protein are among the fastest yet measured for any six-coordinate spin-equilibrium compound, the rest of which are all synthetic in nature with spin-interconversion rate constants ranging from ~5 × 10^5 to 1.5 × 10^7 s^{-1}.[20-24] It is likely, however, that there are other systems such as the $[\mathrm{Fe^{III}(dtc)_3}]$, $[\mathrm{Fe^{III}(benzac)_2trien}]^+$, $[\mathrm{Fe^{III}(salmeen)_2}]^+$, and $[\mathrm{Co^{II}(terpy)_2}]^{2+}$ complexes[25] for which the spin-interconversion rates are as fast or perhaps faster than for the present ferric myoglobin hydroxide case. Thus, the low-spin $\rightleftarrows$ high-spin dynamics of this spin-equilibrium heme center in a protein environment does not seem particularly distinguished from those operative in simple inorganic compounds. It is clear, however, that with k's > 10^7 s^{-1} *intra*molecular spin multiplicity changes are not expected to be rate determining in *inter*molecular (second order) electron transfer reactions in which spin-equilibrium enzyme centers might participate.

Finally, it should be noted that ferric myoglobin hydroxide must undergo a stereochemical change upon a change in spin multiplicity. Assuming that the coordination number about Fe(III) remains six[26] (four porphyrin N's, the proximal histidine N and the $\mathrm{OH^-}$ oxygen) during the low-spin $\rightleftarrows$ high-spin-interconversion process, the high-spin form is expected to have the iron atom displaced out of the porphyrin plane (toward the histidine moiety) by $\lesssim$0.5 Å relative to the in-plane low-spin form.[27] Thus, if the rate determining step in the spin conversion process is this geometry change, the rather large k_1 and k_{-1} rate constants reflect a facile (in-plane) ↔ (out-of-plane) movement of the iron atom. Future studies will focus on obtaining k_1 and k_{-1} spin-interconversion rate constant data for synthetic spin-equilibrium porphyrin compounds to establish the influence (if any) of the protein environment on the spin-interconversion kinetics.

Acknowledgment. Support of this work by The Robert A. Welch Foundation and the Energy Research and Development Administration is gratefully acknowledged. E.V.D. wishes to especially thank ERDA for a summer fellowship and travel grant (Grant T-179) to the Brookhaven National Laboratory as administered under the auspices of the Associated Universities Program. We also thank Dr. Mitchell Hoselton for assistance with the laser T-jump apparatus and Professor John Olsen for the myoglobin sample.

References and Notes

(1) M. Sharrock, E. Munch, P. G. Debrunner, V. Marshall, J. D. Lipscomb, and I. C. Gunsalus, *Biochemistry*, **12**, 258 (1973).
(2) J. P. Collman, T. N. Sorrell, and B. M. Hoffman, *J. Am. Chem. Soc.*, **97**, 913 (1975).
(3) J. P. Collman and T. N. Sorrell, *J. Am. Chem. Soc.*, **97**, 4133 (1975).
(4) C. K. Chang and D. Dolphin, *J. Am. Chem. Soc.*, **97**, 5948 (1975).
(5) C. K. Chang and D. Dolphin, *J. Am. Chem. Soc.*, **98**, 1607 (1976).
(6) J. H. Dawson, R. H. Holm, J. R. Trudell, G. Barth, R. E. Linder, E. Bunnenberg, C. Djevassi, and S. C. Tang, *J. Am. Chem. Soc.*, **98**, 3707 (1976).
(7) J. H. Dawson, J. R. Trudel, G. Barth, R. E. Linder, E. Bunnenberg, C. Djevassi, R. Chiang, and L. P. Hager, *J. Am. Chem. Soc.*, **98**, 3709 (1976).
(8) S. C. Tang, S. Koch, G. C. Papaefthymiou, S. Foner, R. B. Frankel, J. A. Ibers, and R. H. Holm, *J. Am. Chem. Soc.*, **98**, 2412 (1976).
(9) C. D. Coryell, F. Stitt, and L. Pauling, *J. Am. Chem. Soc.*, **59**, 633 (1937).
(10) P. George, J. Beatlestone, and J. S. Griffith, "Haematin Enzymes", Vol. 19, Pergamon Press, New York, N.Y., I.U.B. Symposium Series, 1961, pp 105–141.
(11) I. Morishima and T. Iizuka, *J. Am. Chem. Soc.*, **96**, 5270 (1974).
(12) See for example: R. L. Martin and A. H. White, *Transition Met. Chem.*, **4**, 113 (1968); E. K. Barefield, D. H. Busch, and S. M. Nelson, *Q. Rev.* (*Chem. Soc.*), **22**, 457 (1968).
(13) D. H. Turner, G. W. Flynn, N. Sutin, and J. V. Beitz, *J. Am. Chem. Soc.*, **94**, 1554 (1972).
(14) J. K. Beattie and R. J. West, *J. Am. Chem. Soc.*, **96**, 1933 (1974).
(15) A. Ehrenberg, *Ark. Kemi*, **19**, 119 (1962).
(16) D. H. Turner, Ph.D. Thesis, Columbia University, New York, N.Y., 1972.
(17) While a deconvolution least-squares approach for obtaining τ is probably better in the general case, it has not been employed here for two reasons. First, although the least-squares approach has proven successful in our hands for long lifetime traces, the parameters obtained for the "shorter" MbOH traces here were disappointing in their instability while converging, and frequently could not be made to converge at all. Secondly, it is suspected that for the relatively small 2-ns intervals used in the MbOH calculations the "random" error in each reading is not independent from those of adjacent readings; i.e., it is not random at all. This independence is essential for a least-squares approach but is not nearly so crucial for the method of moments approach.
(18) S. S. Brody, *Rev. Sci. Instrm.*, **28**, 1021 (1957); D. H. Cooper, *ibid.*, **37**, 1407 (1966); J. N. Demas and G. A. Crosby, *Anal. Chem.*, **42**, 1010 (1970).
(19) The molar susceptibility for myoglobin in aqueous solution at pH 11.0 (88% hydroxide form) has been measured to be $\chi_M = 10\,780 \times 10^{-6}$ cgsu and that for a solution at pH 6.5 (7% hydroxide form) $\chi_M = 14\,180 \times 10^{-6}$ cgsu at 1.7 °C (J. Beetlestone and P. George, *Biochemistry*, **3**, 707 (1964)). Extrapolation to 100% hydroxide form yields $\chi_M = 10\,260 \times 10^{-6}$ cgsu, corresponding to $\mu = 4.75\ \mu_B$. Assuming low-spin and high-spin limits of 2.20 and 5.92 μ_B (as for myoglobin $\mathrm{CN^-}$ and myoglobin $\mathrm{F^-}$, respectively), myoglobin hydroxide is calculated to possess an equilibrium constant ($K_{eq} = [\mathrm{hs}]/[\mathrm{ls}] = (\mu^2 - \mu_{ls}{}^2)/(\mu_{hs}{}^2 - \mu^2)$) of 1.42 at 1.7 °C.
(20) J. K. Beattie, N. Sutin, D. H. Turner, and G. W. Flynn, *J. Am. Chem. Soc.*, **95**, 2052 (1973).
(21) M. F. Tweedle and L. J. Wilson, *J. Am. Chem. Soc.*, **98**, 4824 (1976).
(22) M. A. Hoselton, R. S. Drago, L. J. Wilson, and N. Sutin, *J. Am. Chem. Soc.*, **98**, 6967 (1976).
(23) E. V. Dose, K. M. M. Murphy, and L. J. Wilson, *Inorg. Chem.*, **15**, 2622 (1976).
(24) M. G. Simmons and L. J. Wilson, *Inorg. Chem.*, **16**, 126 (1977).
(25) And as discussed in ref 22 and 23.
(26) It has usually been assumed that Fe(III) porphyrin complexes must undergo a coordination number change of six → five upon low-spin → high-spin conversion (for example, see ref 14). This is *not* a necessary requirement in the present case, since the ferric myoglobin fluoride and cyanide compounds are apparently both six-coordinate, and yet, high-spin and low spin, respectively. It, therefore, seems reasonable that ferric myoglobin hydroxide is a $^2\mathrm{T} \rightleftarrows {}^6\mathrm{A}$ spin-equilibrium species which remains six-coordinate throughout the spin conversion processes. Furthermore, the azido derivative, which is also a spin-equilibrium case (P. George, J. Beetlestone, and J. S. Griffith, *Rev. Mod. Phys.*, 441 (1964); J. Beetlestone and P. George, *Biochemistry*, **3**, 707 (1964)), undergoes formation/dissociation of the sixth $\mathrm{N_3^-}$ ligand more slowly than the spin interconversion rates reported here (D. E. Goldsack, W. S. Eberlain, and R. A. Alberty, *J. Biol. Chem.*, **240**, 4312 (1965); **241**, 2653 (1966)). At present, however, there is insufficient evidence to show that, in the substrate-bound oxidized form of $\mathrm{P_{450}}$ enzymes, different spin states are associated with the same coordination number (Sharrock et al., *Biochim. Biophys. Acta*, **420**, 8 (1976)).
(27) Based on a comparison of x-ray structures for the low-spin six-coordinate species $[\mathrm{Fe(Im)_2(TPP)}]^+$ (R. Countryman, D. M. Collins, and J. L. Hoard, *J. Am. Chem. Soc.*, **91**, 5166 (1969); **94**, 2066 (1972)), and the high-spin five-coordinate species, [Fe(Cl)(proto-IX)] (J. L. Hoard, G. H. Cohen, and M. D. Glick, *J. Am. Chem. Soc.*, **89**, 1992 (1967)), where Δ(Fe–porphyrin plane) ~ 0.46 Å.
(28) Robert A. Welch Foundation Predoctoral Fellow and ERDA Research Fellow under the Associated Universities Program (summer 1976).
(29) Robert A. Welch Foundation Predoctoral Fellow.

Eric V. Dose,[28] Michael F. Tweedle,[29] Lon J. Wilson*
Department of Chemistry, William Marsh Rice University
Houston, Texas 77001

Norman Sutin*
The Department of Chemistry, Brookhaven National
Laboratory, Upton, Long Island, New York 11973

Received December 14, 1976

Organic Reactions of Oxide-Free Carbon Surfaces, an Electroactive Derivative[14]

Sir:

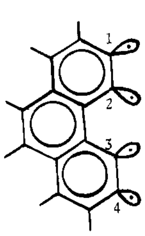
In the absence of the usual oxides, surfaces of graphite and related forms of carbon are quite reactive. For example, at room temperature a highly exothermic reaction occurs with molecular oxygen to regenerate surface oxides.[1,2] This chemistry is known to occur exclusively in prismatic regions, that is, surfaces other than the basal plane, {0001}.[3] Although the structure of the reactive centers is unknown, those represented by I, II, and III seemed to us more likely on thermodynamic grounds than the various alternative possibilities. By analogy with structurally similar molecules, these surface functions might be expected to undergo cycloaddition reactions with olefinic substrates. Preliminary evidence presented below tends to support this description.

Synthetic carbon fiber was freed of surface oxides by pyrolysis at 1020 °C in a vacuum system (10^{-5} Torr).[4] Samples were allowed to cool to room temperature and were then exposed to vapors of a variety of potential substrates. The total quantity of substrate adsorbed was determined manometrically

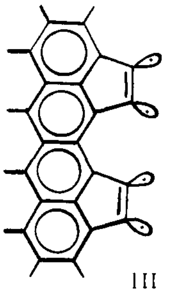
Table I. Irreversible Adsorption on Oxide-Free Carbon Fiber[a] (VYB fiber, B.E.T. surface area 240 m^2/g)

Substrate	Irreversible adsorption, mmol/g of fiber
Oxygen	0.62
Propane	0.00
Ethylene	0.02
Propylene	0.02
Isobutylene	0.25
followed by oxygen	0.43
Allene	0.63
followed by oxygen	0.09
Cyclopentadiene	0.35
followed by oxygen	0.30
Methyl acrylate	0.83
followed by oxygen	0.00
Acrylyl chloride	0.72
Vinyl bromide	0.48

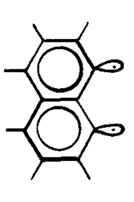
[a] All values were measured at room temperature and were corrected for reversible adsorption at an ambient pressure of 20 Torr.



I



III

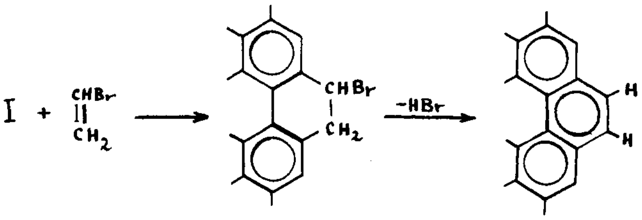


II

at pressures <30 Torr. Correction was made for reversible, physical adsorption by subsequent measurements following periods of outgassing to 10^{-5} Torr. The residual, irreversible adsorptions are reported in Table I.

The extent of reaction with O_2 compares well with values reported for other graphitic carbons.[1] The resulting surface oxides can only be removed by repeating the pyrolytic decomposition which results in liberation of CO and CO_2. (We estimate that ~20% of the total surface area is reactive.) Reactivity toward organic substrates is remarkably selective. Some of these react in quantities comparable with O_2. That the same surface functions are involved in all cases is indicated by the fact that reaction with O_2 is inhibited, roughly stoichiometrically, by prior addition of the reactive organic compounds. Moreover, the properties of the adducts are consistent with the conclusion that C–C bonds are formed with atoms in the lattice. Once "irreversibly adsorbed", the substrates could not be recovered by extraction with organic solvents though they were shown to be accessible to reagents in solution. Heating to 200 °C at 10^{-5} Torr likewise failed to liberate the substrate or reactivate the surface toward O_2.

More specific evidence for the formation of cycloadducts was obtained from detailed studies of the vinylbromide adduct. Of the total 0.48 mmol of vinyl bromide irreversibly adsorbed by 1 g of fiber, all but 0.11 mmol of the initial adduct was found to decompose spontaneously with loss of HBr.[5] The remaining 0.11 mmol/g was significantly more stable, being unaffected by heating for more than an hour at 200 °C and 10^{-5} Torr. If the major fraction of initial reaction involved cycloaddition across the C_2, C_3 positions of surface function I, the facile loss of HBr would be a reasonable consequence as the reaction would create a new aromatic nucleus at the lattice edge:



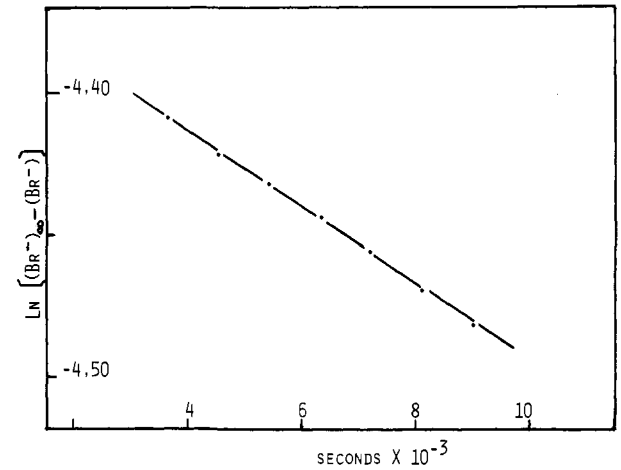


Figure 1. Kinetics of solvolytic loss of Br^- from vinyl bromide treated carbon fiber (reaction in 0.1 M $NaNO_3$, 1 M HNO_3, 75% aqueous ethanol at 25 °C).

Since the occurrence of I is limited to surfaces with crystallographic orientation $\{11\bar{2}l\}$ (or the equivalents), the observed heterogeneity among the vinyl bromide adducts is not surprising. Cycloaddition to either II or III would each provide derivatives with bromine α to the conjugated π system of the lattice although not located on a six-membered ring. The "benzylic" reactivity predicted by this description was in fact confirmed by experiment.

The rate of solvolytic loss of Br^- from the more stable of the two vinyl bromide adducts was found to follow first-order kinetics. Kinetic plots retained excellent linearity over more than 4 half-lives (see Figure 1). The rate constant, $9.8 \times 10^{-5}\ s^{-1}$, is close to that for solvolysis of α-methylbenzyl bromide under similar conditions ($1.36 \times 10^{-5}\ s^{-1}$ in aqueous acetic acid).[6] The fact that all of the more stable adduct reacts accordingly to a simple first-order rate law requires that all of it was bound in a single kind of structural unit on the surface.

In recent years several approaches have been devised to prepare electrodes with reagents covalently bound at the surface.[7-10] Previous work on graphite[8,9] has involved attempts to derivatize the surface oxides. Such experiments are complicated by the highly inhomogeneous nature of the oxide functionalities.[1] Indeed, beyond analytical evidence for the presence of chemisorbed heteroatoms,[8,9] the constitution of the chemically modified surface oxides remains entirely ambiguous. Olefinic addition reaction to the oxide-free surface provides a new route to the preparation of derivatized graphite electrodes.

Acrylyl chloride reacts with the oxide-free surface of synthetic carbon fibers in good yield. A pressure-annealed, highly oriented pyrolytic graphite crystal,[11] more suitable than the fiber for use as an electrode, was treated similarly. A sample was heated at 1020 °C for 10 h at 10^{-5} Torr to remove surface oxides. Following this it was cooled to room temperature, exposed to acrylyl chloride vapor at 20 Torr, and finally excess reagent was removed by outgassing at 10^{-5} Torr for several

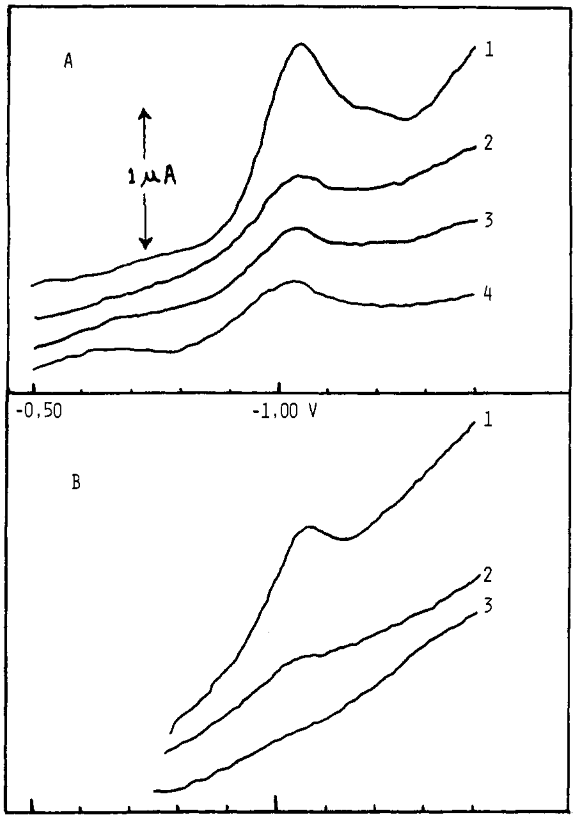
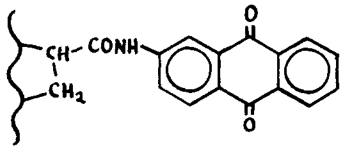


Figure 2. Alternating current voltammetry[12] of β-aminoanthraquinone adsorbed on acrylyl chloride treated oxide-free graphite, A, and untreated graphite with normal surface oxides, B (potential vs. Ag/AgCl(s); numbers indicate scan sequence).

hours. The sample was next immersed in a stirred solution of β-aminoanthraquinone, IV, in dry pyridine and finally washed with fresh pyridine and acetone. The successful preparation of surface derivative V was evidenced by the electrochemistry.



V

The sample was mounted in a kel-F holder which masked all but a single, edge-oriented surface. Voltammetric measurements were made using the graphite as working electrode in a three-electrode cell filled with 0.1 $\overline{\text{M}}$ tetrabutylammonium perchlorate in acetonitrile. In conventional cyclic voltammetry the faradaic current was poorly resolved from the very large capacitive component. Consequently, first-harmonic ac voltammetry was employed[12] using phase-selective rectification of the faradaic current.

Figure 2A shows the first four consecutive scans of freshly prepared sample (dc potential was scanned from −0.5 to −1.4 V). The ac current peaks correspond closely to the reversible half-wave potential for one-electron reduction of IV. (Measurements with an independent working electrode could detect no quinone free in solution before or after the experiment.) The peak diminishes significantly between the first and second cathodic cycles but remains constant for all successive scans. The change is due to the fact that a fraction of quinone is only physically adsorbed on the surface and is desorbed following reduction to the anion radical, as demonstrated by the following control experiment.

The exposed surface of the sample was renewed by brief polishing on a ground glass plate. This process exposes fresh edge-oriented surface which immediately becomes covered with surface oxides, and results in complete disappearance of the cathodic peak. A freshly resurfaced sample was treated with the pyridine solution of IV, washed, and remounted as before. Figure 2B shows the first three cathodic cycles. Physically adsorbed IV is detected on the first scan and is completely removed by the third. These experiments demonstrate that the covalently bound acid chloride is required for the chemisorption of IV.

Our continuing efforts are directed at more detailed characterization of these new surface derivatives and at extending the variety of functional groups which may be incorporated by means of reactions at the oxide-free surface.

References and Notes

(1) Yu. A. Zarafyanz et al., *Carbon*, 7, 127 (1967); S. S. Barton et al., *ibid.*, **10**, 395 (1972).

(2) The particular surface oxides obtained by this method are nonacidic; see, for example, H. P. Boehm and M. Voll, *Carbon*, **8**, 227, 741 (1970).

(3) G. R. Hennig, *Proc. Carbon Conf., 5th*, 143 (1961); J. M. Thomas and E. E. G. Hughes, *Carbon*, **1**, 209 (1964); B. McCarroll and D. W. McKee, *ibid.*, **9**, 301 (1970).

(4) Union Carbide VYB carbon yarn was used. Following treatment as described it was found to have a surface area of 240 m^2/g (B.E.T. method using N_2). This material consists of dense fibers, about 10μ in diameter. Deep grooves run parallel to the fiber axis but no significant pore structure is evident to 10^5X magnification by scanning electron microscopy.

(5) Decomposition occurred at room temperature contemporaneous with the addition of vinyl bromide. A trace of Br_2 was liberated as well.

(6) A. H. Fainberg and S. Winstein, *J. Am. Chem. Soc.*, **79**, 1603 (1957).

(7) R. F. Lane and A. T. Hubbard, *J. Phys. Chem.*, 77, 1401, 1411 (1973).

(8) R. W. Murray et al., *Anal. Chem.*, 47, 1882 (1975); **48**, 1247 (1976); *J. Am. Chem. Soc.*, **98**, 7435 (1976).

(9) L. L. Miller et al., *J. Am. Chem. Soc.*, **97**, 3549 (1975); **98**, 8271; 8727 (1976).

(10) T. Kuwana et al., *Anal. Chem.*, **48**, 741 (1976).

(11) Generously provided by Mr. Y. Z. Leger of Union Carbide Corp.

(12) dc scans were made at 2 mV/s from positive to negative potentials with a 5-Hz ac modulation of 10-mV root mean square amplitude. Phase angle was optimized so as to diminish ac current in regions of no faradaic activity.

(13) While on leave from the University of München, K.C. wishes to thank the Deutsche Forschungsgemeinschaft for financial support.

(14) This work was supported by U.S. Army Research Office Grant DAHCO-75-0064-G.

Stephen Mazur,* Tomislav Matusinovic, Karl Cammann[13]
Department of Chemistry, University of Chicago
Chicago, Illinois 60637
Received January 25, 1977

Isoenergetic Hydride Transfer. A Reversible, Phase Transition Mediated tRNA Modification

Sir:

Transfer RNAs contain an abundance of modified nucleosides, the possible functions of which have been studied intensively for years.[1] In spite of such studies, and the availability of a detailed, three-dimensional model of one tRNA which illustrates the relative orientations and possible interactions of the modified nucleosides, the role of these species remains a central, unsolved problem. We wish to report a selective, reversible modification of tRNA by a novel, energetically degenerate process which is accompanied by a reversible alteration of the biochemical activity of the nucleic acid. The nature of the chemical and biochemical changes are in accord with predictions based on the x-ray crystallographic structure of yeast tRNA^{Phe}[2] and permit verification of the importance of a single tertiary interaction in the maintenance of structure and biochemical activity of tRNA.

7-Methylguanosine (**1**) is an unusual modified nucleoside in that it exists as a zwitterion at physiological pH. The nucleoside occurs naturally in ribosomal[3] and messenger[4] RNAs,