Spin-orbit effects have been neglected for the MP2 dissociation energies, since these are assumed to be small for these compounds.[5,13] Only $AuMe_2^-$ shows a relativistic increase in the dissociation energy. The trend in dissociation energy is opposite to the trend in force constants discussed above, i.e., $D_e(HgMe_2) > D_e(TlMe_2^+) > D_e(PbMe_2^{2+})$. The dissociation energy of $C_2H_6 \rightarrow 2CH_3$ has been calculated to be 395 kJ/mol at the MP2 level (exp 368 kJ/mol[28]). According to the reaction $[M(CH_3)_2]^n \rightarrow M^n + C_2H_6$ (e.g., $n = -1$ for M = Au), the methyl complexes are not very stable at the relativistic level (Table I). Also entropy effects clearly would shift the reaction to the right hand side. This agrees with the fact that Hg–C bonds in organomercury compounds can be easily broken homolytically. On the other hand, such compounds are relatively air- and water-resistant and therefore kinetically stable. The calculated MP2 dissociation energy for $HgMe_2$ of 286 kJ/mol is in relatively good agreement with the experimental value of 234 kJ/mol.

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Observation of a Peroxyl Radical in Samples of "Purple" Lipoxygenase[1]

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We report the existence of a peroxyl radical, analogous to a proposed mechanistic intermediate, in samples of the metastable purple form of soybean lipoxygenase-1. This enzyme[2] catalyzes the production of 13-hydroperoxy-9,11-*cis,trans*-octadecadienoic acid (13-HPOD[3]) from linoleic acid. Mammalian lipoxygenases catalyze similar reactions of arachidonic acid and are important in the production of leukotrienes and lipoxins, messengers involved in the inflammatory and immune responses.[4] One hypothetical mechanism of the oxygenation reaction, similar to that of the autoxidation of polyunsaturated fatty acids,[5] proposes that the active-site Fe^{3+} oxidizes the 1,4-diene unit of the substrate to a pentadienyl radical, which should react smoothly with dioxygen, giving a peroxyl radical.[6] Reduction by the iron (now Fe^{2+}) would result in the peroxide anion. A second mechanism proposes that the Fe^{3+} facilitates the deprotonation of the substrate, yielding a Fe^{3+}–alkyl complex. Insertion of dioxygen into the Fe–C bond, then, is envisioned as giving the coordinated peroxide anion.[7] Radicals derived from the substrate have been trapped in experiments in which the reaction is prevented from going to completion by anaerobiasis.[8] However, such radicals may result from homolytic Fe–C bond cleavage of the proposed Fe^{3+}–alkyl complex as well as from simple release of free-radical intermediates.[7] Recently, broad EPR spectra consistent with fatty acid peroxyl radicals were reported in samples of lipoxygenase under turnover conditions at room temperature.[9] In that case, the peroxyl radical was thought to be in solution, rather than enzyme-bound, and thus conclusions of mechanistic relevance are somewhat tenuous.

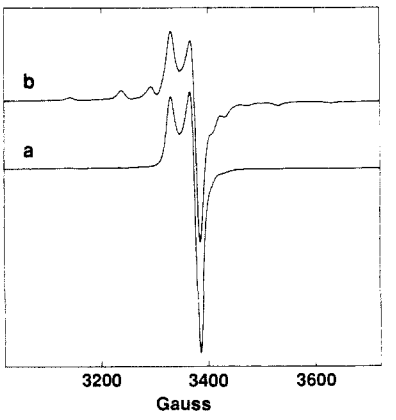

Figure 1. EPR spectra of "purple" lipoxygenase generated by addition of linoleic acid under oxygen. (a) Natural-abundance O_2. (b) 36% enriched $^{17}O_2$. Samples prepared as stated in the text. EPR parameters: microwave frequency, 9.52 GHz; power, 1 mW; modulation amplitude, 0.5 mT; temperature, 10 K.

Treatment of ferric soybean lipoxygenase-1 with 13-HPOD or with linoleic acid and oxygen results in the formation of a metastable purple enzyme.[10] This form reverts slowly to the native ferric enzyme with release of 12,13-epoxy-11-hydroxy-9-octadecanoic acid,[11] suggesting that it represents an intermediate complex on the isomerization pathway. The origin of the purple color, endowed by a band in the visible spectrum at 585 nm, has never been adequately explained, but CD spectra suggest that the environment of the metal ion is significantly different in the native ferric and purple states.[12]

EPR spectra[13] of purple lipoxygenase prepared by treating ferrous or ferric lipoxygenase[14] with linoleic acid in the presence of oxygen at 4 °C show an axial signal in the $g = 2$ region (Figure 1a). When the experiments were repeated with 36% ^{17}O-enriched

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(14) Soybean lipoxygenase-1 was purified and assayed as published;[15] the enzyme used had specific activities between 250 and 270 units/mg. Stock solutions of linoleic acid (Sigma Chemical Co.) were prepared by evaporating an ethanolic solution to an oil under N_2, then redissolving in 0.1 volume of 1 M NH_4OH, and adding 0.9 volume of 0.1 M sodium borate, pH 9. Concentrated ferrous or ferric lipoxygenase was diluted into oxygen-saturated buffer in a serum-stoppered cuvette thermostated at 4 °C in an HP4150a diode-array spectrophotometer. The spectrum was obtained after each aliquot of linoleic acid was added. Addition was terminated after the absorption at 585 nm no longer increased upon further addition of substrate. Samples were transferred with chilled syringes to EPR tubes on ice and rapidly frozen in liquid nitrogen.
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oxygen (Mound Laboratories), the spectra revealed strong coupling to two ^{17}O nuclei (Figure 1b). The g values and ^{17}O hyperfine splitting parameters obtained by simulating the spectra (g = 2.039, 2.012, 2.003; $A_x(^{17}O)$ = 9.9, 6.1 mT)[16] agree well with those published for oleyl peroxyl radical (g = 2.035, 2.008, 2.003; $A_x(^{17}O)$ = 9.5, 5.9 mT).[17] Thiol peroxy radicals ($RSO_2^{\bullet}$)[18] and the superoxide anion[19] have very similar EPR parameters, but the difference between the ^{17}O hyperfine splittings of the two oxygen nuclei is significantly smaller in the former ($A_x(^{17}O)$ = 8.1, 6.4 mT) and negligible in the latter. The large (3.8 mT) difference we observe suggests that this spectrum represents an alkyl peroxyl radical, logically 13-dioxy-9,11-octadecadienoic acid.

Changes in the line shape and apparent hyperfine splittings begin to be seen as the temperature is raised through about 150 K (data not shown). In contrast, the spectra of 13-dioxy-9,11-*cis,trans*-octadecadienoic acid in a protein-free frozen matrix begin to show similar effects at about 110 K.[17] These spectral changes are thought to arise from the onset of both rotation about the C–O bond and molecular tumbling; thus, the peroxyl radical in the enzyme sample appears to be more conformationally constrained than that in the frozen matrix and probably is indeed bound to the enzyme.

After a few minutes at 4 °C, the sample recovers the visible and EPR spectra of the native ferric enzyme;[10] in particular, the spectrum of the peroxyl radical is no longer apparent. During this time, the intensities of the 585-nm absorption band and the peroxyl radical EPR spectrum decline in parallel. Treatment with more linoleic acid and oxygen regenerates both the purple color and the peroxyl radical signal. On the other hand, addition of 10 mM ethanol (known to bind in or near the active site of lipoxygenase[20]) eliminates the radical signal without affecting the intensity of the purple color. Thus, the purple state appears to be necessary but not sufficient for the existence of the radical.

The mechanistic origin and importance of the peroxyl radical are not certain. We hypothesize that it is either a trapped intermediate in the oxygenation pathway or the result of reduction of the active-site Fe^{3+} by endogenously produced 13-HPOD. The stability of the radical at 4 °C is inconsistent with its being an intermediate of the oxygenation reaction (reduction of an intermediate peroxyl radical must occur at a rate $> k_{cat} > 200$/s at 25 °C[21]) *unless* that reduction is driven by dissociation of the product hydroperoxide from the enzyme. In that case, the relatively high enzyme concentrations used in these experiments (in excess of 0.1 mM) may favor formation of the lipoxygenase–13-HPOD complex, which then may seek an equilibrium between Fe^{3+}–ROOH and Fe^{2+}–ROO$^{\bullet}$. The relatively high reduction potential of the active-site iron (≈0.6 V vs NHE) favors this possibility.[15] We are testing it by quantitation of the Fe^{3+} and peroxyl radical EPR signals as a function of enzyme and linoleic acid concentrations. If indeed this latter explanation is true, it will provide strong evidence for the existence of radical intermediates in the mechanism of fatty acid oxygenation by lipoxygenase.

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(16) The relative intensities of the features corresponding to splitting by the two oxygen nuclei were faithfully reproduced by assuming equal contribution by each nucleus. However, simulation of the relative intensities of the spectra from ^{17}O-enriched and natural-abundance peroxyl radicals required assuming approximately 25% ^{17}O enrichment, implying some contamination by unenriched oxygen either in the stock ^{17}O-enriched gas or during the experiment.

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Are Fluorocarbon Chains "Stiffer" Than Hydrocarbon Chains? Dynamics of End-to-End Cyclization in a C_8F_{16} Segment Monitored by Fluorescence

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Bending and folding of hydrocarbon chains has been probed by using fluorescence methods since the discovery by Hirayama[1] of the intramolecular excimer formation process in 1,3-diphenylpropanes. The dynamic flexibility of hydrocarbon chains depends upon the chemical type and length of the chain, the solvent or environment in which it is immersed, and the temperature.[2] Cyclizations of polymer chains have been examined by this method, especially using α,ω-dipyrenyl probes: poly(styrene),[3] poly(ethylene oxide),[4] poly(tetramethylene oxide),[5] poly(dimethylsiloxane),[6] and poly(bisphenol A–diethylene glycol carbonate).[7] Fluorocarbon polymers constitute an industrially important class of polymers whose properties are quite distinct from those of the hydrocarbon analogues.[8] We report kinetic data on a fluorocarbon and hydrocarbon pair of model compounds that show significant differences in the rates and kinetic barriers to cyclization between simple $-(CF_2)_8-$ and $-(CH_2)_8-$ chain segments.

The 18-atom-chain species bis(1-pyrenylmethyl) dodecanedicarboxylate (**1**)[2d] and analogue **2** [1,8-bis[[(1′-pyrenylmethoxy)carbonyl]ethyl]perfluorooctane] containing a C_8F_{16} core in the center of the molecule were prepared by dicyclohexylcarbodiimide coupling of 1-pyrenylmethanol with the corresponding hydrocarbon and fluorocarbon diacids. Monopyrene-containing model compounds **3** and **4** were obtained similarly from the monomethyl ester acids. Steady-state fluorescence spectra[10] of **1** and **2** in 2-methyltetrahydrofuran (MeTHF) at various temperatures in the range −10 to +50 °C (λ_{ex} = 340 nm) are shown in Figure 1. Several features are notable. First, at all temperatures, both structured pyrene-localized emission (LE) at short wavelength (370–425 nm) and broad, structureless emission

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