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Di-π-cyclooctatetraenethorium

Sir:

The relative stability of the recently synthesized di-π-cyclooctatetraeneuranium from cyclooctatetraene (COT) dianion and uranium tetrachloride suggests that significant stabilization is afforded by overlap of the highest occupied (E_2) molecular orbitals of the two rings with vacant f orbitals (f_{xyz},$f_{z(x^2-y^2)}$) of the central metal.[1] It is clearly important to compare the chemistry of similar complexes with other actinide and with lanthanide rare-earth metals. We report here the preparation and some of the chemistry of the thorium analog.

A suspension of dry thorium tetrachloride in tetrahydrofuran was added to 2 equiv of K_2COT in THF cooled to Dry Ice temperature. The mixture was allowed to warm to room temperature with stirring overnight. The solvent was evaporated from the yellow reaction mixture and the residue was sublimed at 0.01 Torr and 160° to produce the product as fine bright yellow crystals. The mass spectrum showed a parent peak at *m/e* 440 ($Th(COT)_2$) with important fragment peaks at *m/e* 336 (ThCOT), 111 and 109 (unassigned), and 104 (COT). Resublimation gave crystals suitable for X-ray analysis. The compound is isomorphous with di-π-cyclooctatetraeneuranium[2] (uranocene), and the thorium compound therefore also has the D_{8h} sandwich structure.[3]

$Th(COT)_2$ ("thoracene") is decomposed readily by water. It is unstable in air but does not enflame, as does uranocene; crystals of the thorium compound change in color from yellow to brown after a few minutes' exposure to air. The compound decomposes without melting at temperatures above 190° and explodes if heated to red-hot. Thoracene is insoluble in most organic solvents, *e.g.*, $CHCl_3$, CCl_4, THF, benzene, acetone, etc.; it is soluble in DMSO, but the nmr spectrum of this solution shows a complex multiplet at 6.2 ppm rather than the sharp singlet expected for a D_{8h} sandwich structure. On exposure to air for several seconds the nmr spectrum changes to the sharp singlet of COT at 5.75 ppm. We suggest that thoracene forms a complex with DMSO which destroys the symmetry of the rings and changes the structure to that of a diene–transition metal type. This chemistry may be rationalized as follows if bonding interaction with 5f orbitals is important in the di-π-cyclooctatetraeneactinide structures. We have suggested[1] that the two highest energy electrons in uranocene are in a back-bonding $f_{x(x^2-3y^2)}$, $f_{y(3y^2-x^2)}$–E_{3u} combination. These MO's are vacant in thoracene and could produce a Lewis acid capability that would lead to the observed reactions with Lewis bases such as DMSO and water. The extension of this chemistry to other actinides and to the lanthanide rare earths is in progress.

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The Reaction of a Sulfonyl-Chymotrypsin with Hydrogen Peroxide. Generation of a Hydroperoxy Enzyme

Sir:

Generating a reactive free radical at a specific site on a protein and allowing the radical to react with its surroundings would be useful as a chemical probe for three-dimensional structure in solution.[1] The present work was designed to produce a peroxide moiety (which could then be decomposed by photolysis) at the active site of the serine protease chymotrypsin. In this report the preparation of an apparently catalytically active hydroperoxy enzyme is described.

Benzylsulfonyl-chymotrypsin (modified at serine-195) was prepared according to the published procedure.[2] In all experiments it had less than 0.8% catalytic activity. When incubated at pH 7 with H_2O_2 as shown in Figure 1, catalytic activity was fairly rapidly restored until deactivation of enzyme became a significantly competing process (about 12 hr). As previously reported,[2] benzylsulfonyl-chymotrypsin was completely stable under the same conditions without H_2O_2. Native enzyme under the reaction conditions lost activity only very slowly, while the same reaction with the sulfonate and *n*-propyl hydroperoxide resulted in an extremely slow restoration of catalytic activity, probably due to H_2O_2 impurity.[3]

Variation in pH of the reactivation mixture resulted in a regular increase in the rate of reactivation from pH 4 to about pH 7 (the optimum). At much above pH 7 activity was lost at a rate comparable to its restoration.

The product of the H_2O_2 reaction was isolated by exhaustive dialysis (2 to 5 ml *vs.* two changes of 2 l. of 10^{-3} *M* HCl, then four changes of double distilled water) followed by lyophilization. Further experiments were performed on this hydrogen peroxide free material.

From the simple organic system we would expect that the dominant process might be (1) by an SN2 displace-

$$E\text{–}CH_2OSO_2CH_2C_6H_5 + {}^-OOH \longrightarrow E\text{–}CH_2OOH + C_6H_5CH_2SO_3^- \quad (1)$$

(1) A carbene approach to this problem has been taken by Westheimer: J. Shafer, P. Baronowsky, R. Laursen, F. Finn, and F. H. Westheimer, *J. Biol. Chem.*, **241**, 421 (1966); R. Vaughan and F. H. Westheimer, *J. Am. Chem. Soc.*, **91**, 217 (1969).
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(3) *n*-Propyl hydroperoxide was prepared from *n*-propyl methanesulfonate and H_2O_2: H. R. Williams and H. S. Mosher, *J. Am. Chem. Soc.*, **76**, 2984 (1954). After two distillations, analysis still showed *n*-propyl alcohol and H_2O_2 impurities. Work is in progress to clearly define the alkyl hydroperoxide reaction.

ment reaction. Three separate chemical approaches have been taken to characterize the recovered material. (A) Photolysis (>300 nm) in the presence of 5×10^{-3} M β-naphthoic acid ($K_i = 1.4 \times 10^{-3}$ M)[4] produced a considerably (about fivefold) faster loss of catalytic activity for reactivated material than for α-chymotrypsin. β-Naphthoic acid has the correct energy levels and known properties to be an efficient photosensitizer (by energy transfer) for peroxide decomposition[5] and can also saturate the active sites of the enzyme. Side reactions may be photooxidation of a histidine[6] (the samples were only made partially anaerobic) or direct photoreaction at another group. (B) Incubation with ferrous sulfate for several days resulted in a slow loss of activity (leveling off at about 60%) for α-chymotrypsin but a much faster loss (leveling off at 10%) for peroxide-reactivated material. This is probably reasonable for an alkyl hydroperoxide, since the dominant reaction (2) may be accompanied by (3), and we would also expect

$$\mathrm{ROOH} + \mathrm{Fe^{2+}} \longrightarrow \mathrm{RO\cdot} + \mathrm{OH^-} + \mathrm{Fe^{3+}} \quad (2)$$

$$\mathrm{ROOH} + \mathrm{Fe^{2+}} \longrightarrow \mathrm{RO^-} + \mathrm{OH\cdot} + \mathrm{Fe^{3+}} \quad (3)$$

RO· to gain a hydrogen atom to give ROH (serine) partly *via* reaction that does not destroy activity. (C) N,N,N′,N′-Tetramethyl-*p*-phenylenediamine was also used as a titrant.[7] With H_2O_2 or *t*-butyl hydroperoxide only about 10% equivalent Wurster's blue is formed (followed at 622 nm[7b] under optimum conditions (pH 5, anaerobic)). Controls showed less than 1% oxidation, and the 10% figure is quite reproducible in many experiments. Numerous other reagents are reported in the literature to be no more quantitative for hydroperoxides, and the Wurster's blue method is very sensitive. Native and benzylsulfonyl-chymotrypsin, as well as native enzyme treated with H_2O_2 as in the reactivation experiments, showed no titer (<0.04 absorbancy). Reactivated material gave a value 10% (0.2 absorbancy) of that calculated assuming 100% hydroperoxy enzyme (based on active sites as determined by cinnamoylimidazole titration). This value is in agreement with the hydroperoxide structural assignment. That dialysis was removing all H_2O_2 was shown by (a) a complete lack of titer for solution outside the dialysis membrane after the third buffer change (six were done) and (b) the lack of titer of recovered chymotrypsin which had been put through the reaction conditions with H_2O_2 and worked up as in the sulfonate experiments.

Native chymotrypsin was incubated for 22 hr with 0.6 M H_2O_2 under the usual conditions, dialyzed exhaustively, and then treated with benzylsulfonyl fluoride (1.2 equiv) to produce a protein with less than 1% activity. This material was then tested for reactivation with and without hydrogen peroxide at pH 7. The results were essentially the same as for sulfonyl enzyme that had not been pretreated with H_2O_2 (the buffer solution did not regain activity, while the H_2O_2 solution reactivated at about the same rate as shown in Figure 1). It would thus seem that H_2O_2 must be acting directly at the active serine sulfonate ester. These experiments, taken

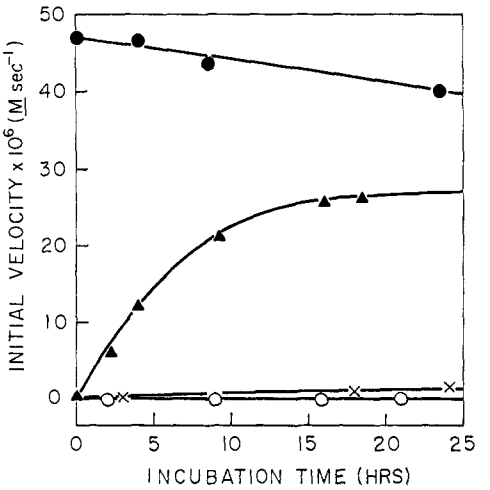

Figure 1. Assays toward ethyl N-acetyl-L-tryptophanate (pH 8.00, 0.1 M tris buffer, 25°, 1.6% (v/v) acetonitrile, $S_0 = 2.9 \times 10^{-3}$ M, enzyme = 1×10^{-6} M) with various incubation mixtures *vs.* time. All incubation mixtures have 10 mg of protein/ml (3×10^{-4} M) and are 0.1 M phosphate buffer, adjusted to pH 7.0 after addition of all reagents; ●, α-chymotrypin + 0.60 M H_2O_2; ▲, benzylsulfonyl-chymotrypsin + 0.60 M H_2O_2; ×, benzylsulfonyl-chymotrypsin + 0.55 M *n*-propyl hydroperoxide; ○, benzylsulfonyl-chymotrypsin alone.

with the reasonable chemistry,[8] point to the likelihood that OOH^- has displaced sulfonate to produce chymotrypsin-Ser-195-OOH, and that this material is catalytically active. *n*-Butyl hydroperoxide, in agreement with the literature,[8] was stable in aqueous solution for weeks, considerably longer than the course of our experiments. Reactivation of [α-^{14}C]benzylsulfonyl-chymotrypsin[2] by H_2O_2 showed a loss of 70% of the label by the time maximum activity had been reached.

On the basis of the "α effect"[9] hydroperoxy enzyme might be expected to be more reactive toward substrates than is native chymotrypsin (with an –OH), but with the changed spatial requirements for efficient multifunctional catalysis the reverse might obtain. Cinnamoylimidazole titration[10] of three separately prepared samples gave 56 ± 2% active sites based upon the same titration with chymotrypsin (titer was compared to absorbancy at 282 nm for each). Using this value for active site concentration, preliminary kinetics show that toward ethyl N-acetyl-L-tryptophanate k_{cat} increases from pH 6 to about pH 8, and is constant from pH 8 to pH 10 with a value of 80 ± 20 sec^{-1} (compared to 47 sec^{-1} for chymotrypsin).[11] K_m is pH dependent, rapidly rising above pH ~8.5.

We interpret the striking efficacy of hydrogen peroxide to be an example of the high nucleophilicity of hydroperoxide anion toward sp^3 carbon, as reported by Pearson for benzyl bromide.[12] Additionally, the

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(8) Except for sulfonate esters of some phenols, there appear to be no documented cases of other than simple C–O scission of sulfonate esters by nucleophiles or oxidizing agents. Indeed, a standard preparation of hydroperoxides or mixed dialkyl peroxides involves displacement of sulfonate from an ester by H_2O_2 or RO_2H (respectively) in dilute alkaline solution (A. G. Davies, "Organic Peroxides," Butterworth & Co. (Publishers) Ltd., London, 1961, Chapters 9 and 10).
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higher acidity of hydrogen peroxide than water (pK = 11.6 *vs.* 15.6) more than compensates for the difference in concentrations between H_2O_2 and H_2O in our experiment (about 100-fold), so that at pH 7 there is about 100-fold more OOH^- than OH^-. There are other concurrent reactions of hydrogen peroxide with the protein.[13] These will be reported in the full paper.

Acknowledgments. We are grateful to the Public Health Service (GM 15100) and the Research Corporation (Cottrell Fund) for partial support of this work and to Mr. Michael MacLean for able assistance at the outset.

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The Oxidation of Iron(II) Porphyrins by Organic Molecules

Sir:

A knowledge of the nature of bond types capable of effecting the oxidation of iron(II) to iron(III) porphyrins is a requisite for formulating mechanisms of hemoprotein function. Moreover, low-valent metalloporphyrins, by virtue of their unique coordination, should be highly efficient reagents for organic synthesis.

The unusually rapid rates of oxidation of iron(II) deuteroporphyrin and iron(II) protoporphyrin by alkyl halides and the stoichiometric conversion of DDT to DDD have been noted.[1] Most recently, a report of the oxidation of iron(II) porphyrins by olefins and acetylenes has appeared.[2]

We wish to outline the general nature of some rapid, quantitative room-temperature reactions of iron(II) deuteroporphyrin ($Fe^{II}D$) and its dimethyl ester with alkyl halides, quinones, and nitro and nitroso compounds and present a brief discussion of our findings. The selected illustrative cases (eq 1–6) are representative of a broad range of molecules possessing these reactive groupings. Typical reaction concentrations employed were $\sim 2 \times 10^{-3}$ M in $Fe^{II}D$ and $\sim 10^{-3}$ M substrate in 1:1 n-methylpyrrolidone–acetic acid. Calculated yields range from 97 to 100% in all cases. No other products are detectable.

It is to be emphasized that the reactive bond types reported herein were found by a very broad scan of a wide array of organic substances. In addition to peroxides, they represent the only reactive kinds of molecules encountered. The scan was conducted by injecting substrate (initial concentration 10^{-3} to 10^{-2} M) into a 10^{-4} M $Fe^{II}D$ solution under nitrogen and monitoring for the appearance of the iron(III) band at 630 mμ. The reactivity of bond types indicated by the scan were corroborated by scaling up to reaction conditions and establishing the stoichiometry.

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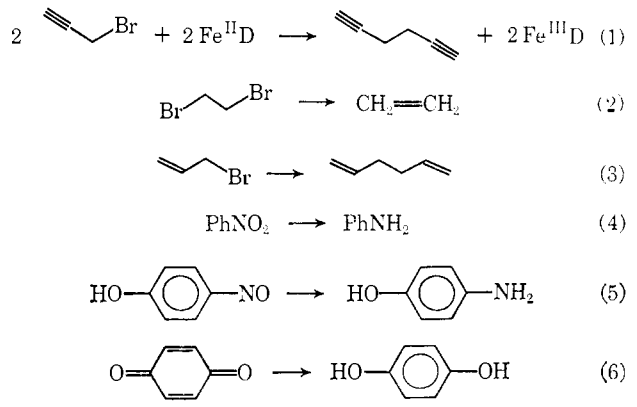

In our system, the solvent is not so highly coordinating as to preclude any association that may be necessary for reaction. Consequently, these results *do not* accord with the reported[2] oxidative reactivity of olefins and acetylenes toward iron(II) porphyrins. Indeed we have examined some 30 olefins and acetylenes of widely differing linkage without observing oxidation by these substances. For example, the spectrum of a solution of 0.1 M methylcyclohexene and 10^{-4} M iron(II) deuteroporphyrin dimethyl ester was not altered in 8 hr.[3]

The typical coupling of halides (eq 1 and 3) is striking in contrast to the total reduction of these substances (to propylene) by "uncomplexed" metal ions like chromium(II).[4] Furthermore, the quick oxidation of $Fe^{II}D$ by quinones (eq 6), and in particular ubiquinone, is instructive. The reverse reduction of $Fe^{III}D$ by ubihydroquinone does not occur. However, this hydroquinone is a known physiological reductant of some cytochromes.[5] These results suggest that ubiquinone can function as a long-range "electron-transfer" agent at the cellular level and, further, that it is the nature of the axial ligands and the conformation of the protein about the porphyrin complex that control the redox capacities of hemoproteins toward this quinone–hydroquinone pair.

Amplified studies of each of these reactions will be reported later. Investigations of the parallel reactions with a variety of hemoproteins are under way.

Acknowledgment. We are grateful to the National Science Foundation for generous support.

(3) For comparison, at 0.01 times the substrate concentration, the reaction with benzoquinone is complete in less than 1 sec. A trace of peroxide impurity, however, will cause oxidation and degradation of the porphyrin. With no other substrates have we observed porphyrin degradation. Hence, we believe the results reported in ref 2 are due to peroxidic impurities in the unsaturated substrates employed.

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Photosensitized Aquation of Some Chromium(III) Complexes

Sir:

We wish to report the observation that aquation of various Cr(III) complexes can be photosensitized by organic compounds known to have relatively stable