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Original article

Computational study of the electronic structure characterization of a novel anti-inflammatory tripeptide derived from monocyte locomotion inhibitory factor (MLIF)-pentapeptide

Carolina Barrientos-Salcedo [a], Guadalupe Rico-Rosillo [b], Juan Antonio Giménez-Scherer [b], Catalina Soriano-Correa [c,*]

[a] Unidad de Investigación Médica en Genética Humana, Hospital de Pediatría, Centro Médico Nacional Siglo XXI (CMN-SXXI), Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[b] Unidad de Investigación Médica en Inmunología, Hospital de Pediatría, CMN-SXXI, IMSS, Mexico City, Mexico
[c] Laboratorio de Química Computacional, FES-Zaragoza, Universidad Nacional Autónoma de México (UNAM), C.P. 09230 Iztapalapa, Mexico City, Mexico

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ABSTRACT

The structure and properties of molecules are determined by their charge-density distribution. Several works have shown that electron delocalization along the peptide backbone and side-chain modulates the physical and chemical features of peptides and protein properties. Research on *Entamoeba histolytica*-soluble factors led to the identification of the pentapeptide Met-Gln-Cys-Asn-Ser, with anti-inflammatory *in vivo* and *in vitro* effects. A synthetic pentapeptide, Met-Pro-Cys-Asn-Ser, maintained the same anti-inflammatory actions in experimental assays. A previous theoretical study allowed proposing the Cys-Asn-Ser tripeptide (CNS tripeptide) as the pharmacophore group of both molecules. This theoretical hypothesis was recently confirmed experimentally. The aim of this study was to characterize the electronic structure and physico-chemical properties of the CNS tripeptide through a theoretical study at the second-order Møller–Plesset perturbation theory (MP2) and density functional theory (DFT) theoretical levels. Our results in deprotonation energies show that the hydrogen atom (H2) of the serine-amide group possesses acidic characteristics. This result was confirmed by means of a study of bond order. Atomic charges, dipole moment, frontier molecular orbitals (Highest occupied molecular orbital [HOMO − 1] and Lowest unoccupied molecular orbital [LUMO + 1]), and electrostatic potential isosurface and its geometric parameters permitted to characterize its electronic structure and physico-chemical features and to identify some reactive sites that could be associated with this tripeptide's anti-inflammatory activity.

1. Introduction

Tissue damage caused by a wound or invasion by a pathogenic microorganism induces a complex sequence of events collectively known as the inflammatory response. Inflammatory-response events are initiated by a complex series of interactions involving several chemical mediators; these interactions continue to be at present only partially understood. Some of these interactions derive from the invading organism, some are released by the damaged tissue, several are generated by various plasma enzyme systems, and others are the products of several white blood cells participating in the inflammatory response [1,2]. Recently, works have shown that electron delocalization along the main chain bond of amino-acid side-chain peptides can be considered as a substituent group along the peptide backbone; both situations are important in modulating peptide chemical and physical features and therefore, the protein properties [3–6]. Amino-acid side-chain electronic properties, such as inductive and field effects, have not yet been characterized in any detail. Quantum mechanical calculation and fundamental equations that account for substituent effects may provide insight into these important properties [3,4]. Also, hydrophobicity and steric effects comprise two major factors that govern protein folding [3,7]. In addition, the weakness of the individual bond is such that it is frequently not sufficient for providing the strength and specificity necessary for biological processes. If hydrogen-bond donors or acceptors are arranged in particular geometries, hydrogen-bonding interactions become very specific, with additive—and often cooperative—strengths. Thus, hydrogen bonding between functional

* Corresponding author. Tel./fax: +52 (55) 5573 6333.
E-mail address: socc@puma2.zaragoza.unam.mx (C. Soriano-Correa).

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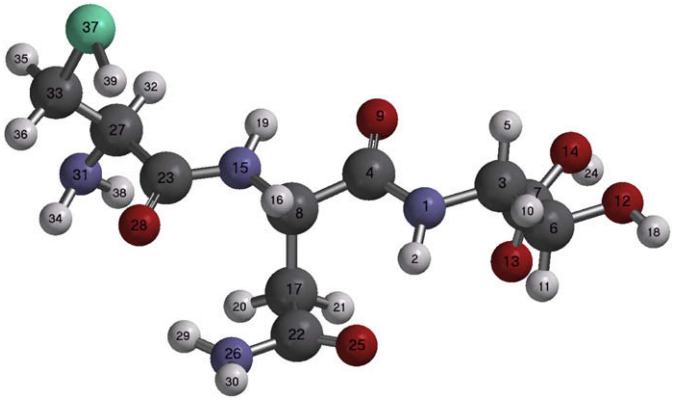

Fig. 1. Convention used in numbering atoms for analysis of atomic charges for the Cys-Asn-Ser (CNS) tripeptide.

groups occurring in biological molecules plays an important role in the electronic density of these bonds, as well as in their physico-chemical and structural features [3–7]. From a theoretical point of view, we are interested in the electronic, physico-chemical, and structural properties of small peptides with anti-inflammatory activity. Thus, Kretschmer and co-workers [8] investigated anti-inflammatory factor production by means of the parasite *Entamoeba histolytica* [9], which produces a thermostable pentapeptide (Met-Gln-Cys-Asn-Ser) that *in vitro* inhibits mononuclear phagocyte locomotion; the original name assigned is human monocyte locomotion inhibitory factor (MLIF). These authors established that the same synthetic sequence possesses similar anti-inflammatory properties to those of native MLIF [10], while another analogous pentapeptide, i.e., the proline amino acid, was substituted with glutamine in the second position (Met-Pro-Cys-Asn-Ser, pMLIF), presented anti-inflammatory activity; nevertheless, another pentapeptide with the same amino acids but a different arrangement (Gln-Cys-Met-Ser-Asn, sMLIF) did not present anti-inflammatory activity. These findings allowed studying the relationship between physico-chemical and structural properties with the anti-inflammatory activity of the three pentapeptides (MLIF, pMLIF, and sMLIF) by Soriano-Correa et al. [11,12]. These investigators carried out a theoretical study that related the importance of structural, physico-chemical, and electronic features with MLIF, pMLIF, and sMLIF anti-inflammatory activity. They found that MLIF and pMLIF pentapeptides maintained a great structural similarity [11,12], particularly among the last three amino acids (Cys-Asn-Ser, CNS) (Fig. 1). Later, Rico et al. carried out experimental studies in *in vivo* and *in vitro* models on the novel CNS tripeptide's anti-inflammatory activity with >95% purity and synthesized by the American Peptide Company [13]; they found that the Cys-Asn-Ser (CNS) tripeptide's carboxyl-terminal end group maintained 100% of its biological and anti-inflammatory properties, similar to the MLIF, thus experimentally verifying the pharmacophore group's anti-inflammatory activity proposed in the MLIF molecule predicted theoretically [11,12].

Therefore, the focus of this work was to perform calculations employing second-order Møller–Plesset perturbation theory (MP2) and density functional theory (DFT) theoretical levels to characterize electronic structure, physico-chemical properties, and geometric parameters utilizing standard and complementary density descriptors, with the purpose of better understanding the inflammatory processes and in this manner, design new small peptides with specific anti-inflammatory features.

2. Computational details

The electronic structure calculations were carried out employing the Gaussian 03 software program suite [14]. Also, with the purpose to gauge the effects of electron correlation, the structures were analyzed at the second-order Møller–Plesset perturbation theory (MP2) and density functional theory (DFT) theoretical levels. Because, we considered that the results of both methods are complementary between themselves, i.e., MP2 results of frequency calculations are more reliable, such that the optimized geometries are characterized by harmonic vibrational frequencies, which confirmed that the structures obtained are minima on the potential-energy surface [15]. In addition, the DFT-B3LYP theoretical level presents good descriptions of chemical reactivity. Also, in order to check the usefulness of DFT results, frequently important for large molecules for which more sophisticated methods require a long CPU time [16]. All neutral structures were optimized at MP2 and DFT-B3LYP levels utilizing Becke's three-parameter hybrid functional and the Lee–Yang–Parr correlation [17–20], while deprotonated (anionic) structures were optimized at unrestricted UMP2 and DFT-UB3LYP levels, all these with a 6–311 + G(d,p) basis set [21,22]. Optimized geometries are characterized by harmonic vibrational frequencies, which confirmed that the structures obtained are minima on the potential-energy surface. Energies were corrected to include zero-point vibrational energy (ZPVE) at B3LYP/6–311 + G(d,p) levels [14,21,22]. It is important to mention that the RMP2 and DFT-RB3LYP to UMP2, and DFT-UB3LYP SCF solution stability were tested for anionic molecules. Single-point calculations were performed on optimized structures (neutral and anionic) at MP2 and DFT (B3LYP) levels with a 6–311++G(2d,2p) basis set. To determine serine (Ser)- and cysteine (Cys)-amino acid relative acidity, deprotonation energy (ΔE) was calculated by deprotonating H2 and H39 hydrogen atoms, respectively (Fig. 1), according to the following reaction:

$$MXH \rightarrow MX^- + H^+ \quad (1)$$

where X = N1 or S37, and H = H2 or H39, respectively. Then, small ΔE values imply more acidic amide [23–25] (COHNH2) and thiol (SH39) groups. In addition, atomic and group charges fitted to the electrostatic potential (ESP) [26] were obtained to examine amide- and thiol-group acidic character and CNS-tripeptide active sites. Additionally, a bond-order analysis employing Natural bond orbital (NBO) scheme [27–29] was performed to provide an alternative insight into amide- and thiol-group relative acidity with the purpose of analyzing reactive sites. Also, dipole-moment analysis (μ) was undertaken at the same theoretical level to assess the charge's electronic distribution. Furthermore, we determined frontier molecular orbitals (Highest occupied molecular orbital [HOMO − 1] and Lowest unoccupied molecular orbital [LUMO + 1])

Table 1
Deprotonation energies, ΔE, for the CNS tripeptide at MP2 and B3LYP/6–311++G(2d,2p)//B3LYP/6–311 + G(d,p) levels.

Method	$-E$ (a.u.) MXH	$-E^-$ (a.u.)MN1$^-$	ΔE (kcal/mol)	$-E^-$ (a.u.) MS37$^-$	ΔE (kcal/mol)
MP2	1457.78570	1457.24429	330.52	1457.23907	336.30
B3LYP	1460.90395	1460.35790	333.40	1460.35401	338.37

Where X = N1 or S37 and H = H2 or H39. CNS tripeptide, Cys-Asn-Ser tripeptide.