

Carnosine Conformers

S. A. Klyuev

Belgorod State University, ul. Pobedy 85, Belgorod, 308015 Russia

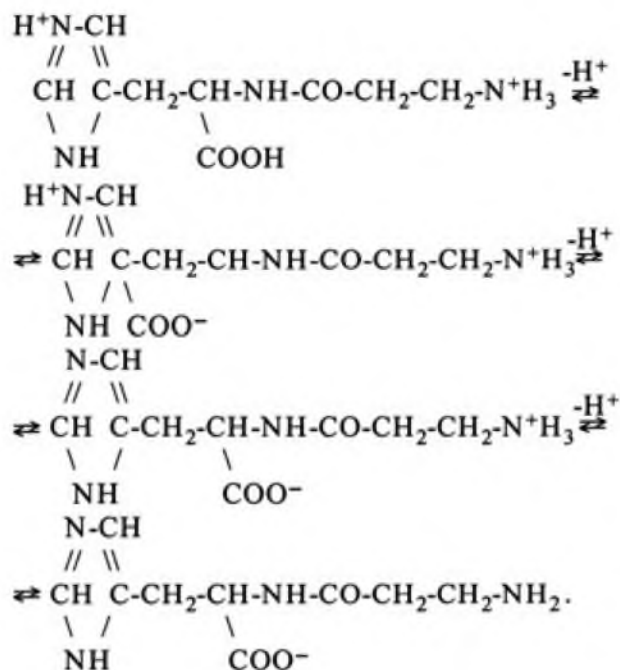
Abstract—A semiempirical quantum chemical method PM3 was used to calculate the geometry and energy parameters of the most stable conformations of carnosine and its clusters with water and zinc.

Key words: carnosine, semiempirical quantum chemical calculation, hydration

INTRODUCTION

Carnosine (β -alanyl-L-histidine) is a natural dipeptide. This compound is a main source of β -alanine in human organism. It forms complexes with metal ions (Zn, Mg, Mn, Cu, Co, etc.), reacts with carbonyl compounds and radical species, and shows biological activity [1–3].

Acid–base interactions in carnosine solution can be represented by the following scheme [4, 5]:



In aqueous solution, the ^1N tautomeric form of the imidazole fragment is present. The imidazole group in solid carnosine exists in the ^3N tautomeric form [5]. In alkaline medium, hydrolysis of the dipeptide takes place along with anion formation, which hampers the study of the anionic form of carnosine [6].

The anionic and uncharged forms of carnosine take part in complex formation. The anionic form reacts with carbonyl compounds. The efficiency of reaction of one or another form of the peptide with a reagent also depends on peptide conformation. Obviously, the most stable conformations can be isolated for peptides.

The aim of this work is to calculate the parameters of different forms of carnosine (bipolar ion and anion) by quantum chemical method. The task of the study was to isolate the most stable carnosine conformations and to calculate their parameters by semiempirical PM3 with different optimization algorithms, to consider the effect of hydration and complex formation (formation of zinc complex) on carnosine geometry (within the framework of supramolecular approximation, discrete solvation model), and to compare calculated and experimental data.

CALCULATION METHOD

The geometric and energetic (E is total energy) parameters of carnosine were determined with the

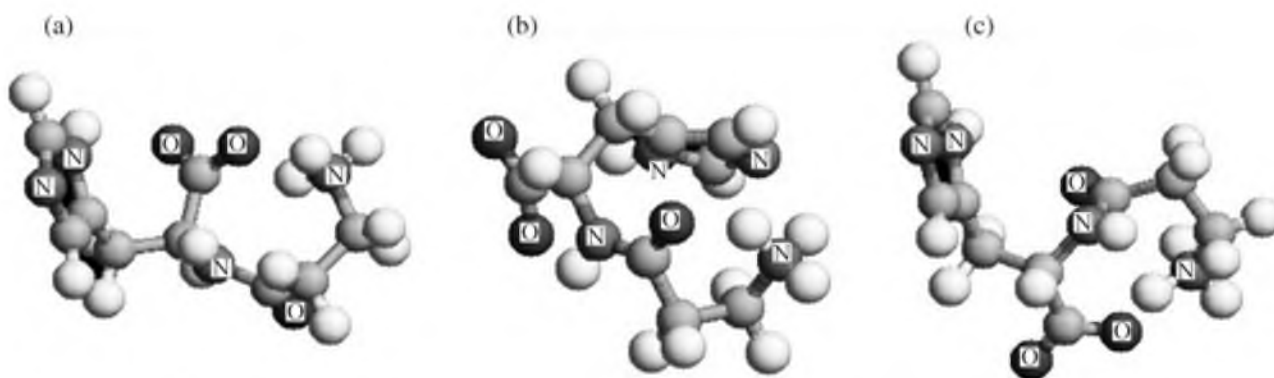


Fig. 1. (a) Conformer with the bend of the β -alanine fragment toward the imidazole ring and the deprotonated carboxy group, $E = -2811,498$ eV; (b) conformer with the bend of the β -alanine fragment toward the imidazole ring and away from the deprotonated carboxy group, $E = -2810,797$ eV; (c) conformer with the bend of the β -alanine fragment toward the deprotonated carboxy group and out of the imidazole ring, $E = -2811,485$ eV.

PM3 semiempirical parametric method (software package MOPAC, v. 7.00). This method allows calculations with the set of atoms necessary for this system and to describe well hydrogen bonds. The success of optimization was determined by testing. Semiempirical methods are known to underestimate the height of rotation barrier for the peptide bond; therefore, we performed correction (on the basis of molecular mechanics). Restricted Hartree-Fock method was used because the studied systems belonged to the systems with closed electron shells.

RESULTS AND DISCUSSION

Figure 1 shows the conformers of carnosine bipolar ion (ball and stick models). Conformers (a) and (c) are characterized by close positions of ^-OOC and H_3N^+ groups. The interatomic distance in conformer



Fig. 2. Hydrated bipolar ion of carnosine (five water molecules).

(a) between one of oxygen atoms of the carboxy group and the nearest hydrogen atom of the amino group is 1.61 \AA . The charged groups in peptides are arranged similarly according to X-ray diffraction data and NMR spectroscopy (visualization with RasMol software) [7]. Overlapping of the spherical surfaces for the hydrogen of the H_3N^+ group and for the oxygen of the ^-OOC group was observed in the models with atoms represented as spheres with van der Waals radii.

The hydration of conformer with a bend of β -alanine fragment toward the imidazole ring and deprotonated carboxy group (a) did not lead to considerable change in its geometric parameters (we calculated supermolecules containing from one to five water molecules, the arrangement of water molecules in clusters varied, water molecules introduced between charged groups were moved aside). The distance between the oxygen and the hydrogen atom of ^-OOC and H_3N^+ groups slightly increased (from 1.61 to 1.69 \AA) when passing from bipolar ion to cluster containing five water molecules. Figure 2 shows the hydrated bipolar ion of carnosine. According to electrostatic notions, increase in the dielectric constant of a medium (water is a solvent with high dielectric constant) should weaken interaction between the charged groups within the framework of continual solvation model. In this work, use was made of a discrete solvation model, considering the interaction of carnosine with each solvent molecule. The weakening of interaction between the charged groups did not lead to conformational transitions.

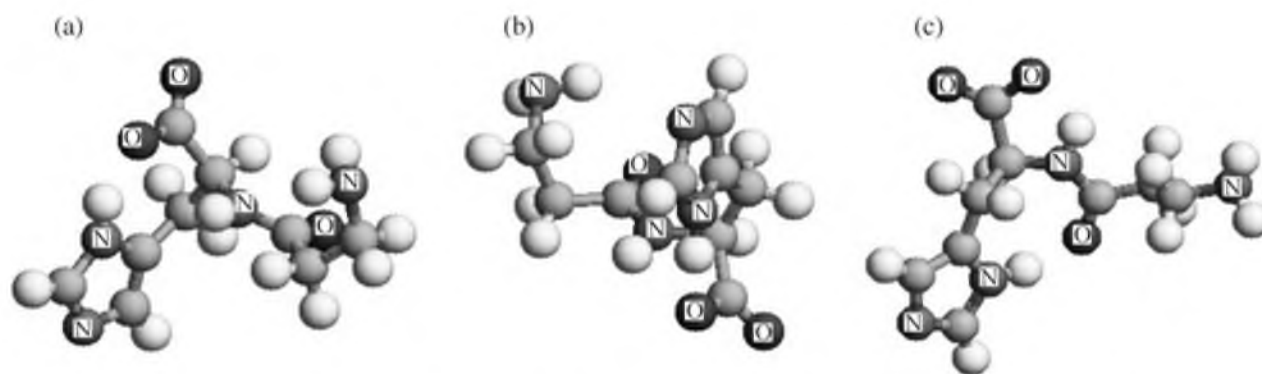


Fig. 3. (a) Conformer with the bend of the β -alanine fragment toward the deprotonated carboxy group and away from the imidazole ring, $E = -2799.504$ eV; (b) conformer with the bend of the β -alanine fragment toward the imidazole ring and away from the deprotonated carboxy group, $E = -2799.525$ eV; (c) conformer with the bend of the β -alanine fragment away from the imidazole ring and the deprotonated carboxy group, $E = -2799.135$ eV.

The data of ^{13}C and ^1H NMR spectra indicate the predominance in aqueous medium of a conformation with the bend of β -alanine fragment toward the imidazole ring [8].

The conformers of carnosine anion are shown in Fig. 3. The removal of one proton from the amino group alters substantially the geometry of the species. In the conformers (a), (b), and (c), the deprotonated amino group is distant from other carnosine groups that excludes interaction with them. A hydrogen bond between the hydrogen atom bound to the ^1N atom of the imidazole ring and the oxygen atom of the peptide bond is observed in conformer (c). The calculated length of the H bond was found to be 1.73 Å. The length of hydrogen bond usually varies within 1.6 to 2.3 Å.

It was noted earlier that carnosine has the ^3N tautomeric form in solid state. The calculations for the conformer of bipolar ion in this form where atom groups are arranged similarly to conformer (a) of ^1N form gave 0.182 eV (1 eV = 23.061 kcal/mol = 96.497 kJ/mol) higher value of total energy. One can suppose that the stability of the ^3N tautomeric form is associated with the aggregation of carnosine species and their strong interaction with each other.

The complexes of zinc with carnosine were studied intensively on account of their interesting and important pharmacological activity. It is noted that zinc complex in solid state has a polymeric structure [5]. The study of $\text{Zn}(2+)$ -carnosine systems in aqueous medium is possible only within very narrow pH range (6–7) [9]. According to experimental data, it is

supposed that carnosine in solution can form a complex where $\text{Zn}(2+)$ is bound to the nitrogen atom (^3N) of the imidazole ring (carnosine in this complex is a monodentate ligand) and a complex where the nitrogen of the amino group and the nitrogen of the peptide bond are involved in coordination interaction.

Figure 4 shows the calculated complex of monodentate type. It comprises seven water molecules, bipolar carnosine ion in conformation (a), and zinc($2+$) ($E = -5099.832$ eV). The difference (ΔE) between the total energy of hydrated $\text{Zn}(2+)$ -carnosine (7 water molecules) and the sum of the total energies of hydrated $\text{Zn}(2+)$ ($[\text{Zn}(\text{H}_2\text{O})_4]^{2+}$, $E = -1308.086$ eV) and carnosine (carnosine + $3\text{H}_2\text{O}$, $E = -3787.802$ eV) was -3.9 eV. Similar difference between ΔH (change in heat content (enthalpy)) is -89.25 kcal/mol. The large magnitude of ΔE is caused by the considerable energy of $\text{Zn}(2+)$ interaction with the ^3N atom of the imidazole fragment in this calculation method. Variation of carnosine conformers and mutual arrangement of water molecules led to small changes in ΔE .

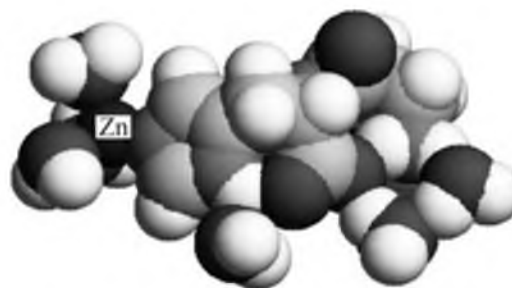


Fig. 4. Carnosine complex with $\text{Zn}(\text{II})$ where the ligand is monodentate.

CONCLUSIONS

As a result of this study, the stable conformations of carnosine were determined. The most stable conformation has a bend of the alanine fragment toward the carboxy group and the imidazole fragment, which agrees well with experimental data. It is shown that hydration does not lead to considerable alteration of stable conformation. The formation of complex in the zinc–carnosine–water system is an energy-favorable process.

REFERENCES

1. M. A. Babizhayev, M. C. Seguin, J. Gueyne, et al., *Biochem. J.* **304**, 509 (1994).
2. A. M. Wang and J. Dong, *Basic Med. Sci. Clin.* **15**, 73 (1995).
3. C. R. Scriver and K. M. Gibson, *The Metabolic and Molecular Basis of Inherited Disease*, 7th ed. (Mc. Graw Hill, N.Y., 1995) Vol. 1.
4. C. R. Cantor and P. R. Schimmel, *Biophysical Chemistry* (Freeman, San Francisco, 1980; Mir, Moscow, 1984), vol. 1, p. 50 [in Russian].
5. E. Kh. Beran, *Biochemistry* **65** (7), 928 (2000).
6. P. C. Daniele, P. Amico, and G. Ostacoli, *Inorg. Chim. Acta* **66**, 65 (1982).
7. *The Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank* (www.rcsb/pdb).
8. E. Gaggelli and G. Valensin, *J. Chem. Soc. Perkin Transact.* **2**, 401 (1990).
9. E. Farkas, I. Sovago, and A. Gergely, *J. Chem. Soc. Dalton Trans.* 1545 (1983).