibid.* **1981**, *74*, 5737-5743.

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