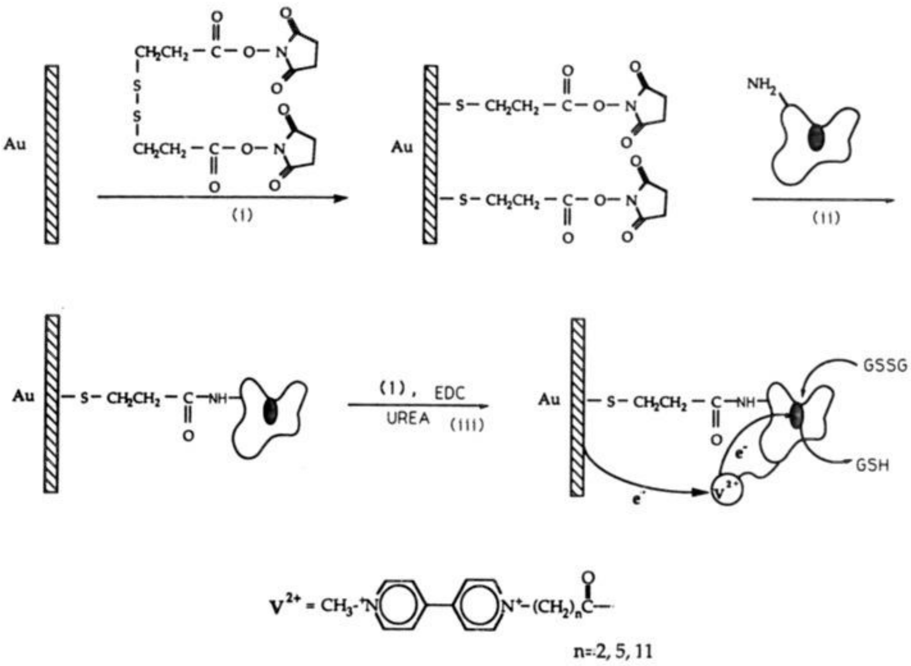
Scheme I. Sequence for Assembling the Monolayer of Glutathione Reductase Exhibiting Electrical Communication



−0.255 V at pH 7.0,[15] and thus the bipyridinium units are thermodynamically capable of mediating electron transfer to the active site.

It can be seen that electroreduction of GSSG proceeds with all bipyridinium-functionalized enzyme assemblies. Nevertheless, the rates of GSH formation depend on the alkyl chain length bridging the bipyridinium component with the protein backbone: As the chain length is increased, the electroreduction of GSSG is improved. Control experiments reveal that in the absence of functionalization of the protein by bipyridinium components no reduction of GSSG occurs. Similarly, when the modification of glutathione reductase by the bipyridinium components is performed without unfolding of the protein with urea, an electrically inactive protein assembly toward reduction of GSSG is formed, although the bipyridinium units are electroactive. These results clearly indicate that the bipyridinium units covalently linked to glutathione reductase mediate electron transfer from the Au electrode to the enzyme redox site. Unfolding of the protein by urea[9] during the redox modification is essential to substitute inner shell lysine residues with bipyridinium components acting as electron-transfer-mediating stations for electron tunneling to the active site. The improved electrical communication upon lengthening the alkyl chain bridges is attributed to enhanced intraprotein electron-transfer rates as a result of electron-donor distance shortening.[16] Namely, the flexible alkyl bridging "arms" generate shorter intraprotein electron-transfer distances, resulting in enhanced electrical communication (Scheme I). The fact that the electron-transfer rate from the electrode to the bipyridinium components is similar for **1b** and **1c** implies that the effectiveness of the electrical communication in the protein assemblies is controlled by the intraprotein electron-transfer process from the reduced anchored relays to the enzyme active site.

We thus conclude that tailored monolayer assemblies composed of redox-modified proteins linked to thiol-derivatized Au electrodes provide novel configurations for mediated electron transfer in enzymes. We find that other functionalized disulfides such as dimethyl 3,3′-dithiobis(propionimidate) can be applied as the monolayer microstructuring component for attachment of the enzyme to Au electrodes.

Further experiments to apply this concept to other redox enzymes and to design polymeric enzyme layers associated with thiolate monolayer structures are underway in our laboratory.

Acknowledgment. This research is supported by the Bundesministerium für Forschung und Technologie, Germany, and the Israeli Council for Research and Development, Ministry of Science and Technology, Israel.

Supplementary Material Available: Experimental procedure for the preparation of Au electrodes comprising self-assembled thiolated monolayers and glutathione reductase modified by bipyridinium tethers as electron-transfer mediators (1 page). Ordering information is given on any current masthead page.

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Effects of Hydration on the Claisen Rearrangement of Allyl Vinyl Ether from Computer Simulations

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The synthetic importance of the Claisen rearrangement in organic chemistry and biochemistry has stimulated numerous mechanistic studies.[1-6] A fundamental issue is the extent of dipolarity in the transition state (TS).[3-6] Substantial rate increases are observed in protic solvents for substituted cases that enhance putative enolate/allyl cation character; however, solvent effects on Claisen rearrangements in general and especially for the parent allyl vinyl ether (AVE) are often thought to be modest.[1,3,7] The present results challenge this notion and the importance of ion-pair development. Enhanced hydrogen bonding that accompanies more subtle polarization appears as the alternative explanation, and a desirable feature for catalysts is noted.

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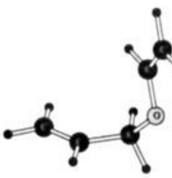
The initial aim was to obtain the change in free energy of hydration (ΔG_{hyd}) along a likely reaction path. Following an established procedure,[8] the minimum energy reaction path (MERP) in the gas phase was constructed first from ab initio calculations. Vance et al. previously supported the RHF/6-31G(d) level for this reaction.[9] Reaction path following[10] was performed[11] with this basis set to generate a 143 frame "movie" along the MERP from AVE (**1**) through the chair TS (**3**) to 4-pentenal (**5**). Five frames are illustrated, **1**–**5**. The conformers shown for **1** and **5** are ca. 1 kcal/mol above the global minima.



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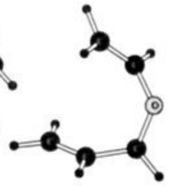

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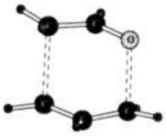

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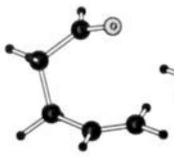

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The ΔG_{hyd} profile for the MERP then comes from Monte Carlo (MC) simulations. The intermolecular interactions are represented by Coulomb and Lennard-Jones terms with all atoms explicit.[12] The TIP4P model was adopted for water,[13] while standard OPLS Lennard-Jones parameters were used for the solute and scaled as the hybridization changed.[12] Most importantly, the partial charges for the solute were obtained by fitting to the 6-31G(d) electrostatic potential surfaces via CHELPG[14] calculations on the frames. Mulliken populations were considered; they yielded a similar $\Delta\Delta G_{hyd}$ between **1** and the TS, but provided the wrong ΔG_{hyd} order for **1** and **5**. The Mulliken charges also showed more variation in going to the 6-31+G(d,p) basis set. The simulations were executed in the NPT ensemble at 25 °C and 1 atm with Metropolis and preferential sampling.[15] The system consisted of 838 water molecules and the solute in a periodic cell, ca. 30 Å on a side. The BOSS program[16] perturbed the system between frames and computed the change in ΔG_{hyd} via statistical perturbation theory.[15,17] In all, 59 of the 143 frames were used, which required 29 separate MC simulations with double-wide sampling.[18] Each simulation entailed 1×10^6 configurations of equilibration followed by 4×10^6 configurations of averaging. Solute–water interactions were included for waters with an oxygen within 10.5 Å of any solute atom, and the water–water cutoff was set at 10.5 Å on the basis of the O–O distance.

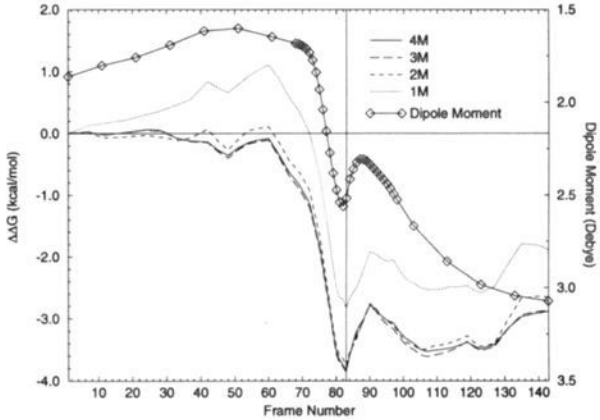

Figure 1. Computed changes in ΔG_{hyd} by averaging for $(1–4) \times 10^6$ configurations and in the 6-31G(d) dipole moment.

The convergence of the ΔG_{hyd} profile is illustrated by the four curves in Figure 1, which correspond to averaging for $(1–4) \times 10^6$ configurations. The TS at frame 83 has the lowest ΔG_{hyd}, 3.85 ± 0.16 kcal/mol below the reactant. The implied rate increase in water is a factor of 644 over the gas phase. This assumes no solvent dynamical effects on the barrier crossing, as in classical TS theory.[19] Other computed $\Delta\Delta G_{hyd}$ values for the AVE TS in water are 2.1 kcal/mol from Still's GB/SA treatment[20] with the present structures and charges and 0.75 kcal/mol from an AM1-SM2 study.[21] Perusal of the experimental data starts with the relative rates measured at Cornell for AVE, 1:4:58 in di-*n*-butyl ether, ethanol, and 2:1 methanol/water at 75 °C.[4] These give a Grunwald–Winstein *m* value of 0.4, which leads to a predicted relative rate of 876 in water; however, kinetic data on 4-substituted AVEs in solvents from cyclohexane to water suggest a value closer to 150.[5] Furthermore, the gas-phase kinetics indicate a rate of 0.031×10^{-6} s^{-1} at 75 °C,[22] while the rate in di-*n*-butyl ether extrapolated to 75 °C is 0.276×10^{-6} s^{-1},[2] a factor of 9 higher. Thus, when the range of media is extended from the gas phase to water, a substantial rate acceleration for AVE indeed emerges, ca. 10^3 at 75 °C. Another potential comparison is for $\Delta\Delta G_{hyd}$ between **1** and **5**, −2.88 kcal/mol in Figure 1. Experimental data are not available for these compounds; however, aldehydes are well-established to have lower ΔG_{hyd}'s than analogous ethers by 1–2 kcal/mol.[23]

The origin of the computed acceleration in not partial ionization. The net CHELPG charges on the $H_2C{=}CHO$ unit in AVE and the TS are −0.287 and −0.273 e, although solvent polarization of the solute was not included.[21] The simulations do show enhanced hydrogen bonding to the oxygen in the TS. Analysis gives average numbers of hydrogen bonds of 0.9, 1.9, and 1.7 for the reactant, TS, and product with average strengths of −3.4, −4.7, and −4.5 kcal/mol, respectively.[24,25] Typical examples from MC configurations are shown in **6**–**8**. The one and two hydrogen bonds with water for ether and carbonyl oxygens are normal.[8,24,25] There is enhanced polarization of the HC2O unit in proceeding from AVE to the TS that promotes hydrogen-bond acceptance; the O becomes more negative by 0.07 e, which more than offsets the +0.04 e change for HC2.[26] Continuing to **5**, the O gains an additional 0.05 e; however, HC2 loses 0.17 e. The lengthening of the C4—O

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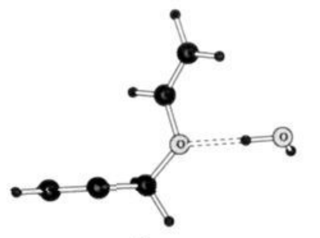

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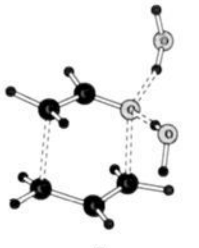

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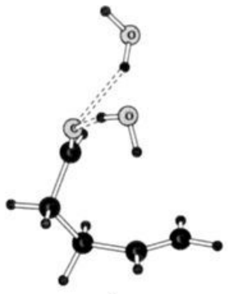

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bond to 1.92 Å in the TS from 1.41 Å in AVE also increases the solvent accessibility of the O. Simultaneously, the dipole moment increases from 1.9 *D* for **1** to 2.5 *D* for the TS; it continues to increase to the product, while $\Delta\Delta G_{\text{hyd}}$ levels off (Figure 1). Overall, the results promote catalyst designs that incorporate two or more hydrogen-bond-donating groups positioned to interact with the oxygen in the TS (**7**).

Acknowledgment. This work was sponsored by the National Science Foundation.

Supplementary Material Available: Listings of 6-31G(d) geometries in *Z*-matrix format and the potential function parameters for the reactant, TS, and product (2 pages). Ordering information is given on any current masthead page.

Cation Radical Probes. Development and Application to Metalloporphyrin-Catalyzed Epoxidation

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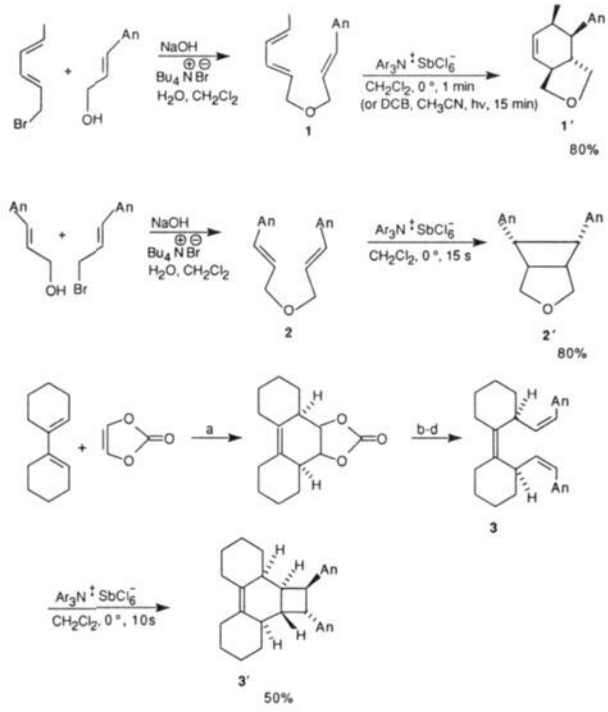
Radical probes such as the cyclization of the 5-hexenyl radical have proved highly effective in diagnosing free radical mechanisms.[1] Analogous cation radical probes would constitute a powerful tool for detecting short-lived cation radical intermediates in the burgeoning area of hole catalytic chemistry.[2,3] The synthesis of conveniently accessible cation radical probe substrates and the characterization and calibration of their rapid intramolecular probe reactions as cation radical clocks are now reported. The new probes have further been used to investigate the hypothetical electron-transfer mechanism for metalloporphyrin-catalyzed epoxidation.[4,5]

Scheme I. Synthesis of Probe Substrates and Characterization of the Probe Reactions



An = 4-MeOC$_6$H$_4$- ; Ar = 4-BrC$_6$H$_4$- ; DCB = 1,4-Dicyanobenzene

a. Δ, 24h, 270°C ; b. LAH, Et$_2$O, 1 h, r.t. ; c. Pb(OAc)$_4$, benzene, 1h, r.t.;

d. AnCH$_2$PPh$_3^+$ Cl$^-$, n-BuLi, THF, 3 hr, r.t.

The cation radical probe reactions developed in this study are based upon the intramolecular cation radical Diels–Alder reaction[6] and analogous intramolecular cyclobutanation.[7] The choice of substrates **1** and **2** was based upon synthetic accessibility and reactivity considerations. Both are conveniently prepared by phase-transfer-catalyzed coupling of readily available alcohols and bromides (Scheme I). Although the probe reaction of **2** (cyclobutanation) would appear to be inherently less favorable than that of **1** (Diels–Alder addition), it is significant that the trapping moiety of $\mathbf{2}^{\bullet+}$ (a neutral moiety of the *trans*-anethole type) is much more reactive toward a cation radical moiety than is the neutral diene moiety of $\mathbf{1}^{\bullet+}$.[8] In both instances the cation radical moiety is of the *trans*-anethole type. Further, $\mathbf{1}^{\bullet+}$, but not $\mathbf{2}^{\bullet+}$, requires prior s-trans to s-cis isomerization of the diene moiety.[9] Conformational contributions to the activation free energy of cyclization are further minimized in the preorganized probe molecule **3**. The efficient cyclizations of $\mathbf{1}^{\bullet+}$, $\mathbf{2}^{\bullet+}$, and $\mathbf{3}^{\bullet+}$ were characterized synthetically under standard aminium salt conditions [tris(4-bromophenyl)aminium hexachloroantimonate, CH_2Cl_2, 0 °C]. The already well-characterized Diels–Alder cycloaddition of 1,3-cyclohexadiene (**4**) has also been exploited as a bimolecular probe reaction.[10]

Calibration of the probe reaction of $\mathbf{1}^{\bullet+}$ was accomplished via standard quenching studies, using 1,4-dicyanobenzene as the photosensitizer and 1,2,4-trimethoxybenzene as the quencher in acetonitrile ($k_{\text{DA1}} = 3 \times 10^7\ \text{s}^{-1}$).[11] The intramolecular cyclobutanation of $\mathbf{2}^{\bullet+}$ (rate constant ≡ k_{CB2}) was then calibrated via

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