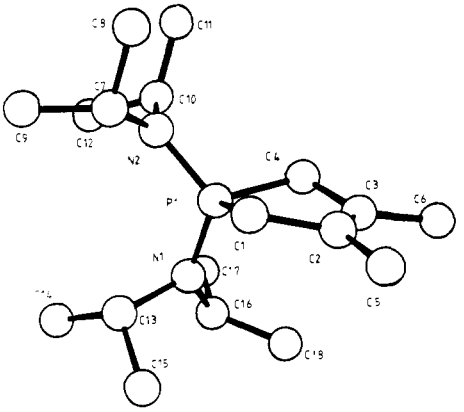

Figure 1. Structure of the 3-phospholenium cation **2a** showing the atom numbering scheme. Important parameters: C(2)-C(3) 1.313 (5), C(1)-C(2) 1.509 (5), C(3)-C(4) 1.526 (5), P(1)-N(1) 1.633 (3), P(1)-N(2) 1.620 (3) Å; N(1)-P(1)-N(2) 116.1 (2)°. The angle between the planes C(1)-P(1)-C(4) and C(1)-C(2)-C(3)-C(4) is 31.4°.

reactions of phosphenium ions with alkenes and alkynes are in progress.

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Registry No. 1, 68880-45-5; **2a**, 87712-41-2; **2b**, 87712-43-4; **2c**, 87712-45-6; **2d**, 87712-47-8; **2e**, 87712-49-0; **3a**, 87712-51-4; **3b**, 87712-53-6; **4a**, 87712-56-9; $[(Me_2N)_2P]^+$, 61788-01-0; $[(i\text{-}Pr_2N)(Cl)P]^+$, 87712-54-7; 2,3-dimethyl-1,3-butadiene, 513-81-5; isoprene, 78-79-5; 1,3-butadiene, 106-99-0; *trans*-1,3-pentadiene, 2004-70-8; *trans*-2,-*trans*-4-hexadiene, 5194-51-4.

Supplementary Material Available: Tables of atomic coordinates, thermal parameters, bond angles, and bond lengths for **2a** (5 pages). Ordering information is given on any current masthead page.

Electron Spin Echo Modulation Demonstrates P-450$_{scc}$ Complexation

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The initial structural information needed for understanding the chemical mechanism of an enzymatic reaction includes the topological relationship between the substrate and the protein active site. Unfortunately, most present pictures of such states come from indirect measurements: crystallographic studies of enzyme-inhibitor complexes[1] or examinations of reaction products derived enzymatically from mechanism-based inhibitors.[2] In a few cases, it has been possible to visualize enzyme-substrate complexes stabilized at low temperature, using crystallography.[3] Herein we report the first application of electron spin echo spectroscopy (ESE) to the estimation of interatomic distance in an enzyme-substrate complex.

This novel approach takes advantage of the sensitive detection of weak hyperfine interactions, between an unpaired electron and its environment, afforded by electron spin echo envelope modulation (ESEEM). In this technique, the application of an intense microwave field reorients the individual magnetic dipoles associated with an electron spin system. Sequences of pulses can be used to interrogate residual magnetic polarization after varying ensemble phase decay intervals. In rephasing after multiple perturbations the system emits an "echo" signal, which reflects the relaxation processes of the system. Thus, a plot of echo magnitude as a function of time between pulses can reveal not only the decay associated with magnetic relaxation mechanisms (T_m) but also periodic modulations resulting from coupling between the electron and neighboring nuclear spins. The depth and frequency of this modulation convey information as to the identity of nearby nuclei, their number, and the distance separating them from the electron spin. Consequently, one can probe directly the immediate magnetic environment of a paramagnet.[4]

Here we investigate the bovine adrenal side-chain cleavage enzyme, cytochrome P-450$_{scc}$. This membrane-bound hemoprotein catalyzes the transformation of cholesterol to pregnenolone via three successive hydroxylations, in the rate-determining step of adrenocorticosteroid synthesis.[5] P-450$_{scc}$ acts as well on a variety of related steroidal substrates; all of these reactions exhibit a high degree of regio- and stereospecificity.[6] We are concerned both with understanding the molecular basis for this specificity and in its implications for the mechanism of catalysis.

We examined the first step in the catalytic cycle of P-450$_{scc}$, i.e., the binding of substrates to the oxidized form of the enzyme. In order to distinguish substrate-derived modulations from those due to the proton background of solvent and protein residues, we have used steroids selectively deuterated at specific positions.[7] Our present concern has been with the initial site of cholesterol activation, carbon 22, and we have examined thus far three different substrates deuterated at that position: cholesterol-*22,22-d*$_2$, 22-hydroxycholesterol-*22-d* (the first intermediate in cholesterol turnover), and 20-azacholesterol-*22,22-d*$_2$. The results of three-pulse echo experiments[8] carried out at $g = 2.2$ for these enzyme-substrate complexes are shown in Figure 1. In the presence of unlabeled substrate, little modulation of the echo decay can be detected. A clear periodicity, however, is obvious in each of the deuterated samples. Analysis of the data for 22(*R*)-hydroxycholesterol-*22-d* (Figure 1a) is the most straightforward, since only one deuterium is involved. Fourier transformation of this spectrum reveals a modulation frequency of 1.9 MHz, well within the range expected for deuterium (with a free precession frequency of 1.93 MHz at 2950 G). Comparisons with simulated spectra,[9] as illustrated in Figure 2, imply a separation of 4 ± 1 Å between the deuteron at C22 and the unpaired spin. Inter-

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(9) Simulations were carried out by using the echo decay compensation analysis of Suryanarayana et al.;[10] in this technique, a best fit is obtained through a comparison of the ratio of maxima and minima in the echo amplitudes (as a function of time between pulses) in the experimental and simulated data. Our analysis assumes a 0.1 isotropic hyperfine term and a quadrupolar term of 0.05 MHz, based on nuclear quadrupole resonance data.[11]
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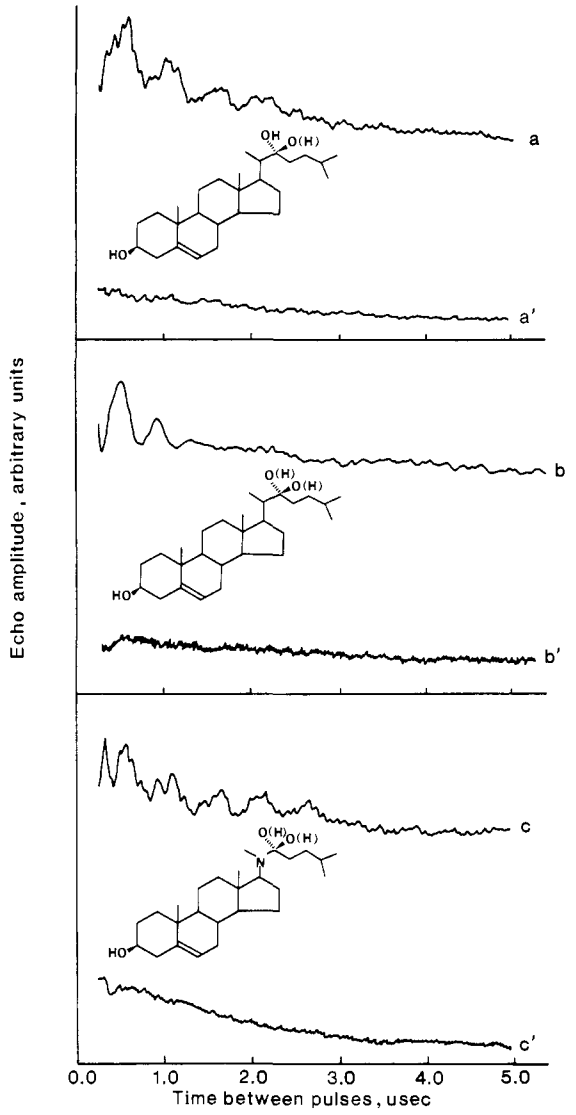

Figure 1. Three-pulse ESE modulation ($\pi/2-\tau-\pi/2-T-\pi/2-\tau$... echo) for ferric P-450$_{scc}$-substrate complexes, recorded at $g = 2.2$ (9.2 GHz), 6-7 K, $\tau = 250$ ns. Spectra are displaced for clarity. Substrates: (a) 22(*R*)-hydroxycholesterol-*22-d*; (a') 22(*R*)-hydroxycholesterol; (b) cholesterol-*22,22-d*$_2$; (b') cholesterol; (c) 20-azacholesterol-*22,22-d*$_2$; (c') 20-azacholesterol. Concentrations: P-450, 400–700 μM; steroids, 0.5–1 μM (1.5-fold over heme); in GPED (20% glycerol, 1 mM ethylenediamminetetraacetic acid disodium salt, 0.1 mM dithiothreitol, and 0.1 M potassium phosphate, pH 7.4).

estingly, we find no sign of ^{2}H modulation for the "unnatural" isomer 22(*S*)-hydroxycholesterol-*22-d*; this suggests strongly that the deuteron in this complex does not couple significantly to the electron spin and is thus presumably more than 6 Å from the heme.

In the cases of cholesterol-*22,22-d*$_2$ and 20-azacholesterol-*22,22-d*$_2$, slightly different modulation patterns are observed. Detailed analyses of these spectra are more difficult given the presence of two $I = 1$ nuclei and are presently under way. Clearly, however, both cholesterol and the 20-azacholesterol bind in close proximity to the heme.

These experiments demonstrate the potential of electron spin echo spectroscopy to probe the structural aspects of substrate binding to paramagnetic enzymes. The method may be of particular interest with membrane-bound systems (such as P-450$_{scc}$), which are not presently amenable to crystallographic investigations. By examining a more complete set of deuterated steroids it should be possible to approximate the relative position of a substrate with respect to the catalytic site of P-450$_{scc}$ under nonperturbing conditions; these studies are in progress.

Similar deductions for other enzymes have been derived from NMR relaxation measurements on substrate molecules in equilibrium with paramagnetic E·S complexes.[12] The NMR method requires the evaluation of multiple spin relaxation times and chemical exchange rate constants but can be done at room temperature. The ESEEM method requires no such additional information but usually must be carried out at very low temperatures. Both methods give interpretable data only when dipolar, Fermi contact and quadrupolar contributions can be estimated properly.

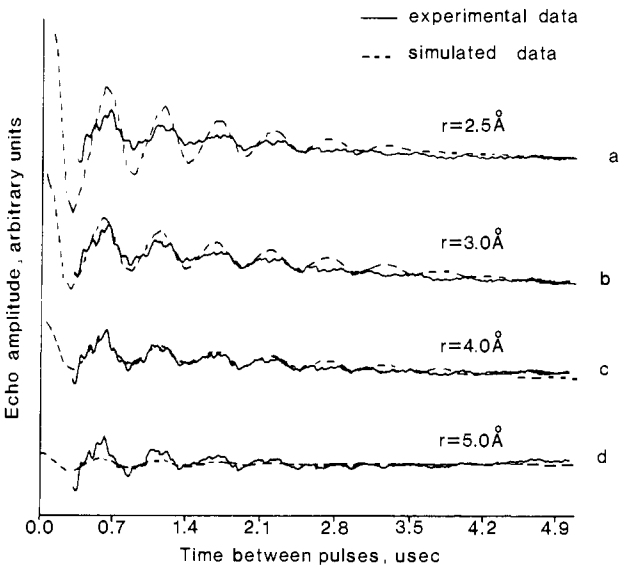

Figure 2. Comparison of experimental and simulated three-pulse ESE deuterium modulation for the data of Figure 1a. Simulations were according to the method of Suryanarayana et al.,[10] using a 0.1 isotropic hyperfine term and a quadrupolar term of 0.05 MHz.[11]

Acknowledgment. This work was supported by grants from the USDA (No. 5901-0410-9-0226-0) and the NIH (No. GM28358). S.E.G. acknowledges an NIH postdoctoral fellowship.

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Investigations of the Biosynthesis of Furanomycin. Unexpected Derivation from Acetate and Propionate

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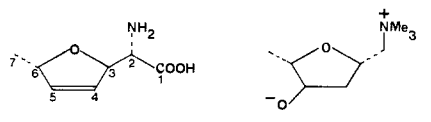
In 1967, Katagiri et al. reported the isolation of the novel antibiotic furanomycin (**1**) from *Streptomyces threomyceticus* (ATCC 15795).[1] The compound was found to be a competitive antagonist of L-isoleucine and to inhibit the growth of T-even coliphage. Furanomycin was synthesized in 1980 by Joullié and co-workers who also revised the stereochemistry to that shown in **1**.[2] The structure of furanomycin bears some resemblance to



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