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A Theoretical Investigation of the Preferred Conformations of Glutathione and Its Constituent Amino Acid Residues

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The preferred conformations of the tripeptide glutathione have been investigated by performing quantum mechanical calculations using the PCILO method. A series of model compounds representing fragments of the tripeptide has been studied as well as the complete molecule. The results are compared with the available experimental data.

Key words: PCILO – Glutathione – Conformations.

1. Introduction

Glutathione, γ-glutamyl-cysteinyl-glycine, is an important constituent of most if not all living cells. One of its chief functions is to protect the SH groups of proteins, which it does by keeping them in a reduced form. It also serves as a specific reducing agent for hydrogen peroxide and the oxidised form of ascorbic acid, and acts as a coenzyme for certain enzymic systems including the glyoxalase system [1, 2].

It is this latter function which is of particular interest in this laboratory in view of our interest in Szent-Györgyi's electronic theory of cancer [3]. The glyoxalase system is thought to play a vital role in controlling cell proliferation [3] and theoretical calculations on methyl glyoxal, which forms an adduct with glutathione in this system, are currently being carried out in this laboratory. We therefore decided also to attempt a theoretical study of the preferred conformations of glutathione.

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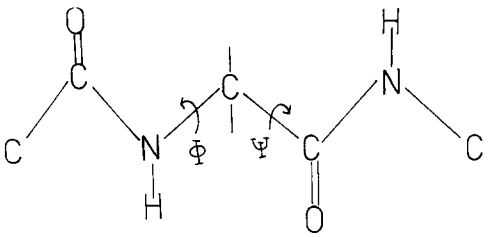


Fig. 1. An example of a typical fragment illustrating torsional angles

The size of this molecule precludes a thorough investigation of all possible conformations and for this reason the present study is necessarily incomplete. However it was hoped that some insight into the generally preferred shape of the molecule would be gained as well as further information regarding the influence of adjacent amino acids on the conformational preferences of their neighbours.

We present therefore the results of this preliminary study in this paper.

2. Procedure

The method used was the Perturbative Configuration Interaction Procedure using Localized Orbitals (PCILO) devised by Diner, Malrieu and Claverie [4]. This method has been widely used for conformational studies on peptides and nucleic acids with considerable success [5, 6].

The majority of results in this study are presented in the form of conformational energy "maps"[1]. These are produced by the simultaneous variation of two torsional angles, Φ, Ψ, usually in 30° steps (Fig. 1). The total energy of the molecule is calculated for each value of Φ, Ψ and "contours" linking points of equal energy are then drawn. A "global" map may be produced by combining a series of related "sub-maps". This is done by taking for each value of Φ, Ψ the lowest energy presented by any sub-map. In this way it is possible to combine the results of varying more than two torsional angles simultaneously. (The torsional angle τ about bond B–C in the sequence of bonded atoms A–B–C–D is the angle between the planes ABC and BCD. This angle is positive for clockwise rotation around B–C when looking from B to C (Fig. 2).)

In constructing the energy maps the convention is adopted that zero values of torsional angles correspond to fully eclipsed conformations [7], thus the fully extended peptide chain is represented by $\Phi = \Psi = 180°$.

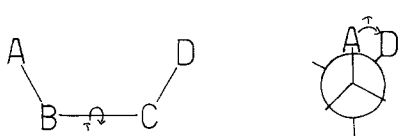


Fig. 2. Definition of torsional angle τ as seen (a) perpendicular to the bond being rotated, and (b) looking along the bond

[1] For reasons of space only a few maps are presented here. If required the remainder may be obtained from the authors by private request.

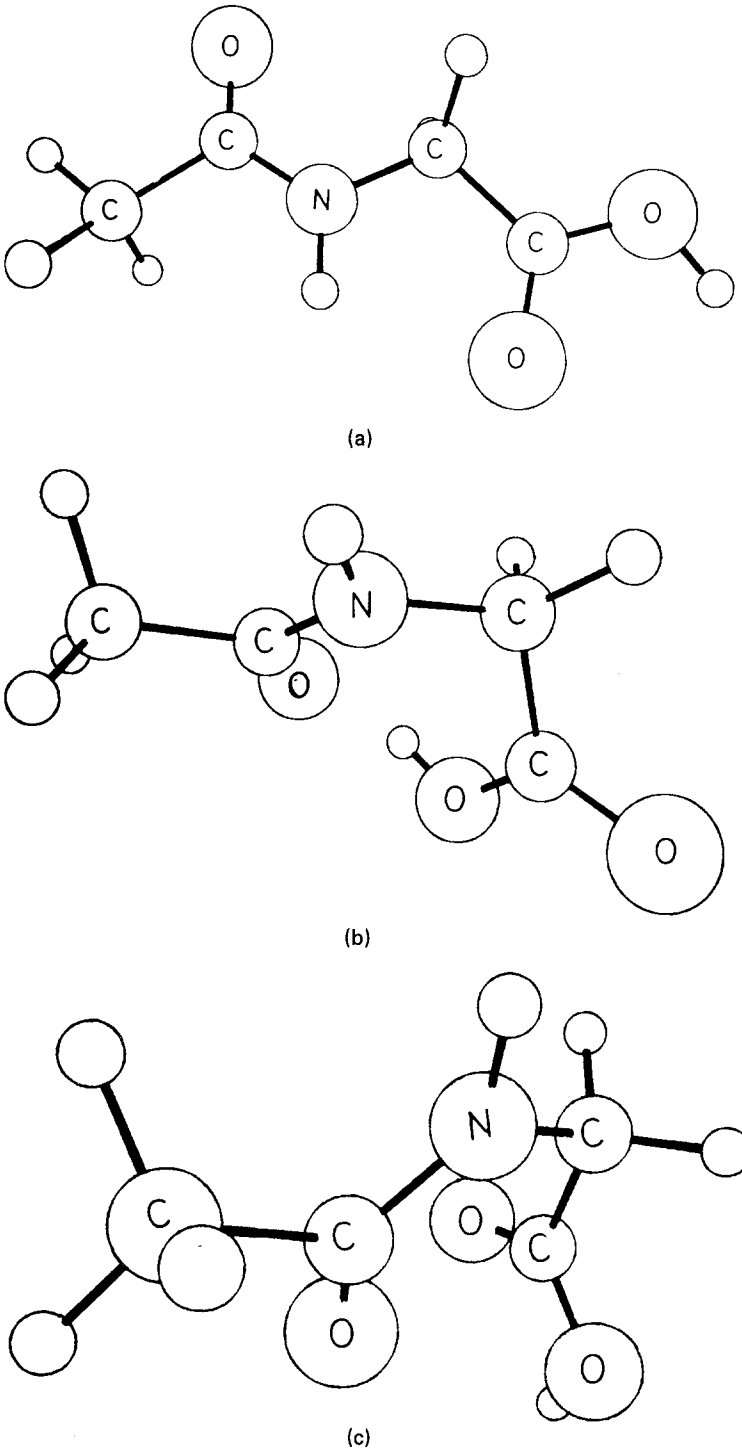


Fig. 3. Stable conformations of the glycyl model (a) C_5, (b) C_7, (c) M

Stable conformations are described as C_5, C_7, M etc. following Pullman and Maigret [8] where C_n denotes an n-membered hydrogen-bonded ring and M conformations have adjacent peptide groups approximately perpendicular. C_5, C_7 and M conformations are illustrated for the glycyl model in Fig. 3.

Standard values were used for bond lengths and angles [9, 10]. The calculations were performed on the Honeywell 66 at Aberdeen University and the IBM 360 at St. Andrews using a modified version of the PCILO program obtained from Professor A. Pullman [11].

All energies are quoted in kcal mol^{-1}.

3. Results

Since the complete molecule contains 37 atoms and 162 electrons, these preliminary calculations have been performed by considering a series of model compounds representing fragments of the molecule. (These models are similar, but except in the case of cysteine not identical, to the "residues" used extensively by Pullman et al. in studies of di- and tri-peptides [8, 12].) The division of the molecule into fragments is illustrated in Fig. 4.

The glycyl, cysteinyl and glutamyl fragments were first studied individually. Ionic as well as neutral species were considered for the glycyl and glutamyl models. The cysteinyl and glycyl fragments were then combined to give a cysteinyl–glycine model, which was again studied in neutral and ionic forms. A limited number of calculations were performed on a glutamyl–cysteine model formed by combining the glutamyl and cysteinyl fragments. Since this model is only slightly smaller than glutathione itself, it was thought more useful to perform calculations on the complete molecule and the calculations on this model are not reported here. A restricted number of calculations were performed on the complete glutathione molecule in both neutral and ionic forms.

The results of these calculations are presented in the sections which follow.

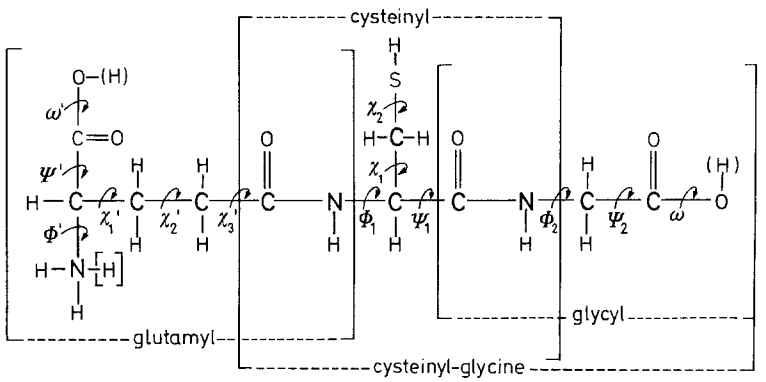


Fig. 4. Division of glutathione molecule into fragments. [H] present only in ionized form; (H) present only in neutral form

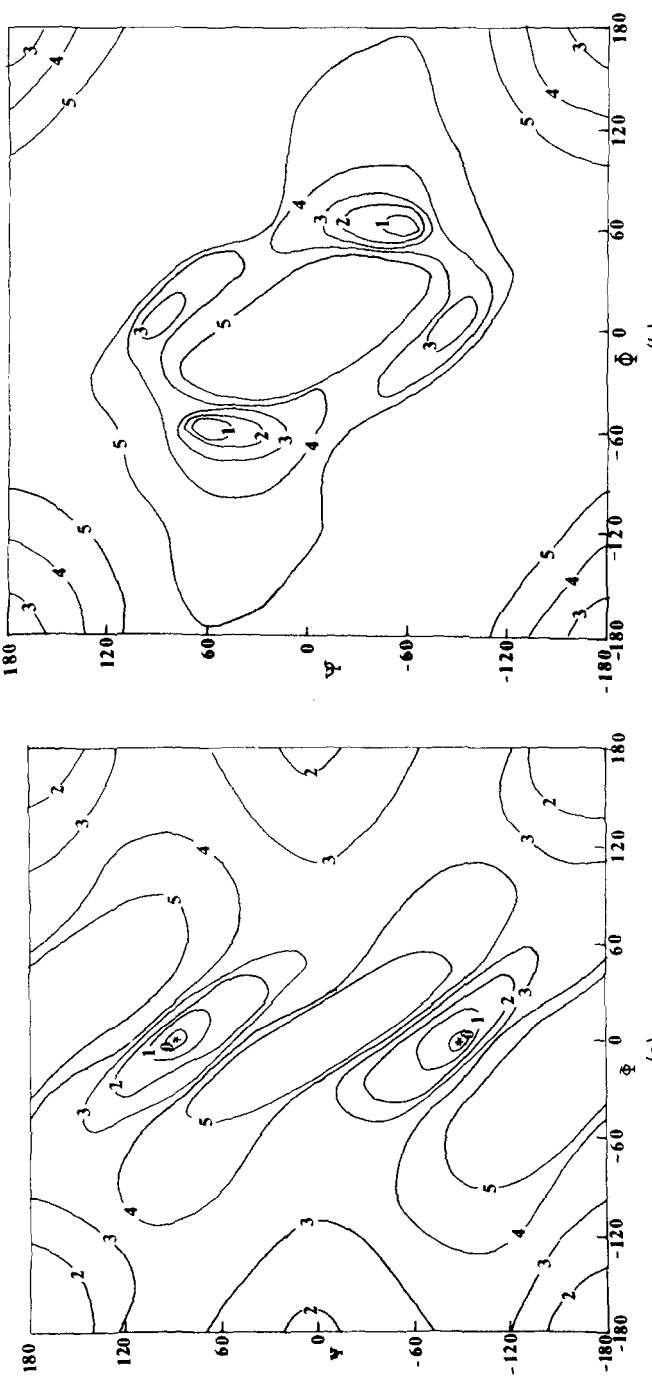


Fig. 5. Conformational energy maps for glycyl model (a) with $\omega = 0°$, (b) with $\omega = 180°$. Isoenergy curves (kcal/mole) with respect to the global *energy minimum* (†) taken as *energy zero*

Table 1. Optimum conformations of glycyl model. Equivalent conformations are indicated }

Values of torsional angles (°) Φ	Ψ	ω	Description of conformation
180	180	0	C_5
180	0	0	C_5
0	90	0 }	M
0	−90	0 }	M
180	180	180	C_5
0	−90	180 }	M
0	90	180 }	M
60	−60	180 }	C_7
−60	60	180 }	C_7

3.1. Glycyl Model

The neutral form of this molecule has three degrees of rotational freedom; however the only important values of ω appear to be 0° and 180°. Accordingly two surfaces for Φ, Ψ rotation were produced corresponding to $\omega = 0°$ and 180° respectively (Fig. 5a, b). Conformations giving rise to minima on the energy surfaces are listed in Table 1 and illustrated in Fig. 3.

Ab initio calculations (using the Gaussian 70 program) performed on this molecule emphasize the importance of the C_5 and C_7 rings. (These calculations will be the subject of a separate paper [13].)

The ionized form of this molecule was also considered. There are problems in using PCILO to study such a molecule since the structures (a) and (b) (Fig. 6) do not give rise to equal energies. Strategies that have been suggested [4, 14] to overcome this difficulty are to take the structure giving the best zeroth order energy and use it to calculate the third order energy, or to use the structure giving the best third order energy, or to take a mean of the third order energies. The first two approaches were used and resulted in similar energy maps. These indicate that the only important conformation is a planar "C_5" arrangement in which there is hydrogen-bonding between −NH and O^-. The barrier to Ψ rotation is only ~4 kcal. However the barrier for Φ rotation is considerably higher (~70 kcal).

Ab initio calculations confirmed these results.

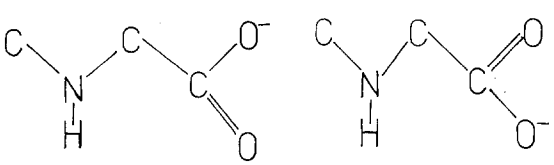


Fig. 6. Non-equivalent representations of ionized carboxylic acid group

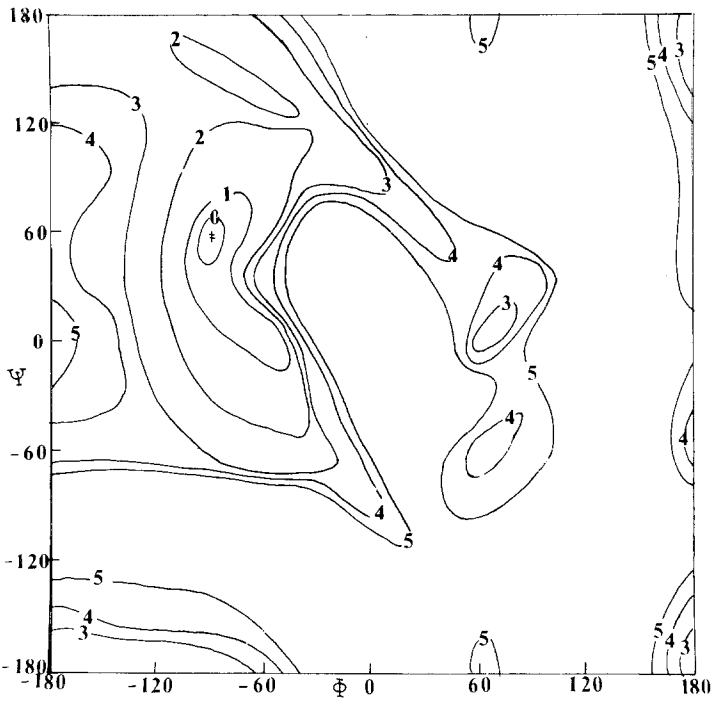


Fig. 7. Global conformational energy map for cysteinyl model

3.2. Cysteinyl Model

This is equivalent to the cysteinyl residue studied by Perahia, Pullman and Claverie [15].

The side chain angles χ_1, χ_2 were restricted to staggered values i.e. 60°, 180°, 300°. Nine sub-maps were constructed corresponding to the possible combinations of these angles. The global energy map is presented in Fig. 7.

The results of this study follow closely those obtained by Perahia et al. Thus the global minimum occurs for $\Phi_1 = -90°$, $\Psi_1 = 30°$–$60°$, which is the preferred conformation for all values of χ_1, χ_2 (Fig. 8). Perahia predicts $\chi_1 = \chi_2 = 60°$ to be the most stable combination of side chain angles with $\chi_1 = 60°$, $\chi_2 = 180°$ 0.3 kcal higher; the results of this study indicate $\chi_1 = 60°$, $\chi_2 = 180°$ to be the more stable arrangement with $\chi_1 = \chi_2 = 60°$ 0.12 kcal higher. Within the error limits of the method (0.5 kcal) these two conformations must be regarded as equivalent. The arrangement with $\chi_2 = 60°$ makes possible the formation of a hydrogen-bond between SH and O_2, but possibly this is too weak to be of any significance. Both conformations can form a weak hydrogen-bond between S and N_1H as well as the bond between N_2H and O_1. Other stable combinations of χ_1, χ_2 for these values of $\Phi_1\Psi_1$ are $\chi_1 = 300°$, $\chi_2 = 180°$ which offers the possibility of a hydrogen bond between S and N_1H, and $\chi_1 = 180°$, $\chi_2 = 300°$ which could involve a bond between SH and O_2.

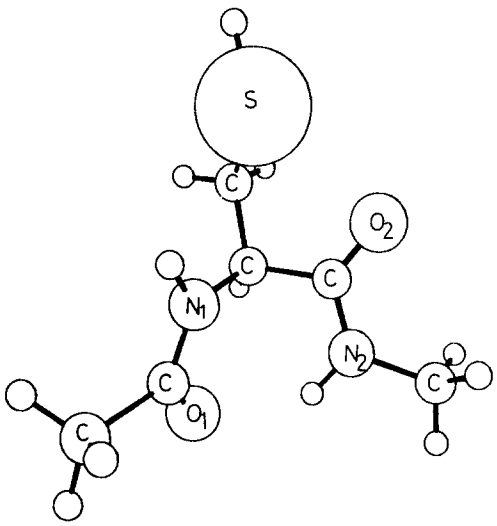


Fig. 8. Optimum conformation of cysteinyl residue with $\chi_1 = 60°$, $\chi_2 = 180°$.

Other stable conformations are an "M" conformation at $\Phi_1 = -30°$, $\Psi_1 = 120°$ for $\chi_1 = 300°$, $\chi_2 = 180°$; and a number of conformations associated with $\Phi_1 = \Psi_1 = 180°$ for various values of χ_1, χ_2, corresponding to the formation of a C_5 ring.

3.3. Glutamyl Model

In studying this molecule the aim was to find the preferred conformations of the glutamyl chain. Keeping the peptide link fixed in the trans planar arrangement with the methyl group eclipsed there remained six torsional angles to consider. Each of these was varied in turn.

Table 2. Optimum conformations of glutamyl model

Values of torsional angles (°) ($\phi' = 60°$, $\omega' = 0°$ for all conformations)			
Ψ'	χ'_1	χ'_2	χ'_3
30°	60°	0°	−120°*
−150°	60°	60°	120*
−150°	60°	0°	−120°
−150°	60°	30°	120°
−150°	60°	90°	120°
−30°	60°	60°	180°
30°	60°	30°	120°
0°	60°	60°	−150°*
180°	60°	60°	90°*
0°	60°	0°	−120°
0°	60°	60°	−120°

The preferred value of Φ' was 60° and of ω' was 0°. Ψ' variation showed a twofold minimum at $\Psi' = 0°$ and 180° with values close to this (±30°) also being stable. χ_1' variations showed the usual threefold minimum but the pattern for χ_2' and χ_3' was not so simple. Accordingly, for each preferred value of χ_1' and Ψ', χ_2' and χ_3' were varied simultaneously.

The most stable conformation occurred for $\Psi' = 30°$, $\chi_1' = 60°$, $\chi_2' = 0°$, $\chi_3' = 240°$ (illustrated in Fig. 9a). Some of the other stable conformations are listed in Table 2 and a typical example is illustrated in Fig. 9b.

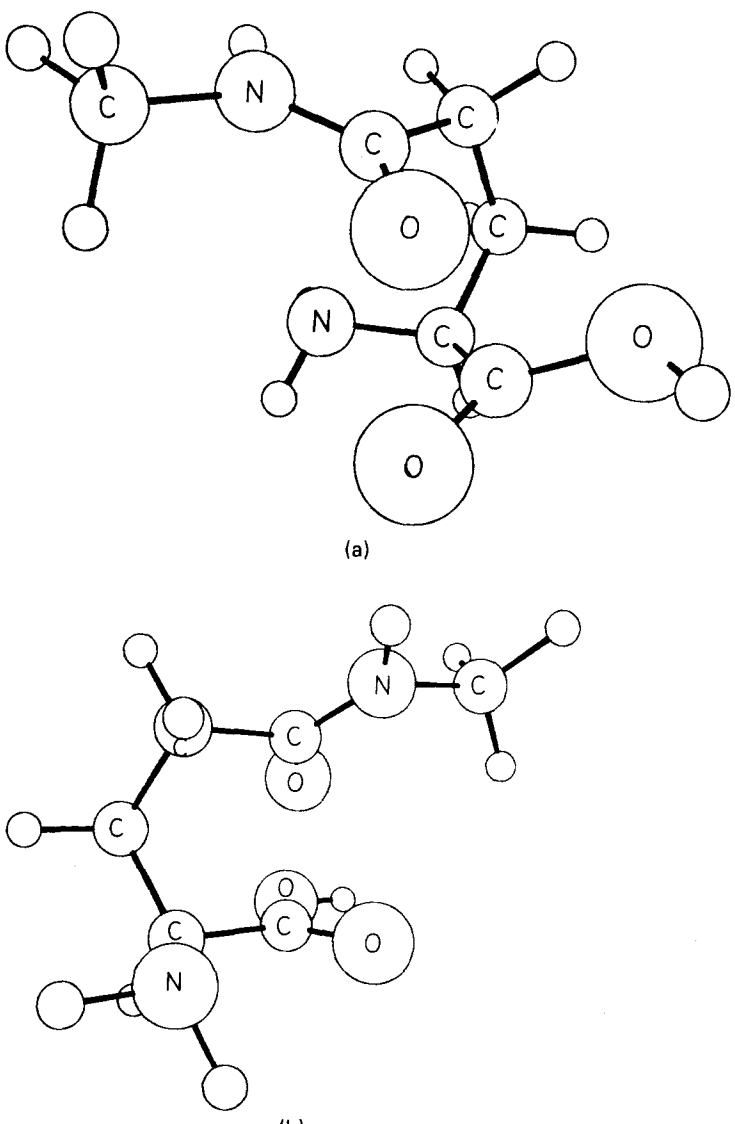
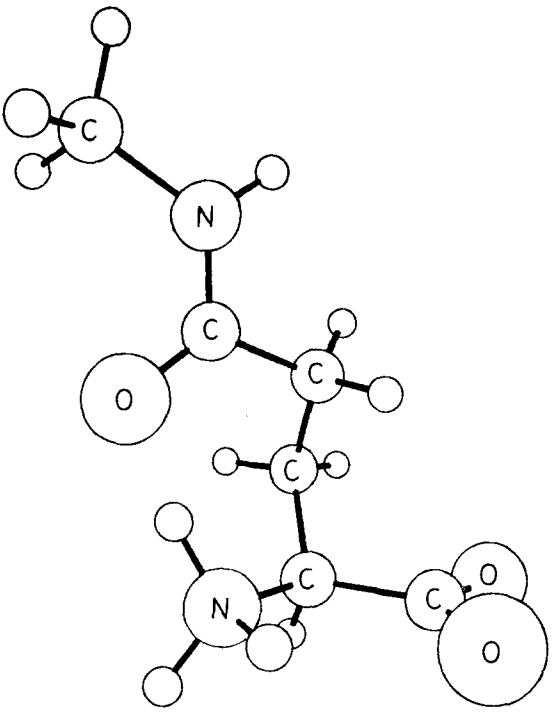
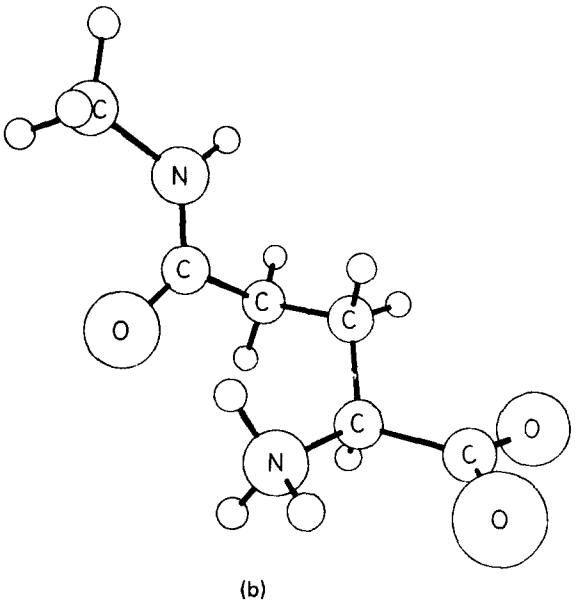


Fig. 9. Stable conformations of glutamyl model

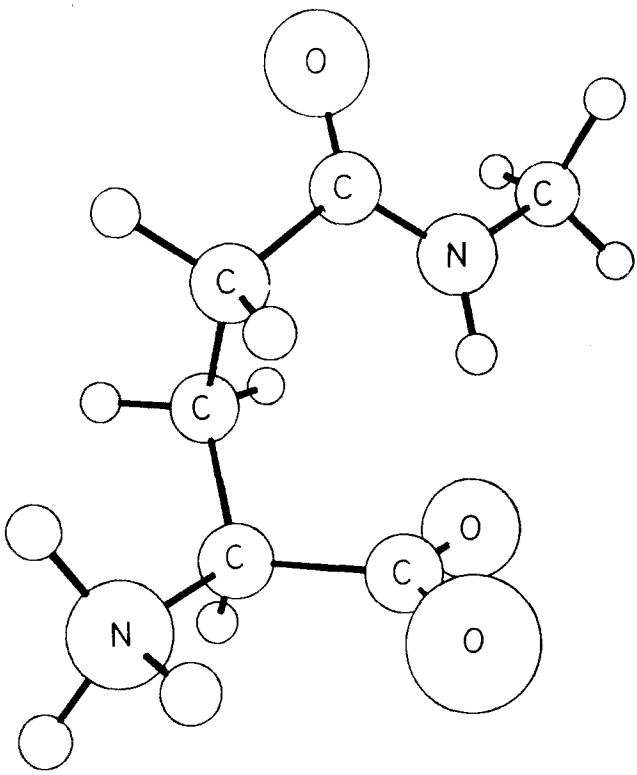


(a)

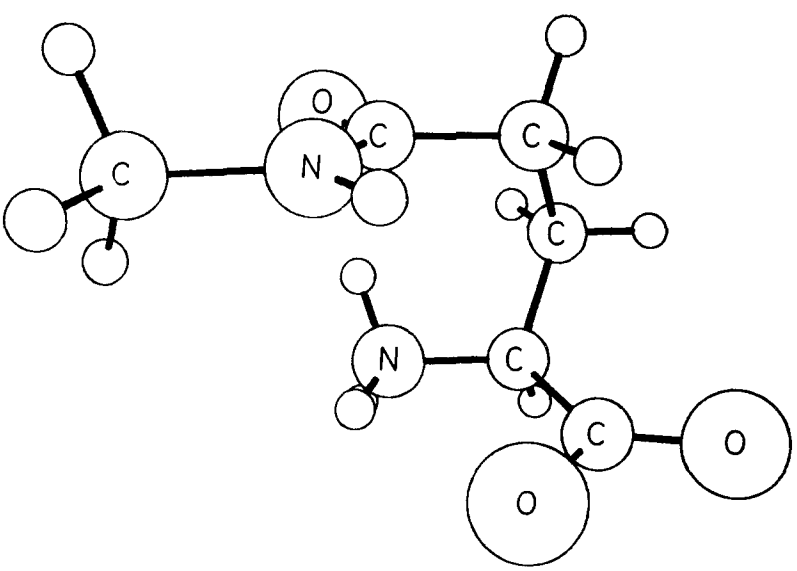


(b)

Fig. 10. Stable conformations of glutamyl model ion



(c)



(d)

Fig. 10. *contd.*

Obviously there are a good many conformations that have not been studied and it may even be that some important conformations have been overlooked. However it is perhaps possible to conclude that "closed" structures involving some interaction between the amino acid and peptide groups are favoured over "open" structure in which these groups are far apart.

The zwitterion of this molecule was studied in a similar way to the neutral species. $\Phi' = \Psi' = 0°$ were the only stable values for these angles. Stable conformations occurred as follows (Fig. 10a, b, c, d):

$$\chi_1' = 60°, \quad \chi_2' = 270°, \quad \chi_3' = 180° - 270°$$
$$\chi_1' = 300°, \quad \chi_2' = 90°, \quad \chi_3' = 90° + 180°$$
$$\chi_1' = 60°, \quad \chi_2' = 120°, \quad \chi_3' = 300°$$
$$\chi_1' = 60°, \quad \chi_2' = 300°, \quad \chi_3' = 120°.$$

The first two conformations represent arrangements in which hydrogen-bonding can occur between the NH_3^+ group and the carbonyl of the peptide. The third conformation involves hydrogen-bonding between the −NH of the peptide and the carboxylate oxygens, while the fourth has the two groups parallel so that both interactions may occur.

3.4. Cysteinyl–Glycine Model

As well as ω and the two side-chain angles χ_1 and χ_2 there are two pairs of backbone angles Φ, Ψ to consider. Ideally a sub-map for $\Phi_1\Psi_1$ rotation should be produced for each set of values $\Phi_2\Psi_2$ and vice versa. This would be very time-consuming so a select number of conformations only were considered. Thus $\Phi_1\Psi_1$ rotations were performed for the preferred values of $\Phi_2\Psi_2$ determined by the study of the glycyl model; and similarly for $\Phi_2\Psi_2$. The results were combined on "global" energy maps. These maps cannot be regarded as complete descriptions of the conformational space, however they do illustrate the following general points.

To a large extent the maps resemble those of the individual residues. The map for $\Phi_2\Psi_2$ variation with $\omega = 0°$ is virtually identical to that obtained for the glycyl model. The map for $\omega = 180°$ retains the main features of the corresponding glycyl model map but shows two new areas of low energy: $\Phi_2\Psi_2 = 120°$ to 150°, −60° to 0° and, less noticeably, $\Phi_2\Psi_2 = -90°$, 0° to 30°. These areas represent the formation of C_{10} rings (Fig. 11a, b). The corresponding values of Φ_1, Ψ_1 are: $\Phi_1, \Psi_1 = -30°$ to 0°, 90° to 120° and Φ_1, $\Psi_1 = -60°$, −30° to 0° respectively. The global map for $\Phi_1\Psi_1$ variation does not indicate the areas represented by these values to be of particularly low energy; however an inspection of the component sub-map reveals that such low energy areas do exist for certain conformations.

Low energy areas corresponding to the formation of C_9 rings involving the S atoms (Fig. 11c) also appear on some sub-maps. The fact that these rings do not show up on the global energy maps possibly implies that they are of minor

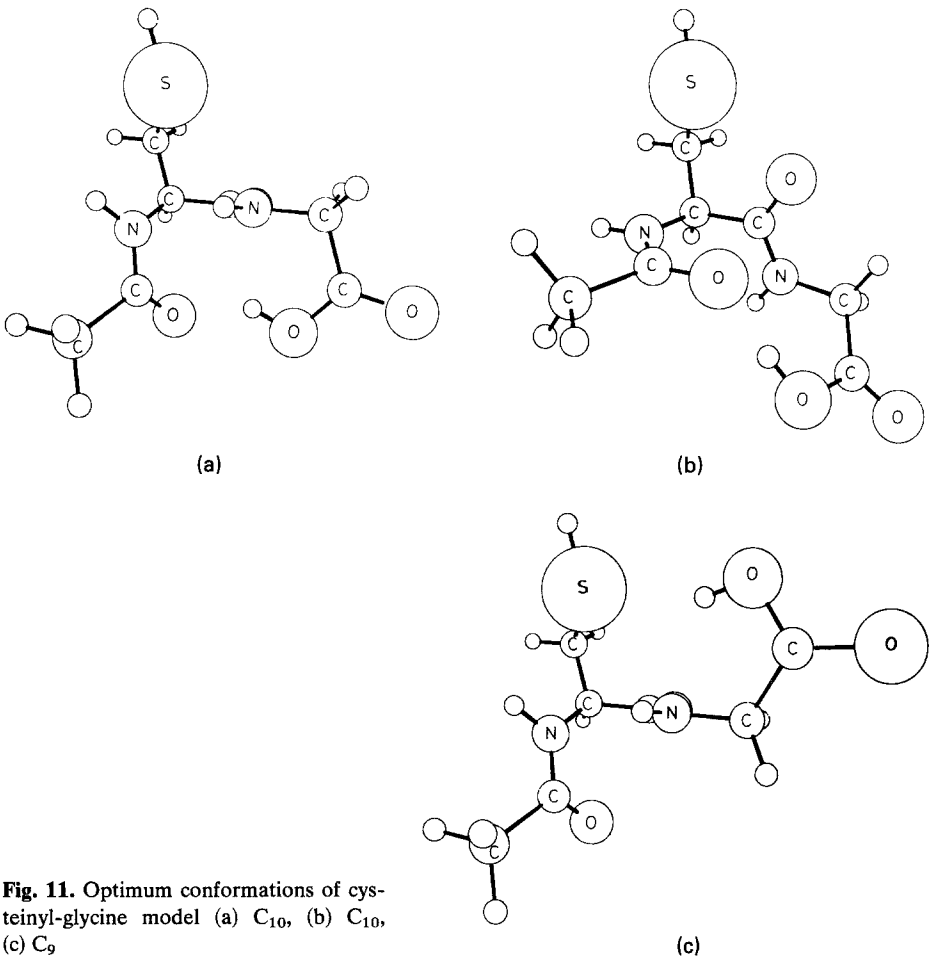


Fig. 11. Optimum conformations of cysteinyl-glycine model (a) C_{10}, (b) C_{10}, (c) C_9

importance, however it is not possible to assess the relative importance of the various rings that may form without a thorough study of the entire conformational space.

The ion of the cysteinyl–glycine model was also considered. This was studied in a similar way to the neutral molecule. All the sub-maps for $\Phi_2\Psi_2$ variation were very similar to those obtained for the ion of the glycyl model, showing a marked preference for a planar arrangement. Any gain in stability from the formation of hydrogen bonds between the carboxylate oxygens and –SH or –NH of cysteine is apparently insufficient to overcome the loss of stability that would result from a non-planar arrangement.

The map for $\Phi_1\Psi_1$ variation is rather different from that obtained for the cysteinyl residue or the neutral cysteinyl–glycine molecule. The previously preferred "C_7" conformation ($\Phi_1 = -90°$ $\Psi_1 = 60°$) is destabilised in favour of an arrangement with $\Phi_1 = -150°$, $\Psi_1 = 30°$. The C_5 conformation is still stable and represents the

global minimum. There is a further low energy area around $\Phi_1 = 120°$ to $-150°$, $\Psi_1 = -60°$ to $-30°$. The reason for the lower stability of the C_7 arrangement could be a repulsion between the negatively charged carboxylate oxygens and the oxygen of the peptide link, similarly the increased stability of the $\Phi_1 = -150°$ $\Psi_1 = 30°$ arrangement may be due to attraction between O^- and the NH group of cysteine.

3.5. Glutamyl–Cysteinyl–Glycine

This is the complete neutral glutathione molecule. It was studied by taking five arrangements of the glutamyl chain and for each selecting the preferred values of Φ_1, Ψ_1 and performing Φ_2, Ψ_2 variations. The five arrangements chosen are the four indicated by * in Table 2 and the fully extended chain.

"Global" maps were constructed for $\Phi_2\Psi_2$ variation with $\omega = 0°$ and 180°, and for $\Phi_1\Psi_1$ variation. Discussion of these is a little difficult since they represent only a few of the many possible conformations.

The map for $\Phi_2\Psi_2$ variation with $\omega = 0°$ shows the same principal features as that for the glycyl model, M and C_5 still being the preferred conformations. The map for $\omega = 180°$ shows C_7 to be still of major importance while the relative energy of the C_5 and M conformations has increased. A low energy area occurs around Φ_2, $\Psi_2 = 120°$, $-90°$ to $-30°$ representing the formation of a C_{10} ring involving the glu–cys peptide link; this is given added stability by interaction with the glutamyl

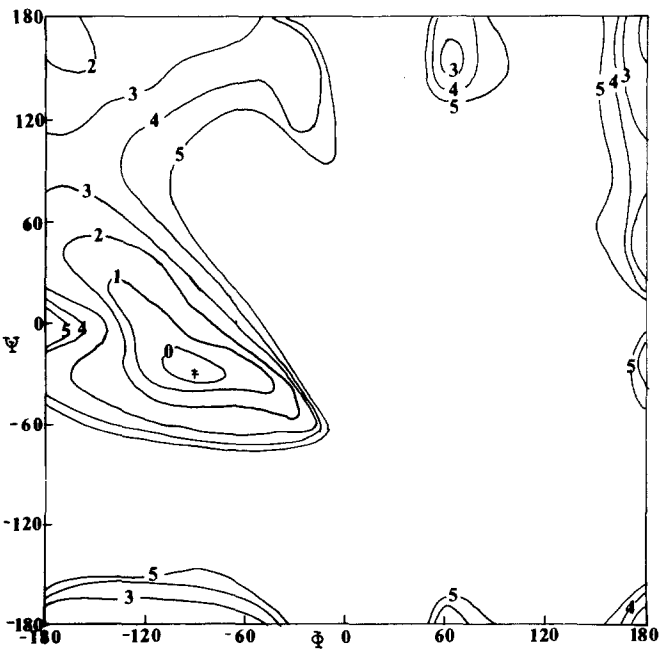


Fig. 12. Global conformational energy map for ionized glutathione Φ_1, Ψ_1 variation

amino and carboxylic acid groups. A further low energy area occurs at Φ_2, $\Psi_2 =$ 90° to 120°, 60° to 90° also involving interaction with the glutamyl chain.

On the $\Phi_1\Psi_1$ map the C_7 conformation still appears as a local minimum; this conformation is sterically hindered for some arrangements of the glutamyl chain. C_5 also appears as a local minimum but the global minimum now occurs in the "C_{10} area" with $\Phi_1\Psi_1 = -60°$ to $-30°$, 90° to 120°. Again added stability is provided by interaction with the glutamyl chain.

3.6. Ionized Glutathione

This was studied in a similar way to the neutral molecule. Five arrangements of the glutamyl chain were considered, these being the extended chain and the four stable conformations found in the study of the ionised glutamyl model (Sect. 3.3). Since the preferred values of $\Phi_2\Psi_2$ are always 180°, 0° respectively (from the study on the cysteinyl–glycine ion) these angles were kept at these values and variation of Φ_1, Ψ_1 was performed. The results are presented in a global map (Fig. 12). Once again this map cannot be regarded as fully representative of all possible conformations, however there does seem to be a marked preference for the conformation represented by $\Phi_1\Psi_1 = -90°$ to $-60°$, $-30°$ to 0°. This is illustrated in Fig. 13 for the most stable arrangement of the glutamyl chain. This conformation

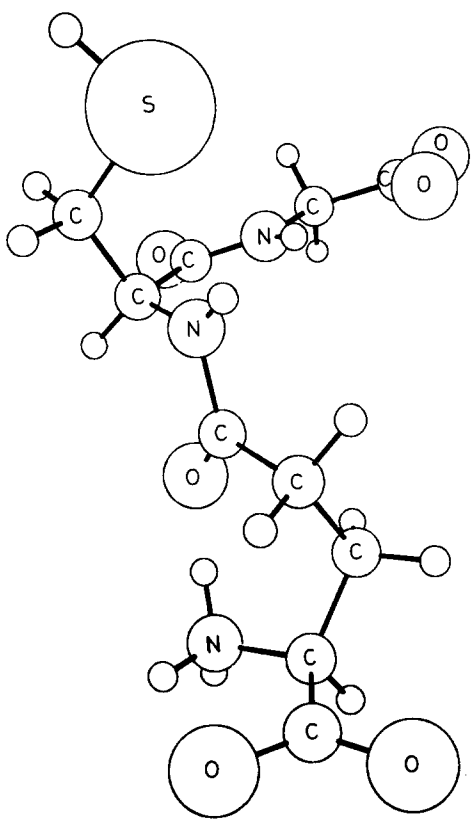


Fig. 13. Optimum conformation of ionized glutathione

does not appear to involve any significant "new" hydrogen bonds i.e. hydrogen bonds other than those already present within the glutamyl chain and the glycyl residue. Its particular stability must thus be due to other factors.

The map also shows a local minimum for the C_5 conformation ($\Phi_1, \Psi_1 = 180°$, 180°) and for a conformation with $\Phi_1, \Psi_1 = 60°$, 150°–180°. This latter conformation only occurs for a particular arrangement of the glutamyl chain and involves interaction between the NH_3^+ group and the carbonyl oxygen of the cys–gly peptide link.

4. Discussion

Experimental data on the structure of glutathione is not extensive. The crystal structure was determined by W. B. Wright in 1958 [16]. A more recent study by F. B. Cole [17] gives no details but mentions that the results are largely in agreement with those obtained by Wright. The molecule is described as being in an S-shaped configuration with an angle between the two peptide links of 94.4°. There are no intramolecular hydrogen bonds.

The conformation of the central part of the molecule is in close agreement with that obtained in the calculations on the ionized molecule. The arrangement of the glutamyl chain and of the glycyl fragment is rather different. This is not surprising since in the crystal the terminal carboxylic acid and amino groups are involved in intermolecular hydrogen bonds. The calculations are performed on an isolated molecule where no such intermolecular bonds can form, intramolecular bonds are thus of greater importance.

Two n.m.r. studies have been carried out on glutathione [18, 19] and the two groups of authors come to quite different conclusions. The first group concludes that the molecule must be in a "Figure 8" conformation with interaction between the glutamyl NH_3^+ group and the glycyl COO^- as well as between the glutamyl COO^- and the peptide –NH groups. Attempts to find a stable conformation corresponding to such a structure were unsuccessful.

The other group of authors concludes that the preferred conformation of the fully dissociated molecule is one in which the carboxyl and amino groups of glutamic acid are far from the peptide backbone. This is in better agreement with the X-ray study but again rather different from the results of the calculations which always indicate a preference for structures with the terminal groups of glutamic acid interacting with the peptide link. However it must be remembered that the n.m.r. study refers to a completely dissociated molecule, i.e. an NH_2 rather than NH_3^+ group is involved. The paper also concludes that changes in the functional groups of glutamic acid have an important influence on the preferred conformation of the molecule. This is supported by the calculations since the preferred conformation of neutral glutathione is rather different from that of the ionized molecule.

In conclusion we may say that while our results do not reproduce exactly the available experimental data, this system is one of the largest tripeptides to be

studied in this way, and it is gratifying that there is a substantial degree of agreement with experiment, particularly with the X-ray results. It may also be noted that the experimental data is somewhat sparse and not completely consistent. Further experimental work, particularly n.m.r., could provide some useful results. Theoretical calculations taking account of solvent effects are not practicable for such a large molecule, however it might be possible to study the effect of adding water molecules around the carboxylic acid groups of the glutamyl and glycyl residues.

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