

# Computational investigation on microsolvation of the osmolyte glycine betaine [GB (H<sub>2</sub>O)<sub>1-7</sub>]

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**Abstract** The preferential interactions of glycine betaine (GB) with solvent components and the effect of solvent on its stability have been examined. In particular, the microsolvation of organic osmolyte and widely important osmoprotectant in nature as glycine betaine has been reported by using M06 method. A number of configurations ( $b_x$  (a-z)) of the clusters for one to seven water molecules ( $\times=1-7$ ) have been considered for the microsolvation. Structures of stable conformers are obtained and denoted as b1a, b2a, b3a, b4a, b5a, b6a and b7a. It is observed from the interaction energy difference ( $\Delta E$ ) that only seven water molecules can be accommodated in the first solvation shell to stabilize GB. It is also observed that the calculated relative energy using M06 is in close agreement with calculations at the MP2 level of theory.

**Keywords** Betaine · DFT · Glycine betaine · Microsolvation · M06

## Introduction

Organic osmolytes are utilized to maintain cell volume, when these small solutes are used by cells of numerous water stressed organisms and tissues [1, 2]. Osmolytes also help the evolutionary adaptation of most cells in a dehydrated or concentrated ion solution [3]. They have been

reported as being highly soluble compounds which carry no net charge at physiological pH and these are nontoxic at high concentrations [4]. Determination of the conformational details of biological osmolytic macromolecules is important to understand their biological functions. The N-methylated derivatives of the glycine family are known as osmoprotectants or osmolytes which also affect the melting behavior of proteins and nucleic acids [5–8].

Glycine betaine (GB) has been reported as a highly polar but neutral zwitterionic compound and their cationic functional group cannot be deprotonated even at high pH [9, 10]. GB (N,N,N-trimethylglycine) has been shown as an effective *Escherichia coli* osmolyte which allows the cell to efficiently retain intracellular water and to maintain cytoplasmic volume. As a result of which *E.Coli* grows well under dehydrating conditions [11]. Under low water accumulation GB supports the growth of *Lactococcus lactis*, an industrially important microorganism which is used in manufacturing a variety of dairy products [12]. GB has sustained the growth of human pathogen *Listeria monocytogenes* [13]. GB has also been reported for increasing the cold tolerance of plants [14]. A number of bacterial pathogens accumulate cytoplasmic GB to adapt to osmotic stress, increasing their growth rate and thus affecting colonization and infectivity [11, 15]. Such efficient osmoprotectants have been studied not only in bacteria but also in eukaryotic cells [16–21]. In vitro studies of GB have shown strong stabilizing sitespecific protein-DNA complexes [22–25] and moderately stabilize the globular (folded) conformation of protein [26, 27]. Betaine (in common with most osmolytes) is a “compensatory” or “counteracting” solute [28] that enhances protein stability. GB is particularly effective at countering the denaturing effect of urea [29–32] which is an important function in the renal medulla [31].

In 1990 Mak reported the crystal structure of betaine monohydrate [33]. Later Fornili et al. studied hybrid QM/MM molecular dynamics simulation of GB and reported the

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first solvation shell using the cage model. They have also revealed that the number of water molecules residing in 3.5 Å of first solvation shell in GB could be seven [34].

The zwitterionic forms of amino acids are unstable in gas phase. Most of the zwitterionic conformers are stable when water molecules are added explicitly [35]. So, far, microsolvation has been reported for Glycine [36–38], Cysteine [39], Alanine [40, 41], Tyrosine [42, 43], and Proline [44]. Naturally occurring zwitterionic form of neutral organic osmolyte GB (N-methylated glycine) has not yet been discussed in detail. Earlier reports suggest that the osmolytes increase the protein stability via their preferential exclusion from the protein surface, which in turn is preferentially hydrated [45, 46].

In this present study we have investigated the hydration properties of Glycine betaine through microsolvation. We have extensively searched the configurations for the clusters from one to seven water molecules, intensively looking for the most stable conformer. **Computational details** are briefly given in section 2 and **Results and discussions** are in section 3. **Summary** and **Conclusions** are given in section 4 and 5, respectively.

#### Computational details

Geometry optimizations were performed at M06/6-31 + G\* and corresponding single point energy calculation have also been performed using 6-311++G\*\* basis set at M06 and MP2 level of theory. We optimized the geometry of clusters labeled as bx(a-z), where b is defined as Glycine Betaine, x indicates the number of water molecules and a-z designates the number of configurations. M06 is known to describe the hydrogen bonding strength in a better way than other DFT methods [47]. Numerous successful results compared with other DFT functional using M06 method for the non-covalent interactions have been reported in the literature [47–51]. Single point energy calculation at MP2/6-311++G\*\*//M06/6-31 + G\* level has been computed to estimate the hydrogen bonding strengths [52, 53]. Single point energy calculation at CCSD(T)/6-311++G\*\*//M06/6-31 + G\* level has also been computed for the bench mark study. The betaine-water interaction energy ( $E_{\text{int}}$ ) with BSSE correction has been calculated using the following equation:

$$E_{\text{int}} = E_{\text{complex}} - E_{\text{betaine}} - nE_{\text{water}} \quad (1)$$

at M06/6-311++G\*\* level. Where  $E_{\text{complex}}$  is the total energy of the betaine-water complex,  $E_{\text{betaine}}$  is the total energy of the betaine in betaine-water complex and  $E_{\text{water}}$  is the total energy of water in betaine-water complex. We have optimized the number of betaine-(water)<sub>1-7</sub> clusters and investigated the stability of complexes using the hydrogen bonding strengths and relative energy. All calculations have been computed by using the Gaussian 09 suite of programs [54].

We justified the M06 method by comparing the relative energies of configurations of GB up to two water molecules obtained with M06/6-311++G\*\*//M06/6-31 + G\*, MP2/6-311++G\*\*//M06/6-31 + G\* and CCSD(T)/6-311++G\*\*//M06/6-31 + G\* level. We believe this benchmark is sufficient to justify use of this method to investigate the microsolvation of GB.

The benchmark occupies comparison of the relative energies of GB-one water and GB-two water clusters. We have optimized a number of these clusters (Figs. 2 and 3) at M06/6-31 + G\*. We have compared the calculated relative energies of these clusters computed at M06/6-311++G\*\*//M06/6-31 + G\*, MP2/6-311++G\*\*//M06/6-31 + G\* and CCSD(T)/6-311++G\*\*//M06/6-31 + G\*, values are listed in Tables 1 and 2. The results shows a good agreement between CCSD(T) and MP2 relative energies. The stability ordering is the same at both levels and the energy difference not more than one kilocalorie per mole. Moreover M06 calculations predict the same energetic ordering with MP2 and CCSD(T) level of GB with one and two water clusters. Optimization of these clusters at CCSD(T) and MP2 is expensive and especially for the larger water clusters is unaffordable. From our studies we conclude, this benchmark is sufficient to utilize the M06 functional for the larger clusters. Therefore we have decided to use M06 results to analyze the preferential interaction of water with GB and its stability.

We have opted for a semirational approach to select a number of configurations for the GB-(water)<sub>1-7</sub> clusters, using the possible number of hydrogen bonds, we build up from the smaller water clusters with GB. Initially the water molecules are associated with the carboxylate group then they can form the cage like structure for the larger clusters.

## Results and discussion

### Glycine betaine

The optimized structure for the Glycine betaine (GB) at M06/6-31 + G(d) is shown in Fig. 1. Mak [33] has reported

**Table 1** Relative energies (kcal mol<sup>-1</sup>) of the betaine-one-water clusters

System	M06//M06 <sup>a</sup>	MP2//M06 <sup>b</sup>	CCSD(T)//M06 <sup>c</sup>
b1a	0.00	0.00	0.00
b1b	0.44	0.67	0.60
b1c	1.58	1.72	1.56

<sup>a</sup> M06/6-311++G\*\*//M06/6-31 + G\*

<sup>b</sup> MP2/6-311++G\*\*//M06/6-31 + G\*

<sup>c</sup> CCSD(T)/6-311++G\*\*//M06/6-31 + G\*

**Table 2** Relative energies (kcal mol<sup>-1</sup>) of the betaine-two-water clusters

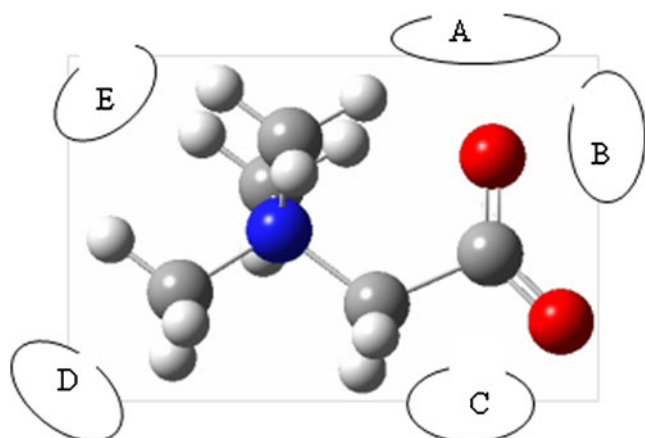
System	M06//M06 <sup>a</sup>	MP2//M06 <sup>b</sup>	CCSD(T)//M06 <sup>c</sup>
b2a	0.00	0.00	0.00
b2b	0.07	0.33	0.57
b2c	1.38	1.01	0.96
b2d	1.78	0.73	0.83

<sup>a</sup> M06/6-311++G\*\*// M06/6-31 + G\*<sup>b</sup> MP2/6-311++G\*\*// M06/6-31 + G\*<sup>c</sup> CCSD(T)/6-311++G\*\*//M06/6-31 + G\*

the crystal structure of GB-mono hydrate. Fornili and co-workers have reported the optimized conformation of GB using *ab initio* method and investigated water interaction with GB using the QM/MM MD simulation approach based on modified AM1 parameters and AMBER force field [34]. They have explained GB to be hydrophilic in nature but the methylene group of GB was specifically hydrophobic in nature. Most recently Stangret et al. [55] have performed FT-IR spectroscopic studies on the interactions of water with glycine and its N-methylated derivatives. In our calculation we have considered several possible interactions between betaine-water and the number of configurations to find the most stable conformer.

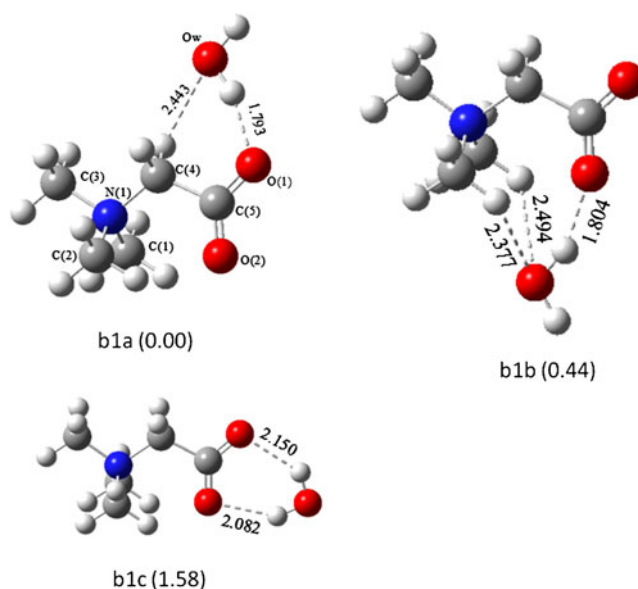
#### Betaine: one water clusters

Five configurations are identified for betaine-one water cluster at M06 level, shown in Fig. S1 (see Supporting information). There are five regions (A-E) about a GB to interact with water shown in Fig. 1. Regions A-C have been observed to be the strong interaction regions and formed strong hydrogen bonds with water molecules. The betaine-water interaction energy for the betaine-one-water molecule can be observed from Table 3. b1a, b1b and b1c have been observed ca. -12 kcal mol<sup>-1</sup> as

**Fig. 1** Optimized structure of Betaine at M06/6-31 + G(d) with water interaction regions of GB (a-e)**Table 3** Interaction energy ( $E_{\text{int}}$ ) (kcal mol<sup>-1</sup>) for betaine-one-water complexes at M06/6-311++G\*\* level

System	$E_{\text{int}}$	Interaction regions
b1a	-12.35	C
b1b	-12.66	A
b1c	-11.34	B
b1d	-5.46	E
b1e	-3.66	D

interaction energy due to their strong interaction regions of A-C. Similarly the weak interaction regions of D-E have been observed to be the lowest interaction energies  $\sim -3$ -5 kcal mol<sup>-1</sup> for b1d and b1e clusters. Three lowest energy configurations with hydrogen-bonding lengths are shown in Fig. 2 and relative energies are listed in Table 1 and Table S1 (see Supporting information). From the relative energy, it has been observed that the three lowest energy configurations b1a, b1b and b1c possess at least one hydrogen bond between COO<sup>-</sup> of betaine and H of water. In the most stable b1a, a cyclic hydrogen-bonding network is observed through the strong O...H-O at 1.793 Å and a weak C-H...O type hydrogen bond at 2.443 Å. This agrees with earlier reports by Bachrach et al. that the cyclic hydrogen bond holding configurations were the most stable [36]. The b1a configuration is well matched with the reported structure [33]. The geometrical parameters of the crystal such as hydrogen-bond lengths (Å), bond angles (°) and selected torsion angles (°) obtained in the present study are in good agreement with literature (Table 4). Though we have observed three hydrogen bonds in b1b configuration: one O...

**Fig. 2** Optimized structures of betaine-one-water clusters and relative energy (kcal mol<sup>-1</sup>) at M06 level. Dashed lines indicate hydrogen bond distances with their values (Å)

**Table 4** Experimental and theoretical results (at M06) of bond lengths (Å), bond angles (°) and torsion angles (°) in betaine-one-water (b1a)

Parameter	betaine-one-water <sup>a</sup>	b1a
N(1)-C(1)	1.495(3)	1.496
N(1)-C(3)	1.497(3)	1.487
C(4)-C(5)	1.533(3)	1.561
C(5)-O(2)	1.239(3)	1.244
N(1)-C(2)	1.491(3)	1.495
N(1)-C(4)	1.508(3)	1.522
C(5)-O(1)	1.251(3)	1.245
C(1)-N(1)-C(2)	110.8(2)	109.7
C(1)-N(1)-C(3)	108.3(2)	109.1
C(2)-N(1)-C(3)	108.2(2)	109.2
N(1)-C(4)-C(5)	117.6(2)	116.6
C(4)-C(5)-O(2)	120.1(2)	117.9
C(3)-N(1)-C(4)-C(5)	-179.2(2)	-178.8
C(1)-N(1)-C(4)	110.4(2)	110.1
C(2)-N(1)-C(4)	111.3(2)	110.2
C(3)-N(1)-C(4)	107.8(2)	108.5
C(4)-C(5)-O(1)	112.6(2)	111.0
O(1)-C(5)-O(2)	127.2(2)	131.1
N(1)-C(4)-C(5)-O(1)	-177.5(2)	177.6
Ow...O(1)	2.814(4)	2.753

<sup>a</sup> From reference [33]

H-O type at 1.804 Å and two weak C-H...O types at 2.377 Å and 2.494 Å, the longer O...H-O hydrogen bond length reflects the relative higher energy. The third most stable b1c configuration has two O...H-O type hydrogen bonds and is observed to be 1.58 kcal mol<sup>-1</sup> higher in energy than b1a conformer. Two protons of a water molecule in b1c conformer are connected to the carboxylate group of betaine and as a result, b1c shows higher relative energy. In the betaine-one water clusters, b1d and b1e are observed to be the least stable configurations compared with b1a. Whenever the clusters have a greater number of hydrogen bonds with the methyl group, such clusters are not favored as compared to hydrogen bonds observed with the carboxylate group. From the energetic view point, the stability order and relative energies at M06, MP2 and CCSD(T) levels are very close to each other. The conformers stability order is b1a > b1b > b1c > b1d > b1e at M06, MP2 and CCSD(T) level.

#### Betaine: two water clusters

Six configurations of betaine-two water clusters are located at M06/6-31 + G(d) level, shown in Fig. S2 (see Supporting information). There are four low lying configurations within 2 kcal mol<sup>-1</sup>, shown in Fig. 3 and relative energies are listed in Table 2 and Table S2 (see Supporting information). Betaine-water interactions and water-water interactions

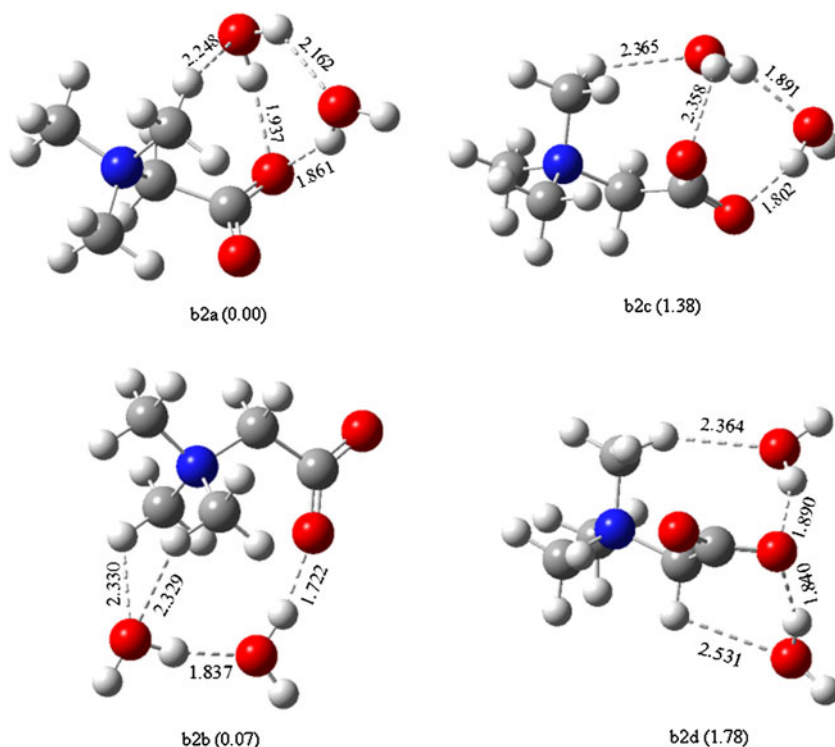
(except b2f and b2d) have been observed in most of the clusters with strong and weak hydrogen bonds. Such additional water-water interaction aids in the stability of clusters. Among the six configurations b2a has been observed to be the most stable configuration. The second most stable and the nearest energy to the b2a is b2b configuration. We have observed four hydrogen bonds with cyclic nature in b2a and b2b configurations. The b2a is more stable than b2b which might be due to the three strong O-H...O type hydrogen bonds at 1.861 Å, 1.937 Å, 2.162 Å between betaine-water and one weak C-H...O type hydrogen bond at 2.248 Å contributed by the methyl group which is attached to the quaternary nitrogen. Rather b2b configuration has two strong O-H...O type hydrogen bonds at 1.722 Å and 1.837 Å observed between betaine-water and water-water, respectively. Additionally, b2b has two weak C-H...O type hydrogen bonds at 2.329 Å and at 2.330 Å contributed by the two different methyl groups, which are attached to the quaternary nitrogen of betaine. The b2c configuration has three O-H...O type hydrogen bonds at 1.802 Å, 1.891 Å and 2.358 Å and a C-H...O type hydrogen bond at 2.365 Å. The longer O-H...O hydrogen bond length reflects a higher relative energy. It is 1.38 kcal mol<sup>-1</sup> higher in energy than b2a. Also, b2d configuration is close in energy with b2c. The b2d shows four hydrogen bonds, in which two are strong O-H...O type at 1.890 Å and 1.840 Å and two are weak C-H...O type at 2.364 Å and 2.531 Å. The additional water-water interaction has not been observed for b2f and b2d configurations. The b2f has been observed to be the least stable configuration in the betaine-two water clusters, because of the interaction of water with the carboxylate and methyl group of betaine independently. From the energetic view point, the stability order and relative energies at M06, MP2 and CCSD(T) levels are very close to each other. The configurations stability order is b2a > b2b > b2c > b2d > b2e at M06 level and b2a > b2b > b2d > b2c > b2e at MP2 and CCSD(T) level.

#### Betaine: three water clusters

Seven configurations of betaine-three water clusters optimized at M06 level are shown in Fig. S3 (see Supporting information). Among them, four lower energy configurations with hydrogen-bonding lengths are shown in Fig. 4 while the relative energies are listed in Table S3 (see Supporting information). Among the betaine-(water)<sub>3</sub> clusters, b3a is the most stable configuration. The five hydrogen bonds, including the intramolecular hydrogen bonds, together play a role in the stability compared with the b3b and other configurations. In b3a, three hydrogen bonds are located between betaine-water (O-H...O; 1.811 Å, 1.943 Å and C-H...O 2.198 Å) and two are between the water molecules (O-H...O; 1.883 Å, 1.740 Å). Also the water-



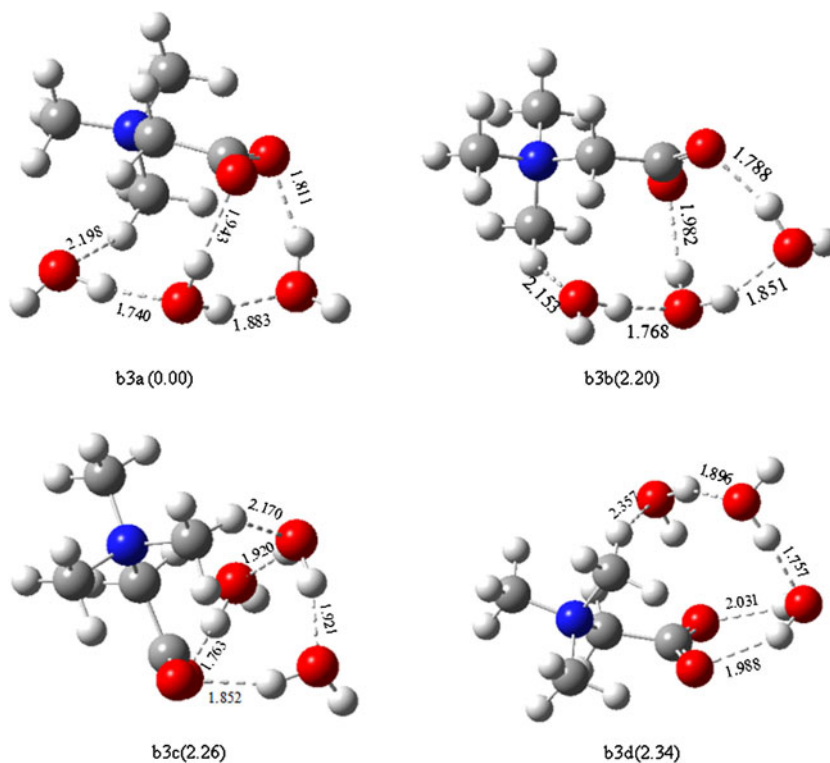
**Fig. 3** Optimized structures of betaine-two-water clusters and relative energy ( $\text{kcal mol}^{-1}$ ) at M06 level. Dashed lines indicate hydrogen bonds distances with their values ( $\text{\AA}$ )



water interactions are evidently preferred over the betaine-water interaction when the number of water molecule increases. A slight distorted form of b3a, b3b also has five hydrogen bonds, which resembles b3a, but is  $2.20 \text{ kcal mol}^{-1}$

relatively higher in energy. Next to b3a and b3b configurations, b3c is shown as the third most stable configuration. The b3c structure also possesses five hydrogen bonds (O-H...O;  $1.852 \text{ \AA}$ ,  $1.763 \text{ \AA}$ ,  $1.920 \text{ \AA}$ ,  $1.921 \text{ \AA}$  and C-H...O;  $2.170 \text{ \AA}$ )

**Fig. 4** Optimized structures of betaine-three-water clusters and relative energy ( $\text{kcal mol}^{-1}$ ) at M06 level. Dashed lines indicate hydrogen bonds distances with their values ( $\text{\AA}$ )



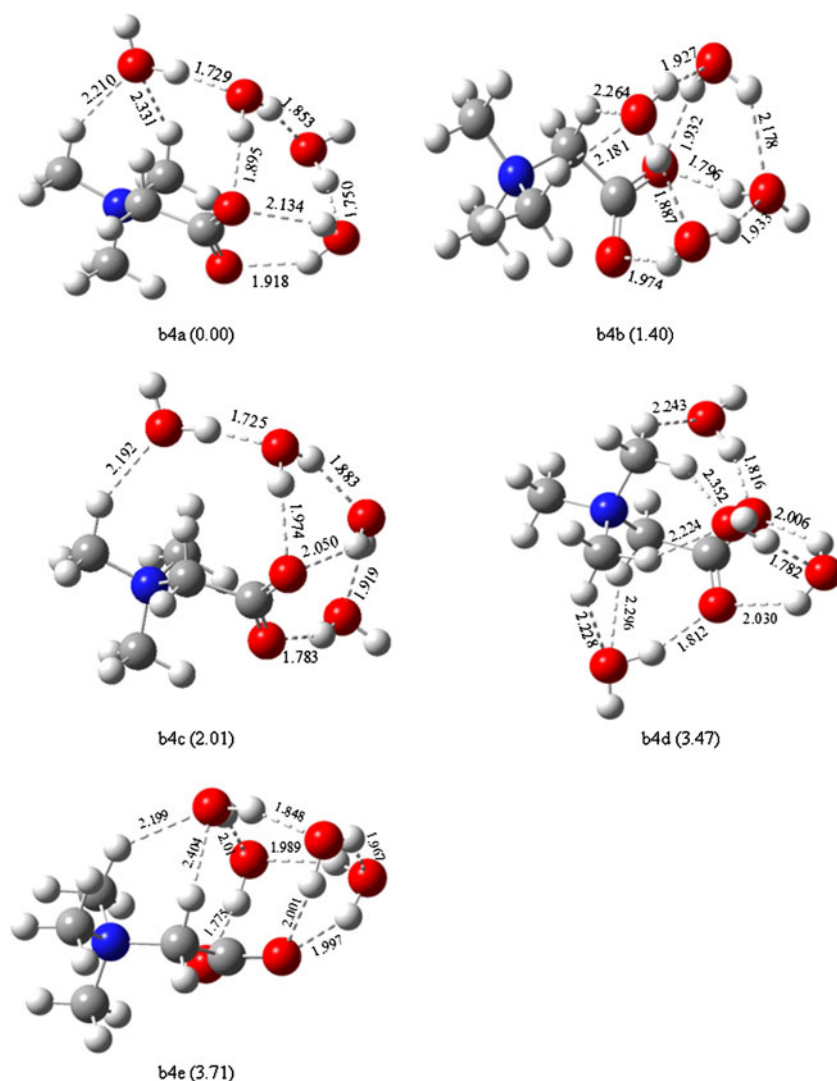
without a cage between carboxylate group and its hydrogen bonded water molecules, but  $\sim 2.2$  kcal mol<sup>-1</sup> relatively higher in energy. From an energetic view point, the stability order is b3a > b3b > b3c > b3d > b3e at M06 level and b3a > b3b > b3c > b3f > b3e > b3d at MP2 level.

#### Betaine: four water clusters

Twenty five configurations are optimized at M06 level for the betaine-four water clusters, shown in Fig. S4. Five configurations are observed to possess relatively lower energy, shown in Fig. 5 and others are  $>4$  kcal mol<sup>-1</sup> higher in energy listed in Table S4 (see Supporting information). Among them b4a is the most stable configuration, which has eight hydrogen bonds including five hydrogen bonds between betaine-water (O-H...O; 1.895 Å, 2.134 Å, 1.918 Å and C-H...O; 2.210 Å, 2.331 Å) and three hydrogen bonds (O-H...O; 1.729 Å, 1.853 Å, 1.750 Å) between water-water forming cyclic hydrogen bond network. Both

the protons of a water molecule are bonded to the two oxygens of carboxylate group in b3a. The interaction of water molecule begins with carboxylate group and ends with the methyl group of betaine. The strong intramolecular forces between the methyl and carboxylate group are also additional support for the complex stability when we increase the number of water molecules. The formation of cage like hydrogen-bonding structure, due to the water-water interaction along with the betaine-water interaction, is also responsible for the betaine-water complex stability. In b4d, one of the water molecules interacting with the carboxylate group with two hydrogen bonds resembles b4a, but these two hydrogen bonds are weaker (O-H...O; 2.006 Å, 2.030 Å), and thus reflects a higher relative energy. However, other water molecules having a hydrogen-bonding network with the methyl groups also explains the higher relative energy. The second most stable configuration shown by b4b, which has nine hydrogen bonds including five hydrogen bonds between betaine-water (O-H...O; 1.932 Å,

**Fig. 5** Optimized structures of betaine-four-water clusters and relative energy (kcal mol<sup>-1</sup>) at M06 level. Dashed lines indicate hydrogen bonds distances with their values (Å)



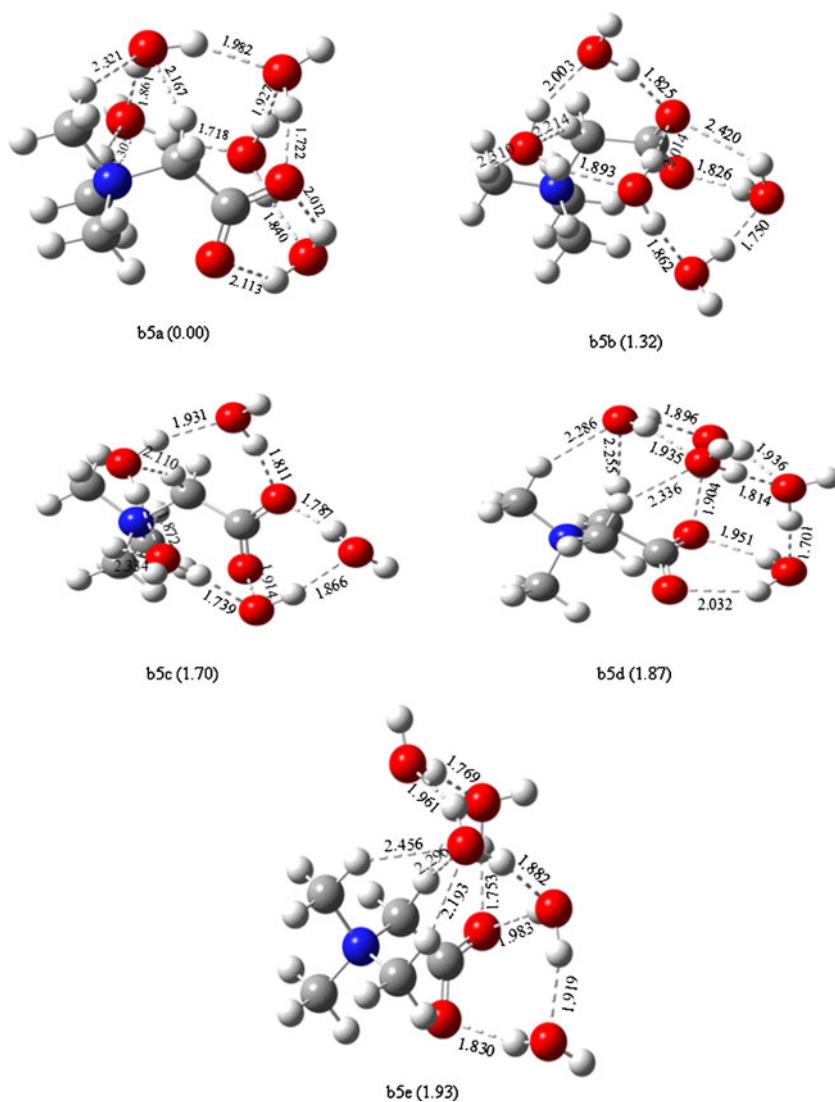
1.796 Å, 1.974 Å and C-H...O; 2.264 Å, 2.181 Å) and four hydrogen bonds (O-H...O; 1.927 Å, 2.178 Å, 1.933 Å, 1.887 Å) between water-water forming cyclic-hydrogen-bonding network. Similarly, b4c, b4d and b4e have been observed to possess seven, ten, nine hydrogen bonds with 2.01, 3.47, 3.71 kcal mol<sup>-1</sup>, respectively, but relatively higher in energy. From an energetic view point, the stability order is b4a > b4b > b4c > b4d > b4e at M06 level and b4a > b4c > b4b > b4d > b4f at MP2 level.

#### Betaine: five water clusters

Forty eight configurations of betaine-five water clusters are optimized at M06 level, shown in Fig. S5 (see Supporting information). The five lowest energy configurations are shown in Fig. 6 and relative energies are listed in Table S5 (see Supporting information). Among them b5a has been identified as the most stable configuration having 11 hydrogen bonds including six hydrogen bonds between betaine-water (O-H...

O; 1.722 Å, 2.012 Å, 2.113 Å C-H...O; 2.321 Å, 2.305 Å, 2.167 Å) and five hydrogen bonds (O-H...O; 1.982 Å, 1.861 Å, 1.927 Å, 1.840 Å, 1.718 Å) between water-water with cyclic-hydrogen-bonding network. Water molecule interaction starts with carboxylate group and ends with methyl group that resembles a cage. Two water molecules directly interact with the carboxylate group and the rest interact among themselves, and with the methylene and methyl groups. It clearly explains that water molecules interact with every group of betaine. So, water surrounds the betaine instead of interacting with the carboxylate group only. The b5c and b5e have four membered rings of water molecules that are hydrogen bonded to the carboxylate group and form a bridge between methyl and methylene groups. In b5b, one of the water molecules interacting with the carboxylate group with two hydrogen bonds resembles the b5a and one among the two hydrogen bonds is weaker (2.420 Å), it reflects a higher relative energy. The configurations stability order is b5a > b5b > b5c > b5d > b5e at M06 level and b5a > b5c > b5f > b5d > b5b at MP2 level.

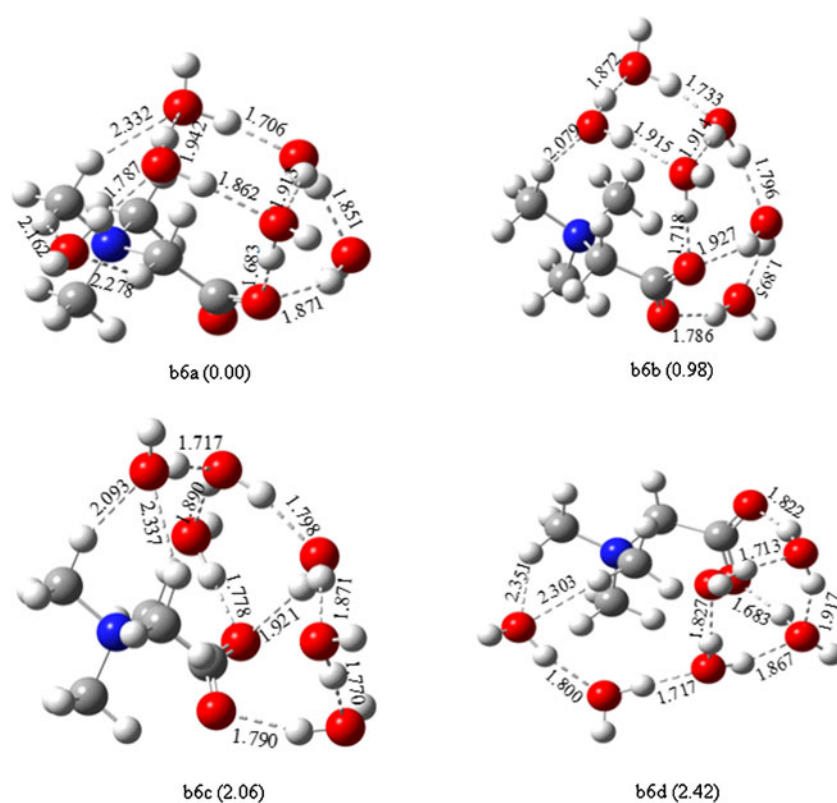
**Fig. 6** Optimized structures of betaine-five-water clusters and relative energy (kcal mol<sup>-1</sup>) at M06 level. Dashed lines indicate hydrogen bonds distances with their values (Å)



## Betaine: six water clusters

Forty eight configurations have been identified when betaine interacts with six water molecules, shown in Fig. S6 (see Supporting information). Four lowest energy configurations are shown in Fig. 7 and relative energies are listed in Table S6 (see Supporting information). Among the four configurations b6a has been observed to be the most stable configuration. The configuration b6a has 11 hydrogen bonds, specifically, five hydrogen bonds between betaine-water (O-H...O; 1.683 Å, 1.871 Å and C-H...O; 2.332 Å, 2.162 Å, 2.278 Å) and six hydrogen bonds (O-H...O; 1.787 Å, 1.942 Å, 1.706 Å, 1.862 Å, 1.913 Å, 1.851 Å) between water-water forming cyclic-hydrogen-bonding network. In b6c and b6e one part of the configuration forms a pentagon like cage structure, where the two corners are occupied by carboxylate oxygen and the remaining three oxygens are from water molecules. From b6a to b6e, multiple hydrogen bonds between the water molecules and between water molecules and carboxylate group, and minimum one hydrogen bond have been observed ( $>2$  Å) between water and methyl group. The b6a itself provides the information that six water molecules are almost sufficient, due to their interaction with all the parts of betaine and its surface. The configurations stability order is  $b6a > b6b > b6c > b6d > b6e$  at M06 level and  $b6a > b6d > b6b > b6c > b6e$  at MP2 level.

**Fig. 7** Optimized structures of betaine-six-water clusters and relative energy ( $\text{kcal mol}^{-1}$ ) at M06 level. Dashed lines indicate hydrogen bonds distances with their values (Å)

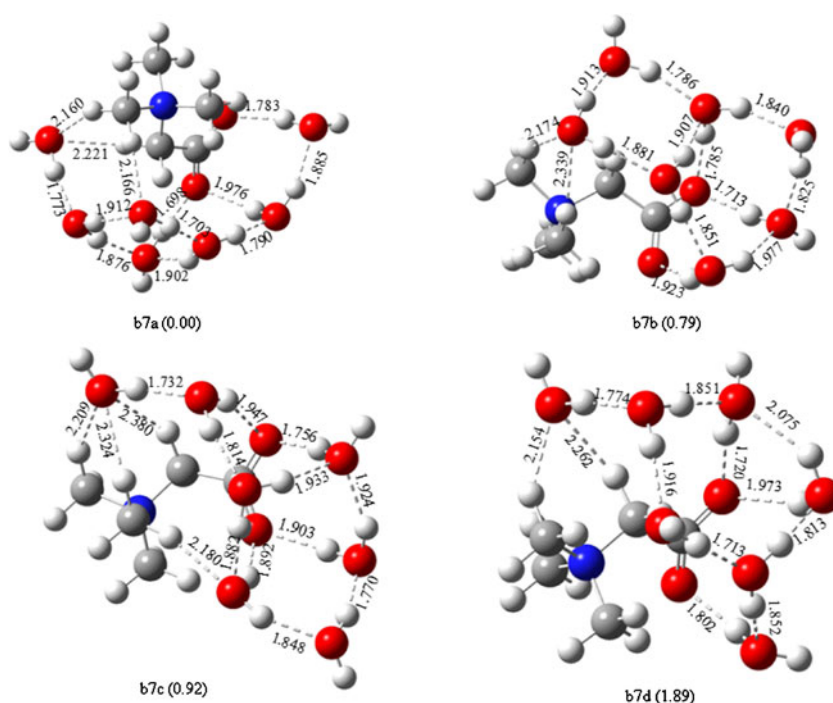


## Betaine: seven water clusters

Seventy one configurations of betaine-seven water clusters are located at M06 level, shown in Fig. S7 (see Supporting information). Four lowest energy configurations are shown in Fig. 8 and relative energies are listed in Table S7 (see Supporting information). b7a has been observed to be the most stable configuration among the 71 GB( $\text{H}_2\text{O}$ )<sub>7</sub> complexes. The b7a possesses 13 hydrogen bonds, specifically six hydrogen bonds are formed between betaine-water (O-H...O; 1.783 Å, 1.976 Å, 1.698 Å and C-H...O; 2.160 Å, 2.221 Å, 2.166 Å) and seven hydrogen bonds between water-water (O-H...O; 1.885 Å, 1.790 Å, 1.902 Å, 1.876 Å, 1.773 Å, 1.912 Å, 1.703 Å). Two proton donors from a methyl group to water, with weak interaction ( $>2$  Å) has been observed in b7a. A number of water molecules interact with the hydrophilic region of betaine surface. The larger number of water-water interactions are evidently preferred for the complex stability. Two water molecules are directly hydrogen bonded to the carboxylate group as a donor while the other two are hydrogen bonded to the methyl group as acceptor. The remaining water molecules extensively form a water-water hydrogen-bonding network. Two water molecules bridging the carboxylate group pattern have been observed in b7a to b7e, but remaining hydrogen-bonding network has different pattern. Four lowest energy configurations were observed with  $\sim 2$   $\text{kcal mol}^{-1}$  relative energy of difference in our calculation. When we increase



**Fig. 8** Optimized structures of betaine-seven-water clusters and relative energy ( $\text{kcal mol}^{-1}$ ) at M06 level. Dashed lines indicate hydrogen bonds distances with their values ( $\text{\AA}$ )



the number of water molecules one can observe that water acts as donor as well as acceptor. Betaine-seven water clusters have shown that water molecules interact with betaine surface sufficiently with all parts of betaine. Overall the qualitative trend for betaine-seven water complexes are  $b7a > b7b > b7c > b7d > b7e$  at M06 level and  $b7a > b7b > b7d > b7c > b7e$  at MP2 level.

### Summary

Bachrach [36] and Wang et al. [56] reported that the zwitterionic cluster with one water molecule is not stable due to the proton transfer from ammonium group to the carboxylate group, but in the case of cysteine [39] they were able to observe two lowest energy conformers stabilized by weak intramolecular interaction between ammonium and thiol group. Independently, interactions of ammonium-water and carboxylate-water have also been reported [36].

For GB-one-water we have identified three among five clusters shown as low-lying configurations (Fig. 2). It might be attributed to the absence of free proton in the quaternary nitrogen of betaine and additional intramolecular hydrogen bond between bulky methyl group and carboxylate group of betaine. Additionally the bulky methyl groups do not allow the water molecule to interact with the nitrogen. In general, for neutral or zwitterionic form of amino acid clusters, the formation of cyclic-hydrogen-bonding network proved to be a key factor for the stable configurations [36–44]. Moreover the GB clusters having cyclic-hydrogen-bonding network

configurations are observed to be the most stable (b1a, b2a, b3a etc.).

Numerous configurations have been considered for betaine-(water)<sub>1-7</sub> clusters in this study. By adding water molecules to betaine, the geometry has been varied and water has formed a ring around the betaine surface, where water is connected to the carboxylate and methyl group of betaine. In GB clusters, water molecule is associated with the carboxylate group then to the methyl group, building water-water hydrogen bonding networks to cover these distanced groups. The carboxylate group accepts hydrogen from three different water molecules. The remaining water molecules build the cage of hydrogen-bonding network between the carboxylate and methyl groups.

Preferential interaction of betaine-(water)<sub>1-7</sub> in the first solvation shell has been discussed above. We have shown all the possible interaction regions in betaine-water clusters and the stability of complexes. The water bridging has started from the carboxylate oxygen atoms and ended with the methyl group. Also in b7b and b7c the three water molecules interacting with the carboxylate oxygen atoms have been observed. In b7b the water chain has started and ended with the methyl groups, which is different from b7a. In b7a the hydrogen bond pattern with the water chain is below the carboxylate group whereas the same is above in b7b and b7c. Whenever the methyl groups donate a proton independently to water without forming a water chain invariably leads to higher energy structures (b1d, b1e, b2f, b4w, b4x, b5as, b6at, b7br). Two water molecules donating

protons to carboxylate group have been observed in betaine-two water and betaine-three water with two hydrogen bonds, whereas three hydrogen bonds with two water molecules have been observed in betaine-four water (b4a) and betaine-five water (b5a) clusters. However b6a has only two water molecules that are interacting directly with the carboxylate group, but three water molecules interacting with the carboxylate group have been observed in b6b and b7a.

We have calculated the betaine-water interaction energy ( $E_{\text{int}}$ ) for the most stable complexes and the interaction energy differences ( $\Delta E$ ) between the stable complexes. The interaction energy and  $\Delta E$  values for the most stable complexes are summarized in Table 5. The sequential decrease of  $\Delta E$  clearly indicates the strong water-water interaction compared to betaine-water interactions. It can also be noticed that when we add seventh water molecule at GB, it has reached the maximum of water for their interactions in the first solvation shell. Further, one can go more than seven water molecules to obtain the nil value of  $\Delta E$  with the high level of computations to investigate the water-water interaction in GB.

When we increase the water molecules more than seven, for example, eight water molecules (b8a in Supporting information), it has remarkably formed water cage through water-water interaction rather than betaine-water interaction. Therefore it has been concluded that the seven water molecules are sufficient enough to occupy GB surface in the first solvation shell. The seven water molecules accommodation in GB has good agreement with the earlier report [34].

## Conclusions

The studies on osmolytic GB show that it protects protein from the biologically unfavorable consequences of dehydration and thermodynamic perturbation [45, 46]. We have discussed the microsolvation of such osmolytic GB structural

properties using M06 method. Our study can help to understand the behavior of GB in aqueous phase and provides insight regarding the interplay of water between GB and protein. The water molecules have been observed to interact with the carboxylate group in the lower clusters like betaine-one-water and when we increased the number of water molecules to seven, water occupy around the surface of GB. It can be clearly concluded that GB consists of mostly hydrophilic region ( $-\text{CH}_3$ ,  $-\text{COO}$ ) and the water molecules attenuated the hydrophobicity ( $-\text{CH}_2$ ). In the present study, a maximum of two methyl groups of GB have been observed in the hydrogen bonded complexes (b2b, b4a, b5a, b7a, b7b, b7c) with water rather than three methyl groups. It was also observed that a single water molecule can stabilize the GB, however that is not sufficient for the zwitterionic glycine [36]. We have considered a number of possible configurations for each betaine-(water)<sub>1-7</sub> cluster to find the most stable complex for better understanding of the osmolyte GB in aqueous phase. In all the configurations, the energy difference between M06 and MP2 level is observed to be  $\sim 0.1$  kcal mol<sup>-1</sup>. The conformation of betaine in aqueous phase has been revealed through our study. The benchmark for the M06 level has been estimated at MP2 and CCSD(T) level of theory. It can also be noticed that the calculated relative energy using M06 is in close agreement with calculations at the MP2 level of theory. Thus, it can be concluded that seven water molecules are sufficient enough for the preferential interaction of the zwitterionic nature of organic osmolyte GB in aqueous phase, which has also been reported through QM/MM MD approach for the 3.5 Å of the first solvation shell [34].

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**Table 5** Interaction energy ( $E_{\text{int}}$ ) (kcal mol<sup>-1</sup>) for the most stable betaine-water complexes and interaction energy difference ( $\Delta E$ ) between the most stable complexes of betaine-water at M06/6-311++G\*\* level

System	$E_{\text{int}}$	$\Delta E$ ( $b_{(n+1)a} - b_{na}$ )
b1a	-12.35	-12.35
b2a	-27.41	-15.06
b3a	-34.57	-7.16
b4a	-44.44	-9.87
b5a	-51.55	-7.11
b6a	-55.77	-4.22
b7a	-64.28	-8.51
b8a	-67.23	-2.95

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