Russian Journal of General Chemistry, Vol. 72, No. 6, 2002, pp. 976–978. Translated from Zhurnal Obshchei Khimii, Vol. 72, No. 6, 2002, pp. 1043–1045.
Original Russian Text Copyright © 2002 by Rogachevskii, Shchegolev, MacKey, Krylov, Baranovskii.

Quantum-Chemical Study of the Equilibrium Geometry and Electronic Structure of the Defensin Molecule

I. V. Rogachevskii, B. F. Shchegolev, M. L. MacKey, B. V. Krylov, and V. I. Baranovskii

St. Petersburg State University, St. Petersburg, Russia
Grebenshchikov Institute of Silicate Chemistry, Russian Academy of Sciences, St. Petersburg, Russia
Auburn University, USA
Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia

Received December 4, 2000

Abstract—The molecular geometry of rabbit NP-1 defensin is fully optimized by means of molecular mechanics and MNDO methods. Atomic charges are calculated, and an estimate for the dipole moment of the molecule is obtained (μ 4.0 D). The possible mechanism of defensin–receptor interactions is discussed.

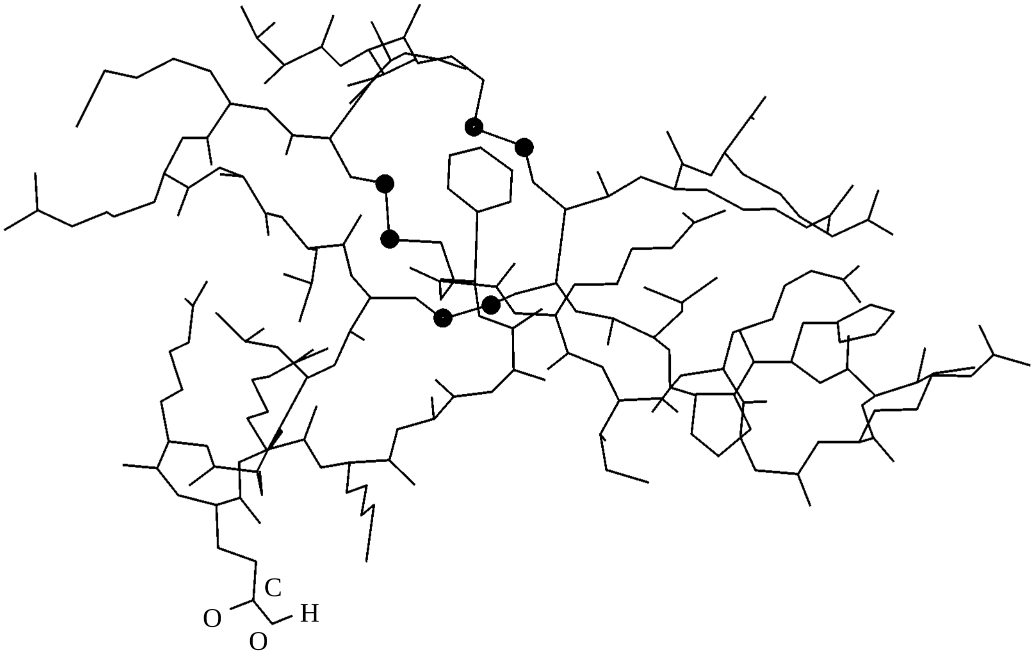
Defensins are low-molecular cationic proteins. The term, as such, reflects the most important function of such peptides, namely, to protect a macroorganism from pathogens [1]. At present, a variety of mammal defensins has been isolated, and their primary structure has been assessed [1, 2]. The high antibiotic activity of defensins has given impetus to their wide physicochemical exploration. However, regardless of small molecular dimensions of defensins and defensin-like proteins, experimental (NMR and X-ray diffraction) geometries are now available for only few such molecules [3–9], which is explained by severe difficulties associated with their isolation and purification. On the other hand, unclear are reasons for the lack of reported quantum-chemical simulations of the steric structure of defensin molecules, since knowledge of their geometry and electronic structure would give one insight into the mechanism of action of these endogenous antibiotics.

In the present work we performed a quntum-chemical study of rabbit NP-1 defensin whose antimicrobial properties are well-documented [10].

The molecular geometry of NP-1 was fully optimized by means of molecular mechanics, as well as MNDO semiempirical calculations by the GAUSSIAN 98 program [11] realized on Cray-J90-Unicos. The starting geometry was constructed on the basis of the primary structure Val–Val–Cys–Ala–Cys–Arg–Arg–Ala–Leu–Cys–Leu–Pro–Arg–Glu–Arg–Arg–Ala–Gly–Phe–Cys–Arg–Ile–Arg–Gly–Arg–Ile–His–Pro–Leu–Cys–Cys–Arg–Arg [1, 10] with three intramolecular S–S bonds: Cys^3–Cys^{31}, Cys^5–Cys^{20}, and Cys^{10}–Cys^{30} (numbering from Val^1 to Arg^{33}).

As follows from our calculation results, the NP-1 molecule has a closed ellipsoidal shape (linear dimensions 30 × 18 × 16 Å), which is consistent with the reported experimental molecular dimensions of the monomer of human HNP-3 defensin (26 × 15 × 15 Å) [8]. On the outside of the NP-1 molecule (see figure), there are side radicals of arginyl residues, which are basic and readily protonated guanidine groups. These groups determine the hydrophilic properties of the molecule and are responsible for its interaction with negatively charged phospholipids of cell membranes. The NP-1 molecule contains a total of ten arginine fragments, six of which form two groups by three: Arg^7, Arg^{14}, Arg^{16} and Arg^{23}, Arg^{25}, Arg^{32}, arranged on the opposite ends of the molecule. The internal surface of the NP-1 molecule are formed by hydrophobic amino acids, such as leucine (Leu), alanine (Ala), and phenylalanine (Phe). Thus, hydrophilic and hydrophobic functional groups are spacially separated, and this explains the amphipathic properties of the molecule.

The molecular volume of the NP-1 defensin molecule is 4100 ± 100 $Å^3$. This estimate is given by all the calculation methods used. However, the conformation of the molecule and some its geometric parameters depend on the approximation used for optimization. In the structure of NP-1, optimized by molecular mechanics, six O···H–N hydrogen bonds are present, with O···N distances of no less than 2.1 Å, which seems unrealistic. The semiempirical calculation gives no hydrogen bonds shorter than 2.5 Å. The MNDO optimization is disadvantageous in that it slightly underestimate C–S and S–S bond lengths. According to our calculations, they are 1.74 and 1.93–1.94 Å, respectively, whereas the experimental lengths of analogous bonds in the *N*,*N'*-diglicylcystine are 1.87



Steric stucture of the NP-1 defensin molecule, optimized by the MNDO method. The sulfur atoms of disulfide bridges are shown by black circles. Atoms of the γ-carboxy group of Glu^{14} are shown. Hydrogen atoms are omitted.

and 2.04 Å and in dimethyl sulfide, 1.78 ± 0.03 and 2.04 ± 0.03 Å [12]. However, it is hardly probable that this underestimation will considerable affect the conformation of the peptide as a whole.

One undeniable advantage of semiempirical calculations in our case is that they gave us estimates for the charge distribution in and the dipole moment of the defensin molecule. As would be expected, the negative charge is mostly localized on the guanidine fragments of arginine residues [q(N) –0.3 to –0.5 e], while sulfur atoms bear a small positive charge [q(S) 0.01–0.04]. The dipole moment of the NP-1 molecule is μ 4.0 D. Regardless of the fact that the defensin molecule has a considerable molecular volume and a nonuniformly distributed arginine fragments, the dipole moment is not too high, implying that the mechanism of interaction of the defensin with cell membranes is chemical rather than electrostatic.

Knowing the steric structure of the NP-1 defensin, we can draw some conclusion concerning the mechanism of its biologic action. Recently [13] we demonstrated the ability of the NP-1 molecule to selectively bind (stoichiometry 1 : 1) an unidentified receptor of a sensor neuron membrane. This result suggests that the NP-1 molecule has only one functional group involved in donor–acceptor interactions forming the ligand–receptor complex. In this connection it is quite important that the single free hydroxy group incorporated into the γ-carboxy group of glutamic acid (Glu^{14}) points away from the NP-1 globule (see figure). Such steric arrangement of Glu^{14} allows us to propose that it is this amino acid residue that ensures, due to intramolecular hydrogen bonding, ligand–receptor interaction with, apparently, one of the membrane glutamate receptors. Importantly, Glu^{14} is present in most studied α-type mammal defensins [2], which implies that the γ-carboxyl hydroxyl of Glu^{14} is generall involved into the mechanism of recognition of defensins by excitable membrane. Evidence for this assumption comes from the fact that the ethanol molecule, being capable of interacting with receptors via intramolecular hydrogen bonding only, too, binds in a 1 : 1 ratio [14].

Thus, the MNDO method provides a more realistic steric structure of the NP-1 defensin molecule compared with molecular mechanics. Moreover, based on the results of semiempirical calculations, one can examine the electronic structure of the protein molecule and gain insight into the mechanism of its biologic action.

REFERENCES

1. Lehrer, R.I., Lichtenstein, A.K., and Ganz, T., *Ann. Rev. Immunol.*, 1993, vol. 11, no. 1, p. 105.
2. Kokryakov, V.N., *Biologiya antibiotikov zhivotnogo*

proiskhozhdeniya (Biology of Animal Antibiotics), St. Petersburg: Nauka, 1999, p. 11.

3. Zimmermann, G.R., Legault, P., Selsted, M.E., and Pardi, A., *Biochemistry*, 1995, vol. 34, no. 41, p. 13 663.
4. Torres, A.M., Wang, X., Fletcher, J.I., Alewood, D., Alewood, P.F., Smith, R., Simpson, R.J., Nicholson, G.M., Sutherland, S.K., Gallagher, C.H., King, G.F., and Kuchel, P.W., *Biochem. J.*, 1999, vol. 341, no. 3, p. 785.
5. Fant, F., Vranken, W.V., and Borremans, F.A.M., *Proteins: Struct., Funct., Genet.*, 1999, vol. 37, no. 1, p. 388.
6. Fant, F., Vranken, W.V., Broekaert, W., and Borremans, F.A.M., *J. Mol. Biol.*, 1998, vol. 279, no. 1, p. 257.
7. Torres, A.M., De Plater, G.M., Doverskog, M., Birinyi-Strachan, L.C., Nicholson, G.M., Gallagher, C.H., and Kuchel, P.W., *Biochem. J.*, 2000, vol. 348, p. 649.
8. Hill, C.P., Yee, J., Selsted, M.E., and Eisenberg, D., *Science*, 1991, vol. 251, no. 5000, p. 1481.
9. Bontems, F., Gilquin, B., Roumestand, C., Menez, A., and Toma, F., *Biochemistry*, 1992, vol. 31, no. 34, p. 7756.
10. Selsted, M.E., Szklarek, D., and Lehrer, R.I., *Infect. Immun.*, 1984, vol. 45, no. 1, p. 150.
11. Frisch, M.J., Trucks, G.W., Schlegel, H.B., Gill, P.M.W., Johnson, B.G., Robb, M.A., Cheeseman, J.R., Keith, T., Petersson, G.A., Montgomery, J.A., Raghavachari, K., Al-Laham, M.A., Zakrzewski, V.G., Ortiz, J.V., Foresman, J.B., Cioslowski, J., Stefanov, B.B., Nanayakkara, A., Challacombe, M., Peng, C.Y., Ayala, P.Y., Chen, W., Wong, M.W., Andres, J.L., Replogle, E.S., Gomperts, R., Martin, R.L., Fox, D.J., Binkley, J.S., Defrees, D.J., Baker, J., Stewart, J.P., Head-Gordon, M., Gonzalez, C., and Pople, J.A., *GAUSSIAN 98*, Pittsburgh: Gaussian, 1995.
12. *Tables of Interatomic Distances and Configurations in Molecules and Ions*, Special Publication, London: Burlington House, 1958, vol. 1, no. 11.
13. Plakhova, V.B., Shchegolev, B.F., Rogachevskii, I.V., Nozdrachev, A.D., Krylov, B.V., Podzorova, S.A., and Kokryakov, V.N., *Fiziol. Zh.*, 2000, vol. 86, no. 11, p. 1471.
14. Vilin, Yu.Yu., Krylov, B.V., and Podzorova, S.A., *Sensorn. Sistemy*, 1997, vol. 11, no. 3, p. 323.