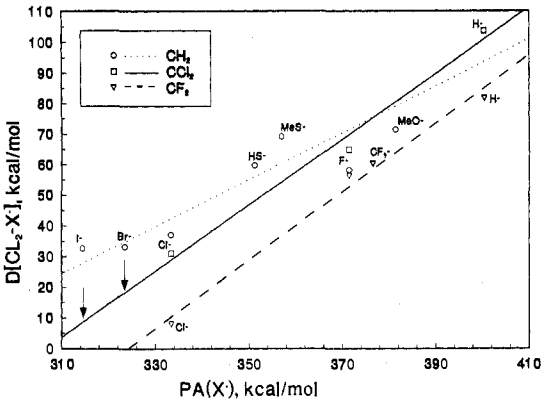

Figure 2. Correlation of proton affinities and singlet carbene binding energies for negative ions.

on the Lewis acidity of the carbene carbon. Reaction between CH_3OCH_2Cl and OH^- in the flow tube yields several products, among which is an $[M - H]^-$ ion. Exclusive deprotonation at the chloromethyl group is indicated by the absence of any $[M - D]^-$ product in the analogous reaction with CD_3OCH_2Cl. The CH_3OCHCl^- ion exhibits a Cl^- appearance plot from CID that closely resembles that for CF_2Cl^-, and it is observed to undergo exothermic transfer of Cl^- to c-C_5H_{10} and CS_2 ($D[CS_2-Cl^-]$ = 11.7 kcal/mol).[28] Low-level (4-31G) optimization of the geometry of CH_3OCHCl^- yields a C_1 "carbanion" structure with an unusually long C-Cl bond (2.6 Å). However, this carbanion structure is unstable at higher levels of theory with respect to Cl^- loss and rearrangement to the minimum energy geometry shown in Figure 1B. This remarkable structure incorporates a free carbene moiety and a Cl^- ion electrostatically bound to the backside of the methoxy group in a manner reminiscent of gas-phase S_N2 intermediates.[29] The computed Cl^- binding energy (ΔH_{298}) for this structure at the MP3/6-31+G(d)//6-31+G(d) and MP2(full)/6-31+G(d)//MP2(full)6-31+G(d) levels is 11-12 kcal/mol, in good agreement with our experimental results.

The Cl^- binding energies of singlet carbenes provide a direct measure of their relative Lewis acidities. We[30] and others[31] have previously noted the good linear correlations that often can be found between the binding energies of a series of negative ions to specific neutral Lewis acids and to the proton, i.e., between their Lewis and Bronsted basicities. Figure 2 illustrates a set of such correlations for singlet carbenes, where the requisite thermodynamic data for the various carbanions (CL_2X^-), negative ions (X^-), and carbenes (CL_2) are taken from results from our laboratory[19] or established literature data.[32] The linear relationship between $PA(X^-)$ and $D[CL_2-X^-]$ for singlet CH_2, CF_2, and CCl_2 extends over a wide range of anion basicities and provides a predictive capability for identifying additional candidates for gas-phase carbene/anion complexes. Thus, one predicts from the correlations that neither Br^- nor I^- will exhibit any binding other than weak electrostatic attraction to singlet CF_2. Moreover, Br^- should possess a ca. 20 kcal/mol bond strength toward singlet CCl_2, whereas I^- should have a CCl_2 bond energy of about half as much. After formulating these predictions, we generated a sample of the CCl_2Br^- ion in the flowing afterglow by deprotonating $CHCl_2Br$ and carried out energy-resolved CID studies. An appearance energy for Br^- of 0.9 eV (21 kcal/mol) was obtained, in excellent agreement with the predicted $D[CCl_2-Br^-]$ value from the correlation.

We are currently examining the gas-phase reactions of these unusual species with olefins and other reagents in pursuit of further evidence for their carbene character.

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Electron-Transfer Reaction of Cytochrome *c* Adsorbed on Carboxylic Acid Terminated Alkanethiol Monolayer Electrodes

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Protein electron-transfer (ET) kinetics is a topic of intense current interest.[1] Recent experimental progress has been due largely to the use of donor-acceptor species for investigating diffusionless intramolecular ET reactions.[1,2] Significant developments have also been reported for self-exchange reactions[1,3] and for direct electrochemical ET reactions.[4] Recently it has become clear that the ET kinetics of strongly adsorbed native proteins can be investigated from an *intramolecular* perspective using voltammetry, as shown for cytochrome *c* (cyt *c*) (sub)monolayers on tin oxide[5] and bis(4-pyridyl) disulfide modified gold.[6] This type of voltammetric approach is well suited for probing fundamental kinetic aspects of ET reactions[7] and was recently used to determine the reorganization energy of cyt *c* adsorbed on tin oxide.[5d] A highly desirable goal in this regard would be the development of electrode/adsorbed protein systems of controllable

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and well-defined geometry. We report here preliminary results on the use of self-assembled alkanethiol monolayers on gold to study the ET reaction of horse heart cyt *c*.[8] In this approach, cyt *c* was irreversibly adsorbed on carboxylic acid terminated alkanethiol monolayers, and the redox behavior of the cyt *c*/monolayer complex was examined by using cyclic voltammetry.

Self-assembled monolayers formed by the chemisorption of long-chain alkanethiols on gold are receiving much attention because of the control they offer for the facile formation of chemically and structurally well-defined organic surfaces.[9] Several groups have explored the influence of structure and distance on diffusionless ET reactions by covalently attaching electroactive species to the alkanethiol adsorbate.[10] The densely packed hydrocarbon chains anchor the electroactive group to the electrode and rigidly hold it a well-defined and controllable distance from the electrode surface. Using a similar approach, we hoped to further exploit the structural control afforded by the alkanethiol/Au system to construct a surface that would irreversibly bind cyt *c* in an active state. A carboxylic acid terminated monolayer surface was selected to provide favorable electrostatic binding with cyt *c*.[11] At pH 7, the monolayer surface is expected to have a net negative charge which would lead to attractive interaction with positively charged lysines on the surface of cyt *c*.[12]

Freshly sputter deposited Au films (~2000 Å) on silicon wafers were equilibrated in ethanol solutions of $\sim 10^{-3}$ M 16-mercaptohexadecanoic acid [$HS(CH_2)_{15}COOH$] (MHDA) for 1 day.[13] Characterization of the resultant MHDA monolayers by ellipsometry, infrared spectroscopy, and X-ray photoelectron spectroscopy gave results, in close agreement with previous studies,[9e,f,i] that demonstrated the formation of densely packed, crystalline-like assemblies with the surface consisting primarily of carboxylic acid functionalities. MHDA films were used immediately for cyt *c* adsorption experiments because prolonged exposure to the laboratory ambient atmosphere appeared to result in deleterious surface contamination. Figure 1A shows a typical background cyclic voltammogram obtained from an MHDA/Au electrode in pH 7, 4 mM potassium phosphate buffer. The charging current is about a factor of 50 less than that for bare Au and reflects the dense packing of the MHDA monolayer.[14]

The cyt *c* adsorption process was performed by filling the cell with ca. 0.5 mL of 30 μM purified ferricyt *c*[15] in 4 mM, pH 7 phosphate buffer and allowing 20 min for equilibration, after which the cell was emptied, rinsed three times, and filled with the same buffer. Figure 1A shows a typical cyclic voltammogram obtained for cyt *c* adsorbed on an MHDA/Au electrode. The peaks are due to the one-electron oxidation and reduction of adsorbed cyt *c*. Rinsing the cell with buffer four additional times resulted in nearly identical current–voltage traces, which demonstrates that desorption is negligible. We estimate the surface coverage of electroactive cyt *c* to be 0.5–1 monolayers by integration of the charge under the anodic peak.[16] The cyt *c*/MHDA surface formal potential, taken as $(E_{p,c} + E_{p,a})/2$, was +0.19 V vs NHE, which is some 60–70 mV negative of the solution $E^{\circ\prime}$.[17] This adsorption-induced negative shift is greater than that previously observed for binding to tin oxide electrodes[5c] or to other proteins,[18] but agrees well with $E^{\circ\prime}$ shifts for cyt *c* bound to mitochondrial membranes.[19] These results suggest the interesting possibility that the interaction of cyt *c* with MHDA may be similar to that found in physiological environments.

Figure 1B shows cyclic voltammograms of the cyt *c*/MHDA system acquired at different potential scan rates. Peak separations were used to calculate an apparent ET rate constant (k_{et}°) at zero overpotential of 0.1 s^{-1}.[20] This value is 10–100 times smaller than k_{et}° for the cyt *c*/tin oxide system and indicates an extremely nonadiabatic reaction. Using the conventional expression,[1] $k_{et}^{\circ} = \nu \exp[-\beta(d - d_0)] \exp(-\Delta G^*/RT)$, with $\nu = 10^{13}$ s^{-1},[1a,c] $\beta = 1.0$ $Å^{-1}$,[1c] $d_0 = 3$ Å,[1a] and $\Delta G^* = 11$ kJ/mol (i.e., the intrinsic activation free energy determined previously for cyt *c* adsorbed on tin oxide[5d]), we calculate an ET distance of $d = 31$ Å, which agrees well with $d = 29$ Å estimated from the MHDA and cyt *c* structures. That is, if we assume that cyt *c* is electrostatically oriented with its exposed heme edge facing the film, the calculated tunneling distance is 24 Å for the MHDA film[9e] (along the chain direction) plus an additional 5 Å[1a] to the heme edge.[21] The close correspondence between the structural and kinetic determinations of ET distance may be somewhat fortuitous in light of the uncertainties regarding protein orientation and the assumed kinetic parameters.[22] Furthermore, it should be noted that the experimental cyclic voltammograms do not exhibit the ideal wave shape predicted by Laviron's model.[20] The broadness of these peaks suggests an adsorbate distribution[20b] perhaps involving film defect sites.[10a,23] Although these issues require closer scrutiny, the general features of the experimental strategy and kinetic analysis appear sound.

In summary, we have demonstrated the feasibility of using carboxylic acid terminated alkanethiol monolayers to study the

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(13) MHDA was synthesized and purified according to published procedures.[9e] A Cr layer of ~50 Å was deposited on the silicon to improve adhesion of the Au layer.

(14) Differential capacitance calculated from the background cyclic voltammogram is 1.3 $\mu F/cm^2$ (at 0.0 V vs NHE, scan rate of 50 mV/s), which is comparable to values reported for $HO_2C(CH_2)_{10}SH/Au$[9i] after accounting for the difference in monolayer thickness.

(15) Sigma type VI horse heart cyt *c* was chromatographically purified according to the following: Brautigan, D. L.; Ferguson-Miller, S.; Margoliash, E. *Methods Enzymol.* **1978**, *53D*, 128–164.

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(21) A tunneling distance of 29 Å would appear to be the minimum possible assuming that the alkanethiol monolayer is compact and that the adsorbed cyt *c* does not penetrate to any significant extent. The maximum possible tunneling distance would be ca. 40 Å for very unfavorable orientations.

(22) (a) The value of β used here was obtained from protein tunneling experiments;[1c] β for the MHDA part of the tunneling path may be different. (b) ΔG^* may be somewhat different for MHDA compared to tin oxide[5d] in light of the fact that adsorption appears to be stronger on MHDA. We plan to measure the actual value for cyt *c*/MHDA. (c) The correct value for the frequency factor (ν) remains controversial.[2d]

(23) ET at defect sites undoubtedly occurs; however, the high cyt *c* coverage indicates that the major part of the faradaic current results from ET through intact MHDA domains.

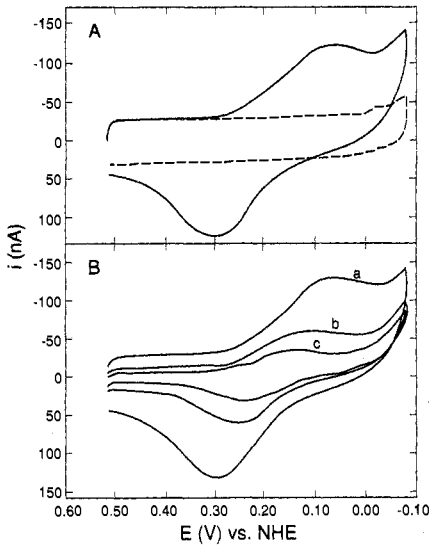

Figure 1. (A) Cyclic voltammograms acquired at a scan rate of 50 mV/s of an MHDA monolayer covered Au electrode (dashed) and the same electrode after adsorption of cyt *c* on the MHDA monolayer (solid). In both cases, the supporting electrolyte is 4 mM potassium phosphate buffered at pH 7. See text for details of cyt *c* adsorption procedure. (B) Cyclic voltammograms of cyt *c* adsorbed on an MHDA monolayer at scan rates of (a) 50, (b) 20, and (c) 10 mV/s. Solution conditions are the same as in part A. Electrode area = 0.32 cm^2.

ET reaction of adsorbed cyt *c*. Through synthetic manipulation, we expect that the self-assembled alkanethiol monolayer system will be very useful for controlling protein/monolayer surface interactions as well as the nonadiabaticity of protein ET reactions. Moreover, the monolayers may serve as useful analogues of biological membranes as suggested by the redox thermodynamic data given above.

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Biosynthesis of Coronatine: Investigations of the Biosynthesis of Coronamic Acid

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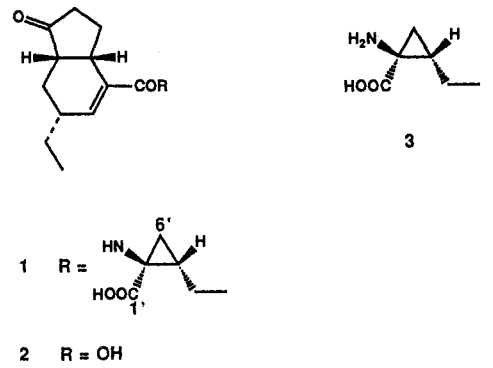
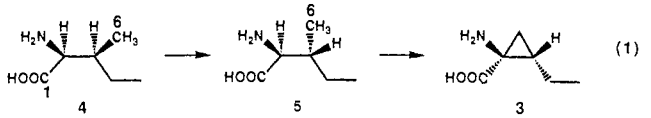
Coronatine (**1**) is a phytotoxin of novel structure isolated from liquid cultures of the plant pathogens *Pseudomonas syringae* pv. *atropurpurea*[1,3] and *Ps. syringae* pv. *glycinea*.[2,3] Infection of the host plants by these bacteria induces chlorosis on the leaves due to the production of coronatine.[1,2] The coronatine molecule is constructed from two moieties of distinct biosynthetic origin, coronafacic acid (**2**) and coronamic acid (**3**). Previous investigations in our laboratory have shown that coronafacic acid (**2**) is a polyketide derived from five acetate units and one pyruvate unit and that coronamic acid (**3**) is derived from isoleucine.[4] We now report the results of investigations that provide some insight into the mechanism of conversion of isoleucine into coronamic acid.



In previous studies, a mixture of (1-^{13}C)-DL-isoleucine and (1-^{13}C)-DL-alloisoleucine was found to label coronatine specifically at C-1′. This experiment provided no information on the stereochemistry of the amino acids that serve as precursors of coronamate. The absolute configuration of coronamic acid[5] suggests that the compound should be derived from L-isoleucine (**4**) via L-alloisoleucine (**5**) (eq 1). This hypothesis was evaluated by



administration of commercially available (1-^{13}C)-L-isoleucine and (1-^{13}C)-L-alloisoleucine to *Ps. syringae* pv. *glycinea* and isolation of coronatine as its methyl ester. The results (Table I, experiments 1 and 2) show that both of these amino acids are specifically incorporated into coronamic acid, but that L-alloisoleucine is a much more efficient precursor. Since the cyclization of the amino acid L-alloisoleucine to a cyclopropane derivative is a highly unusual process, we decided to verify that the methyl group of the amino acid is indeed the source of the cyclopropane bridge. This was accomplished by administration of a mixture of (6-^{13}C)-DL-isoleucine and (6-^{13}C)-DL-alloisoleucine[6] to *Ps. syringae*. The results (Table I, experiment 3) clearly show that the cyclopropane bridge is derived from the methyl group of L-alloisoleucine. These observations set the stage for a mechanistic probe of the cyclization mechanism. A mixture of (6-^{13}C,6-$^{2}H_3$)-DL-isoleucine and (6-^{13}C,6-$^{2}H_3$)-DL-alloisoleucine was synthesized[7] and administered to *Ps. syringae*. Examination of the carbon-13 NMR spectrum of the coronatine methyl ester obtained from this fermentation while carrying out simultaneous ^{1}H and ^{2}H decoupling revealed the presence of a new ^{13}C resonance shifted upfield by 0.59 ppm relative to the signal for C-6′ of coronatine. The magnitude of the upfield shift indicates that two deuterium atoms are located at C-6′.[8] The conversion of L-alloisoleucine to coronamic acid therefore proceeds with the removal of one hydrogen atom from C-6 of the precursor. It is also noteworthy that the level of incorporation observed in this experiment (Table I, experiment 4) was dramatically lower than that observed when no deuterium was present at C-6 (Table I, experiment 3). This difference in incorporation efficiency is probably due to the operation of a substantial deuterium isotope effect.

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