

Directional Preference for a Catalytically Important N...S Contact Seen in Acyl-thiolproteases

K. I. Varughese, A. C. Storer, and P. R. Carey*

Contribution from the Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6. Received February 27, 1984

Abstract: X-ray crystallographic and Raman studies were carried out on two *N*-benzoylglycine dithio esters which are good models for the corresponding acyl groups in acylpapains. The crystals of *N*-benzoylglycine ethyl dithio ester contain two crystallographically independent molecules in the unit cell, with both molecules assuming a B-type conformation in which the glycinic N atom comes into close contact with the thiol S atom. (*p*-Chlorobenzoyl)glycine ethyl dithio ester also takes up a B-type conformation, but differs in the orientation of the end methyl group. The (ϕ',ψ') values (the CNH-CH₂C and NHCH₂-CS(thiol) torsional angles, respectively) of these three molecules, as well as those of *N*-acetylglycine ethyl dithio ester (Huber et al. *Biochemistry* 1982, 21, 3109), which is also in the B form, lie along a straight line in a (ϕ',ψ') plot. The significance of this correlation is that there is a strongly preferred line of approach of N to S in the N...S nonbonding contact and that this line represents the conformational pathway with maximal nitrogen-sulfur orbital interactions. The details of conformer B stereochemistry assume a special importance for understanding the mechanism of cysteine proteases since the substrate in dithioglycine acyl enzymes takes up a B-type conformation. Additional insight into the active-site conformation stems from the differential methyl group orientations of the unsubstituted and *N*-(*p*-chlorobenzoyl)glycine ethyl dithio esters. These permit us to establish a Raman spectrum-structure correlation for conformation in the SCH₂CH₃ fragment. The correlation, when transferred to the corresponding native (dithioacyl)papains, indicates that the C-S-C-C linkages of cysteine 25 assume a nonplanar conformation with the modulus of the torsional angle about S-C probably in the region of 90°. However, upon denaturing the dithioacyl enzyme the C-S-C-C bonds appear to assume a more relaxed planar zigzag state.

Considerable advances have been made in recent years in the correlation of chemical structure with reactivity. One approach pioneered by Bürgi and Dunitz¹ has required very precise structural measurements, obtained by X-ray crystallographic techniques, on small molecules. For example, consideration of interatomic distances in a series of related compounds has led to the concept of favored lines of approach for a nucleophile approaching a carbonyl group.² In another pioneering study, examination of a series of pentacoordinate cobalt complexes led to the proposal that each species could be regarded as a "frozen" reaction intermediate, each being at a different point along a S_N2 pathway.³ Independently, Jones and Kirby⁴ have shown how in tetrahydropyranyl acetals σ - σ^* and n - σ^* overlap control bond lengths and thence reactivity. Such exquisitely detailed information is at the forefront of present-day chemical knowledge and it would obviously be of great interest to extend the precise chemical approach to complex biochemical systems. For example, an appreciation of important nonbonding contacts, preferred lines of approach, and orbital interactions between a substrate and an active site would place our understanding of enzyme mechanism at the level achieved by the most sophisticated physical-organic studies on small molecules. The present work represents part of a continuing effort of this laboratory to elicit such an understanding.

The approach we have been developing has been reviewed elsewhere.⁵ In essence, it is a blend of spectroscopic, X-ray crystallographic, and kinetic techniques. By reacting substrates, which are thiono esters (C(=S)OR), with cysteine proteases such as papain it is possible to generate dithioacyl enzymes, substrate-C(=S)S-papain, in which the thiol sulfur is donated by papain's cysteine 25. The dithio ester group in the active site has a λ_{\max} near 315 nm, and excitation of the reaction mixture with 324-nm Kr⁺ laser irradiation generates the resonance Raman (RR) spectrum of the dithio ester moiety. By these means we can observe selectively the vibrational spectrum of the group undergoing transformation in the active site. Peak positions in the RR

spectrum are very sensitive to the ϕ',ψ' torsional angles of the substrate and, to a lesser degree, to the torsional angles in cysteine 25's -S-C-C bonds. Thus, we have a precise monitor of certain conformational events during the catalytic process. Although the RR data can be gathered on a rapid time scale⁶ under physiologically meaningful conditions, interpretation of the data is not always unambiguous. The principal means we have of overcoming this drawback is by combined X-ray crystallographic and Raman spectroscopic studies on model compounds that have spectral properties closely resembling those of the enzyme-substrate intermediate.⁷ In this way we are able to set up exact structure-spectra correlations which greatly facilitate interpretation of the dithioacyl enzyme RR spectra. At the same time, we are able to investigate structural phenomena in the model compounds at a degree of precision needed to invoke modern theories of reactivity. By means of the spectra-structure correlations we are then able to transfer the concepts developed for the model compounds to the functioning active site.

For *N*-acylglycine (dithioacyl)papains there is evidence for a single homogeneous conformational population of bound substrate designated conformer B.⁸ The torsional angle about the C-C bond in the glycinic NHCH₂C(=S)S moiety assumes a value such that the amide N atoms comes into close contact with the thiol S atom and comparison with model compounds, facilitated by isotope replacements, shows that the conformation in the glycine bonds is essentially relaxed.⁸ For *N*-benzoyl glycine substrates in the active site there is evidence for, at most, only a small distortion of the glycine torsional angles away from those of the corresponding ethyl dithio ester in its relaxed state in solution. Furthermore, no evidence has been found for major distortion in the C(=O)NH peptide group of the bound substrate. However, there is evidence for a nonrelaxed conformation in the cysteine -S-C-C heavy-atom skeleton for the native dithioacyl enzyme. The present paper provides insight into the nature of this perturbed conformation. The main finding of this work, however, concerns the directional preferences of the N...S nonbonding contact in *N*-acylglycine dithio esters and the consequences this may have for the same contact in the active site of (dithioacyl)papains. The

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Table I. Positional and Thermal Parameters of Non-Hydrogen Atoms (*N*-Benzoylglycine Ethyl Dithio Ester)

code	X/A	Y/B	Z/C	B_{eq}^a
S (1X)	58 453 (9)	25 205 (6)	94 662 (5)	4.7
S (2X)	76 831 (10)	53 030 (7)	96 394 (6)	6.9
O (1X)	62 024 (14)	33 738 (11)	68 516 (9)	4.4
N (1X)	41 833 (15)	32 966 (12)	77 726 (10)	4.0
C (1X)	72 276 (34)	11 625 (22)	107 563 (18)	5.6
C (2X)	73 595 (27)	25 137 (18)	104 329 (14)	5.7
C (3X)	62 725 (17)	41 227 (12)	91 439 (10)	3.7
C (4X)	51 447 (18)	43 982 (13)	83 229 (11)	4.2
C (5X)	47 987 (16)	28 530 (12)	70 607 (10)	3.4
C (6X)	37 247 (16)	17 012 (12)	65 399 (10)	3.4
C (7X)	23 332 (21)	8 766 (15)	69 314 (13)	5.2
C (8X)	13 867 (28)	-1 958 (19)	64 284 (18)	7.5
C (9X)	18 229 (31)	-4 358 (20)	55 320 (17)	7.2
C (10X)	32 025 (33)	3 717 (22)	51 514 (15)	6.5
C (11X)	41 595 (23)	14 500 (18)	56 462 (13)	5.2
S (1Y)	90 944 (9)	62 087 (6)	40 900 (5)	4.8
S (2Y)	72 568 (11)	36 574 (7)	32 838 (7)	8.5
O (1Y)	89 049 (13)	74 140 (11)	15 247 (9)	4.2
N (1Y)	108 016 (14)	67 472 (11)	23 304 (10)	4.0
C (1Y)	78 829 (32)	64 668 (21)	58 013 (16)	6.4
C (2Y)	76 046 (25)	55 386 (17)	49 894 (14)	6.0
C (3Y)	86 624 (18)	50 521 (13)	32 346 (11)	4.6
C (4Y)	97 632 (18)	54 103 (13)	23 772 (12)	4.5
C (5Y)	102 847 (15)	76 693 (12)	19 000 (9)	3.3
C (6Y)	114 095 (16)	90 319 (12)	18 922 (9)	3.3
C (7Y)	128 520 (19)	94 065 (13)	24 445 (11)	4.5
C (8Y)	138 280 (23)	106 871 (16)	24 066 (15)	6.3
C (9Y)	133 595 (27)	115 773 (16)	18 203 (17)	6.8
C (10Y)	119 335 (26)	112 110 (16)	12 665 (17)	6.2
C (11Y)	109 530 (20)	99 342 (14)	13 082 (13)	4.7

^a B_{eq} is the isotropic equivalent of anisotropic temperature factors and is calculated as

$$B_{eq} = 8\pi^2/3\{U_{11}a^2 + U_{22}b^2 + U_{33}c^2 + 2U_{12}ab \cos \gamma + 2U_{13}ac \cos \beta + 2U_{23}bc \cos \alpha\}$$

The fractional coordinates are multiplied by 10^5 . X and Y denote Mol-X and Mol-Y.

Following paper⁹ deals with the stereochemical and kinetic consequences of varying the strength of the N...S contact in the active site. We have been concerned until now with dithio esters and dithioacyl enzymes because the dithio group provides a suitable chromophore for providing RR spectra and kinetic data. However, the natural intermediates of the cysteine proteases are thiolacyl enzymes. In the third paper¹⁰ in the present series the crystal structures of two *N*-acyl thiol esters are described, and the conformations of dioxigen, thiol, and dithio esters are compared. Both thiol esters adopt B-type conformations, suggesting that the concepts we develop for the dithioacyl enzymes may be transferable to the more natural thiol intermediates.

Experimental Section

The crystals of both compounds were obtained by diffusing hexane into solutions in ether. For *N*-benzoylglycine ethyl dithio ester, the crystals were yellow in color and a crystal of size $0.9 \times 0.3 \times 0.2$ mm was cut from a larger crystal and was used for all X-ray measurements. The cell constants were determined using 25 well-centered reflections which have 2θ between 74° and 100° . Crystal data: molecular formula, $C_{11}H_{13}NOS_2$; molecular weight, 239.4; space group, $P\bar{1}$; $a = 8.900$ (1) Å, $b = 11.037$ (1) Å, $c = 13.539$ (1) Å; $\alpha = 85.91$ (1)°, $\beta = 95.56$ (1)°, $\gamma = 109.73$ (1)°; $Z = 4$; volume = 1246.3 Å³; $d_x = 1.275$ Mg m⁻³, d_m (floatation) = 1.28 Mg m⁻³; number of molecules in the asymmetric unit = 2. The intensity data were collected using a CAD4 diffractometer using Ni-filtered Cu K α radiation ($\lambda_1 = 1.54051$ Å, $\lambda_2 = 1.54433$ Å) to a 2θ limit of 150° by $\omega/2\theta$ scan, $\Delta\omega = 0.75 + 0.14 \tan \theta$ (deg), aperture $3.0 + 0.40 \tan \theta$ (mm), and a take-off angle of 2.5° . Intensities were monitored by using three standard reflections. The intensities of these reflections decreased by about 5% during the intensity data measurement. A decay correction was applied as a function of time. An

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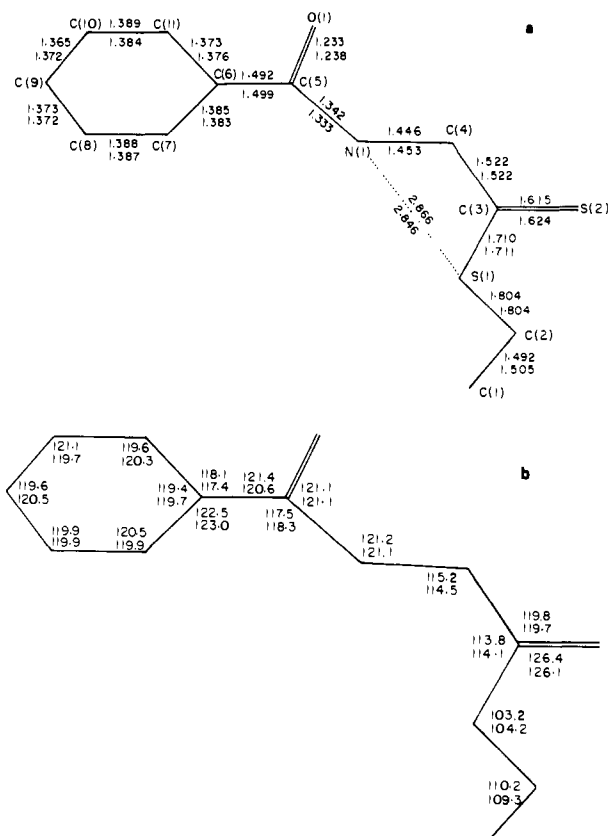


Figure 1. (a) Bond lengths involving non-hydrogen atoms for *N*-benzoylglycine ethyl dithio ester. The upper and lower values are for Mol-X and Mol-Y, respectively. The standard deviations in bond lengths vary from 0.002 to 0.003 Å. (b) Bond angles involving non-hydrogen atoms of *N*-benzoylglycine ethyl dithio ester. The upper and lower values are for Mol-X and Mol-Y. The standard deviations in bond angles are 0.3°.

empirical ϕ absorption correction¹¹ was applied by using measurements on two reflections close to $\chi = 90^\circ$ at 10° interval of azimuth rotation. Out of 5220 unique reflections measured 4441 had $F_0 \geq 1.4\sigma(F)$ and were used in the structure solution and refinement. The structure was solved by the computer program MULTAN 80¹² and refined by block diagonal least squares. Hydrogens were located using stereochemical criteria as well as from the difference electron density map. Hydrogens were refined isotopically while nonhydrogen atoms were refined by using anisotropic thermal parameters. At this stage it was noticed that 14 low-angle reflections were affected by extinction and thus were left out from the refinement. The refinement converged at $R = 0.042$ for 4427 reflections used in the refinement. The atomic scattering factors were taken from the "International Tables for X-ray Crystallography" (1968).¹³ The positional and thermal parameters of non-hydrogen atoms are listed in Table I. The bond lengths and angles of Mol-X and -Y are listed in Figure 1, parts a and b. The bond lengths and angles agree well in most cases. The C(3)-S(2) and C(5)-N(1) distances show the largest differences of 0.009 Å each. The differences in the ψ' , ϕ' angles are discussed under "Directional Preferences of the N...S Contact". For each molecule the standard deviations in bond lengths vary from 0.002 to 0.003 Å and the standard deviations in bond angles are 0.3°.

For *N*-(*p*-chlorobenzoyl)glycine ethyl dithio ester a crystal of size of $0.40 \times 0.35 \times 0.25$ mm was chosen for data measurement. The data measurement, the method of structure solution, and refinement were all very similar to the description provided above. Crystal data: molecular formula, $C_{11}H_{12}NO_2S_2Cl$; molecular weight, 273.8; space group, $P2_1/c$; $a = 8.182$ (1) Å, $b = 9.312$ (1) Å, $c = 17.362$ (1) Å, $\beta = 91.52$ (1)°; $Z = 4$; volume = 1322.4 Å³; $d_x = 1.375$ Mg m⁻³, d_m (floatation) = 1.37 Mg m⁻³; number of molecules per asymmetric unit = 1.

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Table II. Positional and Thermal Parameters of Non-Hydrogen Atoms (*p*-Chlorobenzoyl)glycine Ethyl Dithio Ester

code	X/A	Y/B	Z/C	B_{eq}^a
Cl(1)	77 987 (12)	87 568 (12)	109 625 (5)	6.5
S(1)	18 856 (12)	100 568 (13)	70 262 (5)	6.7
S(2)	23 700 (13)	95 314 (11)	53 519 (5)	6.6
O(1)	61 371 (20)	111 913 (16)	74 061 (9)	4.8
N(1)	51 174 (21)	89 677 (17)	72 618 (9)	4.2
C(1)	-2 221 (43)	120 932 (42)	64 070 (28)	9.2
C(2)	-1 142 (34)	105 492 (41)	66 255 (21)	8.1
C(3)	29 918 (24)	95 810 (19)	62 439 (10)	4.3
C(4)	47 360 (24)	91 416 (22)	64 484 (10)	4.6
C(5)	58 286 (20)	100 028 (18)	76 853 (10)	3.6
C(6)	62 584 (20)	96 702 (17)	85 058 (10)	3.5
C(7)	55 823 (23)	85 177 (20)	89 009 (10)	4.2
C(8)	60 506 (25)	82 389 (22)	96 516 (11)	4.7
C(7)	71 939 (23)	91 189 (22)	100 181 (10)	4.5
C(10)	78 557 (24)	102 679 (22)	96 432 (12)	4.9
C(11)	73 832 (23)	105 462 (19)	88 891 (11)	4.3

^a B_{eq} is the isotropic equivalent of anisotropic thermal parameters and is calculated as

$$B_{eq} =$$

$$8\pi^2/3\{U_{11}/\sin^2\beta + U_{22} + (U_{33}/\sin^2\beta) + 2U_{13}(\cos\beta/\sin^2\beta)\}$$

The fractional coordinates are multiplied by 10^5 .

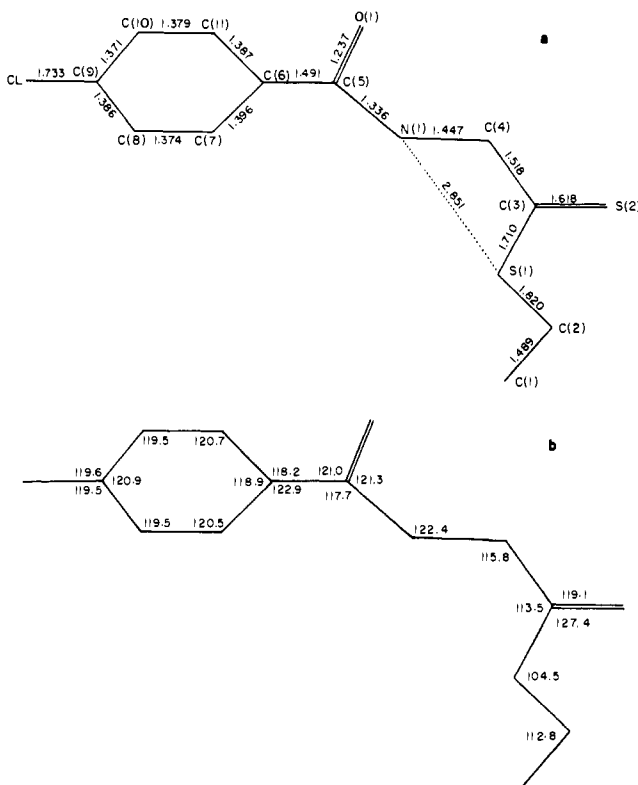


Figure 2. (a) Bond lengths in *N*-(*p*-chlorobenzoyl)glycine ethyl dithio ester. The standard deviations vary from 0.002 to 0.003 Å except for the C(1)–C(2) bond for which it is 0.006 Å. (b) Bond angles in *N*-(*p*-chlorobenzoyl)glycine ethyl dithio ester. The standard deviations are 0.3° except for the S(1)–C(2)–C(1) bond angle for which it is 0.4°.

Three reflections were used to monitor the changes in intensity, and no systematic decrease in the intensity of these reflections was noticed. Out of the 2730 unique reflections measured 395 of them had $F_o < 1.4\sigma(F)$ and were excluded from least-squares refinement. Nine reflections badly affected by secondary extinction were also left out from the refinement. The final R for 2326 reflections used in the least squares was 0.053. Table II gives the positional and thermal parameters of non-hydrogen atoms. The bond lengths and angles are listed in Figure 2, parts a and b.

The listing of the positional and thermal parameters of hydrogen atoms, the anisotropic thermal parameters of non-hydrogen atoms, and structure factors for both compounds is deposited as supplementary material.

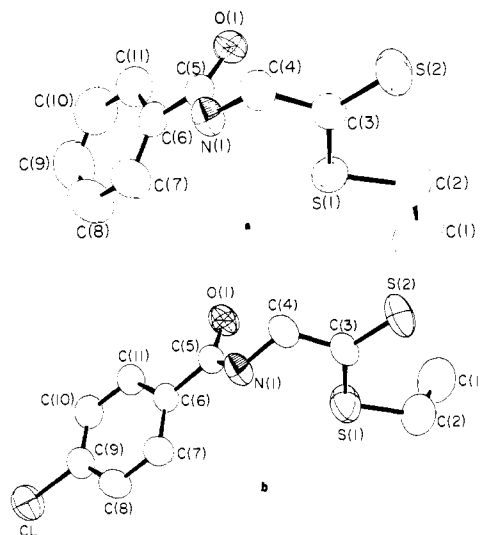


Figure 3. (a) ORTEP plot for Mol-X of *N*-benzoylglycine ethyl dithio ester. Mol-Y has a very similar conformation. (b) ORTEP plot for *N*-(*p*-chlorobenzoyl)glycine ethyl dithio ester.

The 647.1-nm excited Raman spectra of the single crystals were obtained using a Jarrell-Ash 25-400 spectrometer-based system.¹⁴ The 324-nm excited RR spectra of the crystalline materials were obtained by a multichannel system described recently.⁶ Single crystals were ground to a powder which was used to coat the inside of a spinning quartz NMR tube and examined in the backscattering geometry. The method¹⁵ has the advantage of providing reliable UV-excited RR spectra of polycrystalline solids. The normal means of obtaining such spectra, by mixing the powder with KBr, pressing the mixture into a KBr pellet, and rotating the pellet,¹⁶ gave rise to irreproducible results. This was probably due to the formation of different crystalline forms, containing different molecular conformations, at the high pressures used to form the KBr disks.

Results and Discussion

Structural Features. The primary finding of the structural analysis is that all three molecules take up a B-type conformation in the crystalline phase (Figure 3, parts a and b). In conformer B the N–C(4)–C(3)–S(1) torsional angle (designated ψ , by analogy to dipeptide bonds) takes up a value such that the N and S(1) atoms come into close contact. The B form is particularly important in our studies on (dithioacyl)papains, in which the acyl group is normally a *N*-acylglycine derivative, since we have shown that the acyl group takes up a B conformation.⁸ The catalytic consequences of conformer B are not yet fully understood, but it is probable that this form does not confer increased reactivity by virtue of weakening the C–S bond (from –C(=S)S–cysteine) undergoing scission. This conclusion follows from the fact that the C–S(thiol) bond in each conformer B structure analyzed thus far (ref 7 and this work) is not longer than the corresponding bond in *N*-(*p*-nitrobenzoyl)glycine ethyl dithio ester, which lacks the N...S(thiol) contact.⁷ Thus, C(3)–S(1) for *N*-(*p*-nitrobenzoyl)glycine ethyl dithio ester is 1.727 (3) Å, whereas the bond length is 1.700 (6) Å for *N*-acetylglycine ethyl dithio ester⁷ (a B-type conformer) and 1.710–1.711 Å for the molecules examined here.

A second important outcome of the present work occurred fortuitously—in that the major difference in the unsubstituted and *p*-chloro derivatives turned out to be the disposition of the C(1) atom (Figure 3, parts a and b). The approximately 90° difference in the C(3)–S(1)–C(2)–C(1) torsional angle for the two compounds has allowed us to establish a Raman spectra-structure correlation for this fragment which, in turn, offers some insight into the conformation of the cysteine linkages in (dithioacyl)papains.

Other noteworthy observations concerning the structures include the fact that the exocyclic C(11)–C(6)–C(5) angle is less than the C(7)–C(6)–C(5) angle by 4.9° on the average for the three

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Table III. Hydrogen Bonds

atoms	H-H, Å	H...O, Å	N...O, Å	N-H...O, deg	cryst struct
N(1X)-H...O(1Y)	0.94 (2)	1.90 (2)	2.816 (2)	163 (3)	a
N(1Y)-H...O(1X)	0.84 (2)	2.05 (2)	2.847 (2)	158 (3)	a
N(1)-H...O(1)	0.81 (2)	2.07 (2)	2.847 (2)	160 (3)	b

^a*N*-Benzoylglycine ethyl dithio ester. ^b(*p*-Chlorobenzoyl)glycine ethyl dithio ester.

Table IV. Selected Torsional Angles^d

atoms	a	b	c
C(7)-C(6)-C(5)-N(1)	-18.9	-10.9	-17.7
C(6)-C(5)-N(1)-C(4)	179.2	179.0	-176.2
ϕ' C(5)-N(1)-C(4)-C(3)	-78.7	-83.5	-97.1
ψ' N(1)-C(4)-C(3)-S(1)	-15.5	-10.5	9.5
C(4)-C(3)S(1)-C(2)	179.9	179.2	179.3
C(3)-S(1)-C(2)-C(1)	-176.1	178.8	-88.7

^aMol-X *N*-benzoylglycine ethyl dithio ester. ^bMol-Y *N*-benzoylglycine ethyl dithio ester. ^c(*p*-Chlorobenzoyl)glycine ethyl dithio ester. ^dStandard deviations vary from 0.3° to 0.4°.

molecules described here. The widening of the C(7)-C(6)-C(5) angle appears to be due to repulsion between the hydrogen at C(7) and the amide hydrogen. The H...H distances are 2.10 (2), 2.09 (2), and 2.11 (3) Å for Mol-X and Mol-Y of the unsubstituted *N*-benzoyl compound and the *p*-chloro derivative, respectively. Also, the benzene ring is tilted from the amide plane by 19°, 11°, and 18° in Mol-X and Mol-Y of the *N*-benzoylglycine dithioester and in the *p*-chloro analog, respectively.

In all the three molecules the amide nitrogen becomes the donor in the formation of intermolecular N-H...O type hydrogen bonds. The hydrogen bond parameters are listed in Table III.

The hydrogen positions do not have the same precision as the other atoms and hence the bond angles involving hydrogen atoms have a standard deviation of 2°. However, it is seen that the bond angles at the nitrogen add up to 360° in all the three cases described here (359.7°, 360.0°, and 359.9° for Mol-X, Mol-Y and the *p*-chloro derivative, respectively). Thus, there is no evidence for pyramidalization at the N atom. Also there is no appreciable ω distortion. The deviation ($\Delta\omega$) of the torsion angle τ (C(6)-C(5)-N-C(4)) from 180° is 0.8°, 1.0°, and 3.8° for Mol-X, Mol-Y, and the *p*-chloro derivative, respectively.

Directional Preference of the N...S Contact. Each of the three molecules is in the B form with rather short N...S distances because S(1) and N are cis with respect to the C(3)-C(4) bond. The ψ' values are -15.5°, -10.5°, and 9.5° (Table IV) and the S...N distances are 2.866, 2.846, and 2.851 Å in Mol-X, Mol-Y, and the *p*-chloro compound, respectively. These distances are less than the sum of van der Waals radii (3.35 Å),¹⁷ and the main interaction that stabilizes the B conformation appears to be the N...S attraction. Rosenfield, Parthasarathy, and Dunitz¹⁸ have examined the directional preferences of the approach of electrophiles or nucleophiles to divalent sulfur. 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 antibonding orbital σ^* .

For the molecules under consideration here the N...S interaction meets Rosenfield et al.'s geometrical criterion¹⁸ for a nucleophilic nitrogen approaching an electrophilic sulfur. The atoms S(1), C(2), and N(1) lie nearly in a straight line, with the N...S-C angles falling in the range 161° to 165° for the three molecules described here and *N*-acetylglycine ethyl dithio ester.⁷ 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

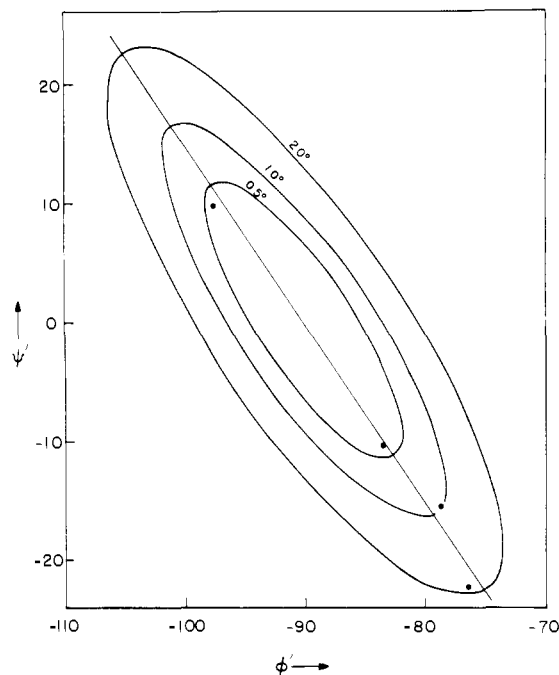


Figure 4. θ values as a function of ϕ' and ψ' . θ is defined as the angle between the normal to the amide plane and the S...N direction. The θ minimum is 20.1° corresponding to $\phi' = -90^\circ$ and $\psi' = 0^\circ$. The contours represent $\Delta\theta$ (deviations from θ minimum) of 0.5°, 1.0°, and 2.0°. The circles represent the observed ϕ', ψ' for the three molecules described here as well as *N*-acetylglycine ethyl dithio ester.⁷

involving a LUMO σ^* based on the S-C(2) fragment, the other involving a sulfur d orbital. A N(HOMO)...S-C(2)LUMO interaction would be expected to lengthen the S-C(2) bond length since electron density would be placed in the S-C(2) σ^* orbital. For the conformer B class this length varies from 1.804 to 1.820 Å and appears to be longer than the bond in crystalline *N*-(*p*-nitrobenzoyl)glycine ethyl dithio ester, which is not a B-type conformer and has a S-C(2) length of 1.791 (4) Å. However, the smallest difference, 0.013 Å, is at the 1% significance level, and the present data cannot be used as definitive evidence for an interaction involving predominantly a σ^* S-C(2) orbital. Thus, this orbital or a S d orbital, or both, may be involved in the N...S interaction. Further data are required for *N*-acylglycine dithio esters in non-B conformers to add to that for the present single structure for an A conformer.

It is difficult to define precisely the HOMO occupied by the nitrogen lone pair. Resonance in the amide C=O and C-N linkages results in nitrogen hybridization between sp^2 and sp^3 . For the three molecules considered here the geometry about the N atom is essentially planar (see above) and thus the hybridization must lie near the sp^2 limit. This being the case, the lone pair occupies a p orbital and some of its electron density is delocalized into the peptide bonds. However, we shall assume that a substantial fraction of the lone-pair density remains in the direction of the p orbital. Support for this assumption is found in quantum mechanical calculations on the amide linkage.^{19,20} For example, Del Bene²⁰ showed, using ab initio SCF calculations, that the π -electron density at the nitrogen atom of formamide was 1.851 electrons. Thus, it is a reasonable working assumption that the HOMO participating in the N...S interaction is a p orbital on the nitrogen and that the orbital population is not much less than 2 electrons.

Although the three molecules described here, as well as *N*-acetylglycine ethyl dithio ester, are all in "B" conformations, the torsion angles ϕ' and ψ' are spread over a range of about 20° and 30°, respectively. This variation helps us to examine the N...S

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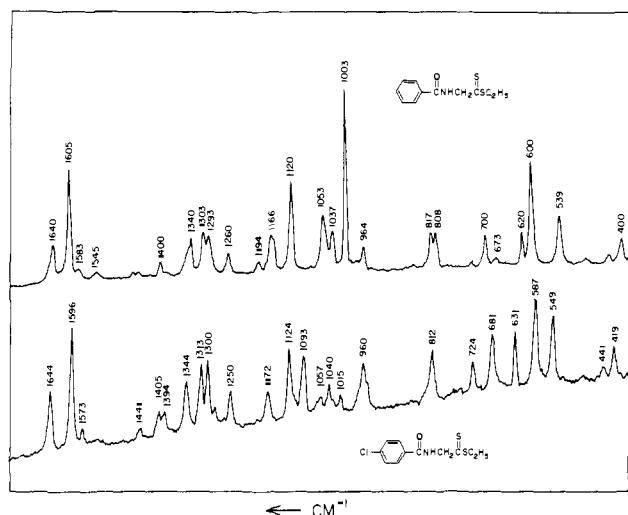


Figure 5. Raman spectra of single crystal of *N*-benzoylglycine ethyl dithio ester (top) and *N*-(*p*-chlorobenzoyl)glycine ethyl dithio ester (bottom). Spectral conditions 647.1-nm excitation, 80 mW, 6-cm⁻¹ spectral slits.

stereochemistry in some detail and to show that ϕ' and ψ' are correlated. The correlation between ϕ' and ψ' is seen in Figure 4, which shows that the four experimentally observed points 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 and which corresponds to the portion of optimal nitrogen HOMO and sulfur LUMO overlap within the constraints of the molecular geometry. The observed ϕ', ψ' correlation cannot be due to the minimization of the repulsive forces between S and H (from the NH group). For the molecules discussed here the S...H distances are greater than 3.0 Å where as the sum of the van der Waals radii for S and H is 2.85 Å.

An interesting question that remains is the importance of the N...S interaction detected in the active site of dithioacyl enzymes. For small molecules HOMO...LUMO interactions are very significant; they form the basis of some of the strongest intermolecular interactions²¹ to the extent that they can influence or dictate crystal packing. Facts such as these, taken with the ψ', ϕ' correlation seen in Figure 4 raise the question of the role of the correlation during the catalytic process. It is believed that the acyl enzyme intermediate lies on the reaction pathway between tetrahedral intermediates for acylation and deacylation. So can the dithioacyl enzyme approach the tetrahedral intermediate for deacylation, for example, along the θ valley seen in Figure 4? At this stage the answers to such questions would be conjectural—presently we have insufficient knowledge of the exact ψ', ϕ' angles in the enzyme-bound substrate. However, the ψ', ϕ' 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.

Raman and Resonance Raman Spectra of the Crystalline Compounds. The 647.1-nm excited Raman spectra of single crystals of *N*-benzoylglycine ethyl ester and *N*-(*p*-chlorobenzoyl)glycine ethyl ester are shown in Figure 5. The corresponding RR spectra, obtained by exciting the powdered crystals with 324-nm irradiation (Experimental Section), are shown in Figure 6. The major features in the RR spectra have corre-

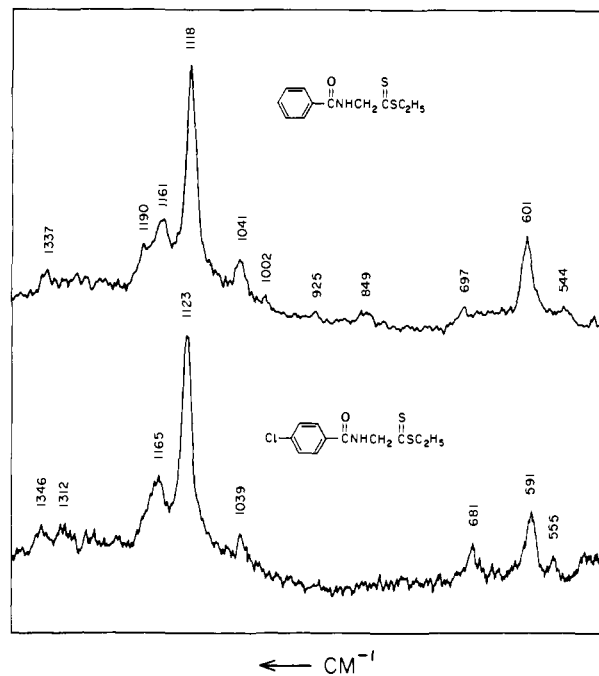


Figure 6. Resonance Raman spectra of crushed crystals of *N*-benzoylglycine ethyl dithio ester (top) and *N*-(*p*-chlorobenzoyl)glycine ethyl dithio ester (bottom). Spectral conditions, 324-nm excitation, 40 mW, 12-cm⁻¹ spectral slit, 20-s data acquisition time.

sponding bands in the Raman spectra of the single crystals, and the approximate origin of each band is understood on the basis of earlier studies.^{5,22,23} In the RR spectra (Figure 6) the peak near 1160 cm⁻¹ is due to a highly coupled mode delocalized throughout the C(=O)NHCH₂C(=S)S fragment, the intense peak near 1120 cm⁻¹ (designated band II²⁴) contains major contributions from C=S and C—C (from CH₂—C(=S)) stretching motions, and the band near 1040 cm⁻¹ has some C—C(ethyl) stretching character. The latter band is believed to be a characteristic B-conformer feature,²³ and it is apparent from the data in Figure 6 that it is not strongly dependent on the conformation about the S(1)—C(2) linkage; the peak is present with about equal intensity in both RR spectra in Figure 6. The peak near 685 cm⁻¹ is the only feature in the spectra that has a group frequency character, it has a major contribution from the S—C (from S—C₂H₅) stretching motion. The peak near 600 cm⁻¹ is due to a highly coupled motion which includes bond stretches in the C—C(=S)—S fragment, and, although less well characterized, the peak near 550 cm⁻¹ may have a similar origin.

The major value of the present study for understanding the RR spectra of dithioacyl enzymes is the insight it offers into the conformation about the S—C—C cysteine linkages. Until this time RR spectra—structure correlations have not been established for the —S—C—C fragment. Now, however, we can explore the spectral consequences of going from a planar zigzag heavy atom C—S—C—C skeleton in the *N*-benzoyl crystal to a nonplanar skeleton in the *N*-(*p*-chlorobenzoyl) analogue in which the CH₂—CH₃ bond is approximately orthogonal to the plane defined by the C—S—C atoms. When the spectral correlation has been established for the geometrical change in *N*-benzoylglycine ethyl dithio esters, it will be possible to go to papain dithio esters on the basis of *N*-benzoyl substrates and to describe the conformation in the —S—C—C bonds of the active site cysteine 25.

Since the RR spectrum is sensitive to the ϕ' and especially the ψ' torsional angles, it is necessary to discuss the dependence of the RR spectrum on these before turning to the effects of rotations about the S—C bond. First, for *N*-benzoylglycine ethyl dithio ester

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it is apparent that the very small changes in ϕ' and ψ' have little or no measurable effect on the RR spectrum. Although band II (the mode near 1120 cm^{-1}) is sensitive to changes in ψ' there is no evidence of conformational heterogeneity from the RR spectrum in Figure 6. The two forms, X and Y, must contribute equally to the 1118 cm^{-1} feature yet it appears as a single un-broadened peak. Similarly, the slightly different values of ψ' and ϕ' for the two forms of the *N*-benzoyl compound have no apparent effect on the RR spectrum in the $500\text{--}700\text{-cm}^{-1}$ range. The better quality normal Raman spectra of the crystals (Figure 5) fully support these conclusions. The *p*-chlorobenzoyl analogue exists as a single homogenous population in the crystalline form. The increase of 5 cm^{-1} in the position of band II compared to the *N*-benzoyl analogues is readily accounted for by the $\approx 20^\circ$ change in ψ' (Table IV). There are also changes of $10\text{--}18\text{ cm}^{-1}$ in the positions of the three peaks in the $550\text{--}700\text{-cm}^{-1}$ region (Figures 5 and 6). It is possible that these changes reflect the minor variation in ψ' as well as the major changes in the S-C torsional angle for the substituted and unsubstituted *N*-benzoylglycine derivatives. However, there is strong evidence from simple diethyl dithio esters that the variation about the S-C torsional angle is the predominant factor. For the molecule $\text{CH}_3\text{C}(=\text{S})\text{SCH}_2\text{CH}_3$ in its *s*-cis form rotational isomerism is only possible about the S-CH₂ bond. Studies from this laboratory^{25,26} revealed that there are two major differences between the Raman spectrum of the neat liquid at 25 and at -132°C . At 25°C there is a pair of bands at 697 and 681 cm^{-1} and a pair of bands at 589 and 579 cm^{-1} , whereas at -132°C there are single sharp features at 682 and 583 cm^{-1} . The disappearance of one band in each pair was ascribed to the fact that only one rotational isomer was present at low temperature. Importantly, this study shows that rotational isomerism involving a methyl group about the S-C linkage causes a 16-cm^{-1} shift in $\nu_{\text{S-C}}$ and a 10-cm^{-1} shift in the complex feature near 580 cm^{-1} . Since these are just the shifts we are seeing in Figures 5 and 6, a good case can be made that the changes in the $550\text{--}700\text{-cm}^{-1}$ region for the *N*-benzoyl- and *N*-(*p*-chlorobenzoyl)glycine ethyl dithio esters results primarily from the difference in the S-C torsional angles.

Conformation of the Cysteine S-C-C Linkages in *N*-Benzoylglycine (Dithioacyl)papains. Recent discussion of the RR

spectra of *N*-benzoylglycine (dithioacyl)papains⁸ centered on the comparison with the spectra of *N*-benzoylglycine ethyl dithio esters,²³ which for the dithio esters in solution were taken as standards for relaxed conformers. The discussion⁸ also dealt with the comparison of the native dithioacyl enzymes in the pH range $4.0\text{--}8.0$ and the denatured dithioacyl enzymes below pH 2.8. On the basis of these comparisons, it was concluded that the conformation in the -S-C-C linkages of cysteine 25 in the native dithioacyl enzyme was in some unknown nonrelaxed state. The present work provides a clue as to the nature of this "nonrelaxed" conformation. In the RR spectra of the native *N*-benzoylglycine(dithioacyl)papains the major peaks in the $500\text{--}700\text{-cm}^{-1}$ range are two bands at 595 and 562 cm^{-1} . The latter band is notable because it does not appear in the RR spectrum of the corresponding ethyl ester in solution. Upon denaturation the 595-cm^{-1} peak shifts to approximately 605 cm^{-1} and the 562 peak shifts to near 550 cm^{-1} and is greatly reduced in relative intensity. The analogy with the RR spectra of the crystalline compounds in Figure 6 is clear. Going from the planar C-S-C-C heavy-atom skeleton found in crystalline *N*-benzoylglycine ethyl dithio ester to the *p*-chloro analogue where there is an approximate 90° rotation about the S-C bond there is a 10-cm^{-1} decrease in the 601-cm^{-1} band and a 10-cm^{-1} increase in the 544-cm^{-1} feature accompanied by a modest increase in relative intensity of the latter. This strongly suggests that the torsional angle about the cysteine S-C bond, in the native (dithioacyl)papain, resembles the 90° found for the *p*-chloro dithio ester although we cannot discriminate between the possibilities of $\pm 90^\circ$ for the dithioacyl enzyme. Upon denaturation the C-S-C-C skeleton relaxes to near planarity accounting for the shifts in the dithioacyl enzyme 595- and 562-cm^{-1} peaks. In addition, $\nu_{\text{S-C}}$ for the nonplanar C-S-C-C *p*-chloro analogue appears at 681 cm^{-1} , 16 cm^{-1} below the value for the *N*-benzoyl analogue (Figure 6). This provides a further rationale for the appearance of $\nu_{\text{S-C}}$ in the (dithioacyl)papain RR spectra⁸ at the relatively low value of $670\text{--}675\text{ cm}^{-1}$.

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Supplementary Material Available: Listing of structure factors, anisotropic thermal parameters, and positional and thermal parameters of hydrogens (44 pages). Ordering information is given on any current masthead page.

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