

## Chapter V

### Discussion and Conclusion

#### Discussion of the hydration shell of glycine zwitterion in aqueous solution.

All calculated water-glycine zwitterion interaction energies have been summarized in table 5.1 . In CNDO optimization all hydrogen bonds are 2.2 - 2.5 Å and the interaction energies are 10.0 - 30.1 kcal/mol, which is surely too large in absolute values, due to artificial stabilization by too short hydrogen bond distances. This artefact is already well known for similar systems, when CNDO calculations have been employed for studying hydrogen bonds (20).

Table 5.1 The interaction energies of glycine zwitterion complexes with one water molecule in nine different conformation

water	CNDO optimization	CNDO H-bond 2.75 Å	ab initio	$\Delta E_{\text{cor}}^{\text{HF}}$
1	-27.1	-10.5	-14.8	-4.7
2	-30.1	-15.7	-20.3	-6.5
3	-22.7	-14.7	-17.6	-5.6
4	-14.9	-11.2	-20.8	-6.7
5	-14.9	-11.2	-20.8	-6.7
6	-10.0	- 4.5	-5.0	-1.6
7	-14.3	- 9.3	-15.2	-4.9

Table 5.1 continued

8	-13.4	- 8.8	-14.2	-4.5
9	-13.9	-10.1	-9.3	-3.0

All interaction energies are reported in kcal/mol

The source of this defect is mainly from the ZDO approximation, which neglects a large part of two electron integrals representing the electron electron repulsion. Therefore, the attractive terms will be overemphasized, so that optimization yields too short bond distances and overestimated interaction energies.

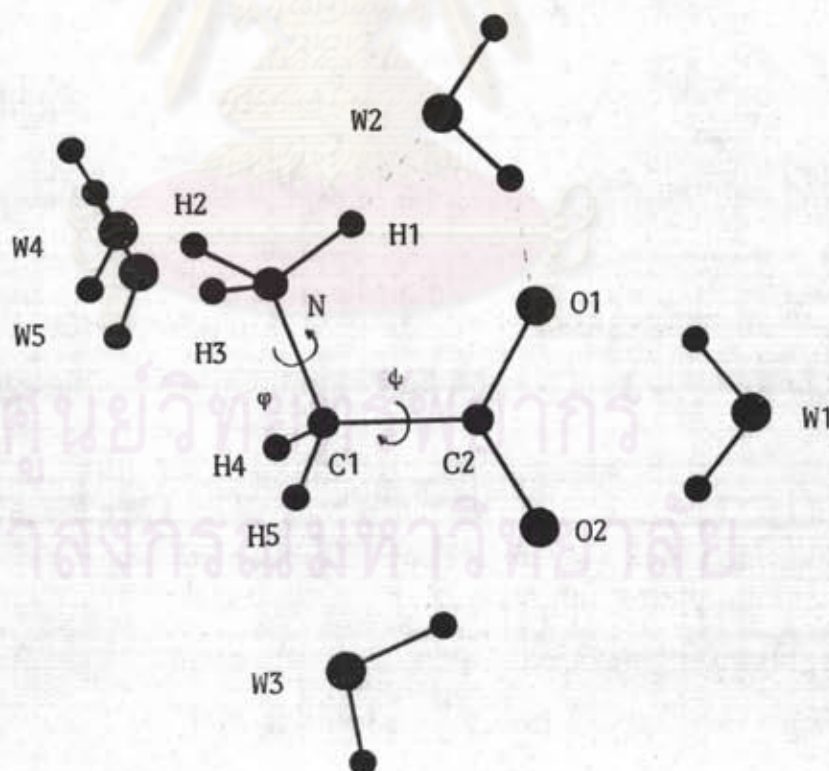


Figure 5.1 The glycine zwitterion with 5 water molecules in the hydration shell by CNDO optimization



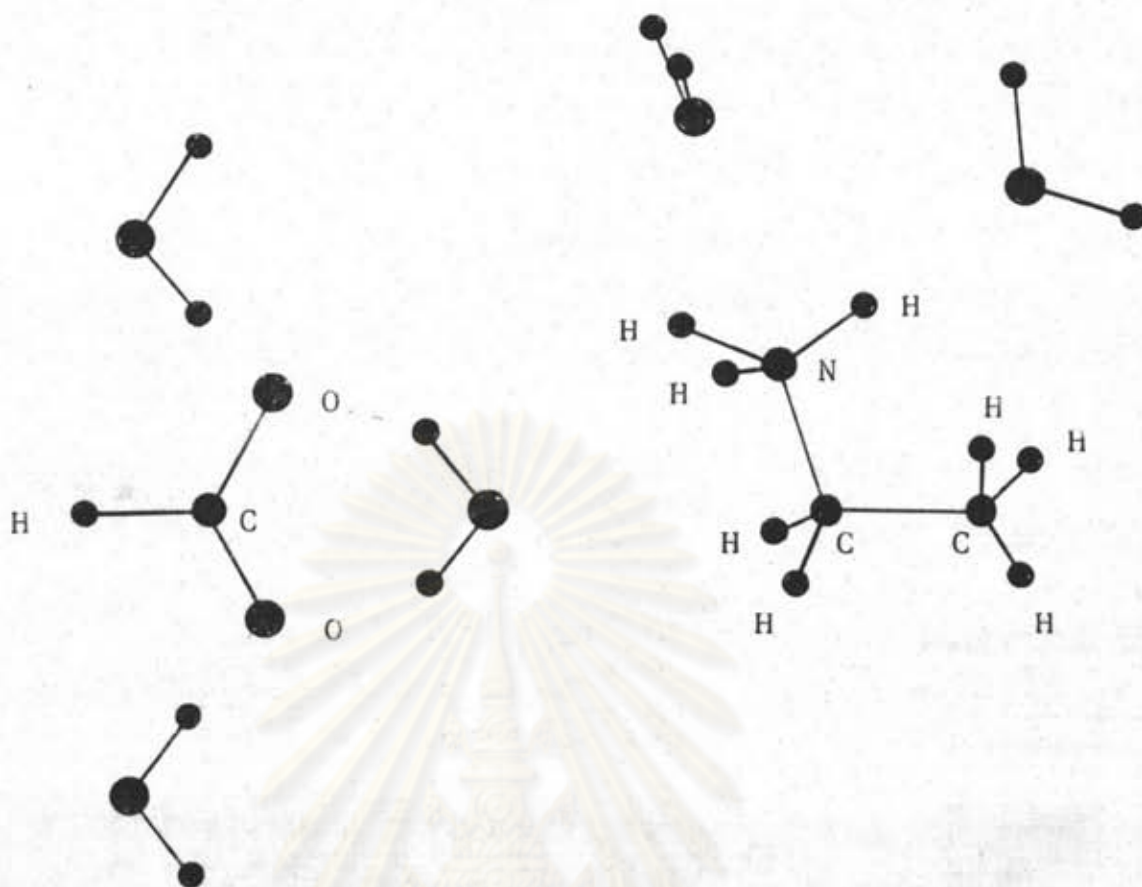


Figure 5.2 The most favored waters around formate ion and ethylamine ion, optimized by ab initio STO-3G (1)

However, a general usefulness of the CNDO optimization procedure is recognized in the case of five most favoured water positions (figure 5.1), when the result is compared with the ab initio STO-3G optimization of water around formate ion and ethylammonium ion (figure 5.2) as a general agreement of geometrical arrangements is found.

Because of the unrealistic hydrogen bond lengths, we had to recalculate all positions of waters at a fixed hydrogen bond distance of 2.75 Å by both CNDO and ab initio methods.

From the CNDO calculations, interaction energies of 4.5 - 15.7 kcal/mol. The results are much more realistic in magnitude when compared with the ab initio calculations. However, when the relative



order of stabilization is compared with ab initio data, some differences are observed. The most favored positions calculated by CNDO are those numbered 2 and 3 (-15.7 and -14.7 kcal/mol). By ab initio calculation positions 2, 4, 5 (-20.3, -20.8, -20.8 kcal/mol) are predicted to be most stabilized. This means that the CNDO calculation overestimates (as usual) a cyclic structure (water 2 leads to a cyclic structure; see figure 5.1). The results of the ab initio calculations will be used for setting up the full hydration shell, as they should be definitely more accurate.

It should be mentioned, however, that the CNDO method has proved a great help to simplify the optimization procedure which could not have been performed by all ab initio calculations.

In order to eliminate possible overestimations in the minimal basis set calculation, a water dimer with hydrogen bond distance 2.75 Å was calculated, using the same minimal basis set as for the glycine-water calculation. Its interaction energy is -11.5 kcal/mol. The results of corrected energy ( $\Delta E_{\text{cor}}^{\text{HF}}$ ) are also reported in table 5.1

In general, formation of a stable hydration shell occurs when the solute-solvent interaction is larger than the solvent-solvent interaction. In our case, from table 5.1 the strongest water-glycine zwitterion interactions correspond to positions 2, 3, 4 and 5. When their interaction energies are compared with that of the water dimer, these four water molecules form a specific hydration shell structure. The other water molecules are at positions 1, 7 and 8, which interaction energies of 14.2-15.2 kcal/mol are also stabilized, but are not much different from the water dimer's interaction energy. The water position 1 was chosen for the full hydration shell, whereas waters 7 and 8 were neglected due to solvent-solvent repulsion effects when the



full shell is formed. The waters 6 and 9 had to be neglected as their interaction energies are lower than that of the water dimer. The waters around the  $-\text{COO}^-$  group, for which the interaction energies are not much different from that of the water dimer, are probably not strongly oriented in the hydration shell and should be easily exchanged. Figure 5.3 shows a model for the first hydration shell for glycine zwitterion in accordance with our results. From figure 5.4 which represents experimental data for the hydration of glycine in aqueous solution it can be seen that our calculated hydration numbers are very likely to be realized in aqueous solution.

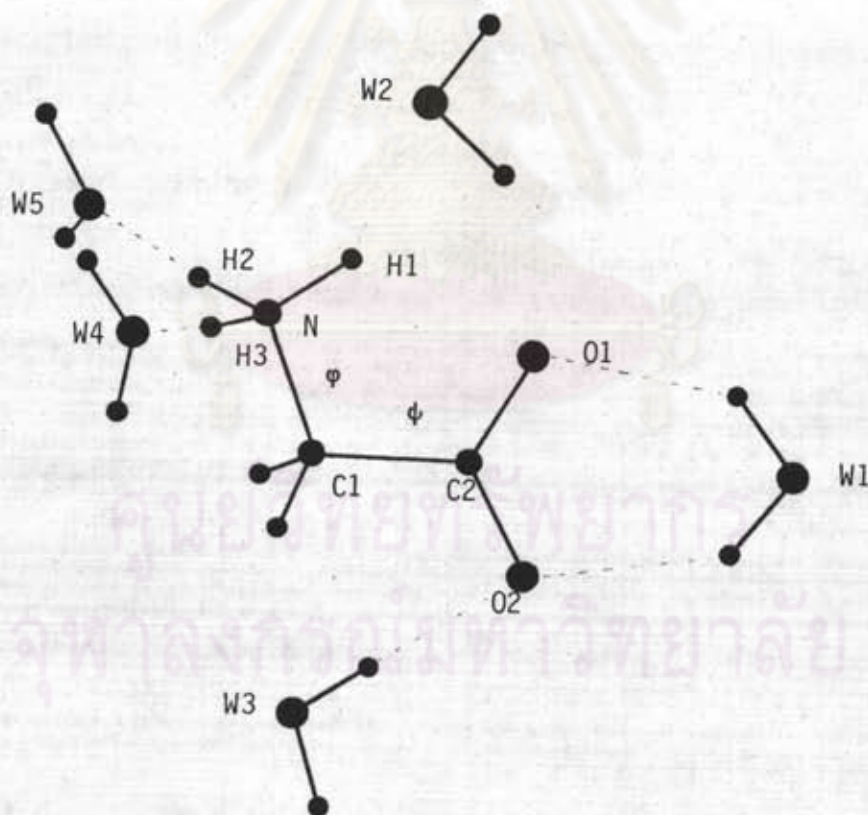


Figure 5.3 The glycine zwitterion with 5 water molecules in the hydration shell. All hydrogen bonds are  $2.75 \text{ \AA}$

(n) hydration numbers

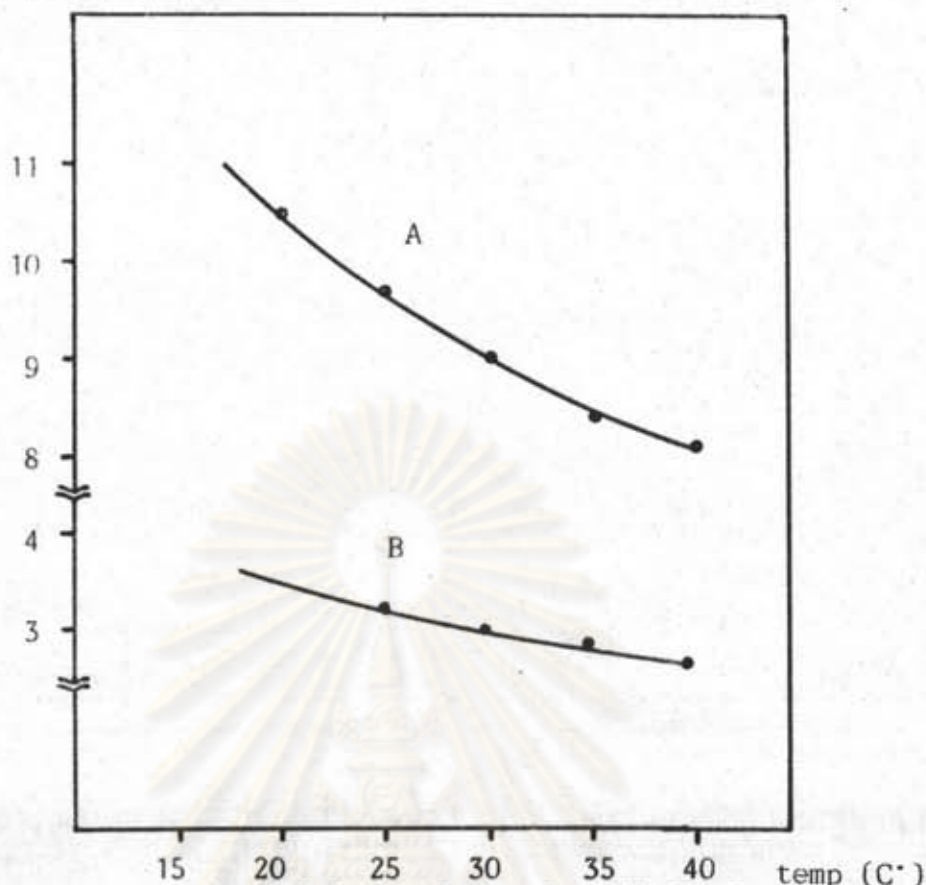


Figure 5.4 The experimental hydration number of glycine zwitterion in aqueous solution. Graph A includes a second hydration shell (26), graph B including only the first hydration shell (27)

We have also evaluated the total hydration energy of glycine zwitterion based on 5 waters with hydrogen bond distances 2.65, 2.75 and 2.85 Å. The results are summarized in table 5.2 In CNDO calculations a change of hydrogen bond of about 0.1 Å leads to a changing of the hydration energy by 10 kcal/mol.

The calculated hydration energy should be considerably too high because of the overestimated stabilization in the ab initio method due to the basis set superposition error. However when we apply the  $f^{\text{HF}}$  correction, the hydration energy is 28.7 kcal/mol, which seems reasonable, as the experimental value ( 52 kcal/mol, reference 28) contains still the transformation energy from neutral glycine to its



zwitterionic form (29 kcal/mol), which was computed by extended ab initio calculations. (29).

Table 5.2 Total energy and hydration energy of hydrated glycine zwitterion, in various hydrogen bond distance

Hydrogen bond	CNDO calculation		ab initio calculation	
	total E	hydration E	total E	hydration E
2.65	-165.8064	-73.9	-	-
2.75	-165.7870	-61.7	-562.7948	-89.7
2.85	-165.7663	-48.8	-	-

Total energies are reported in hartrees, hydration energies are reported in kcal/mol

#### Influence of the hydration on the electron distribution in glycine zwitterion.

Tables 4.14 and 4.15 summarizes the Mulliken populations of glycine zwitterion and glycine zwitterion with first hydration shell as derived from CNDO and ab initio calculations respectively. Figure 5.5 illustrates this change of atomic population of glycine zwitterion upon hydration. Although the atomic population changes of the CNDO calculation are of the same direction as the ab initio data, they are generally lower.

For the change of atomic populations of functional groups of atoms from figure 5.6, the main electron density transfer from waters

to glycine zwitterion is found to occur with waters 4 and 5 and partly from water 2. Transfer occurs to the  $-\text{NH}_3^+$  group (0.063 e) and the other 0.137 e are transferred through the backbone of the glycine zwitterion to waters 1, 2 and 3 which are the charge receivers from glycine zwitterion. The  $-\text{CH}_2$  and  $-\text{COO}^-$  group also display a density decrease, which is transferred to water molecules too.

The total charge of the glycine zwitterion with the hydration shell increases by 0.01 e ( $0.063 - 0.048 - 0.005$ ) according to the ab initio calculation, and 0.012 e ( $0.008 - 0.003 + 0.007$ ), according to the CNDO calculation. In both cases, this means that charge is transferred from the hydration shell to glycine zwitterion.

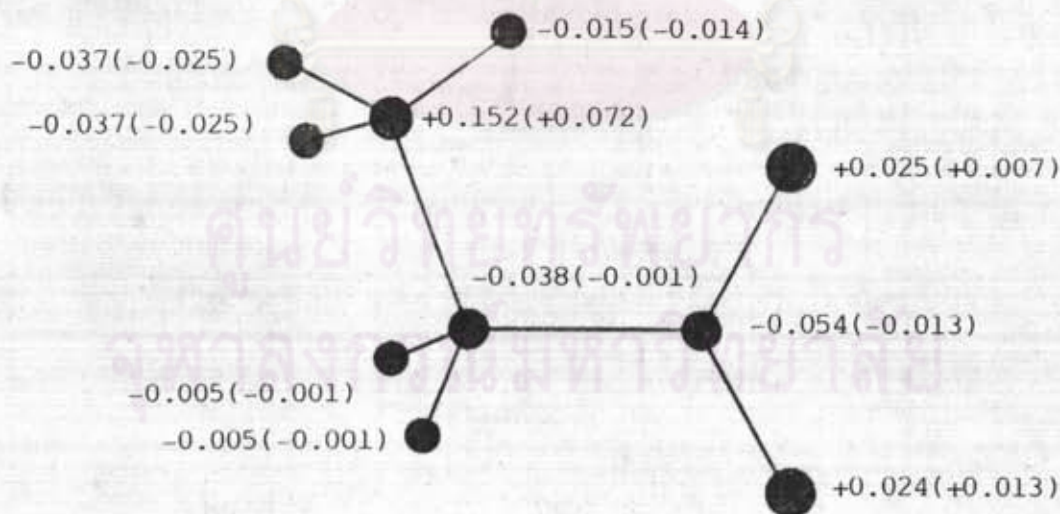


Figure 5.5 The atomic population of glycine zwitterion due to the influence of hydration (the value in brackets are calculated by CNDO)



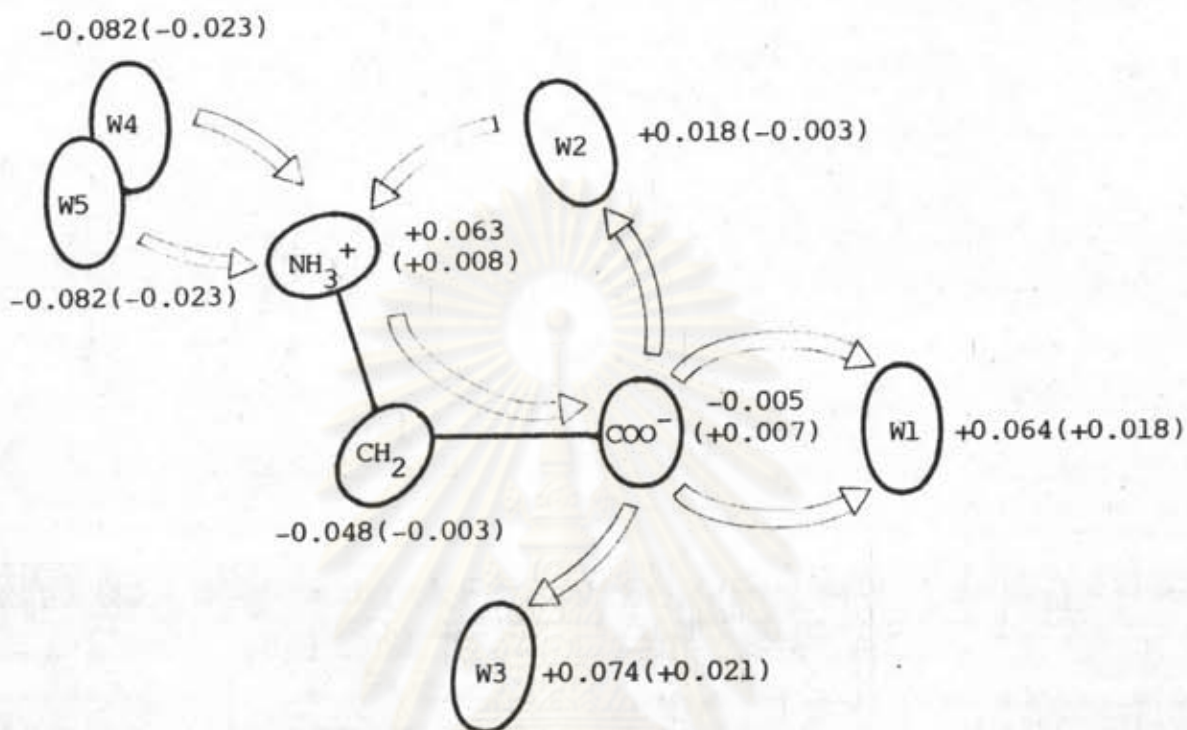


Figure 5.6 The charge of electron distribution of groups due to the influence of hydration in glycine zwitterion. The arrows show the direction of the charge transfer between groups of atoms. (The values in the bracket are CNDO calculation)

The influence of hydration on internal rotation of glycine zwitterion

According to tables 4.3 and 4.4, the minimum energy conformation for glycine zwitterion is  $(\varphi, \phi) = (0, 0)$ . This corresponds to the planar conformation in which an interaction between the two groups  $-\text{NH}_3^+$  and  $-\text{COO}^-$  via an intramolecular hydrogen bond  $\text{NH}\cdots\text{O}$  with bond length  $\text{N}\cdots\text{O} = 2.0 \text{ \AA}$  and an approximate interaction energy  $3.3 \text{ kcal/mol}$  is enabled. The barrier height of the two conformations  $(60, 0)$  and  $(0, 90)$  are  $3.3$  and  $10.47 \text{ kcal/mol}$  respectively.

Two factors which influence conformational changes of glycine zwitterion are the attraction between the two charged ends of the molecule ( $-\text{NH}_3^+$  and  $-\text{COO}^-$ ) and the repulsion between N and C hydrogen atoms. The rotation of the angle  $\varphi$  is affected by both factors, but the rotation of the angle  $\phi$  involves only the attraction between the two charged ends, so that the overall barrier is lower in the case of the angle  $\varphi$ . Figures 5.7 and 5.8 present the minimum pathway for the internal rotation of glycine zwitterion which commences with a rotation of the angle  $\varphi$  first to  $60^\circ$  and then continues by rotating the angle  $\varphi$  to  $90^\circ$ , leading to conformation  $(60, 90)$ . The last conformation has the barrier  $5.8 \text{ kcal/mol}$  (table 4.5).

There have been some less sophisticated calculations predicting other minimum conformations for glycine zwitterion. For example, Molecular Fragment and Extended Hückel methods have predicted the minimum conformation to be  $(60, 0)$  which is the favoured one considering the repulsion between the N and C hydrogen atoms only. The error prediction of these calculations apparently arises from the neglect of electron electron repulsion. The CNDO result is in agreement with the ab initio calculation, so that the internal rotation can reasonably be studied by the CNDO method.



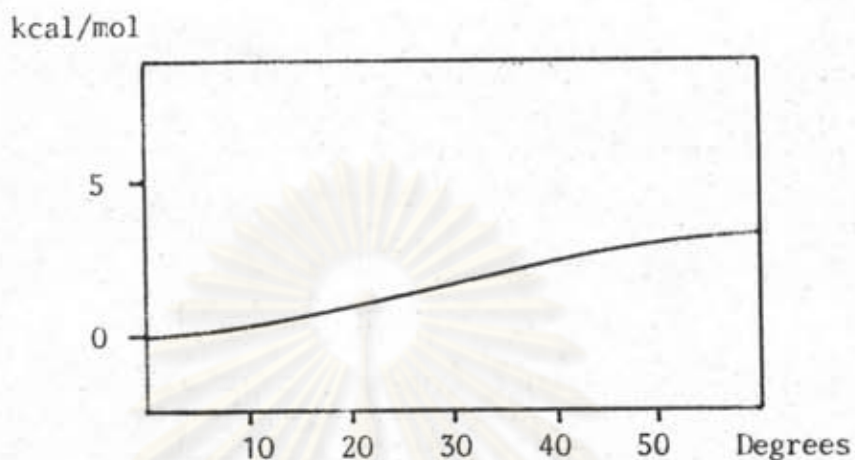


Figure 5.7 The rotation pathway of glycine zwitterion, changing conformation (0,0) to (60,0)

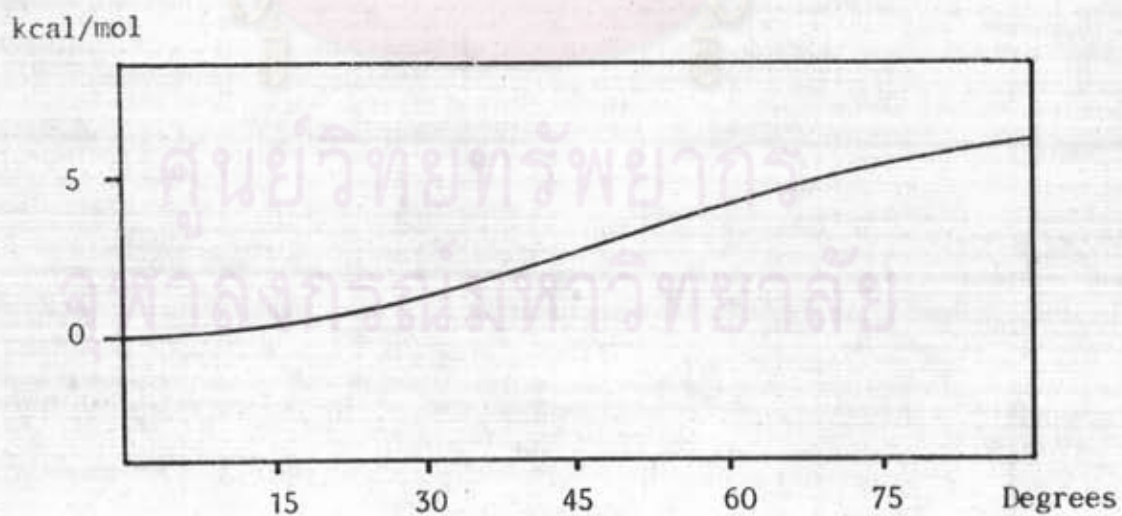
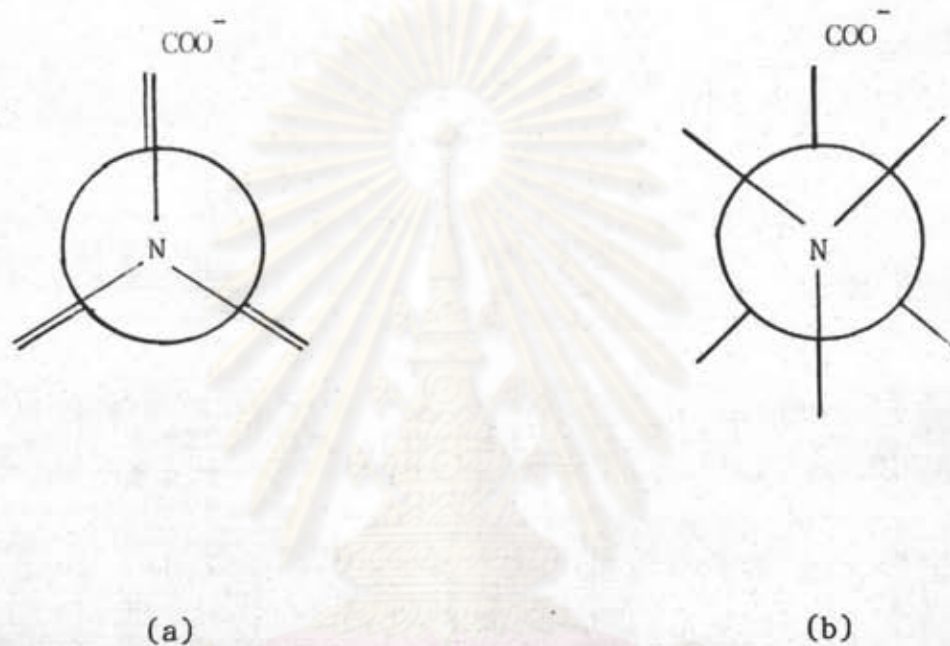


Figure 5.8 The rotation pathway of glycine zwitterion, changing conformation (60,0) to (60,90)



CNDO and ab initio calculation      Molecular fragment and Extended  
Hückel calculation

Figure 5.9 The predicted most stable conformations of glycine zwitterion, (a) results from this calculation and reference 4, (b) is taken from reference 4 .



When the solvent was introduced, only the first hydration shell was considered which includes 5 waters (figure 5.10). From tables 4.17 to 4.24 and figures 5.11 and 5.12, the minimum conformation of hydrated glycine zwitterion remains conformation (0,0). Starting from the minimum conformation, rotation of angle  $\varphi$  is still easier than rotation of the angle  $\psi$ , i.e. the barrier is lower. This indicates that the repulsion of the N and C hydrogens continues to be an important effect for the conformational change of the glycine zwitterion even in an aqueous solution. The pathway in the change of conformation, therefore, remains the same as in the gas phase, i.e. the angle  $\varphi$  is changed first to the conformation (60,0), and the rotation of the angle  $\psi$  to (60,90) follows.

From figure 5.11 when the waters (water 2, 4, 5) are rotated together with the  $-\text{NH}_3^+$  group (graph B), the barriers will be 3.5 kcal/mol lower compared with the rotation of the  $-\text{NH}_3^+$  group fixing these waters (graph A). For water 2, which lies between the two hydrophilic groups, graphs A and B (figure 5.12) show nearly the same shape and barrier height, indicating that the hydrogen bonds from water 2 to glycine zwitterion (NH-O and OH-O) are nearly equivalent in energy (graph A shows that the OH-O bond is broken, and graph B shows that the NH-O bond is broken). Thus the probability for water 2 to follow the rotation of the  $-\text{NH}_3^+$  group should be considered as a reason for the decreased barrier in graph B as compared to that of A.

In the case of rotation of the angle  $\varphi$  graph B of figure 5.11 represents the most suitable pathways. As mentioned above, the rotation of angle  $\varphi$  is easier than that of angle  $\psi$ . Figure 5.14 presents the pathways for rotation of the angle  $\psi$  of hydrated glycine zwitterion, starting from conformation (60,0).

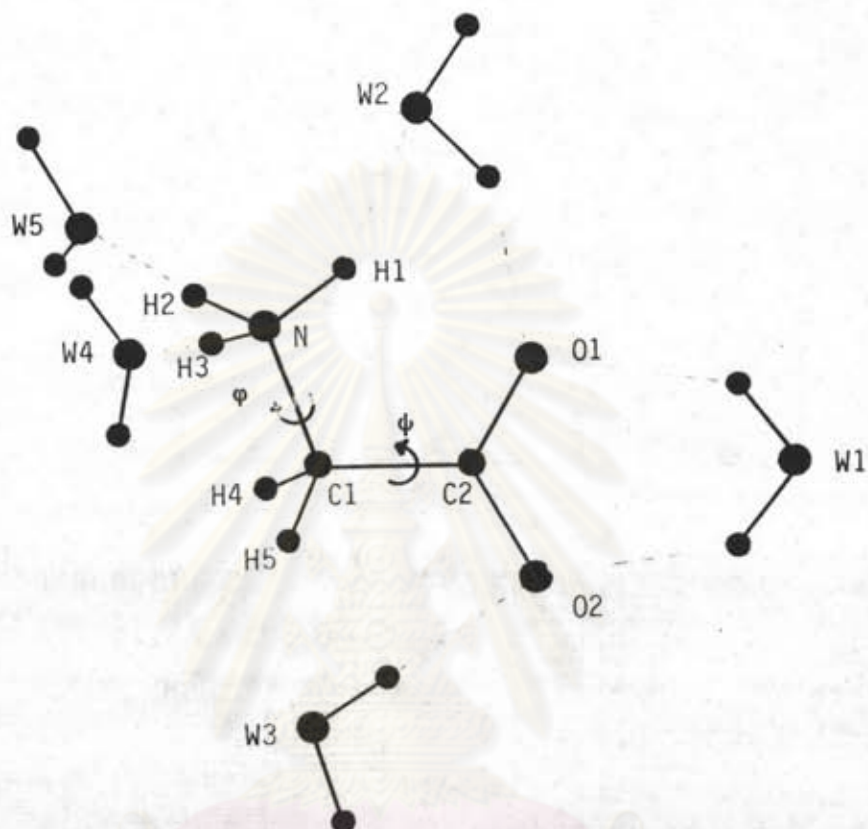


Figure 5.10 The glycine zwitterion with 5 water molecules in the first hydration shell



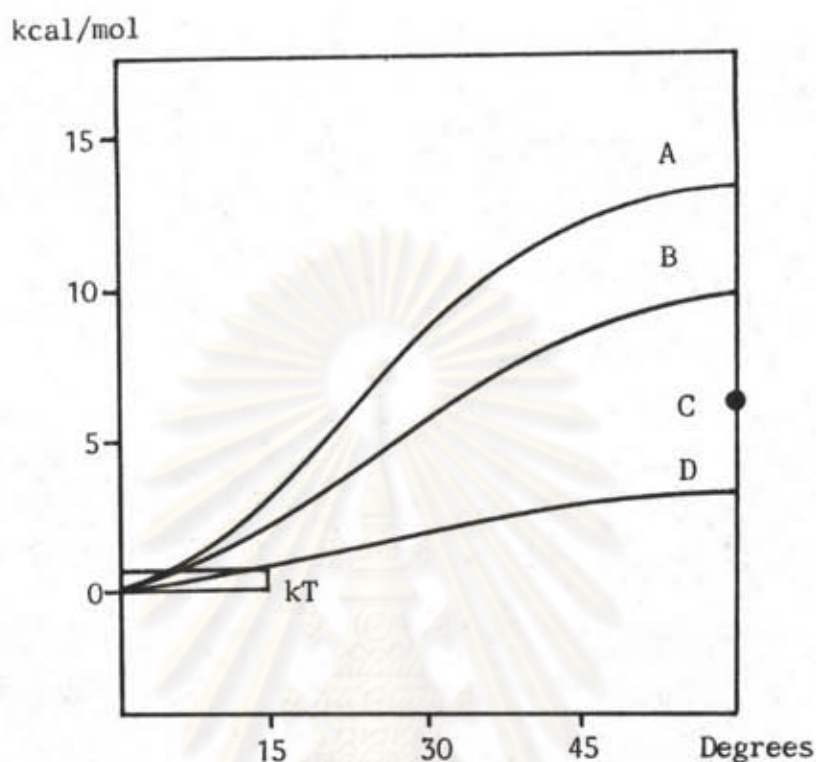


Figure 5.11 The rotation pathway of glycine zwitterion including 5 water molecules in the hydration shell, fixing  $\psi = 0^\circ$ , rotating  $\varphi$  from  $0$  to  $60^\circ$

- A. Fixing the hydration shell, rotating  $-\text{NH}_3^+$  group alone
- B. Fixing waters 1, 3, rotating  $-\text{NH}_3^+$  group, and waters 2, 4, 5 around the C-N bond
- C. After rotating of the  $-\text{NH}_3^+$  group and waters 2, 4, 5 to  $60^\circ$ , adjusting water 2 symmetry with water 5
- D. The rotation of glycine zwitterion neglecting the hydration shell effect, fixing  $\psi = 0^\circ$ , rotating  $\varphi$  from  $0^\circ$  to  $60^\circ$

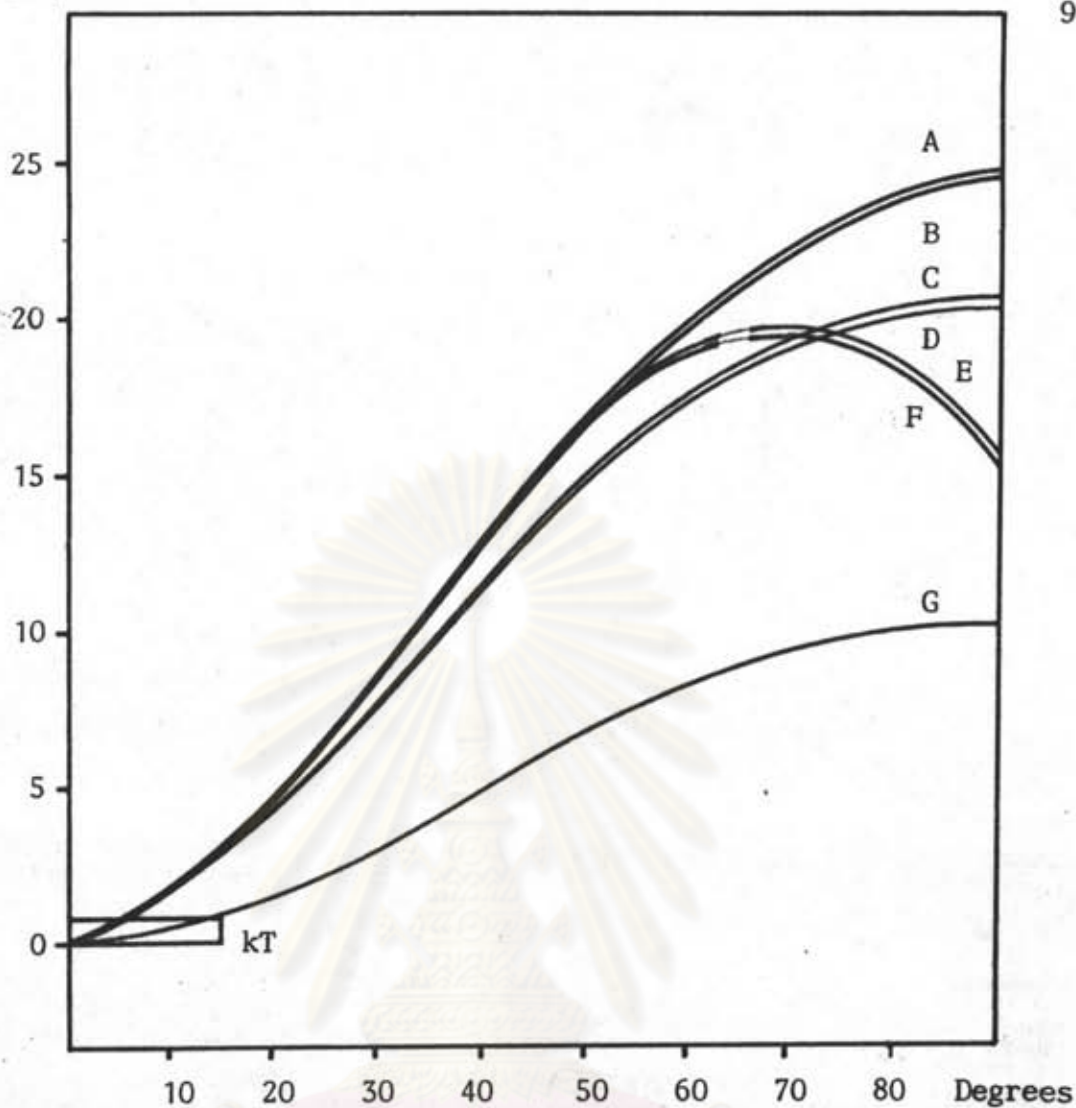


Figure 5.12 The rotation pathway of glycine zwitterion including 5 water molecules in the hydration shell, fixing  $\varphi = 0^\circ$ , rotating  $\psi$  from 0 to  $90^\circ$ .

- A. Fixing the hydration shell, rotating  $-\text{COO}^-$  group alone
- B. Fixing waters 1, 3, 4, 5, rotating  $-\text{COO}^-$  group, and water 2
- C. Fixing waters 3, 4, 5, rotating  $-\text{COO}^-$  group, and waters 1, 2
- D. Fixing waters 2, 3, 4, 5, rotating  $-\text{COO}^-$  group, and water 1
- E. Fixing waters 1, 2, 4, 5, rotating  $-\text{COO}^-$  group, and water 3
- F. Fixing waters 4, 5, rotating  $-\text{COO}^-$  group, and waters 1, 2, 3
- G. The rotation of glycine zwitterion neglecting the hydration shell effect, fixing  $\varphi = 0^\circ$ , rotating  $\psi$  from  $0^\circ$  to  $90^\circ$



Before discussing the rotation of angle  $\phi$  at fixed angle  $\varphi = 60^\circ$ , we have to consider the change of the hydration shell after rotating the  $-\text{NH}_3^+$  group and the waters 2, 4, 5 to  $60^\circ$  (figure 5.11). From the partial glycine-water interaction energies (table 5.1), it was found that the interaction energies of all waters near the  $-\text{COO}^-$  group are in the same range (14.2 - 15.2 kcal/mol) and about 3 - 4 kcal/mol higher than in the water dimer. A change of the water positions around the  $-\text{COO}^-$  group and hence, the hydration shell of glycine zwitterion in the conformation (60,0) as shown in figure 5.13, should be possible.

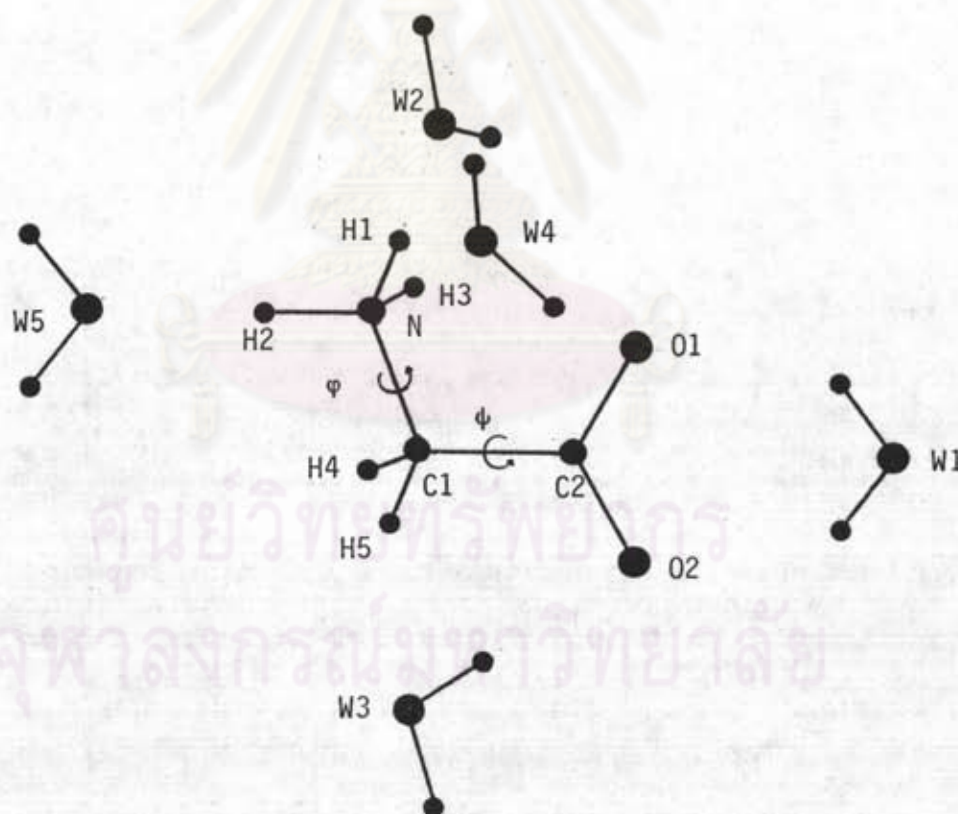


Figure 5.13 Glycine zwitterion including 5 water molecules in the hydration shell after rotating  $-\text{NH}_3^+$  group together with waters 2, 4, 5 to  $60^\circ$

In figure 5.14, which represents the rotation pathways for changing the angle  $\phi$  of hydrated glycine zwitterion, graph A represents the rotation of  $-\text{COO}^-$  group alone within a fixed hydration shell. The corresponding barrier is 13.6 kcal/mol. When water 1 is rotated together with the  $-\text{COO}^-$  group, the barrier decreases to 9.8 kcal/mol (graph B), and when water 1, 3 and the  $-\text{COO}^-$  group are rotated simultaneously the barrier is 9.5 kcal/mol, but the peak of the maximum conformation is shifted to  $65^\circ$  (graph C), because upon rotation of  $\phi$  to  $65^\circ$ , interaction between water 3 and the  $-\text{NH}_3^+$  group will allow some energy gain. The same is valid for the rotation of  $-\text{COO}^-$  group and water 3 (graph E) and the rotation of  $-\text{COO}^-$  group and water, 1, 2 and 3 (graph F) of figure 5.12. They also show the effect of water 3 shifting the maximum peak to  $65^\circ$ . Therefore, graph C of figure 5.14 represents the best rotational pathway of rotating the angle  $\phi$  in hydrated glycine zwitterion.

However, all internal rotations in this work have been calculated assuming rigid molecules. In the case of internal rotation of glycine zwitterion in the gas phase the geometry changes slightly. When a solvent is introduced, a distortion of the solvent shell will also occur. Figure 5.11 illustrates that after rotating the  $-\text{NH}_3^+$  group and water 2,4,5 to  $60^\circ$  (graph B), we have to adjust water 2, which leads to a barrier decrease of 3 kcal/mol (point C). However, adjusting the water geometry to every conformation change would require unreasonable computing times, so that rigid molecules had to be used.

A comparison of the rotational pathways for isolated and hydrated glycine zwitterion shows that, from the minimum conformation (0,0), isolated glycine zwitterion in gas phase at room temperature



( $kT = 0.6$  kcal/mol), can vibrate about  $\pm 15$  around the minimum conformation, i.e.  $(\varphi, \psi) = (\pm 15, \pm 15)$  (figure 5.11 and 5.12). When the solvent is present, this range of vibration will be decreased to  $(\varphi, \psi) = (\pm 7, \pm 7)$  and the overall barrier will increase by the solvation by the factor of 2 .

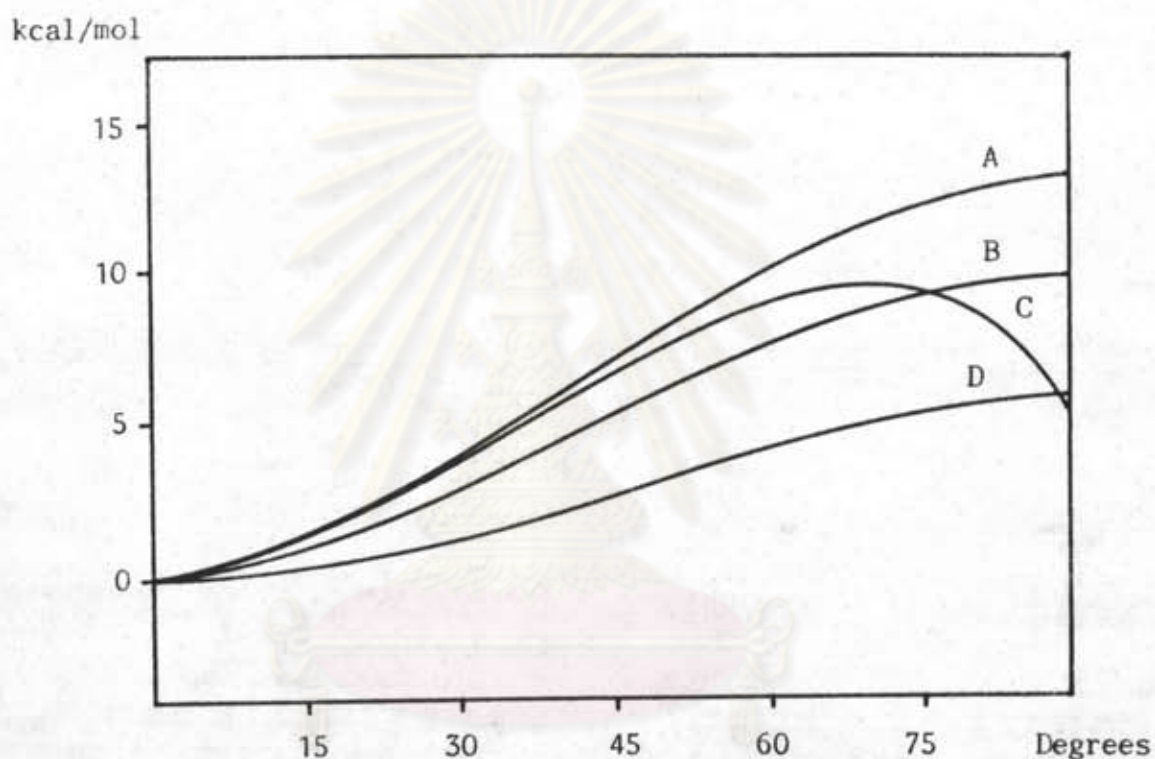


Figure 5.14 The rotation pathway of glycine zwitterion including 5 water molecules in the hydration shell, fixing  $\varphi = 60^\circ$ , rotating  $\psi$  from 0 to  $90^\circ$  (figure 5.13)

- A. Fixing waters 1, 3, rotating  $-\text{COO}^-$  group alone
- B. Fixing waters 3, rotating  $-\text{COO}^-$  group, and waters 1, around the C-C bond
- C. Rotating  $-\text{COO}^-$  group and waters 1, 3
- D. The rotation of glycine zwitterion neglecting the hydration shell effect, fixing  $\varphi = 60^\circ$ , rotating  $\psi$  from  $0^\circ$  to  $90^\circ$

As in the case of the hydration energy, the conformation of hydrated glycine zwitterion is also a function of the hydrogen bond distance. We have tested the rotation of the angle  $\phi$  (including the rotation of  $-\text{NH}_3^+$  and waters 2, 4 and 5) for other hydrogen bond distances (2.65 and 2.85 Å), and the results are summarized in table 5.3. The comparison of the rotation pathways is given in figure 5.15. The shapes are identical and the barrier height at the maximum point differs less than 2 kcal/mol.

Table 5.30 The barriers for internal rotation of hydrated glycine zwitterion are a function of Hydrogen bond distance.

Hydrogen bond (Å)	The rotation of $\phi$ (kcal/mol)	
	conformation (30,00)	conformation (60,00)
2.65	5.8	11.5
2.75	5.0	10.0
2.85	4.3	8.6
glycine	1.3	3.3

$\phi$  rotation means the rotation of  $-\text{NH}_3^+$  group and water 2,4,5,

$\psi$  rotation means the rotation of  $-\text{COO}^-$  group and water 1,3



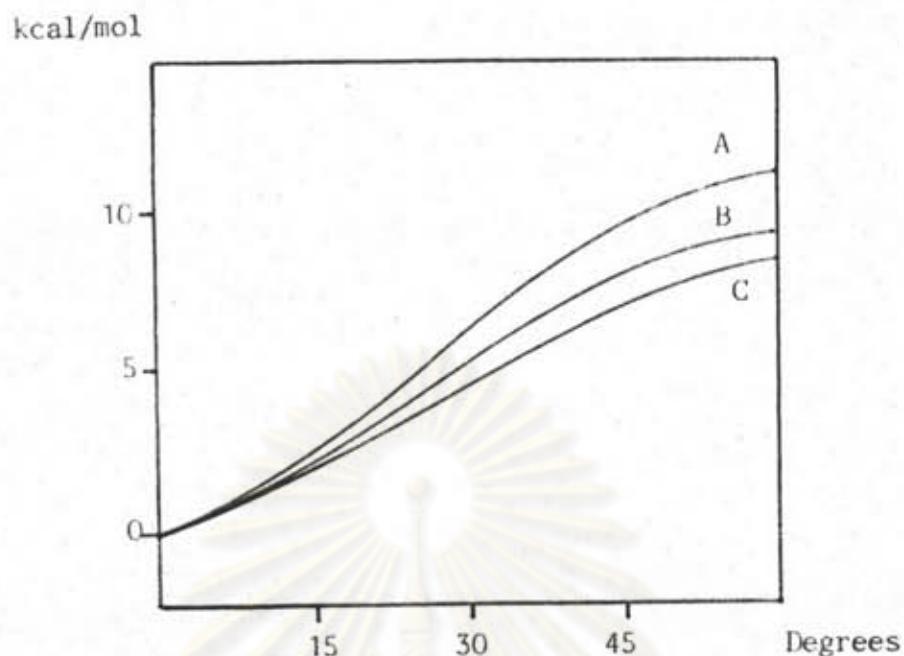


Figure 5.15 The comparison of the rotation pathways for hydrated glycine zwitterion rotating  $-\text{NH}_3^+$  group and water 2,4,5, at varying hydrogen bond distances, A = 2.65 Å, B = 2.75 Å, C = 2.85 Å

Upon rotation of the angles  $\varphi$  and  $\psi$  of glycine zwitterion in water, some water molecules will rotate together with  $-\text{NH}_3^+$  and  $-\text{COO}^-$  groups. We have performed some preliminary calculations of the effect of a second shell on the rotation of  $\varphi$ , which a second shell water distance is 5.5 Å (figure 5.16). The barrier height increases from 10.3 kcal/mol to 19 kcal/mol. The comparison of the rotation pathways is represented in figure 5.17 .

However, for the second shell waters, interaction energies are considerable weaker than the water in the first shell (5.3 kcal/mol per glycine-water, CNDO calculation) so that the second shell waters do not adjust well-orderedly, and will easily be change between being second shell and being bulk water.

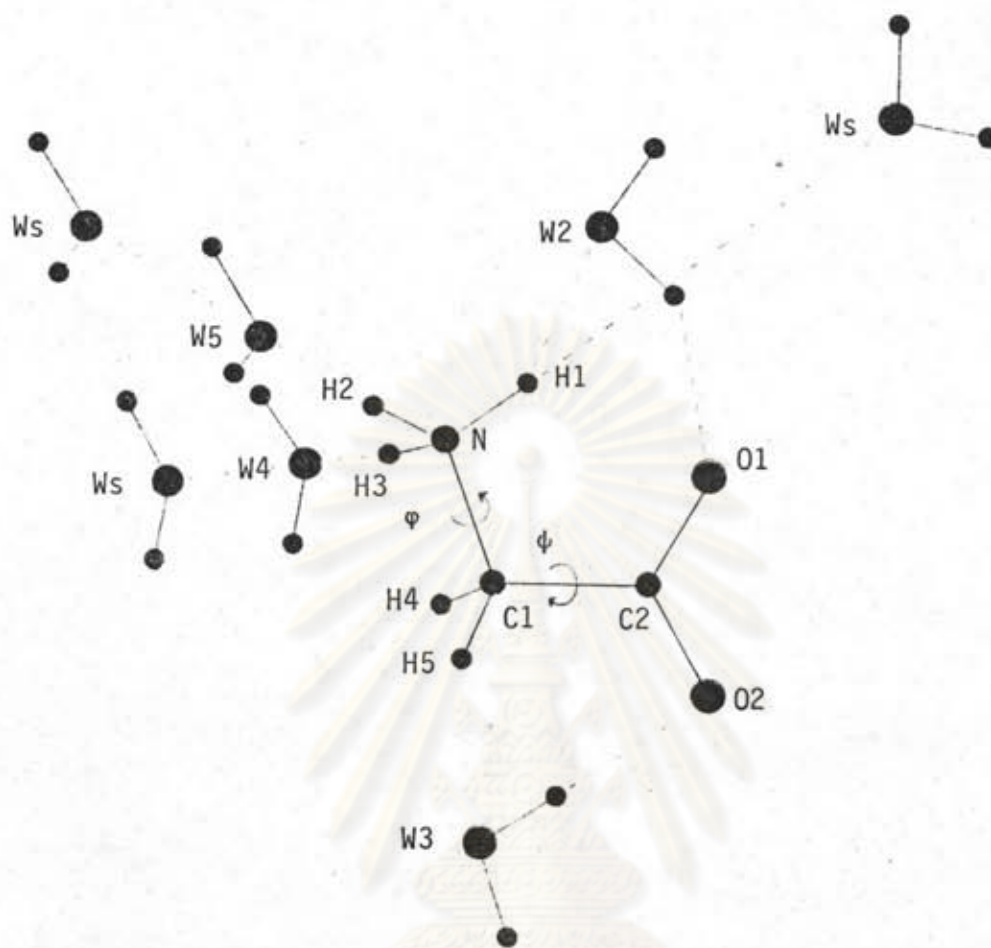


Figure 5.16 Model of glycine zwitterion including 4 water molecules in first hydration shell and 3 water molecules in the second hydration shell.



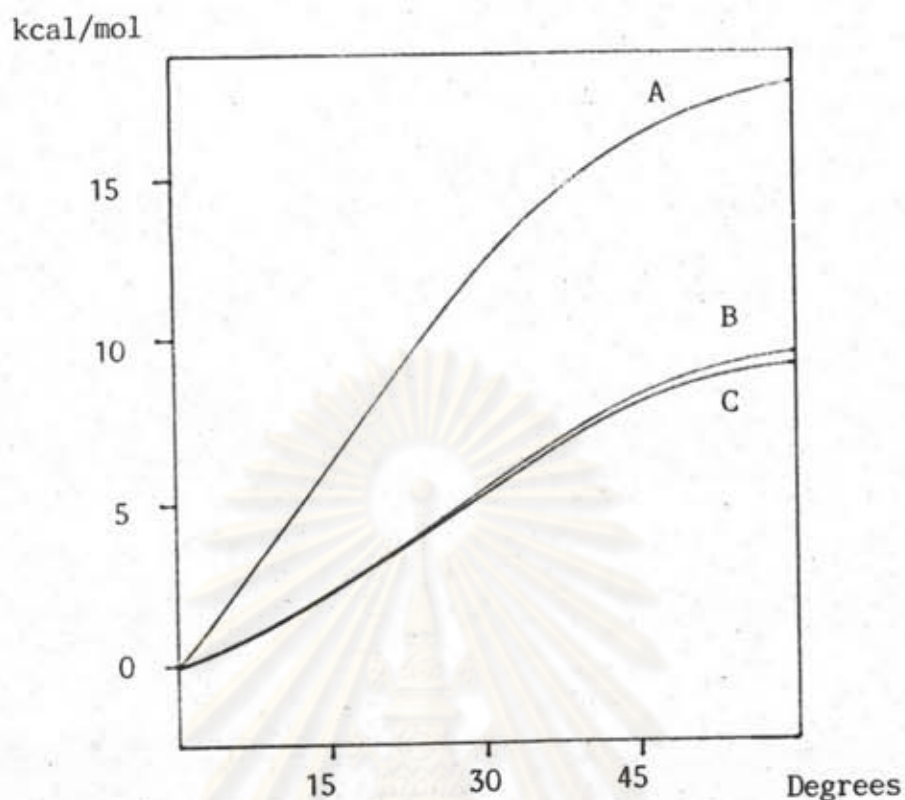


Figure 5.17 The internal rotation pathways of glycine zwitterion including the second shell effect

A. fixing the second shell water and water 3, rotating  $-\text{NH}_3^+$  group and water 2,4,5

B. glycine zwitterion including 4 water molecules in the first shell ( water 2,3,4,5 ), rotating  $-\text{NH}_3^+$  group and water 2,4,5

C. glycine zwitterion including 5 water in the first shell, rotating  $-\text{NH}_3^+$  and water 2,4,5

For the results of all calculations, it can be finally concluded that the water in the first hydration shell exerts a major influence on the internal rotation and hence the conformational stability of glycine zwitterion especially as far as pathways for the conformational changes are concerned.

## Conclusion

The supermolecule model which we have used for studying the influence of solvent on the internal rotation of glycine zwitterion can be only a rough approximation. However, the main influences in aqueous solution should be reflected properly by this approach. Some important solvation effects such as the dynamic exchange and the temperature effects have been neglected. However, our model containing 5 water molecules in the first shell can be expected to describe at least the direction of the influence of the solvent on the conformational stability of glycine zwitterion.

In this work all data on the conformational changes and barriers have been obtained by the CNDO method. Sometimes inadequate description of hydrogen bonding by CNDO will have some effects on the determination of conformation, too. To investigate these effects very extensive ab initio calculations would have been needed. However CNDO at reasonably fixed hydrogen bond distances was shown to work quite reliably and to cover most of the relevant interactions. It was assumed, therefore, that the CNDO method yields correct results on conformations and that much larger ab initio calculations would improve only some of the absolute values for the quantities under investigation. The general effects, which are of importance for the prediction of solvent influence on amino acid conformations and the flexibility of conformations in water, could be evaluated, and this was the objective of this work.