

The structure and dynamics of enzyme-substrate reaction intermediates determined by resonance Raman spectroscopy

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Abstract - The two principal means of obtaining the resonance Raman (RR) spectra of enzyme-substrate complexes are discussed. The first employs substrates which contain extended π -electron systems, such as cinnamic or furylacrylic acid. The spectral data for these substrates bound to serine or cysteine proteinases can be interpreted in terms of a model in which the delocalised π -electrons become polarised. The second method of employing the RR technique creates a dithioester chromophore at the point where the substrate is covalently linked, for a short time, to the enzyme. By this means it is possible to obtain the vibrational spectrum associated with those bonds undergoing catalytic transformation. The RR spectra provide detailed information on the conformation of the enzyme-bound substrate and allow us to discuss conformer selection and substrate distortion and strain. Combined X-ray crystallographic and Raman studies on suitable model compounds are important in setting up the spectra-structure correlations needed for spectral interpretation. These studies also introduce a dynamical concept by suggesting how structural changes occur along the reaction pathway. Complementary dynamical information is elicited from combined kinetic and RR data; the data from these two techniques provide a very detailed structure-kinetic correlation and show how the breaking of a single atom-atom contact in the active site is associated with an individual rate constant for the reaction. Papain is the archetype enzyme used for the dithioester work, but the approach has been extended to other plant cysteine proteinases and to the mammalian enzyme cathepsin B.

INTRODUCTION

Enzymes are responsible for controlling and catalysing the enormous number of reactions needed to maintain a living cell. Thus, enzymes occupy a pivotal position in biochemistry but, at the same time, an understanding of the way in which enzymes perform their chemistry with such selectivity and efficiency provides a fascination and challenge for the chemist. The foundation of our knowledge of the structure of enzymes is laid by X-ray crystallographic studies. However, while this approach is essential for a detailed mechanistic analysis, X-ray crystallography cannot be used to characterise unstable intermediates formed between an enzyme and a substrate. The existence of various enzyme-substrate complexes during a reaction is often inferred from kinetic data or chemical intuition (1) but it is important to develop techniques to characterise such species. Here, it will be shown that the application of resonance Raman (RR) spectroscopy heralds considerable progress in our understanding of some enzyme-substrate complexes. The time scale associated with the RR effect is 10^{-11} sec and the RR data itself can be gathered for enzyme substrate complexes in seconds or less; thus, the method is well-suited for studying transient species. The RR spectra are high in information content (2) and can provide precise data on the conformation and dynamical behaviour of a substrate in an enzyme's active site.

Two factors have helped in the development of the RR-enzymology field over the past decade. The first improvement is technological. Advances in lasers, spectrometers and photon-detectors have expanded both the range of enzyme-substrate complexes that can be studied and the range of experiments that may be considered for each system. The technological innovations which have had the greatest impact are the arrival of reliable CW laser sources in the 325-360 nm range and the advent of multichannel photon detection (3). The second factor which has accelerated progress is the application of a multidisciplinary approach. Of course, spectroscopic skills are required to obtain high quality RR data for enzyme-substrate transients - but this is usually at the end of a series of experiments which have involved substrate synthesis and enzyme purification. Moreover, kinetic studies are often needed to characterise thoroughly the reactions under consideration, and combined X-ray crystallographic and Raman spectroscopic methods are used to set up the structure-spectra correlations needed to interpret enzyme-substrate RR data. The extra techniques are not merely ancillary; in combination with the RR information they provide a much deeper insight into the processes under investigation.

described elsewhere (4-10,11b). Here we shall limit ourselves to discussing the charge polarisation model which is the central theme running through the interpretation of the RR and absorption spectral results.

The π -electron polarisation model

Based on their spectroscopic properties the acyl-enzymes of chymotrypsin fall into a different category compared to the acyl-enzymes of papain. If the methyl ester of the acyl group, dissolved in CH_3OH , is taken as a standard, the spectral properties of the acyl group show only modest changes upon binding to chymotrypsin (4,5,9,10). For example, λ_{max} , the acyl group's absorption maximum, red shifts by approximately 20 nm and in the RR spectrum $\nu_{\text{C}=\text{C}}$, the ethylenic double bond stretching frequency, shows a concomitant drop of 5-10 cm^{-1} . However, upon binding to papain's active site the spectral changes are much larger (6,8). For example, for the 4-dimethylamino, 3-nitrocinnamoyl group there is a large difference in λ_{max} between the product (343 nm) and the acyl-enzyme (410 nm). Moreover, the RR spectra show startling changes as is clear from Figure 2. The RR spectrum of the enzyme-substrate

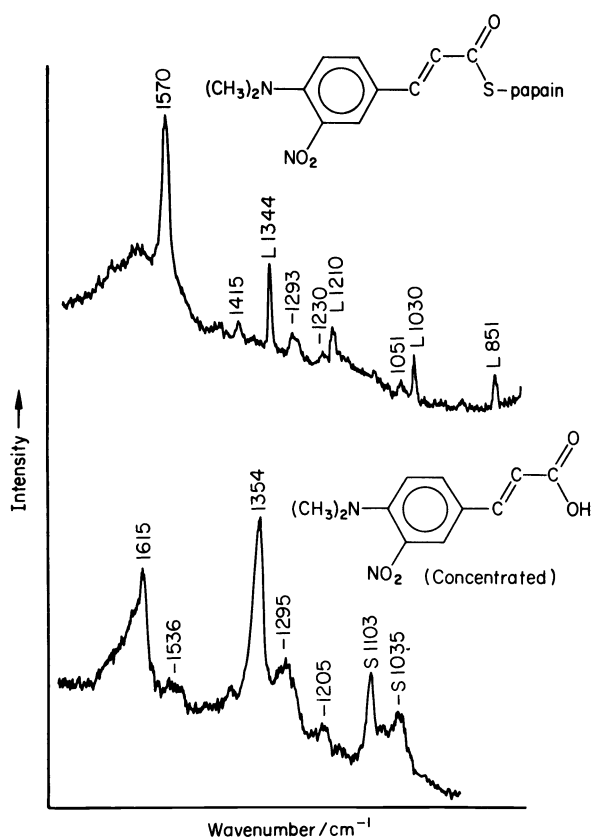


Fig. 2. Comparison of the RR spectra of the acyl-enzyme (top), the product (bottom) of the reaction of 4-dimethylamino, 3-nitrocinnamoyl imidazole with papain. Adapted from ref. (8) with permission.

intermediate is distinct from that of the substrate or the product. The active site obviously produces drastic changes in the properties of the acyl residue. As can be seen in Figure 2, the spectrum of the product is dominated by bands due to ethylenic and ring modes in the 1600 cm^{-1} region and a nitro feature near 1350 cm^{-1} . In contrast, the spectrum of the acyl papain shows a very intense peak at 1570 cm^{-1} . There is little evidence for peaks from the product in the spectrum of the intermediate or vice versa.

The dramatic change in the RR spectra of the cinnamoyl chromophore shown in Figure 2 is thought to be due to the polarisation of π -electrons in the bound cinnamoyl group (8). This idea has been tested using a series of model compounds, such as the imidazole ester of *para*-dimethylaminocinnamic acid, which mimic the absorption and RR properties of the acyl papain (7,8,12,13). For the latter, the overall conclusion is that the acyl group interacts with charged amino acid side chains and/or electric dipoles near the active site with the result the π -electron density in the cinnamoyl moiety becomes depleted near the *para* position in the phenyl ring and enriched near the $\text{C}=\text{O}$ function. Such pronounced π -electron polarisation was first seen in the examples cited above and similar effect probably accounts for the spectroscopic changes observed by Peticolas and his co-workers for *p*-(dimethylamino)benzaldehyde binding to liver alcohol dehydrogenase (14).

π -Electron polarisation is also observed for acyl-chymotrypsins, but the effect is less pronounced and the spectroscopic consequences less dramatic. The differences between acyl-papains and -chymotrypsins have been discussed elsewhere (Ref. 2, Chap. 6; 13, 15).

USING DITHIOESTERS TO OBSERVE THE BONDS UNDERGOING CATALYTIC TRANSFORMATION

By using a dithioester, $-C(=S)S-$, as a chromophore it is possible to obtain the vibrational spectrum of that part of a transient enzyme-substrate complex undergoing catalytic transformation (16). The strategy involves the use of a substrate which is a peptide sequence terminating in a thionoester, peptide- $C(=S)OCH_3$, and a cysteine proteinase where the active site -SH group of the enzyme forms a link to the substrate. With this combination the acyl enzyme intermediate is a dithioacyl enzyme, peptide $-C(=S)S-$ enzyme. A prototype reaction is given in detail in Figure 3.

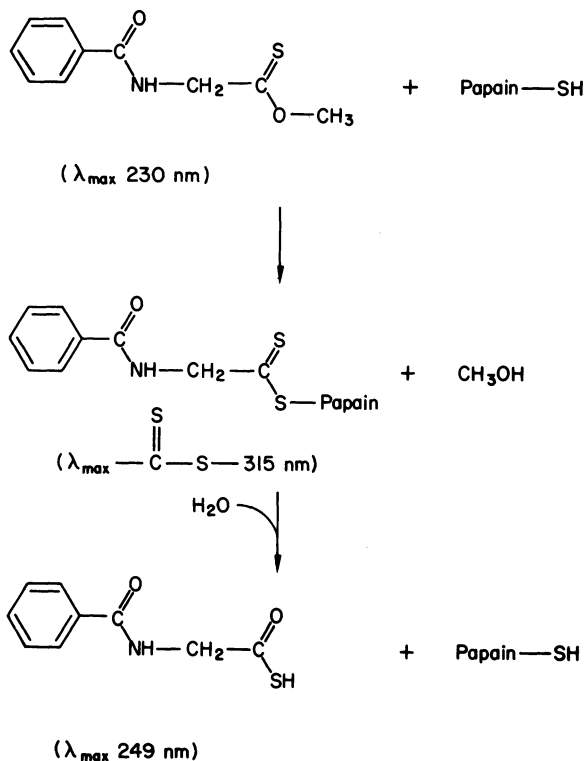


Fig. 3. Reaction scheme involving the formation of a transient dithioacyl papain.

Absorption spectroscopy enabled Lowe and Williams (17) to monitor this process by using methyl thionohippurate as a substrate. They observed the transient appearance of a chromophoric intermediate with a λ_{\max} at 315 nm. Using kinetic evidence and absorption spectral comparisons with model compounds, Lowe and Williams inferred that this intermediate was a dithioacyl enzyme (Figure 3). Importantly, from the point of view of introducing spectroscopic selectivity the intermediate has a λ_{\max} at 315 nm, whereas the substrate and product both absorb below 250 nm. The RR data confirm that the intermediate is a dithioacyl enzyme (18).

As can be seen in Figure 4 by excitation with the 324-nm Kr^+ line, the intermediate gives rise to many bands in the 500-1200 cm^{-1} range. The peaks in Figure 4 contain contributions from the stretching and other motions of the $\text{C}=\text{S}$ and $\text{C}-\text{S}-\text{C}$ bonds, and thus intense modes from the catalytically crucial bonds are observed in the RR spectrum. Moreover, the reaction scheme can be generalized to use, for example, a polypeptide sequence as substrate and any enzyme forming a transient ester linkage involving an active-site thiol. The "natural" reaction involves the formation of the $-\text{C}(=\text{O})\text{S}-$ group, so the RR label now involves a single atom replacement-sulfur for oxygen. Kinetic studies (19) have shown that the reaction mechanisms involving "natural" esters and "model" thionoesters are the same; k_{cat}/K_m 's are similar for the two classes of substrate and k_{cat} 's are 20-30 times slower for the thionoesters. The spectroscopic data are obtained by examining a reaction mixture (in

contrast to the examples discussed above where the acyl enzymes had to be separated from substrate and product) that contains an excess of substrate over enzyme; since for ester substrates acylation is faster than deacylation, there is a buildup of a quasi-steady-state population of intermediate. The RR spectra shows the same dependence as the absorption band at 315 nm; both disappear as the substrate is used up.

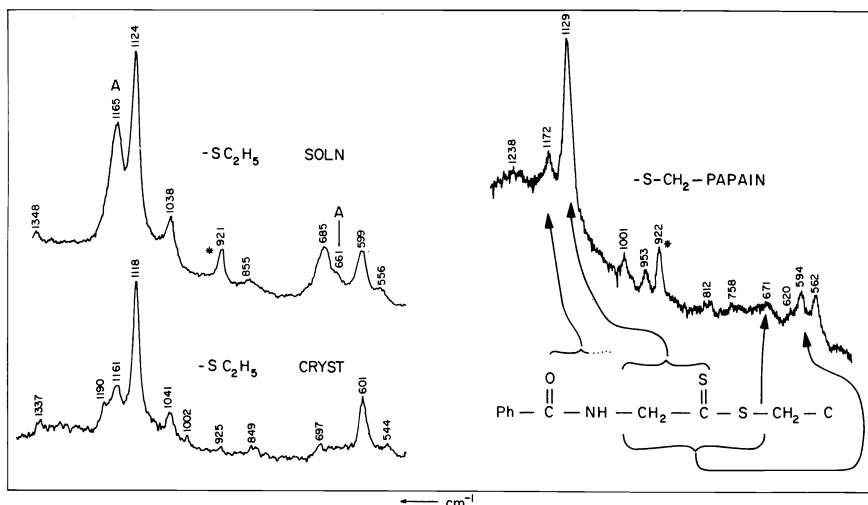


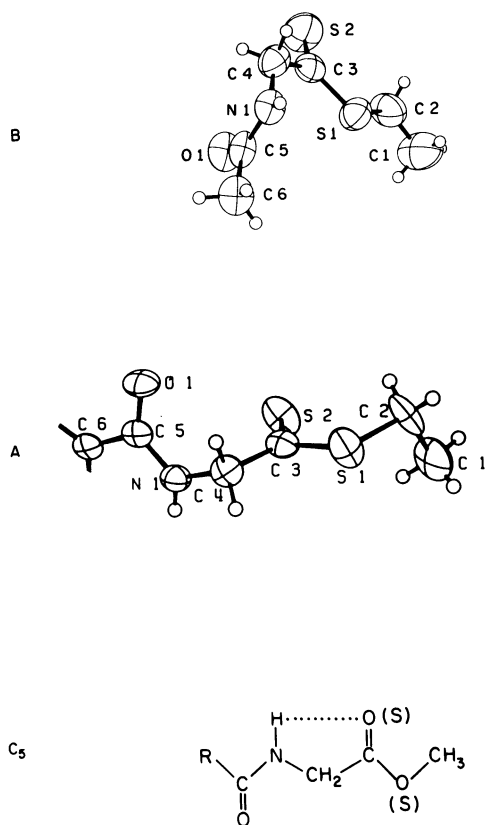
Fig. 4. The 324 nm excited RR spectra of N-benzoylglycine dithioester; ethyl dithioester, left hand side; the dithioacyl papain and an indication of conformationally sensitive peaks, right hand side. Adapted from ref. (15) with permission.

Interpreting dithioester RR spectra: the conformations of N-acylglycine dithioesters and thioesters

In order to understand the RR spectra of dithioacyl papains and other cysteine proteinases, extensive studies have been undertaken on the basic spectroscopy of "simple" dialkyl dithioesters and on the conformational properties of N-acylglycine ethyl dithioesters. Vibrational and theoretical investigations have been carried out for simple dithioesters, such as CH₃C(=S)SCH₃, in order to build a reliable force field for dithioesters, which could then be used as a basis for our understanding of more complex dithioester systems. The work on simple dithioesters included Raman, RR, and infrared analysis coupled with a normal coordinate treatment of methyl and ethyl dithioacetate (20) and several ¹³C- and D-substituted analogs. The approach was extended to molecules such as CH₃CH₂C(=S)SCH₃ and CH₃(C=S)S₂CH₂CH₃, which provided information on the sensitivity of the RR spectrum to rotation about the bonds indicated (21).

Spectroscopic (22,23) and crystallographic (24,25) investigations of N-acylglycine dithioesters, RC(=O)NHCH₂C(=S)SC₂H₅, have provided the key to understanding the RR spectra of the enzyme-substrate transients. Approximately ten different dithioesters of this type, with differing R groups have been analysed to date. The model N-acylglycine ethyl dithioesters give rise to intense RR bands in the 500-700 and 1000-1200 cm⁻¹ regions of the spectrum. In solution, the relative intensities of the bands were found to be very sensitive to temperature and solvent. These facts, taken with other considerations, indicated the presence of more than one conformer. It was found that in aqueous or acetonitrile solutions there are two major conformational states, designated conformers A and B (shown in Figure 5), and that each conformer has a characteristic and separate RR spectrum in both the 600 and 1100 cm⁻¹ regions. In order to form an exact description of conformers A and B, combined X-ray crystallographic-Raman analyses (24,25) were undertaken on single crystals of N-acyl glycine ethyl dithioesters. The combined X-ray crystallographic-RR approach could, in turn, be used to understand the solution RR spectra of the N-acylglycine dithioesters and the RR spectra of the dithioacyl papains. In keeping with the higher thermodynamic stability of conformer B, most of the N-acylglycine dithioesters crystallize in this form.

Figure 4 compares the RR spectrum of crystalline PhC(=O)NHCH₂C(=S)SC₂H₅ with the RR spectrum of this molecule in H₂O. In solution (top spectrum), the spectral signatures of forms A and B are present, but for the crystals only the signature of conformer B is found, e.g. in the upper trace in Figure 4 most of the intensity of the 1165 cm⁻¹ peak is due to a conformer A mode that is absent in the spectrum of the crystalline material. An X-ray diffraction analysis on the crystal provides an accurate structure of conformer B, and this is shown in Figure 5. To date, only pNO₂PhC(=O)NHCH₂C(=S)SC₂H₅ has crystallized in a form giving rise to a conformer A type RR signature [a fact that is related to the effect the pNO₂ substituent

Fig. 5. Conformers A, B and C₅.

has on the basicity of the NH group (26)]; its structure is compared to conformer B in Figure 5. The major conformational difference between conformers A and B is a rotation of $\sim 150^\circ$ about the C(3)-C(4) bond. The rotation about the C-C linkage changes the vibrational coupling in and about the dithioester moiety and accounts, at least in part, for the difference Raman spectral signatures of conformers A and B.

A characteristic of conformer B is that the amide and dithioester groups are in close contact; in fact the N (glycinic nitrogen) \cdots S(thiol) distance is 2.9 Å, somewhat less than the sum of the van der Waals radii, which is 3.35 Å. The amide and ester planes are roughly orthogonal and thus there is no intramolecular H-bonding involving the -NH group.

Recently, it has been shown that *N*-acylglycine thiol esters have similar conformational properties to their dithioester analogues (27). In particular, thiol esters take up B-type conformers typical of dithioesters and which play a vital role in active site geometry. Therefore the discussion and conclusions reached below regarding the "model" dithioacyl enzymes can, in all likelihood, be transferred to the natural thiolester species.

Conformer selection by the active site

***N*-acylglycines as substrates.** The RR spectra of *N*-acylglycine dithioacyl papains, e.g. the RR spectrum of $\text{PhC}(=\text{O})\text{NHCH}_2\text{C}(=\text{S})\text{S}$ -papain seen in Figure 4, demonstrate unequivocally that between pH 4 and 9 the great majority of acyl groups assume a B-type conformation. In Figure 4 the intense peak at 1129 and the peak at 594 cm^{-1} are modes characteristic of a B conformer - the corresponding peaks are seen in the RR spectrum of the crystalline ethyl dithioester. The situation is the same for every *N*-acylglycine papain studied (28,29). There is a very close correspondence between the RR spectrum of each dithioacyl papain and that of its corresponding ethyl ester in the B form. All major peaks and most minor peaks in the acyl enzyme spectra are due to conformer B. Although it is possible that some minor peaks are due to a small population of non-B conformers, there is no convincing evidence to support this motion. However, denaturation of the dithioacyl enzymes below pH 3.0 does result in conformer A peaks appearing in the spectrum (28) and, under these conditions, the conformational population of the covalently linked acyl group reverts to that found for the corresponding ethyl ester. Thus, the first conclusion to emerge is that the native active site exerts conformational selection; it binds one of the conformational states available to *N*-acylglycine dithioesters.

The next consideration is how closely the conformation of the bound substrate resembles that of a standard relaxed dithioester. In other words, does the enzyme bring about any conformational distortion? To approach this question the most stable conformation of the *N*-acylglycine ethyl dithioesters in H_2O/CH_3CN solution, conformer B, is taken as a standard state. Comparison with dithioacyl enzyme RR spectra is facilitated by ND, ^{15}N , and $^{13}C=S$ substitution in the dithioacyl papains (29) and the corresponding ethyl dithioesters (22) for the acyl groups based on *N*-benzoylglycine and *N*-(β -phenylpropionylglycine). For the latter acyl group in the active site there is no evidence for distortion in the $NH-CH_2-C(=S)$ torsional angles that define conformer B. The same is essentially true for the *N*-benzoyl derivative, although there may be a minor perturbation away from the relaxed standard state (29). Conclusions such as these are formed on the basis of the comparison of RR peak positions, which are known to be conformationally sensitive. In Figure 4, for example, the position of the intense band near 1130 cm^{-1} is very sensitive to torsional angles in the glycine $NH-CH_2-C(=S)$ linkages: a $\sim 150^\circ$ rotation about $CH_2-C(=S)$ brings about a 40-cm^{-1} shift in this RR peak. Thus, a slight shift from 1124 to 1129 cm^{-1} (Figure 4), going from the standard state to the active site, is taken as evidence for a minor perturbation. Such conclusions are reinforced by a differential sensitivity to $^{13}C=S$ substitution in the two states.

Multi-peptide substrates. Papain has an extended binding site which can accommodate up to a tetrapeptide on the acyl-binding side. Since it has often been proposed that energy from enzyme substrate contacts removed from the point of catalytic attack may be funnelled to this point to produce rate acceleration (30), it is of considerable interest to see if extending the substrate into the full binding site affects the geometry about the scissile linkages. To this end, the substrates $MeOC(=O)PheGlyC(=S)OCH_3$, $MeOC(=O)GlyPheGlyC(=S)OCH_3$ and $MeOC(=O)GlyGlyPheGlyC(=S)OCH_3$ have been synthesised and reacted with papain (31). The RR spectra of the resulting dithioacyl enzyme intermediates involving the multi-peptide substrates have been compared to those for *N*-acylglycine substrates and to the corresponding dithioethyl ester model compounds. In general the RR spectra for the extended substrate-papain intermediates are similar to each other and to those for the single amino acid substrate bound to the active site. Thus, for multi-peptide substrates the conformational state in the critical $-C(=O)NHCH_2C(=S)S-$ enzyme bonds remains conformer B-like (31). However, there are differences in detail in the RR spectra and an investigation of these differences is being actively pursued.

Other plant and mammalian cysteine proteinases. As a result of its availability and ease of purification from the latex of the papaya fruit, papain is the archetype cysteine proteinase. However, a dozen or more other plant cysteine proteinases have been isolated and purified. For example, the papaya latex also contains chymopapain A and B and papaya peptidase A; actinidin is a cysteine proteinase from the New Zealand kiwi fruit and which, like papain, has been characterised by X-ray crystallography; and bromelain and ficin are cysteine proteinases isolated from the bromeliad and ficus families, respectively. In an initial report, the RR spectra of several *N*-acylglycine dithioacyl enzymes based on chymopapain, ficin and bromelain were compared to those of the corresponding papain intermediates (32). For a given substrate the RR spectra of the four dithioacyl enzymes were found to be similar indicating that the conclusions reached for papain, regarding conformer selection and the presence of conformer B, holds for the other enzymes. These findings were extended by a more detailed study involving the *N*-acylglycine dithioacyl intermediates of papaya peptidase A and actinidin (33). The latter enzyme is of special interest because the X-ray crystallographic analysis of actinidin (34) indicate a very similar active site compared to papain near the cysteine but marked differences in the extended site used to accommodate the substrate's peptide residues. Both RR spectroscopic and stopped-flow kinetic studies were undertaken. From the latter it was possible to determine k_3 , the rate constant for deacylation. The k_3 's for any substrate with papain, papaya peptidase A or actinidin were found to be very similar. This taken with the close parallels in the RR spectra of the corresponding dithioacyl enzymes provided evidence for a strongly conserved deacylation process among the plant cysteine proteases.

Cysteine proteinases are also found in mammalian cells and they represent a biochemical area of great topical concern (35,36). From the evolutionary point of view, it is of interest to see if the mechanisms used by the plant enzymes have been retained by the mammalian cysteine proteinases or whether the pressures of evolution have led to new mechanisms. Combined RR spectroscopic and steady-state kinetic studies have been used to answer this question for that part of the reaction involving the acyl enzyme. Studies were undertaken comparing papain and the mammalian enzyme cathepsin B reacting with the substrate $MeOC(=O)PheGlyC(=S)OCH_3$ (37). The RR spectra of the two dithioacyl enzymes were identical and the deacylation rate constants very similar (37). Thus, the deacylation process appears to be highly conserved in going from the plant proteases to the mammalian cathepsin B.

***N*-acylalanine substrates.** The above discussion of dithioacyl proteinases has been concerned entirely with substrates that are linked to the enzyme via a glycine residue, i.e. of the general formula $RC(=O)NHCH_2C(=S)S-$ enzyme. Substrates with other amino acids in this linking position have kinetic properties distinct from those of the glycine analogues. Moreover the

conformational properties of glycinic amino acids are atypical in that the lack of a side chain leads to a relatively unhindered conformational space (38). Therefore, we have begun to investigate other amino acids at the linking position. An initial study (39) shows that the alanine dithioester DL PhC(=O)NHCHCH₃C(=S)SC₂H₅ takes up a conformer B-type conformation in the crystalline state very similar to that for a glycine derivative. However, unlike the glycine cases, the RR spectra of PhC(=O)NHCHCH₃C(=S)S- and PhCH₂CH₂C(=O)NHCHCH₃C(=S)S-papains (40) cannot be interpreted in terms of relaxed conformer B's. Obviously the addition of the alanine -CH₃ side chain has a profound effect on the conformation in the active site and a detailed interpretation of the alanine dithioacyl enzyme RR spectra is being actively sought.

Dynamics, kinetics and reaction pathways

Much of the foregoing discussion has been concerned with a purely static conformational picture of the enzyme-bound dithioacyl group. However, such a definition is not complete since it ignores the dynamical properties of proteins (41). Even for a chemically well-defined enzyme-substrate intermediate, there are structural fluctuations or rapid interchanges between energetically close conformational states that play a vital role in enzyme action (42,43). Some of the theoretical and practical advantages of using RR spectroscopy to study dynamics in the active site have been outlined elsewhere (15); here some of the first experiments designed to use these inherent opportunities will be discussed. We will begin by showing how a joint RR and kinetic approach can lead to a detailed appreciation of a structural change associated with a single rate constant. Then, it will be shown how combined X-ray crystallographic and Raman measurements provide information on species interconversion along the reaction pathway.

Structure-rate correlation. The structure of the acyl group in N-acylglycine dithioacyl papains has been defined above but it is also possible, by a joint RR and stopped-flow kinetic approach, to learn something about how the conformer B structure changes in the next step of the reaction. For a series of para-substituted N-benzoyl glycine thionoesters reacting with papain the rate constants for deacylation, k_3 's, were derived from stopped-flow data. When the para substituents are -OCH₃, -CH₃, -H, -Cl, -NO₂, the k_3 's are 0.0297, 0.0612, 0.0818, 0.0928, and 0.1783 sec⁻¹ respectively (26). A plot of log(k_3) vs the Hammett parameter σ is a straight line, indicating a strong correlation between k_3 and the electronic nature of the substituent, with an increase in the electron-donating ability of the substituent leading to a decrease in k_3 . The RR data for the para-substituted intermediates show that each dithioacyl papain forms a single conformer B population and that the torsional angles about the glycinic -NH-CH₂-C(=S) bonds are invariant (26). This suggests there is no change in enzyme-substrate contacts throughout the series. In addition, the RR spectra of the corresponding ethyl dithioesters in solution provide insight into the nature and strength of the N...S contact throughout the series of the model compounds. Each of the para-substituted N-benzoylglycine dithioesters in H₂O/CH₃CN solution forms a population of B-conformers having a very similar geometry, and N...S(thiol) interaction, to that selected by the active site. However, the ratio of conformer B to conformer A populations depends on the nature of the para substituent. Again, the population ratio correlates with the Hammett constant σ . Increasing the electron-donating ability of the para substituent increases the basicity of the glycinic nitrogen, which, in turn, leads to an increase in both the strength of the N...S(thiol) interaction and the population of conformer B.

Considering the model and dithioacyl enzyme data together, it appears that, in the active site, the para substituent modulates the basicity of the glycinic nitrogen and thence the strength of the N...S(thiol) interaction. The increase in strength is expressed in a reduction in k_3 , suggesting that the rate-limiting step in deacylation of the dithioacyl papains involves breaking the N...S contact. This is consonant with the view that the acyl enzyme is followed in the reaction pathway by a tetrahedral intermediate, since, in the latter, steric considerations show that the N...S interaction is absent (26).

Reaction pathways. The intellectual underpinnings of our ideas on reaction pathways lie in the pioneering work of Bürgi and Dunitz (44). In one of their approaches to understanding chemical reactivity, these workers have developed the concept of a reaction pathway derived from X-ray data. They have shown that by examining a large number of small molecule structures containing, for example, the molecular fragments X and Y, which are proximal and may be either inter- or intra-molecular, it is possible to discern preferred lines of approach of X to Y. For example, examination of 14 structures containing a nitrogen atom near a C=O group demonstrated a preferred line of approach of the N to the C=O moiety and it was proposed that this represents the line taken by a nucleophile during nucleophilic attack at the C=O.

In an extension of this work to other fragments, Rosenfield, Parthasarathy and Dunitz (45) examined the directional preferences of the approach of electrophiles or nucleophiles to divalent sulfur. They found that electrophiles tend to approach sulfur roughly 20° from the perpendicular to the plane through atoms Y-S-Z with values ranging from 0° to 40°. Nucleophiles, on the other hand, prefer to approach the sulfur along the extension of one of the

covalent bonds to S which is also the direction of the lowest unoccupied molecular orbital (LUMO), the anti-bonding orbital σ^* . These findings can be used to analyse the N...S(thiol) contact found in conformer B for N-acylglycine dithioesters (Fig. 5). Thus far, there are four structures for conformer B-type N-acylglycine dithioesters (24,25); in all of these the N...S interaction meets Rosenfield et al.'s geometrical criterion (45) for a nucleophilic nitrogen approaching an electrophilic sulfur. The atoms S(1), C(2) and N(1) (Fig. 5) lie nearly in a straight line, with the N...S-C angles falling in the range 161° to 165° . In the B conformation the lone pair associated with the N atom can be regarded as being in a HOMO and this interacts with an orbital associated with the thiol sulfur. There are two possibilities for the sulfur orbital, one involving a LUMO σ^* based on the S-C(2) fragment, the other involving a sulfur d orbital.

Although the four molecules, N-acylglycine, N-benzoylglycine (two structures per unit cell), and N-(para-chlorobenzoyl)glycine ethyl dithioesters, are all in B conformations there are considerable variations in the torsional angles about the $-C(=O)NHCH_2C(=S)$ and $NHCH_2C(=S)$ bonds, ϕ' and ψ' , respectively. This variation of $20\text{--}30^\circ$ helps us to examine the N...S stereochemistry in some detail and to show that ϕ' and ψ' are correlated. The correlation between ϕ' and ψ' is seen in Fig. 6 which shows that the four experimentally observed points

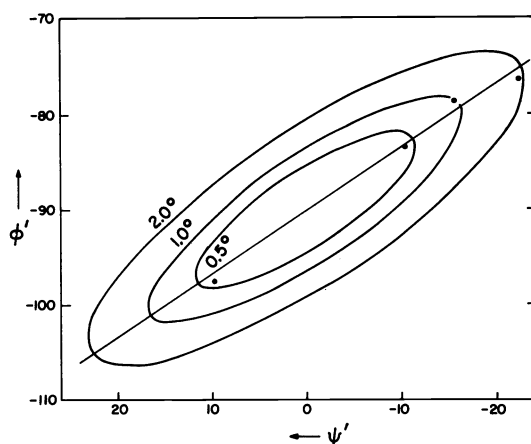


Fig. 6. The four points are the experimental ϕ', ψ' values for four N-acylglycine dithioesters. The contours represent deviations in θ from the minimum value of 20.1° .

lie on a straight line. In order to see whether this line corresponds to any preferred direction of approach, we calculated the orientation of the S(1) atom with respect to the amide plane as a function of ϕ' and ψ' , purely from geometrical considerations. The angle θ is defined as the angle between the N...S direction and the normal to the amide plane (the normal is the direction of the amide lone pair). θ for the four experimentally observed conformations is nearly constant between 20.1° and 22.1° . Thus, we take the view that the ' θ valley' represents a conformational pathway along which the S...N interaction is maximized.

An interesting question that remains is the importance of the N...S interaction detected in the active site of dithioacyl enzymes. It is believed that the acyl-enzyme intermediate lies on the reaction pathway between tetrahedral intermediates for acylation and deacylation. One way for the dithioacyl enzyme to approach the tetrahedral intermediate for deacylation with minimal expenditure of energy would be to change ϕ' and ψ' in a correlated manner and to move along the θ valley seen in Fig. 6. In this way the ϕ', ψ' correlation may serve as a starting point for understanding the pathway of interspecies conversion in the active site and thus provide a link between static and dynamic descriptions.

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