Electron Transfer in the Heme Pocket of Hemoglobin

Michael P. Doyle,* Surendra N. Mahapatro,
Craig M. VanZyl, and Matthew R. Hester

Department of Chemistry, Trinity University
San Antonio, Texas 78284

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Hydrazines, particularly arylhydrazines, cause inhibition or irreversible oxidation of hemoproteins ranging from hemoglobin[1] and myoglobin[2] to cytochrome P-450[3] and lactoperoxidase.[4] The formation of carbon-centered free radicals has been shown to occur during hemoprotein-catalyzed oxidations of monosubstituted hydrazines,[5] and σ-aryliron(III) complexes have been identified as products from reactions of arylhydrazines with hemoglobin and myoglobin.[6,7] The involvement of diazene intermediates in these transformations is becoming increasingly evident[8] but arenediazonium salts, which have recently been detected in enzyme-catalyzed oxidations of naturally occurring aryhydrazines,[9] have been overlooked as causative agents for σ-aryliron(III) formation.

We have recently reported that hemoglobin is oxidized to methemoglobin by *p*-nitrobenzenediazonium tetrafluoroborate with first-order rate dependence on both the hemoglobin and diazonium salts concentrations.[10] Detailed product analyses have suggested that electron transfer occurs via the partially exposed heme edge without penetration of the oxidant into the heme pocket. We now present evidence from substituent dependence in reductive electron transfer to arenediazonium salts for a unique mechanistic duality in reactions with hemoglobin.

Potassium ferrocyanide was employed for comparative evaluation of outer-sphere electron transfer to diazonium salts. Oxidations were performed under anerobic conditions in aqueous phosphate-buffered solutions at pH 7.0 and 25.0 °C. First-order kinetic dependences on both ferrocyanide and diazonium salt were established, and rate constants were obtained from a minimum of four determinations for reactions with each of a representative series of monosubstituted arenediazonium tetrafluoroborates.[11] Figure 1 describes the correlation obtained with Hammett σ-constants[12] (ρ = +4.7) for these second-order constants spread from the *p*-methoxy- to the *p*-nitrobenzenediazonium salts over a range of 10^5.

Stock solutions of human hemoglobin, obtained from Sigma Chemical Co. or prepared from fresh normal human blood[13] by the method of Gibson,[14] were stripped of organic phosphates and deoxygenated as previously described.[15] Second-order rate constants were obtained for oxidations of deoxyhemoglobin with the same series of diazonium salts and under the same conditions as in reactions with ferrocyanide. The plot of the rate constants obtained for hemoglobin oxidations against those from reactions

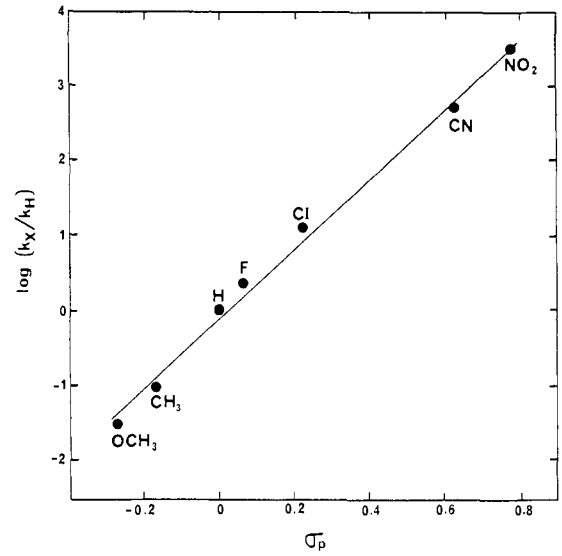

Figure 1. Hammett plot for reactions of *para* substituted benzenediazonium tetrafluoroborates salts with potassium ferrocyanide in 0.05 M phosphate buffered aqueous solution at 25.0 °C.

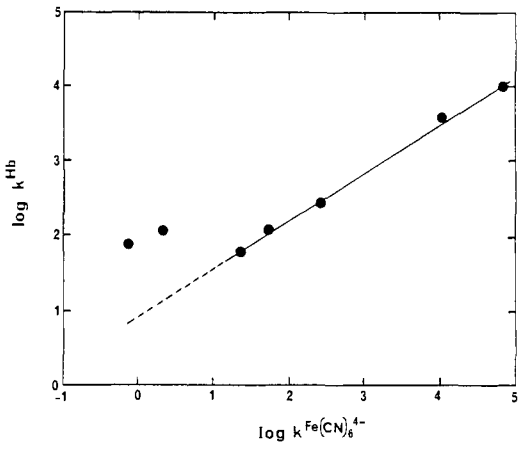

Figure 2. Plot of log *k* for reactions of *para* substituted benzenediazonium salts with deoxyhemoglobin (Hb) against corresponding log *k* for reactions with potassium ferrocyanide. The Hammett plot for the oxidation of Hb exhibits a ρ value of +2.8 (*p*-CH_3 and *p*-CH_3O data excluded).

with ferrocyanide (Figure 2) exhibits a break in this expected correlation that points to a distinct change in mechanism. Consistent with this interpretation, reactions of deoxyhemoglobin with molar equivalent amounts of *p*-nitro- and *p*-cyanobenzenediazonium tetrafluoroborate resulted in the production of only the corresponding arenes, whereas those with *p*-chloro- through *p*-methoxybenzenediazonium tetrafluoroborates formed only the σ-aryliron(III) complex of hemoglobin as well as methemoglobin. The red σ-arylheme complexes were separated from globin by previously reported extraction procedures[6] and identified from their characteristic electronic spectra.[6,16] Exposure of these oxygen-sensitive compounds to air resulted in the formation of their corresponding *N*-arylhemin derivatives whose well-defined green zinc(II) complexes were further confirmed by chromatographic and spectroscopic characterization.[17-19] Identical yields of σ-aryliron(III) complexes of hemoglobin were obtained when reactant $HbFe^{II}/ArN_2^+$ ratios of 2.0 or less were employed, suggesting the reaction stoichiometry of eq 1.

$$2HbFe^{II} + ArN_2^+ \rightarrow HbFe^{III}Ar + HbFe^{III} + N_2 \quad (1)$$

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An attractive explanation for the divergent influence of arenediazonium ion substituents on the rates for oxidation of hemoglobin is that electron transfer occurs in the heme pocket for those diazonium salts that would undergo electron transfer slowly, if at all, outside of this cavity. Once inside the hydrophobic pocket, electron transfer produces a neutral aryldiazo radical that is more likely to expel dinitrogen and combine with iron(III) than to reenter the hydrophilic region outside of the heme pocket where hydrogen abstraction is the product-forming process.[20]

The rate for electron transfer is dependent on the distance between the reaction centers.[21] In the case of diazonium salts, crossover from the hydrophilic region outside of the heme pocket to the hydrophobic region inside the heme pocket reduces the distance for approach of the diazonium salt to hemeiron(II) so that the rate for oxidation becomes a function of the kinetic barrier for entrance of the diazonium ion to the heme cavity. If this kinetic barrier reflects the hydrophilic to hydrophobic crossover, correlation with hydrophobic parameters should be evident. Accordingly, the semilog plot of second-order rate constants for those diazonium salts that produced σ-aryliron(III) complexes against π[22] gave a reasonable correlation (slope = 0.67, corr. coef. 0.90) whereas, as is evident from Figure 2, no correlation exists with σ_p or other electronic parameters. Furthermore, if there is a kinetic barrier to crossover, increasing the hydrophilicity of the diazonium salt should reduce the relative rate for reduction by hemoglobin and cause electron transfer to occur outside of the heme pocket. Indeed, reduction of the diazonium salt derived from (*p*-aminophenyl) acetic acid by deoxyhemoglobin at pH 7.0, which occurs with a second-order rate constant (k_2 = 31 M^{-1} s^{-1}) that is nearly one-quater that for reduction of *p*-toluenediazonium tetrafluoroborate (k_2 = 113 M^{-1} s^{-1}), produces only phenylacetic acid (90% yield). The near identity of second-order rate constants for ferrocyanide reduction of this diazonium salt (k_2 = 2.4 M^{-1} s^{-1}) and *p*-toluenediazonium tetrafluoroborate (k_2 = 2.1 M^{-1} s^{-1}) demonstrates the electronic similarity of the acetate and methyl substituents for electron transfer to the diazonium functional group.

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Stereoselective, Chemodirected Formal S_N2' Addition of Organometallic Reagents to β'-Amino Cyclopentenyl Sulfone Derivatives[1]

D. K. Hutchinson and P. L. Fuchs*

Department of Chemistry, Purdue University
West Lafayette, Indiana 47907

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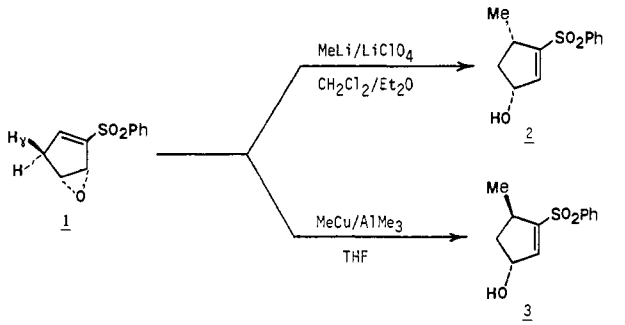
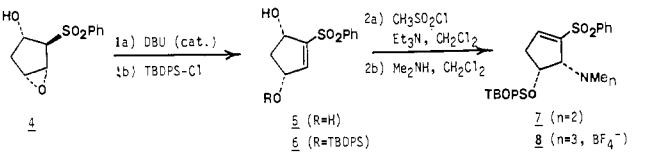
Several years ago we reported a stereoselective method to effect the nucleophilic S_N2' methylation of chiral epoxyvinyl sulfone **1**.[2]

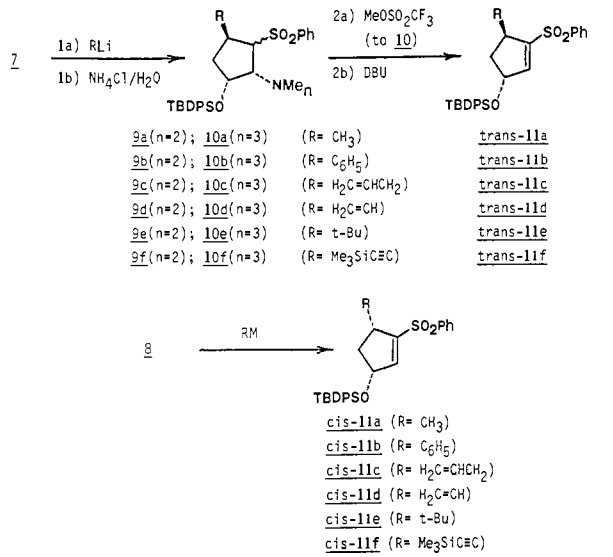
Scheme I



Scheme II



Scheme III



Although the specific procedure worked exceptionally well for the cases required (**1** to **2** and **1** to **3**), our recent attempts to extend this reaction to additional organometallic reagents which encompass tertiary alkyl, alkynyl, aryl, vinyl, and allyl moieties have been most disappointing. The source of the problem seems to be principaly associated with competitive deprotonation of **1** in the γ position, a difficulty that was observed but was correctable in the methyl series[2] (Scheme I).

We now wish to report a *general* and highly efficient solution to this problem. Treatment of β-epoxy sulfone **4**[3] with DBU (to produce the water-soluble vinyl sulfone-diol **5**[3]) followed by in situ silylation with *tert*-butyldiphenylsilyl chloride[4] affords an 86% yield of vinyl sulfone **6**.[5,6] Mesylation[7] of the alcohol moiety

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