cluster stabilizer that prevents aggregation of the gold particles.

The presence of gold clusters in these polymeric composites was also evident from the metal plasmon absorption measured at ~530 nm. This plasmon absorption exhibited a noticeable solvent dependency as a function of time. When Au_x/DPBD/poly-DPBD dispersions were dissolved in either toluene or acetone, brown solutions were initially formed with identical UV/vis spectra. The toluene solution remained brown, and its spectrum did not change, whereas the acetone solution became reddish with time. The spectral changes, reflected in Figure 2, amount to the plasmon absorption band shifting to shorter wavelength and increasing in intensity. Several factors that influence plasmon absorptions are particle size and shape, the dielectric constant of the surrounding media, and the degree of aggregation of the metal particles.[7-9] A TEM study conducted on the same sample revealed that when a Au_x/DPBD/poly-DPBD matrix was dissolved in acetone, the gold clusters aggregated into fractal-like structures. Hence the protective polymeric layer that surrounds the metal cluster was attacked by acetone, leading to metal aggregation and change of the environment surrounding the cluster.

Gold clusters were dispersed in the well-characterized NLO polymer, poly-4-BCMU.[4] Poly-4-BCMU was also found to be very effective at preventing gold clusters from aggregating in solution. This result is indicated in the TEM shown in Figure 1c of Au_x/acetone to which a solution of poly-4-BCMU was added. Figure 1d shows the TEM of Au_x/acetone without poly-4-BCMU. The concentration of Au, relative to poly-4-BCMU, was increased by adding several Au_x/acetone colloids to a solution of poly-4-BCMU until the Au:poly-4-BCMU ratio was 1:1 by weight. Poly-4-BCMU is not readily soluble in acetone; therefore, large volumes of THF were used to ensure that the poly-4-BCMU remained in solution as each successive Au_x/acetone colloid was added. The final solution, although concentrated with respect to the Au:poly-4-BCMU ratio, was still too dilute for the nonlinear optical measurements. Concentration by simple evaporation of the solvent led to the formation of insoluble films on the sides of the glassware. Concentration by distilling away the solvent prevented the formation of films, but the effects of heating on the gold clusters are not known and are still under investigation. Notably, the colloid did not flocculate under reflux conditions. The 1:1 by weight Au_x/poly-4-BCMU solution was successfully concentrated by precipitating the Au_x/poly-4-BCMU composite with methanol and then dissolving it in a minimum amount of THF.

High-optical-quality films of Au_x/poly-4-BCMU were prepared by spin-coating but proved to be too thin (~1 μm) for the NLO measurements at 1.064 μm. The third-order NLO coefficient of solutions of Au_x/poly-4-BCMU was measured by degenerate four-wave mixing (DFWM) at 1.064 μm. For a composite with an estimated gold volume fraction of 7%, a 200-fold enhancement over that of pure poly-4-BCMU[4] was observed. The results of the study of the nonlinear optical properties of these composite materials will be discussed in detail in a future publication.

In summary, we have synthesized and characterized a new composite material, namely, metal cluster laden polydiacetylenes with potential nonlinear optical properties. Gold clusters have been embedded in poly-DPBD and poly-4-BCMU by metal vapor deposition. A large metal volume fraction of ~15% in poly-DPBD was achieved with an average cluster size of 20 Å. Preliminary NLO measurements on Au_x/poly-4-BCMU (metal volume fraction of 7%) indicated a 200-fold enhancement in the third-order optical coefficient of the composite material relative to that of the metal-free poly-4-BCMU.

Acknowledgment. We thank Dr. A. Snow for providing the poly-4-BCMU sample used in this study and Drs. F. Bartoli and R. Lindle for the DFWM measurements conducted on solutions of the composite materials. We also appreciate Dr. R. Pong's valuable contributions to this project. For financial support we acknowledge ONR, ONT, and DARPA.

(8) Kreibig, U.; Genzel, L. *Surf. Sci.* **1985**, *156*, 678.
(9) Papavassiliou, G. C. *Prog. Solid State Chem.* **1979**, *12*, 185.

Competing Redox and Inactivation Processes in the Inhibition of Cysteine Proteinases by Peptidyl *O*-Acylhydroxamates. ^{13}C and ^{15}N NMR Evidence for a Novel Sulfenamide Enzyme Adduct[1]

Valerie J. Robinson, Peter J. Coles, Roger A. Smith,* and Allen Krantz*

Syntex Research (Canada)
2100 Syntex Court, Mississauga, Ontario, Canada L5N 3X4

Received May 23, 1991

We recently reported the rapid inactivation of the cysteine proteinase cathepsin B by peptidyl *O*-acylhydroxamates (e.g., for **1**, inactivation rate k/K = 580000 M^{-1} s^{-1}).[2] This class of compounds was first described by Fischer and co-workers as irreversible inhibitors of serine proteinases such as α-chymotrypsin and dipeptidyl peptidase IV[3] and, more recently, as inhibitors of cysteine proteinases.[4] In this report, we describe ^{13}C and ^{15}N NMR characterization of the papain adduct obtained from the *O*-acylhydroxamate **1** as having a novel sulfenamide structure; as well, we have uncovered a rapid competing inhibition process that is normally masked by the presence of thiol reducing agent.

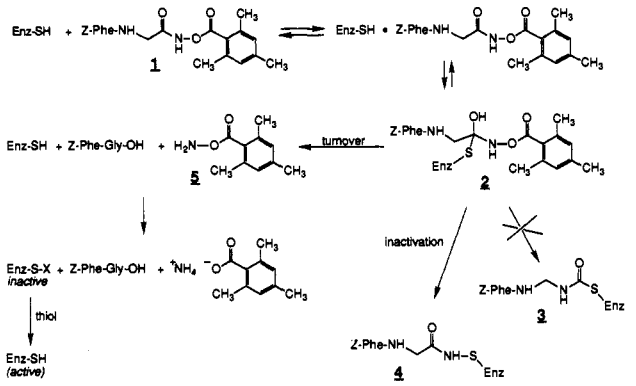
In our report of cathepsin B inactivation,[2] we proposed that the putative tetrahedral intermediate (**2**) could break down to give turnover products or could produce a stable adduct either by (a) migration of the peptidyl group in a manner similar to a Lossen (or related) rearrangement[5] to give a thiolcarbamate (e.g., **3**) or by (b) migration of the enzyme thiol group to afford a sulfenamide (e.g., **4**) (see Scheme I). To characterize the enzyme–inhibitor adduct and help clarify the mechanism of inactivation, ^{13}C- and ^{15}N-labeled *N*-(benzyloxycarbonyl)-L-phenylalanylglycine *O*-mesitoylhydroxamates (**1a**, labeled as $NHCH_2{}^{13}CONH$; **1b**, labeled as $NH{}^{13}CH_2{}^{13}CONH$; and **1c**, labeled as $NHCH_2{}^{13}CO{}^{15}NH$) were synthesized for NMR studies with papain.[6]

As in the case of cathepsin B in the presence of reducing thiol,[2] inactivation of papain was accompanied by some enzyme-catalyzed turnover of inhibitor, so that ca. 12 molar equiv of **1** was required for complete inactivation of the enzyme (pH 7, 5 mM 2-mercaptoethanol, 10 mM potassium phosphate). However, *in the absence of thiol*, papain activity could be completely inhibited with as little as a single molar equivalent of **1**. Inhibition of papain by **1c** in the absence of thiol, followed by removal of small molecules (MW <10000; Sepharose chromatography), provided a sample of inactive papain for which no ^{15}N or ^{13}C label could be detected by NMR measurement. The "small-molecule" fraction from this experiment consisted of $^{15}NH_4{}^+$ (by ^{15}N NMR: −360 ppm relative to nitromethane at 0 ppm), Z-Phe-$NHCH_2{}^{13}COOH$ (by HPLC and ^{13}C NMR: 179 ppm), and mesitoic acid (by HPLC). These byproducts represent an overall hydrolysis and reduction of **1c** by papain. Consistent with this observation, the inactive papain produced behaved as an oxidized enzyme, in that activity could be recovered (up to ca. 80%) by treatment with reducing thiol (e.g., 5 mM 2-mercaptoethanol). As well, treatment

(1) Contribution No. 344 from the Institute of Bioorganic Chemistry, Syntex Research.
(2) Smith, R. A.; Coles, P. J.; Spencer, R. W.; Copp, L. J.; Jones, C. S.; Krantz, A. *Biochem. Biophys. Res. Commun.* **1988**, *155*, 1201-1206.
(3) (a) Fischer, G.; Demuth, H.-U.; Barth, A. *Pharmazie* **1983**, *38*, 249-250. (b) Demuth, H.-U.; Baumgrass, R.; Schaper, C.; Fischer, G.; Barth, A. *J. Enzyme Inhib.* **1988**, *2*, 129-142. (c) Demuth, H.-U.; Neumann, U.; Barth, A. *J. Enzyme Inhib.* **1989**, *2*, 239-248.
(4) Bromme, D.; Schierhorn, A.; Kirschke, H.; Weideranders, B.; Barth, A.; Fittkau, S.; Demuth, H.-U. *Biochem. J.* **1989**, *263*, 861-866.
(5) (a) March, J. *Advanced Organic Chemistry*, 3rd ed.; Wiley-Interscience: New York, 1985; pp 982-990. (b) Groutas, W. C.; Stanga, M. A.; Brubaker, M. J. *J. Am. Chem. Soc.* **1989**, *111*, 1931-1932. (c) The species **3** could also conceivably arise from a Lossen rearrangement (possibly enzyme induced) of enzyme-bound **1**, rather than via intermediate **2**.
(6) Papain serves as a good model for the closely homologous cathepsin B enzyme: Takio, K.; Towatari, T.; Katanuma, N.; Teller, D. C.; Titani, K. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 3666-3670.

0002-7863/91/1513-7760$02.50/0

Scheme I. Proposed Scheme for the Mechanism of Inhibition of Papain-Type Cysteine Proteinases with Peptidyl *O*-Acylhydroxamates (e.g., 1)



of papain (in the absence of thiol) with 1 molar equiv of authentic **5**,[7] the formal hydrolysis product and potential "oxidant" (Scheme I), rapidly provided inactive papain, from which enzyme activity could again be mostly recovered by treatment with thiol.

To discriminate between the alternative adducts **3** and **4** proposed for the inactivation process[2] which occurs *in the presence of thiol*, papain was treated with labeled inhibitors **1a–c**. Using excess inhibitor **1a**,[8] ^{13}C NMR spectroscopy revealed a new, strong signal in the protein fraction for the ^{13}C-labeled carbonyl carbon at 182 ppm. Using **1b**, which has J_{C-C} = 53 Hz (acetone-d_6), an enzyme–inhibitor adduct was obtained that exhibited a carbonyl signal centered at 182 ppm with J_{C-C} = 50 Hz, thereby ruling out the thiolcarbamate structure **3**.[9] Finally, experiments were conducted with ^{13}C,^{15}N-labeled **1c**. In **1c**, J_{C-N} = 9 Hz was observed; in the enzyme–inhibitor adduct from **1c**, the line width of the ^{13}C carbonyl signal rendered measurement of a J_{C-N} coupling constant of ca. 9 Hz as plausible, but not conclusive. Unequivocal evidence for incorporation of the hydroxamate nitrogen atom in the adduct was obtained by measurement of a broad ^{15}N NMR signal at −274 ppm (relative to nitromethane at 0 ppm). This observed ^{15}N NMR chemical shift[10] agrees well with that measured for a model sulfenamide[11] ($CH_3CONHSCH_2CH_2CH_3$; ^{15}N NMR: −284 ppm (neat sample)).

In summary, we have identified two modes of inhibition of cysteine proteinases by peptidyl *O*-acylhydroxamates (Scheme I). *In the absence of a reducing thiol*, treatment of papain with a single molar equivalent of inhibitor **1** affords an inactive, apparently oxidized form of papain; enzymatic activity can be recovered by treatment with reducing thiol. Our evidence suggests that the expected turnover product *O*-mesitoylhydroxylamine **5** is the oxidizing agent in this process. *In the presence of thiol*, the turnover (hydrolysis) of inhibitor is followed by oxidation/reduction to give a complete catalytic cycle; this process competes with the inactivation of papain with a partition ratio of ca. 12. ^{13}C and ^{15}N NMR studies of the covalent inactivation adduct have provided evidence that rules out a thiolcarbamate structure **3**, while providing strong support for the novel sulfenamide adduct structure **4**. Further mechanistic studies are in progress.

Acknowledgment. We are grateful to Leslie J. Copp for her contributions to the enzymology and helpful discussions and to the Biotechnology Research Institute, Montreal, Quebec, Canada, and Bruker (Canada) for use of NMR instrumentation.

Supplementary Material Available: Information for syntheses of **1a–c** and NMR experiments and ^{13}C NMR spectra of papain adducts (2 pages). Ordering information is given on any current masthead page.

(7) (a) Marmer, W. N.; Maerker, G. *J. Org. Chem.* **1972**, *37*, 3520–3523. (c) Carpino, L. A. *J. Am. Chem. Soc.* **1960**, *82*, 3133–3135.

(8) Conditions: 15 equiv of labeled inhibitor **1**, pH 7, 10 mM potassium phosphate, 5 mM 2-mercaptoethanol. Small molecules (MW <10000) were removed from the inactivated enzyme by Sepharose chromatography.

(9) J_{C-C} values for CH_3NHCO are <5 Hz; see p 135 in the following: Wray, V.; Hansen, P. E. *Annu. Rep. NMR Spectrosc.* **1981**, *11A*, 99–181.

(10) Witanowski, M.; Stefaniak, L.; Webb, G. A. *Annu. Rep. NMR Spectrosc.* **1981**, *11B*, 1–486.

(11) (a) Harpp, D. N.; Mullins, D. F.; Steliou, K.; Triassi, I. *J. Org. Chem.* **1979**, *44*, 4196–4197. (b) Kise, H.; Whitfield, G. F.; Swern, D. *J. Org. Chem.* **1972**, *37*, 1125–1128.

Intramolecular Reaction Rate Is Not Determined Exclusively by the Distance Separating Reaction Centers. The Kinetic Consequences of Modulated Ground State Strain on Dyotropic Hydrogen Migration in Systems of Very Similar Geometric Disposition

Leo A. Paquette* and George A. O'Doherty[1]

Evans Chemical Laboratories, The Ohio State University Columbus, Ohio 43210

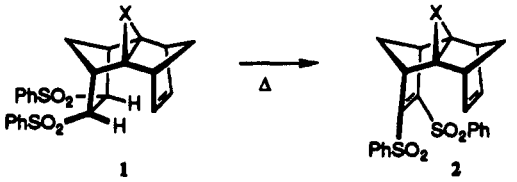
Robin D. Rogers[2]

Department of Chemistry, Northern Illinois University DeKalb, Illinois 60115

Received June 21, 1991

The ability of enzymes to alter state changes within suitable substrates with resultant dramatic acceleration of chemical reactivity has promoted extensive inquiry into the origin of this kinetic phenomenon.[3] The most recent of several controversies to surround this subject finds Menger and Houk as proponents of radically differing viewpoints. The "spatiotemporal hypothesis" holds that rates of enzymatic and intramolecular processes correlate strictly with the time that the reacting centers reside within a critical geometric disposition;[4] "sustained proximity at close distances...generates enzyme-like rates".[4c] The contrary argument holds that "the real determinant of reactivity is the energy required to distort the reacting functional groups into the geometry of the rate-determining transition state."[5,6]

There exists no question that distance can be an important determinant of rate. Relevantly, the first-order isomerizations of **1** to **2** have been shown to correlate very well with the intracavity distance (as determined crystallographically in every instance).[3]



1 **2**

A 0.1-Å change in the gap dimension separating the α-sulfonyl hydrogens from the sp^2-hybridized carbons in **1** translates into a rate spread of 10^4! Despite the excellent correlation observed, it is important and relevant to question whether proximity by itself suffices as a complete explanation. The structural relationships

(1) BP America Fellow, 1991; National Need Fellow, 1989–1990; University Fellow, 1988–1989.

(2) Author to whom inquiries relative to the X-ray crystallographic studies should be addressed.

(3) For a leading reference, see: Paquette, L. A.; Kesselmayer, M. A.; Rogers, R. D. *J. Am. Chem. Soc.* **1990**, *112*, 284.

(4) (a) Menger, F. M.; Venkataram, U. V. *J. Am. Chem. Soc.* **1985**, *107*, 4706. (b) Menger, F. M. *Acc. Chem. Res.* **1985**, *18*, 128. (c) Menger, F. M. *Adv. Mol. Model.* **1988**, *1*, 189. (d) Sherrod, M. J.; Menger, F. M. *J. Am. Chem. Soc.* **1989**, *111*, 2611. (e) Menger, F. M.; Sherrod, M. J. *J. Am. Chem. Soc.* **1990**, *112*, 8071. (f) Sherrod, M. J.; Menger, F. M. *Tetrahedron Lett.* **1990**, *31*, 459.

(5) (a) Dorigo, A. E.; Houk, K. N. *J. Am. Chem. Soc.* **1987**, *109*, 3698. (b) Dorigo, A. E.; Houk, K. N. *Adv. Mol. Model.* **1988**, *1*, 135.

(6) More recently, Sherrod and Menger have recognized that carbon skeleton distortion in a reactant ground state comprises most of the activation energy of a hydride transfer and that "only after a critical distance is reached does actual chemical reaction begin".[4f]

0002-7863/91/1513-7761$02.50/0