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Research paper

Structure and stability of complexes of agmatine with some functional receptor residues of proteins

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ABSTRACT

The paper reports the results of a theoretical study of the conformational behavior and basicity of biogenic amine agmatine. The complexes modelling of agmatine – protein interaction are also under scrutiny of our investigation using the Becke3LYP and B97D levels of the density functional theory. The relative stabilities (Gibbs energies) of individual complexes are by both DFT methods described equally. Hydration has a dramatic effect on the hydrogen bonded complexes studied. The pairing acidic carboxylate group with different agmatine species resulted in charged hydrogen bond complexes containing negatively charged acetate species acting as proton acceptors.

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1. Introduction

Agmatine 1-(4-aminobutyl guanidine) is one of the precursors of arginine containing a guanidine residue and serving as cell-signaling molecule. Agmatine is produced by the enzyme arginine decarboxylase localized in mitochondrial fraction of most mammalian cells and identified in brain, liver, adrenal gland, kidney, small intestine and macrophages [1]. Agmatine was initially investigated as an endogenous ligand at α2-adrenoceptors and imidazoline receptors [2]. However, current knowledge of agmantine pharmacological and physiological functions indicates much greater therapeutic potential of the compound [1–3]. It was shown that agmatine may play important roles in diseases such as diabetes, Alzheimer's disease, anxiety disorder, depression, drug addiction, and cancer [3]. Agmatine itself is a highly hydrophilic compound with ClogP equal to -1.8 [4] and guanidine functionality representing the strongest basicity among the amine derivatives [5]. Toninello et al. examined the structural preference of agmatine species computationally using B3LYP/6–31G** density functional theory (DFT) [6]. The proteins and enzymes commonly use the guanidine group of the guanyl species to recognize and bind anionic sites through ionic and hydrogen bonding interactions [7,8]. The experimental structural data indicate that the agmatine in the active site of biopolymers exists in its diprotonated form [9,10]. Systematic analyses of available structural crystallographic data of the agmatine with proteins have shown that Gly and Asp residues of proteins are in the position to have ionic or hydrogen bond interaction with the positively charged guanidine moiety. The positively charged amino group of agmatine interacts with complementary sites of Glu and Ser. The "spacer" $-C_4H_8-$ forms hydrophobic bonds with Tyr or Val [9,11]. The bulk of these hydrogen bonds are ionic-type interactions. However, despite of their great pharmacological importance, the detailed nature of these interactions still remains one of the structurally and energetically less well characterized.

The present paper reports in detail the structural data for agmatine, its mono- and di-cations and their interaction with amino acid residues typical for binding sites of biopolymers. They are selected to model the typical interaction of agmatine with the hydrogen bonding interaction sites of biopolymers. Model chemistry at the DFT level was applied for this study. The molecular structure of various species of agmatine and overall shape its complexes with selected amino acid residues are examined in this work. Of particular interest is the molecular structure of agmatine and how this structure is changed upon protonation, molecular complexation and/or solvation. The typical receptor fragments are not confined as in real receptor sites, therefore the obtained geometries and thermodynamic quantities cannot be directly applied to interactions in the receptor sites, in which the fragments will embedded in protein structures and have limited mobility and possibly also conformational flexibility and/or bonding ability. Nevertheless,

the study provides useful information about speciation and typical geometries of individual bonds, which will be helpful in further studies.

2. Computational details

The geometry of agmatine species and their molecular complexes (Fig. 1) have been completely optimized with the Gaussian 09 program [12] at the Becke3LYP level of DFT [13–16] and B97D Grimme's functional including dispersion [17] using the polarized triple-ζ 6–311++G(d,p) basis set [18]. Effect of water hydration on the species investigated was computed by means of the conductor-like polarizable continuum model (CPCM) [19,20]. The structures of all gas-phase and condensed-phase (CPCM) species were fully optimized without any geometrical constraint. The gas-phase proton affinity and basicity of agmatine was computed the same way as in our previous publications [21,22].

The macroscopic pK_a values were computed using program SPARC [23–26]. The interaction energy, ΔE, for the interaction of polar groups of agmatine (Agm) with complementary sites of amino acids (AA) in relevant biopolymers is given by the following equation

$$\Delta E = E[\text{Agm} \cdots \text{AA}] - \{E[\text{Agm}] + E[\text{AA}]\} \qquad (1)$$

where $E[\text{Agm}]$ and $E[\text{AA}]$ are the energies of the agmatine species and Lewis acid molecules, respectively, and $E[\text{Agm AA}]$ is the energy of the complex.

3. Results and discussion

3.1. Molecular structure of the agmatine species

In an organism agmatine is formed by enzymatic decarboxylation of ʟ-arginine. The basicity of agmatine is derived from the presence of guanidine and amine moieties at both termini of molecule. The high basicity of the guanidine moiety is derived from the guanidine conjugation system that is formed after protonation. Agmatine contains five rotatable bonds and can be present in different conformations in different structural environments. Neutral species agmatine can be present in three conformational forms (amino tautomers I and II and imino tautomer III). The relative stability of these tautomeric forms is presented in Table 1. Based on the relative Gibbs energies the amino tautomer II is the most stable species (Fig. 1) in both gas-phase and aqueous environment. As regards of imino tautomers two conformers (cyclic structure IIIa and "extended" form IIIb, Fig. 1S of Supplementary information) were considered. The high and negative relative entropy change
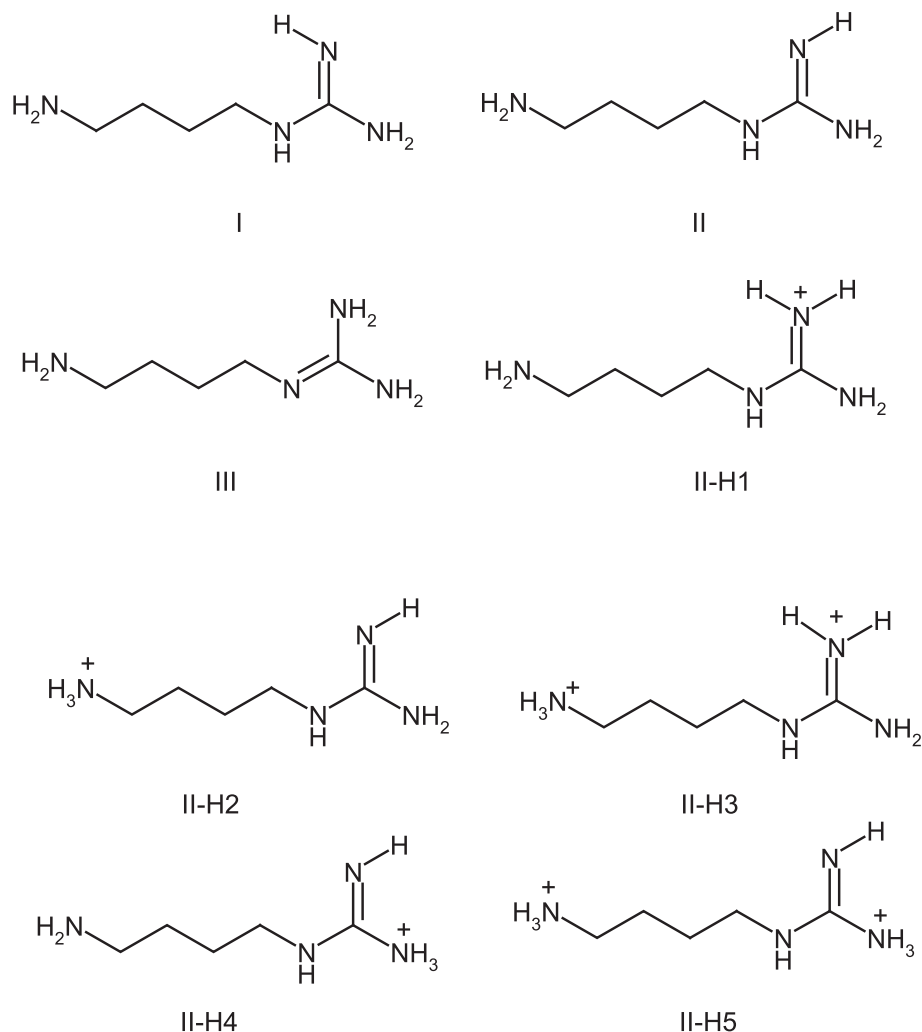

Fig. 1. Structure of the agmatine species studied.

Table 1
Relative stability of the amino and imino tautomers of agmatine computed at the B3LYP/6-311++G(d,p) level of theory.

Agmatine species	ΔE, kJ/mol	ΔH, kJ/mol	ΔS, J/mol.K	ΔG, kJ/mol	ΔG^{CPCM}, kJ/mol
I (agmatin 1), amino	8.07	8.34	−0.97	8.63	6.70
II (agmatin 3), amino	5.62	4.49	16.74	0	0
IIIa (agmatin 2), imino	0	0	−13.85	4.13	4.75
IIIb	5.35	3.84	12.04	0.25	4.75

in cyclic imino tautomer is, however, not sufficient for its stabilization and the extended form IIIb was found in the gas phase the more stable species by about 3.9 kJ/mol. With respect to solvation, theoretical results predict both imino conformers to be present in aqueous solution with equal probability (Table 1). As regards of experimental crystal structure determinations, only the X-ray structure of agmatine sulphate dehydrate was determined [27]. The entire agmatine molecule is nearly planar. In this complex the imino and butylamino groups of agmatine are protonated [27].

3.2. Basicity of agmatine species

The acidobasic properties of agmatine were investigated considering species bearing one (structures II-H1, II-H2 and II-H4) or two positive charges (II-H3 and II-H5 forms), Fig. 1. The geometric characteristics of these species indicate that they exist in nearly planar conformations (species IV – VIII in Fig. 1S of Supplementary information). With respect to the possible existence of several structural forms of amino and imino tautomers of agmatine (Table 1) the proton affinity and basicity may be calculated between two arbitrary structures, however, only the energy differences between most stable protonated groups have a physical meaning. Table 2 contains the gas-phase proton affinity and basicity for individual protonation reactions. As regards of monoprotonation the C=N nitrogen of the cation II-H1 is the most stable species. The addition of the first proton to one of the two basic groups of agmatine represents an intrinsic gas-phase basicity of individual basic moiety. The basicity of both amino groups is distinct. Compared to the guanidine amino group, the butylamino group is computed to be more acidic by about 82 kJ/mol. The relative stability of two bications (II-H3 and II-H5) investigated is dramatically different. The most basic species is the bication II-H3 bearing positive charge on butylamino and the C=N groups of agmatine. This bication is in the gas-phase by 145 kJ/mol more stable (Table 2). The existence of bication II-H3 was also confirmed experimentally in the solid state in the form of its salt with sulphate [27]. The addition of a successive proton in the reaction II-H1 + H$^+$ → II-H3 is a less energy releasing process and results in an lowering of basicity by about 327 kJ/mol.

In water solution the dissociation constant or the pK_a is used as a measure of the strength of acid or base. The computed values of pK_a of agmatine, using the program SPARC indicate that dication and monocation exhibit different basicity (Table 2). Like in the gas phase, the protonation of neutral agmatine II is a much more feasible process (pK_a = 13.71) compared with the protonation of the corresponding monocation II-H3 with pK_a = 9.98.

3.3. Structure and stability of agmatine complexes with selected amino acid residues

The guanidinium group of agmatine and another species containing this moiety is commonly used by proteins to recognize and bind anionic residues of amino acids through ion pairing and hydrogen bonding [8]. The pairing of agmatine species with anionic carboxylate group of Asp (modelled by acetic acid) and the amide moiety of Gly modelled by N-methylacetamide resulted in complexes 1–7 depicted in Fig. 2S of the Supplementary information. Complexes 1–5 model the interaction of the agmatine species with a single interaction group (represented by acetic acid or N-methylacetamide) of amino acid residues of Asp and Gly. Complexes 6 and 7 represent more complex interactions between agmatine and protein residues, pairing agmatine with several associative groups of protein (Fig. 2S of the Supplementary information). Complex 1 exists in the gas phase as a neutral system stabilized by two hydrogen bonds. In aqueous solution proton transfer occurs and results in a stable acetate – charged agmatine complex (complex 1, Fig. 2S). Diprotonated agmatine in complex with acetate ion (complex 2) leaves one proton from the guanidine and it is stabilized by two neutral hydrogen bonds. The stable ion pair complex was found in water solution from DFT calculations only (complex 2, Fig. 2S). In both gas phase and aqueous solution the monoprotonated guanidine moiety of agmatine, complexed with the anionic acetate, exists in the form of the ionized hydrogen bonded complex 3. Complex 4, which models the interaction of the agmatine monoprotonated at the amino end with the acetate moiety, is a stable species in aqueous solution only (complex 4, Fig. 2S).

The pairing of the experimentally detected dication II-H3 of agmatine in systems of growing complexity with acetate and amide functional groups of proteins resulted in the complexes 5–7 with bifurcated hydrogen bonds C=O...H—N between amide oxygen atom and hydrogens of the guanidine moiety (complexes 5–7, Fig. 2S). In the most complex system 7 all basic groups of the dication II-H3 are coordinated by the complementary acidic (carboxylate) and polar (amide) groups, respectively (complex 7, Fig. 2S). The coordinated groups caused appreciable structural changes of the flexible butylamino group of the agmatine dication II-H3. The gradual changing of the molecular structure of dication II-H3 upon complexation is illustrated for the complexes 6 and 7 (Figs. 2 and 3). The coordination of the guanidine moiety by two amides results in a conformational change of the butylamino end group of agmatine. Even more appreciable geometry changes of this flexible group were observed for the pentacoordinated complex 7 (Fig. 3). The molecular structure of the agmatine species

Table 2
Gas-phase basicities (enthalpies ΔH, entropies ΔS and Gibbs energies ΔG) of the agmatine (at 298.15 K) computed at the B3LYP/6–311++G(d,p) level.

Reaction	ΔH, kJ/mol	ΔS, J/mol.K	ΔG, kJ/mol	pK_a
II + H$^+$ → II-H1	−1020.94	−117.89	−985.76	13.71
II + H$^+$ → II-H2	−937.08	−127.59	−899.06	
II + H$^+$ → II-H4	−849.45	−107.96	−817.28	
II-H1 + H$^+$ → II-H3	−696.59	−129.08	−658.12	9.98
II + 2 H$^+$ → II-H3	−1717.53	−247.10	−1643.89	
II + 2 H$^+$ → II-H5	−1569.75	−238.42	−1498.70	

Fig. 2. Molecular superimposition of the complex 5 and most stable gas-phase structure II-H3 of unbound agmatine (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
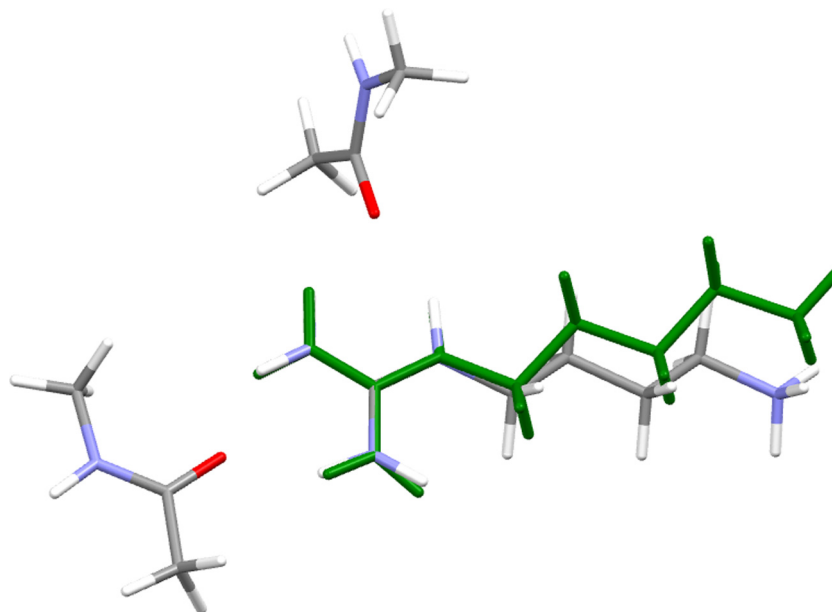

Fig. 3. Molecular superimposition of the complex 7 and most stable gas-phase structure II-H3 of unbound agmatine (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).
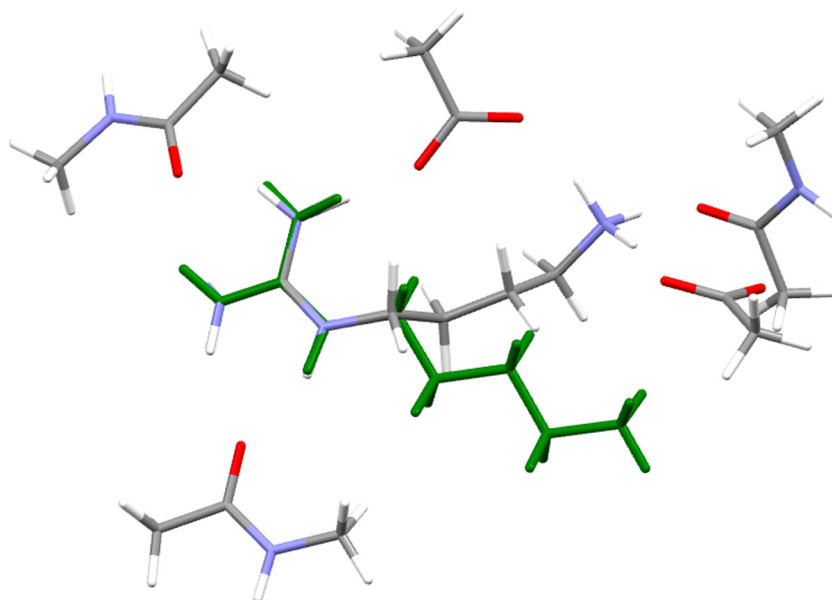
can also be influenced through interactions with solvents such as water. However, the hydration of the charged agmatine II-H3 dication by eight molecules of water representing the first solvation shell did change only slightly the molecular structure of this dication (Fig. 3S), and it is almost the same irrespective of the solvation method used (Fig. 4S).

The computed interaction energies of the gas-phase complexes studied computed at the B3LYP and B97D levels of theory are given in Table 3. The Grimme's B97D functional including dispersion was specifically designed for accurate evaluation of van der Waals complexes [17]. The computed interaction energies and enthalpies of gas-phase complexes are always negative, i.e. the complex formation is an exothermic reaction. However, in real hydrogen bonded systems, the tendency to associate is described by Gibbs energies. It is, therefore, important to know the role of entropy in the

complex investigated. Association of two or more species into a hydrogen-bonded complex necessarily involves a decrease of entropy, since there is more order in the complex. The relative stabilities (Gibbs energies) of individual complexes are by both DFT methods described equally. However, the ΔGs at the B97D level are for most cases substantially more negative. With regards to gas-phase complexes 1 – 8 the entropy changes are negative and cover a broad range (about −130 to−906 J/K mol) and negative. In the case of complex 4 the entropy change is not sufficiently negative and this enthalpically exothermic reaction has a positive ΔG of about 23 kJ/mol and do not proceed towards complex (Table 3). The association of the guanidine cationic head of agmatine by one or two amide functionalities of proteins modelled by complexes 5 and 6 does not show any cooperativity, as indicated by the computed (B3LYP) Gibbs energy for complex 5 (−84.7 kJ/mol)

Table 3

Computed interaction, enthalpies (kJ/mol), entropies (J/K mol), Gibbs energies (kJ/mol) and solvent stabilization interaction energies ΔG^{CPCM} (kJ/mol) of the complexes investigated (T = 298.15 K).

Complex No.	Reaction[a,b]	ΔH^{B3LYP}	ΔS^{B3LYP}	ΔG^{B3LYP}	ΔG^{B97D}	$\Delta G^{B3LYP,CPCM}$	$\Delta G^{B97D,CPCM}$
1	II + AA → II···AA	−70.3	−127.3	−32.5	−33.5		
	II-H2 + AA$^{(−)}$ → II-H2···AA$^{(−)}$					−56.4	−76.4
2	II-H2 + AA → II-H2···AA	−60.7	−153.3	−14.5	−20.6		
	II-H3 + AA$^{(−)}$ → II-H3···AA$^{(−)}$					−57.4	−80.1
3	II-H1 + AA$^{(−)}$ → II-H1···AA$^{(−)}$	−493.5	−161.4	−445.4	−451.7	−57.5	−78.5
4	II + AA → II···AA	−21.5	−152.5	23.5	23.2		
	II-H2 + AA$^{(−)}$ → II-H2··· AA$^{(−)}$					−5.6	−28.1
5	II-H3 + AMD → II-H3···AMD	−125.2	−135.9	−84.7	−87.7	−36.3	−39.8
6	II-H3 + 2AMD → II-H3···(AMD)$_2$	−217.0	−243.3	−144.5	−150.2	−67.1	−80.6
7	II-H3 + 3AMD + 2AA$^{(−)}$ → II-H3···(AMD)$_3$(AA$^{(−)}$)$_2$	−1327.2	−671.8	−1127.0	−1174.6	−168.1	−255.5
8	II-H3 + 8H$_2$O → II-H3···(H$_2$O)$_8$	−464.2	−906.4	−194.1	−211.2	−186.7	−208.9

[a] AA – acetic acid
[b] AMD - N-methylacetamide

and complex 6 (−144.5 kJ/mol), respectively. The tendency to associate in the gas-phase is largest for the complex hydrogen-bonded system 7, with Gibbs energy of −1127 kJ/mol (B3LYP). Hydrogen bonds formed between guanylated derivatives and proteins are responsible for the directionality of such interactions [8].

It is assumed that the lack of such interactions of agmatine with proteins may be linked with some diseases [3,8]. In biological systems such hydrogen bond activity is often determined by environment, and hence the entropy has a significant influence on hydrogen bond properties, particularly in the aqueous solution. Table 3 also contains Gibbs interaction energies ΔG^{CPCM} for the eight complexes investigated in the aqueous environment. Hydration has a dramatic effect on the hydrogen bonded complexes studied. The full optimization of the complexes 1 – 4 pairing acidic carboxylate group with different agmatine species resulted in charged hydrogen bond complexes containing negatively charged acetate species acting as proton acceptors (Table 3). In the complexes 1 – 3 the acetic acid proton is always transferred to the guanidine moiety of agmatine bearing formal positive charge (Fig. 2S). Complex 4 pairing neutral guanidine group of agmatin with an acetate anion is weak, thus the tendency for association of this type is in the solvated state negligible. The Gibbs interaction energy of complex 8, owing to the net positive charge +2 of agmatine, is in both gas phase and aqueous solution almost the same (about 200 kJ/mol). Thus the desolvation penalty may play important part during the pharmacodynamics phase of agmatine action.

4. Conclusions

Based on the relative Gibbs energies the amino tautomer II is the most stable species both in gas-phase and in aqueous environment. The most basic species is bication II-H3 bearing positive charge on butylamino and the C=N groups of agmatine. The existence of bication II-H3 was also confirmed experimentally in the solid state in the form of its salt with sulphate. The computed values of pK_a of agmatine indicate that dication and monocation exhibit different basicity. Like in the gas phase, the protonation of neutral agmatine II is a much more feasible process (pK_a = 13.71) than the protonation of the corresponding monocation II-H3 with pK_a = 9.98. The computed interaction enthalpies of gas-phase complexes are always negative, i.e. the complex formation is an exothermic reaction. Association of two or more species into a hydrogen-bonded complex necessarily involves a decrease of entropy, since there is more order in the complex. In the case of complex 4 the entropy change is not sufficiently negative and this enthalpically exothermic reaction has a positive ΔG of about 24 kJ/mol and do not proceed towards complex. The tendency to associate in the gas-phase is largest for the complex hydrogen-

bonded system 7 with a Gibbs energy of −1127 kJ/mol. Solvation has dramatic effect on these interactions. The pairing acidic carboxylate group with different agmatine species (complexes 1 – 4) resulted in charged hydrogen bond complexes containing negatively charged acetate species acting as proton acceptors. The Gibbs interaction energy of complex 8, owing to the net positive charge +2 of agmatine, is in both gas phase and aqueous solution almost the same (about 200 kJ/mol). Thus the desolvation penalty may play important part during the pharmacodynamics phase of agmatine action.

This work yields quantities that may be inaccessible or complementary to experiments and represents the first quantum chemical approach in which both the gas-phase and solvated phase complexation between agmatine species and complementary hydrogen bonding groups of both acidic carboxylate and polar amide domains of proteins are modelled and absolute values of these interactions in the gas phase and water environment were evaluated.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cplett.2017.02.006.

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