Latimer redox potential of +0.40 V vs NHE for the half reaction[23]

$$O_2 + 2H_2O + 4e^- \rightleftharpoons 4OH^-$$

The drastic anodic shift of a redox potential observed in **2** can be understood in view of the redox potential of the couple Cu(II)/$[Cu^I(CN)_2]^-$ in basic medium which is as high as +1.103 V vs NHE.[23]

The oxidation of *N*-methylaziridine into cyanide is unprecedented. It requires deprotonation steps, proved by the strong increase of the reaction rate in basic media. The ease of the reaction in nitromethane in respect of other solvents is likely due to the participation of the basic $CH_2NO_2^-$ form. The analogy with the function of the EFE in the biological oxidation of ACC[6,7] lies in the production of CN^-, involving two sequential one-electron oxidation steps at positive potentials. The system $[2Cu\subset\mathbf{1}]^{4+}$ plays the role of oxidant, giving the $[2Cu\subset\mathbf{1}]^{2+}$ complex, while *N*-methylaziridine is oxidized in cyanide. $[2Cu\subset\mathbf{1}]^{2+}$, in the presence of the generated cyanide and under air, leads to the mixed valence compound **2**.[8]

Supplementary Material Available: Tables of atomic coordinates and thermal parameters and cyclic voltammograms with related peak parameters (3 pages); tables of observed and calculated structure factors (5 pages). Ordering information is given on any current masthead page.

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Striking Changes Observed in Key Acyl-Enzyme Linkages by Resonance Raman Experiments Near 77 K

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The thermal processes occurring in an enzyme's active site are of high interest in attempting to delineate the efficient action of an enzyme on its bound substrate.[1-3] Questions such as the local temperature, the means of energy migration, the interchange among conformational substates,[4] and the thermalization of vibrational modes along the reaction pathway are often posed without the support of experimental data. Since resonance Raman (RR) spectroscopy provides a vibrational spectrum associated with enzyme-substrate linkages[5,6] and since the vibrational spectrum is sensitive to conformation and dynamical fluctuations, comparison of normal- and low-temperature RR data on enzyme-substrate complexes provides access to some of the changes occurring in the active site upon thermalization.

We report here low-temperature RR data for two acyl-enzyme complexes involving papain,[7] *N*-benzoylglycine dithioacyl- and methoxycarbonyl-L-phenylalanylglycine (PheGly) dithioacyl papain which are from a relatively "poor" and "good" substrate, respectively. For both intermediates, freezing in an ice matrix at 77 K results in a change in the C_α-CS(thiol) torsional angle in a direction away from that expected for the transition state for deacylation—i.e., a change consistent with deactivation. Moreover, at 77 K both intermediates show major RR intensity enhancement of a mode associatedwith the C-S linkage from cysteine-25[6] in the enzyme's active site.

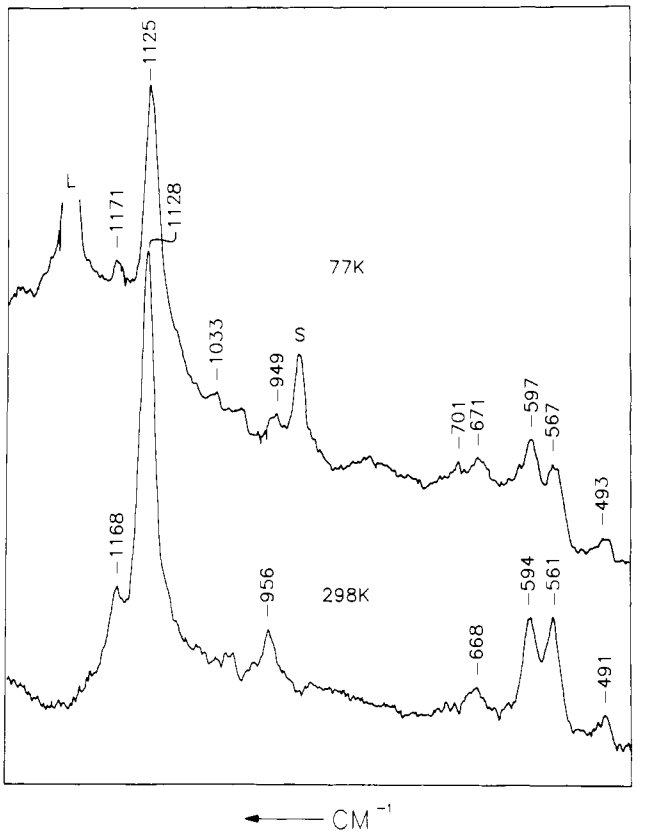

Figure 1. RR spectra of *N*-benzoylglycine dithioacyl papain in solution at room temperature (bottom); in ice matrix near 77 K (top). L = laser plasma line, S = peak due to CH_3CN used to carry substrate into solution.

Upon freezing a reaction mixture containing *N*-benzoylglycine dithioacyl papain to ca. 250 K the intense 1128 cm^{-1} RR peak (Figure 1) moves to 1125 cm^{-1} (note that data at 250 K are *not* shown in Figure 1; we have RR spectra from 12 separate samples at 250 K; all show the small shift in the 1128 cm^{-1} feature). This could be due to the phase change associated with freezing, which commonly brings about changes in vibrational frequencies. An alternative explanation is that the shift to 1125 cm^{-1} is brought about by a 10-15° reduction in Ψ', the NHC-CS (thiol) torsional angle (see below). Upon reducing the temperature to 77 K an additional change occurs in the 668 cm^{-1} feature in the form of major intensity enhancement (≈1.8-fold relative to other RR features), and a shoulder appears near 700 cm^{-1} (Figure 1).[9] Since the 668- and 700-cm^{-1} bands are probably both due to ν_{S-C} (cysteine), the detection of the additional 700-cm^{-1} feature is evidence for conformational heterogeneity in cysteine-25's S-C-C linkages.

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(8) Brocklehurst, K.; Willenbrock, F.; Salih, E. Cysteine Proteinases. A chapter In *Hydrolytic Enzymes*; Neuberger, A., Brocklehurst, K., Eds.; Elsevier: Amsterdam, 1987.
(9) RR spectra are obtained by using 324 nm Kr^+ excitation, a Spex triplemate and intensified diode array detection (Carey, P. R.; Sans Cartier, L. R. *J. Raman Spectrosc.* **1983**, *14*, 271). Low-temperature data were obtained by using back-scattering geometry from the sample in a helium cryostat modified so that the sample could rotate and move in the vertical plane (Sans Cartier, L. R.; Tonge, P. J.; Carey, P. R. *Ind. J. Phys.*, in press).

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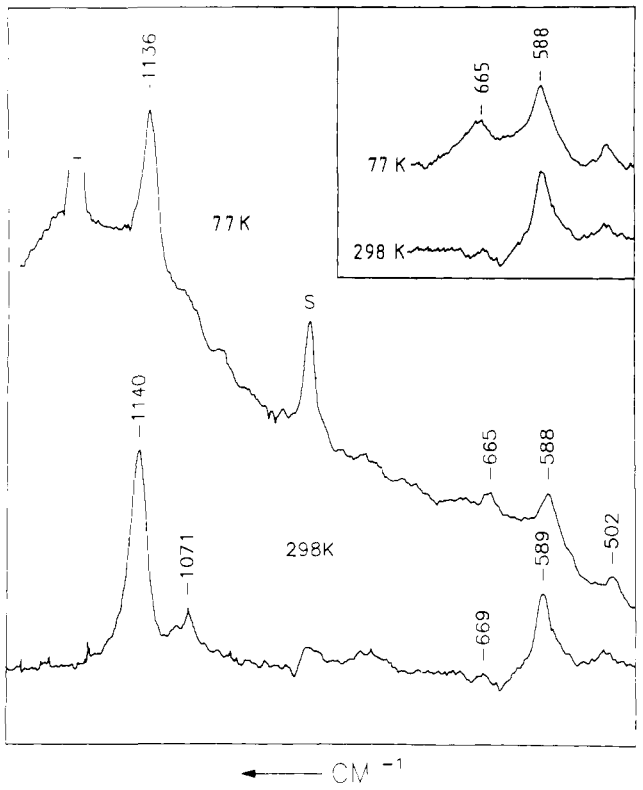

Figure 2. RR spectra of methoxycarbonyl-L-phenylalanylglycine dithioacyl papain in solution at room temperature (bottom); in ice matrix near 77 K (top). L = laser plasma line, S = peak due to CH_3CN used to carry substrate into solution. Inset: 450–750 cm^{-1} spectral regions of room temperature and 77 K traces compared, with intensities of the 588-cm^{-1} peaks equalized.

PheGly dithioacyl papain is a "good intermediate" possessing the correct Phe side chain-enzyme and several enzyme-substrate C=O···H–N H-bond linkages to ensure proper orientation in the active site[10] (where the substrate can be said to "zip-up" the two lobes of papain). Unlike the *N*-benzoylglycine intermediate freezing at 250 K causes no change in the Ψ' sensitive 1140-cm^{-1} band (data not shown; RR spectra from six experiments going from room temperature to 250 K are superimposable). This is a surprising result since it shows that the normal mode pattern of the dithioacyl group in the active site is not measurably perturbed by the phase change in the surrounding solvent. Thus the zippered-up structure seems "protected" from solvent freezing. However, upon lowering the temperature to 77 K[11] the 1140-cm^{-1} band decreases to 1136 cm^{-1} (Figure 2, identical results were obtained for four separate samples). The favored explanation is that removing thermal energy from the protein matrix allows Ψ' to relax by approximately 15° toward a nonstrained (smaller) value found in the corresponding PheGly ethyl dithio ester model compound.[12] The obverse of this inference is that the thermal energy available in the protein at room temperature drives Ψ' toward a value closer to that found in the transition state for deacylation.[13,14]

The PheGly dithioacylpapain, just as for the *N*-benzoylglycine derivative, shows an intriguing increase (3-fold, relative to other RR features) in the 669-cm^{-1} peak intensity at 77 K (Figure 2). The increase could simply correspond to a better mapping of the vibrational mode onto the excited electronic state at low temperature. However, it is remarkable that this feature is from a normal mode associated with an enzyme bond, S–C of cysteine-25,[6] and that the same effect is seen for both "good" (PheGly) and "poor" *N*-benzoylglycine) intermediates. This demonstrates that the vibrational and/or electronic properties of the cysteine-25 S–C bond vary markedly with temperature, and future studies should address the question of whether these properties are used by the enzyme to assist the catalytic process.

Work is in progress to measure the temperature dependence of the RR spectral changes between 77 and 250 K and to extend our observations to liquid helium temperatures.

(10) Reference 8 p 90.

(11) Under the experimental conditions employed, in which the sample is moved in the laser beam, we estimate that the maximum rise in temperature due to the laser beam (≈100 mW power) is 10 K.

(12) Several lines of evidence suggest a relationship exists between Ψ' and the exact position of the 1140-cm^{-1} peak and this peak position has been discussed before in terms of conformational activation upon going from a substrate based on a single amino acid to a di-, tri-, and tetrapeptide: Angus, R. H.; Carey, P. R.; Lee, H.; Storer, A. C. *Biochemistry* **1986**, *25*, 3304. Recent RR and X-ray crystallographic analyses of *N*-(β-phenylpropionyl)-glycine (Huber, C. P. *Acta Crystallogr.* **1987**, *C43*, 902) and *N*-methoxycarbonyl-L-phenylalanylglycine- (Varughese, K. I.; Huber, C. P.; Storer, A. C.; Carey, P. R., manuscript in preparation) ethyl dithio esters show that a change in Ψ' from −19 to −30° changes the position of the intense band from 1134 to 1137 cm^{-1}.

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(14) Increasing Ψ' increases the N···S (thiol) distance and reduces the weak N···S bonding interaction (Varughese, K. I.; Storer, A. C.; Carey, P. R. *J. Am. Chem. Soc.* **1984**, *106*, 8252). For a series of *N*-benzoylglycine dithioacyl papains it was shown that the N···S contact was broken in the rate-determining step for deacylation (Carey, P. R.; Lee, H.; Ozaki, Y.; Storer, A. C. *J. Am. Chem. Soc.* **1984**, *106*, 8258).

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Acid-Catalyzed Electron-Transfer Processes in Reduction of α-Haloketones by an NADH Model Compound and Ferrocene Derivatives

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Nonenzymatic reductions of substrates by reduced nicotinamide adenide dinucleotide (NADH) and NADH model compounds have been extensively studied in order to understand the mechanisms of enzymatic reductions of substrates by NADH.[1,2] However, model studies have hitherto revealed that NADH or NADH model compounds can reduce thermally only activated carbonyl compounds, which are rather strong oxidants, although the excited states of NADH and NADH model compounds can reduce a variety of substrates.[3] Since acid catalysis is known to play an essential role in the enzyme-catalyzed reduction of carbonyl compounds by NADH,[4] it is required to explore the acid-catalyzed reduction of nonactivated carbonyl compounds which would otherwise be reduced only by the presence of an appropriate enzyme.[5] We wish to report herein the first successful reduction of α-haloketones, which are weak oxidants, by an acid-stable NADH model compound, 10-methylacridan ($AcrH_2$),[6,7] in the

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