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Formation of carnosine - an *ab initio* study

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Note: used non-SI units
kcal mol^{-1} = 4.184 $kJ.mol^{-1}$,
debye, D = 3.336 × 10^{-30} Ams,
bohr, a_0 = 5.292 ×10^{-11} m,
angstrom, Å = 10^{-10} m,
atm = 101,325 Pa

Abstract

Formation of carnosine from histidine and β-alanine is studied by *ab initio* MO-LCAO-SCF method followed by the perturbative configuration interaction (MP2) *in vacuo*. After the full geometry optimization at the SCF level, the molecular properties were evaluated and followed by the vibrational-rotational analysis. Consequently, the energy, entropy and free energy were evaluated for the reactants and products of the reaction histidine + β-alanine → carnosine + H_2O and finally, the equilibrium constant was enumerated.

Introduction

Carnosine (*beta*-alanyl-L-histidine) is a simple peptide formed by the condensation reaction of histidine and β-alanine (Eq. 1).

histidine + β-alanine → carnosine + H_2O (1)

The molecular structures are viewed in Fig. 1. Carnosine is a substance with multi-beneficial effects (Boldyrev *et al.* 2013). It is synthesized in organisms by the carnosine-synthase (Eq. 2)

ATP + L-histidine + β-alanine → ADP + phosphate + carnosine (2)

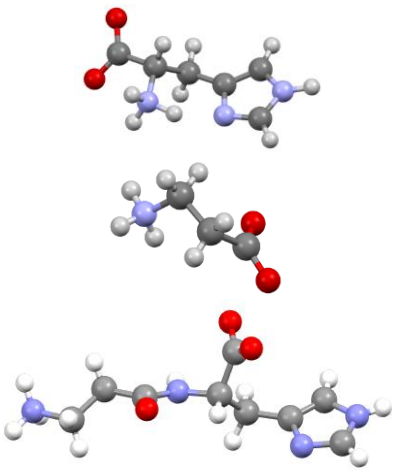


Fig. 1. From top to bottom: structure of the histidine, β-alanine, and carnosine (zwitterionic forms) as extracted from the CCDC (Cambridge Crystallographic Data Centre). CCDC codes: 1889705, 1105698, and 1105666. Colours: C – dark grey, N – blue, O – red, H – white.

and it is metabolized by the canosinase. Beneficial effects of the carnosine were proven in many areas and the most important of them are: prevention of neurodegeneration, improvement of memory, antidepresive action, and suppressing of cancer (Schön *et al.* 2019). As carnosine penetrates the blood-brain barrier (BBB) and causes only little side effects, it possesses high utility potential. It serves also as a reservoir of histidine which is transformed to histamine by L-histidine-decarboxylase; the histamine itself does not penetrate BBB.

Experimental

X-ray structure data for the reactants and products of the reaction (Eq. 1) have been retrieved from the CCDC database (Cambridge Crystallographic Data Centre). These solid-state structures were used as an initial guess for the full geometry optimization *in vacuo*. For such a purpose the *ab initio* MO-LCAO-SCF method was utilized using the 6-31G** basis set (Hyperchem 2008). The dilemma of the zwitterionic *versus* amino forms *in vacuo* was solved by considering both of them. At the SCF level a number of molecular properties were evaluated: the energies of the HOMO (the highest occupied molecular orbital) and LUMO (the lowest unoccupied molecular orbital), the adiabatic ionization energy E_i and electron affinity E_{eg} based upon the positively and/or negatively charged open-shell system after geometry optimization, followed by the evaluation of the Mulliken electronegativity $\chi = (E_i - E_{eg})/2$ and the Pearson hardness $\eta = (E_i + E_{eg})/2$ (Pearson 1977; Sen 1993). For the electroneutral molecule also the dipole moment p and the polarizability volume α (one-third trace of the polarizability tensor) were calculated at the SCF level. The molecular electrostatic potential (Politzer *et al.* 1985; Politzer and Murray 2002) was displayed as a 3D contour map on the isovalue surface of charge density; this shows the acidic/basic sites along the molecular skeleton suitable for nucleophilic and/or electrophilic interactions. The energetic properties were improved by a partial inclusion of the correlation energy by employing the 2nd-order perturbative configuration interaction using the Moller-Plesset partitioning (MP2).

The above results have been compared with those obtained by the enlarged basis set 6-311G(d,p) after the full geometry optimization at the MP2 level.
The optimized geometry has been a starting point for the full vibrational-rotational analysis yielding the corresponding energy levels. They enter the partition function allowing the evaluation of the thermodynamic functions, such as the internal energy U, entropy S, the free energy A and the Gibbs energy G at the room temperature.

Results and Discussion

The MO-LCAO-SCF calculations started from the experimental solid-state geometry that is a zwitterionic form for histidine, β-alanine and also carnosine. The full geometry optimization converged to the zwitterionic form (hereafter Z) as displayed in Fig. 2 for histidine. There exists a five-membered ring H(NH$_2$)CCO with the short contact NH…O forming a bent hydrogen bond. The molecular electrostatic potential shows that the (H$_2$N)H site is positively charged as opposite to the –COO residue.

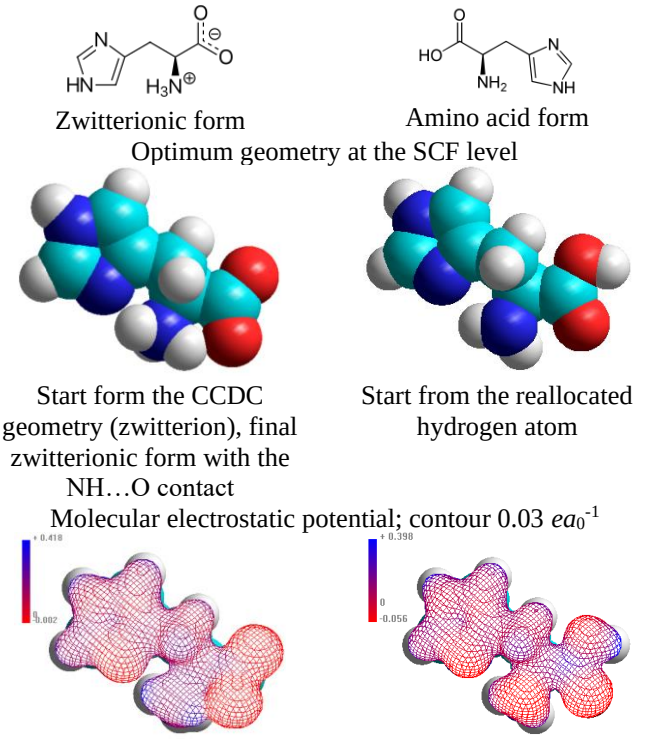


Fig. 2. Optimized structure and molecular electrostatic potential for histidine. (a_0 – bohr).

In addition, the calculations were done for the amino-acid form (hereafter A) in which the

hydrogen atom from the ammonium site was reallocated to the distant oxygen atom. For histidine this canonical form is a bit more stable by 17 kcal.mol^{-1} at the SCF level and 10 kcal.mol^{-1} at the MP2 level (Table 1). The molecular properties of the Z- and A-forms are almost analogous; the only marked difference exhibits the dipole moment that for the Z-form is very high. The Pearson hardness reflects the resistance of the molecule against the electron transfer and for the histidine it is high (155, 161 kcal.mol^{-1}) relative to the series of other amino acids (Vranovičová and Boča 2021).

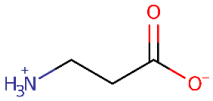
Table 1. Calculated properties of histidine. [a]

	Zwitterion	Amino acid
HOMO	-215	-201
LUMO	101	116
E^+	-342,138	-342,136
E^0 (optimized geometry)	-342,323	-342,340
E^-	-342,220	-342,252
E_i(ΔSCF)	185	204
E_{eg}(ΔSCF)	103	88
χ_M(ΔSCF)	41	58
η_P(ΔSCF)	144	146
E^+(MP2)	-343,183	-343,177
E^0(MP2)	-343,399	-343,409
E^-(MP2)	-343,305	-343,318
E_i(MP2)	216	232
E_{eg}(MP2)	94	91
χ_M(MP2)	61	70
η_P(MP2)	155	161
Dipole moment p/debye	12.99	6.20
Polarizability α/Å^3	75.6	75.6
Surface area S /Å^2	321	325
Volume V/Å^3	475	482

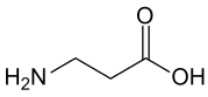
[a] All energy quantities in units of kcal mol^{-1}.

β-alanine belongs to the simplest amino acids and it crystallizes in the zwitterionic form. However, the geometry optimization resulted in travelling of the hydrogen atom from the NH_3^+ site to the $-COO^-$ residue where a six-membered ring $(H_2N)CCCOH$ is formed with the bent N…HO hydrogen bond (Fig. 3). The ground state molecular energy for the Z- and A- forms are almost identical, favouring the A-form only by 0.6 kcal.mol^{-1} (Table 2). The calculated molecular properties are very similar, again except the dipole moment. The molecular electrostatic potential is rather unique with a positive tail at the HO– edge pointing to the negatively charged NH_2– group. The hardness is high: 160 and 167 kcal.mol^{-1}.

For this small molecule the geometry optimization was performed also at the MP2 level. The final geometry is almost identical with that fixed at the SCF level and it is stabilized by only 2 kcal.mol^{-1}.

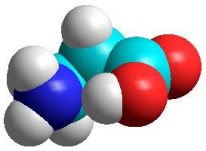


Zwitterionic form

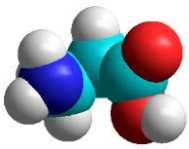


Amino acid form

Optimum geometry at the SCF level



Start from the CCDC geometry (zwitterion), final amino acid form with the N…HO contact



Start from the reallocated hydrogen atom

Molecular electrostatic potential; contour 0.03 ea_0^{-1}

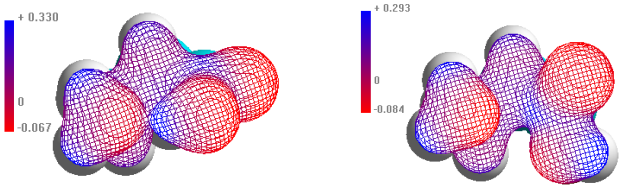


Fig. 3. Optimized structure and molecular electrostatic potential of β-alanine.

Table 2. Calculated properties of β-alanine. [a]

	Zwitterion	Amino acid
HOMO	-261	-247
LUMO	116	124
E^+	-201,788	-201,778
E^0 (optimized geometry)	-201,985.2	-201,985.8
E^-	-201,878	-201,890
E_i(ΔSCF)	197	208
E_{eg}(ΔSCF)	107	96
χ_M(ΔSCF)	45	56
η_P(ΔSCF)	152	152
E^+(MP2)	-202,361	-202,351
E^0(MP2)	-202,593	-202,591
E^-(MP2)	-202,505	-202,496
E_i(MP2)	232	240
E_{eg}(MP2)	88	95
χ_M(MP2)	72	72
η_P(MP2)	160	167
Dipole moment p/debye	6.75	2.97
Polarizability α/Å^3	40.7	40.5
Surface area S/Å^2	242	242
Volume V/Å^3	331	331

[a] All energy quantities in units of kcal.mol^{-1}.

The molecule of carnosine during the geometry optimization becomes folded as shown in Fig. 4.

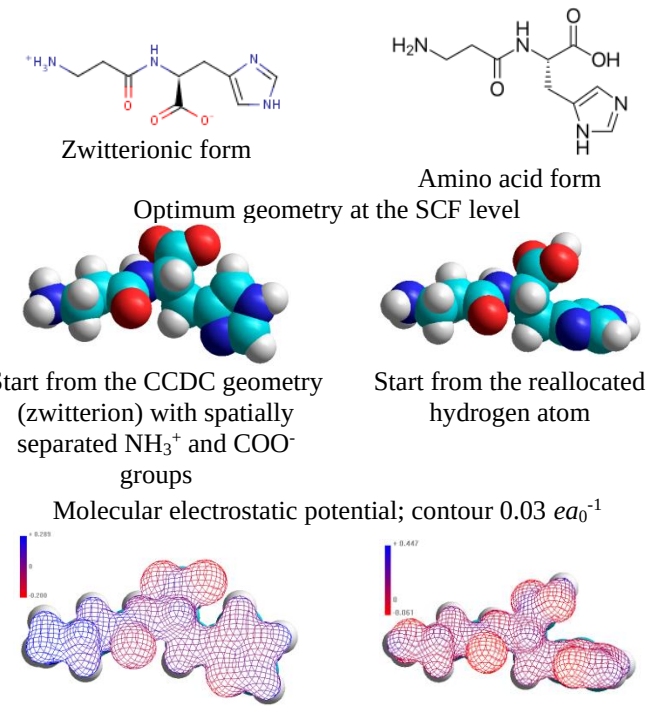


Fig. 4. Optimized structure and molecular electrostatic potential of carnosine.

Table 3. Calculated properties of carnosine.

	Zwitterion [kcal.mol^{-1}]	**Amino acid** [kcal.mol^{-1}]
HOMO	-162	-200
LUMO	38	115
E^+	-496,423	-496,451
E^0 (optimized geometry)	-496,552	-496,619
E^-	-496,526	-496,540
E_i(ΔSCF)	129	168
E_{eg}(ΔSCF)	26	79
χ_M(ΔSCF)	51	44
η_P(ΔSCF)	77	123
E^+(MP2)	-496,846	-496,870
E^0(MP2)	-498,113	-498,171
E^-(MP2)	-496,959	-496,967
E_i(MP2)	1,267	1,301
E_{eg}(MP2)	1,154	1,204
χ_M(MP2)	56	48
η_P(MP2)	1,210	1,252
Dipole moment p/debye	25.24	4.95
Polarizability α/Å^3	113.8	112.3
Surface area S/Å^2	438	445
Volume V/Å^3	677	689

The A-form is preferred against the Z-form by 67 and 58 kcal.mol^{-1}, respectively. The inclusion of the correlation energy via the MP2 method causes a dramatic increase of the ionization energy, electron affinity and consequently the Pearson hardness. The effect to the Mulliken electronegativity is small (Table 3).

The molecule of water possesses rather high ionization energy and electron affinity (Table 4). The calculated ionization energy E_i(MP2) = 283 matches the experimental value of 291 kcal.mol^{-1}. The Pearson hardness η_P(MP2) = 202 kcal.mol^{-1} refers to the class of hard molecules.

Table 4. Calculated properties of water.[a]

HOMO	-312
LUMO	135
E^+	-47,455
E^0 (optimized geometry)	-47,706
E^-	-47,577
E_i(ΔSCF)	251
E_{eg}(ΔSCF)	129
χ_M(ΔSCF)	61
η_P(ΔSCF)	190
E^+(MP2)	-47,547
E^0(MP2)	-47,830
E^-(MP2)	-47,708
E_i(MP2)	283
E_{eg}(MP2)	122
χ_M(MP2)	80
η_P(MP2)	202
Dipole moment p/debye	2.15
Polarizability α/Å^3	4.87
Surface area S/Å^2	116
Volume V/Å^3	117

[a] Experiment: E_i (vertical) = 12.62 eV = 291 kcal.mol^{-1}.
All energy quantities in units of kcal.mol^{-1}.

The free energies of the reactants and products allow determining the overall change $\Delta A(T)$ for the reaction (Eq. 1) which enters the equilibrium constant $\ln K = -\Delta A/RT$. As evident from Table 5, for the amino-acid forms at the SCF level there is $\Delta A \sim 0$ at 300 K which yields $K \sim 1$. This value leads to the conclusion that the formation of carnosine and its hydrolysis via Eq. 1 are equally probable processes. This result gives a thermodynamic (not kinetic) predisposition to the carnosine hydrolysis. Hydrolysis of carnosine has been observed as an enzyme-assisted process (Pegova *et al*. 2000).

The results obtained in an analogous way but using the enlarged basis set 6-311G(d,p) and MP2-optimized geometry gave almost the same results as evident from Table 5. With expectations, all

molecular energies are a bit lower. Amino acid and zwitterionic forms of histidine, β-alanine, and carnosine are close in energy. With ΔG = -0.32 kcal mol^{-1} the equilibrium constant reads $K = \exp(-\Delta G/RT) = 1.14$.

Table 5. Energies, entropies, and free energies of the reactants and products at 300 K.[a]

	Histidine	β-alanine	Carnosine	H_2O	Reaction (Eq.1)
SCF level, 6-31G**					
E^0, Z-form	-342,323	-201,985.2	-496,552	-47,706	50.2
E^0, A-form	-342,340	-201,985.8	-496,619	-47,706	0.8
U, 300 K	-342,225	-201,908	-496,444	-47,689	0
S, 300 K	0.0984	0.0785	0.1283	0.0450	-0.0036
A, 300 K	-342,254	-201,932	-496,483	-47,703	0
E_{vib}	113.4	75.8	172.9	14.6	-1.7
E_{rot}	0.89	0.89	0.89	0.89	0
E_{trans}	0.89	0.89	0.89	0.89	0
S_{vib}	0.0283	0.0138	0.0544	0	0.0123
S_{rot}	0.0290	0.0253	0.0317	0.0103	-0.0122
S_{trans}	0.0411	0.0394	0.0422	0.0347	-0.0036
MP2 level, 6-31G**					
E^0, Z-form	-343,399	-202,593	-498,113	-47,830	49
E^0, A-form	-343,409	-202,591	-498,171	-47,830	-1
MP2 level, 6-311G(d,p) (5D, 7F)					
E^0, Z-form	-344,886	-203,472	-500,247	-48,046	65.4
E^0, A-form	-344,885	-203,470	-500,307	-48,046	1.57
U, 300 K	-344,782	-203,396	-500,141	-48,031	6.73
S, 300 K	0.1084	0.0804	0.1365	0.0465	-0.0058
G, 300 K, 1 atm	-344,805	-203,420	-500,181	-48,044	-0.32

[a] Energies in kcal mol^{-1}, entropies in kcal K^{-1} mol^{-1}. Z – zwitterion, A – amino acid form.

Conclusions

Ab initio MO-LCAO-SCF calculations show that the amino acid forms of the β-alanine, histidine, and carnosine are more stable than the zwitterionic form *in vacuo*. The large Pearson hardness of the carnosine means that it is very resistant against the electron transfer. The formation of the carnosine from the β-alanine and histidine *via* Eq. 1 is an equally probable process as its hydrolysis, $K \sim 1$.

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflict of interest.

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