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A Quantum-Chemical Study of the Steric and Electronic Structure of the Molecules of Endogenic Peptides NP-4 and NP-5

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Abstract — The geometric parameters of the molecules of rabbit defensins NP-4 and NP-5 were fully optimized by the molecular-mechanics and semiempirical (MNDO, AM1) methods. It was suggested that the ligand–receptor binding of the molecules of these defensins occurring, according to the method of local fization of potential, in a 1 : 1 stoichiometric ratio is most probably due to hydrogen bonding involving the carboxy group of the pentant Glu^{14} chain.

Protein and peptide antibiotics of the animal origin (defensins, cecropins, lysozyme, etc.) ensure the general mechanism of protection from pathogens in most of biological species, from insects to mammals [1, 2]. Defensins form a vast family of endogenic peptides with a wide spectrum of effects. These are polar cationic peptides usually consisting of 30–35 amino acid residues. A distinctive feature of their structure is the presence of three intramolecular disulfide bridges and β-folded structure. Defensins exhibit high activity and are characterized by specific effect in micromolar concentrations. Numerous experimental studies concerned the structure and mechanism of the effect of defensins and defensin-like proteins [3–13]. However, quantum-chemical methods furnishing more detailed information on the steric and electronic structure of these compounds are still applied very seldom to studing such complex molecules.

Previously [14, 15] we studied the molecule of rabbit defensin NP-1. In this study we examined the molecules of rabbit defensins NP-4 and NP-5.

The geometric parameters of the NP-4 molecule were fully optimized by the molecular-mechanics method using the MM+ force field and HYPERCHEM program [16], and then by MNDO and AM1 semiempirical methods using GAUSSIAN-98 program [17] with a Cray-J90-Unicos computer (the United States).

The primary structure of the NP-4 molecule is as follows: Val–Ser–Cys–Thr–Cys–Arg–Arg–Phe–Ser–Cys–Gly–Phe–Gly–Glu–Arg–Ala–Ser–Gly–Ser–Cys–Thr–Val–Asn–Gly–Val–Arg–His–Thr–Leu–Cys–Cys–Arg–Arg [2]. It was optimized taking into account the presence of three intramolecular S–S bonds: Cys^3–Cys^{31}, Cys^5–Cys^{20}, and Cys^{10}–Cys^{30} (the numbering order is from Val^1 to Arg^{33}). Taking into account the standard physiological conditions (as polar solvent we used water), we considered not only the electrically neutral form of defensin NP-4 molecule but also its charged form. In the charged form of NP-4, we assumed protonation of the guanidine groups in the pendant chains of the arginine residues, the imidazole ring in the pentant chain of histidine, and the *N*-terminal amino groups, with the carboxy groups of the Glu^{14} pendant chain and C end of the molecule charged negatively. The total charge of the protonated form of NP-4 is +6.

The steric structure of the neutral and charged forms of NP-4 (Figs. 1a, 1b) largely resembles the structure of NP-1. The molecule also has an ellipsoidal shape, but its linear dimensions are larger ($35 \times 30 \times 20$ Å), as the NP-4 molecule contains more amino acid residues with branched pendant chains. The external surface of the molecule is formed by hydrophilic amino acids (Arg, Ser, Thr, His), whereas the hydrophobic aliphatic pendant chains of Val, Ala, and Leu are oriented inwards.

Irrespective of the calculation methods used and the form of the molecule, the volume of the system is 3600 ± 100 $Å^3$, which, however, does not imply that

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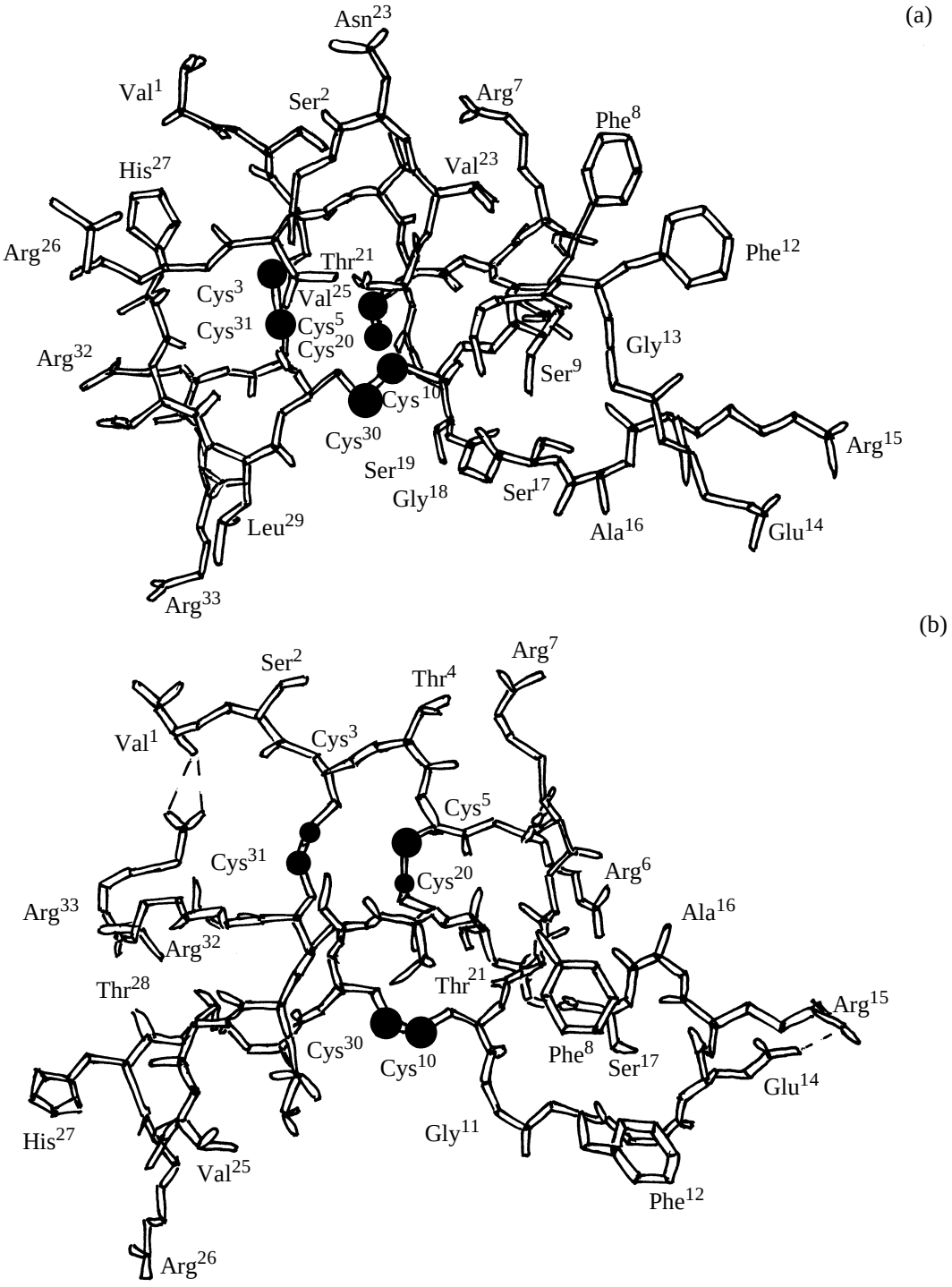

Fig. 1. Steric structures of the (a) neutral and (b) charged forms of the defensin NP-4 molecule, according to AM1 calculations. Black circles denote the sulfur atoms of disulfide bridges, and the dashed lines, intramolecular salt bridges. The hydrogen atoms are not shown.

the structures of different forms of NP-4 are similar. Figure 1 shows that the optimized conformations of the neutral and charged forms of the molecule differ significantly. Furthermore, the conformation obtained depends on the calculation method used for the optimization. According to the MNDO calculations, there are no intramolecular hydrogen bonds in the NP-4 molecule (the shortest distance between a hydrogen

Table 1. Lengths of hydrogen bonds present in the neutral and charged forms of the NP-4 molecule, according to AM1 calculations[a]

Neutral form			Charged form		
bond	$r(O^1–H)$, Å	$r(O^1–X^2)$, Å	bond	$r(O^1–H)$, Å	$r(O^1–X^2)$, Å
$CO(Cys^{31})–NH(Arg^{33})$	2.13	2.98	$CO(Val^{25})–NH^*(Arg^{26})$	2.04	2.97
$CO(Glu^{14})–NH(Ala^{16})$	2.14	2.92	$CO(Cys^5)–NH(Thr^{31})$	2.08	3.06
$CO(Leu^{29})–COOH(C\text{-terminal})$	2.14	2.93	$CO(Glu^{14})–OH^*(Ser^{17})$	2.10	3.04
$CO(Asn^{23})–OH^*(Ser^2)$	2.15	2.94	$CO(Arg^{32})–NH_2^*(Arg^{33})$	2.14	2.87
$OH(Ser^{19})–NH(Cys^{20})$	2.15	3.11	$OH^*(Thr^{28})–NH(Leu^{29})$	2.15	2.93
$CO(Gly^{18})–OH^*(Ser^{19})$	2.16	2.88	$CO(Asn^{23})–NH_2^*(Arg^{32})$	2.15	3.03
$CO(Val^{22})–NH(Gly^{24})$	2.16	2.95	$CO^*(Asn^{23})–NH(Val^{25})$	2.16	3.15
$CO(Arg^{26})–NH(Thr^{28})$	2.17	3.01	$OH^*(Thr^4)–NH_2^*(Arg^7)$	2.19	3.08
$CO(Val^{25})–NH^*(His^{27})$	2.18	3.00	$CO(Ser^9)–OH^*(Thr^{21})$	2.19	3.08
$CO(Ala^{16})–NH(Gly^{18})$	2.18	3.04	$CO(Ser^{17})–NH(Cys^{10})$	2.19	3.09
$CO(Ser^9)–NH(Gly^{11})$	2.19	2.99	$CO(Val^{22})–NH(Arg^{32})$	2.20	3.10

[a] The hydrogen bonds are considered in the form $O^1\cdots H–X^2$, where X = O or N. The groups of pendant chains are marked with asterisks. The same for Table 2.

atom and a heavy atom, not linked by a covalent bond, is 2.37 Å). However, it is improbable that the conformation of such a large peptide molecule is not stabilized by noncovalent intramolecular interactions. In principle, the MNDO method takes into account hydrogen bonding unsatisfactorily [18], which makes it unsuitable for optimization of the geometry of complex protein structures.

The AM1 method, which is a modification of the MNDO method, differs from the basic method in the corrections in the core repulsion integrals for the N–H and O–H atom pairs [19], which significantly improves the account of hydrogen bonds and makes this method more attractive for quantum-chemical analysis of peptide systems. Unfortunately, the version of the GAUSSIAN program that we used does not contain the AM1 parameters for the sulfur atoms; therefore, in these calculations we used a "hybrid" method with the MNDO parameters for the sulfur atoms and AM1 parameters for all the other atoms. Nevertheless, comparison of the S–S and C–S bond lengths and CSS bond angles in the neutral and charged forms of the NP-4 molecule (1.93 and 1.74 Å; $105° \pm 2°$) with the corresponding experimental structural data for the dimethyl disulfide (2.04 ± 0.03 and 1.78 ± 0.03 Å; $107° \pm 3°$) and *N*,*N*'-diglycylcystine (2.04 ± 0.01 and 1.87 ± 0.02 Å; $103° \pm 1°$) [20] molecules shows that the differences are insignificant and hence the use of the mixed set of parameters in optimization of the defensin molecule will hardly alter significantly the results of conformational calculations.

Comparison of the atomic charges shows that the protonation of Arg, His, and *N*-terminal amino group and the deprotonation of the terminal and Glu^{14}-carboxy groups do not significantly alter the charge distribution in the NP-4 molecule; all the changes in charges occur within the ionized functional groups. However, the molecular conformation changes significantly in going from the neutral to charged form. The most essential are changes in the system of hydrogen bonds in the two forms of the peptide.

The calculated lengths of the hydrogen bonds in both forms of NP-4 are given in Table 1. In the uncharged form, the hydrogen bonds are mainly formed between the atoms involved in the peptide bonds. The only exceptions are the oxygen atoms of the hydroxy groups of Ser^2 and Ser^{19}, and also of the C-terminal carboxy group, whereas the proton-donor and proton-acceptor atoms of the *N*-terminal group of the molecule and those of the pendant chains of the other amino acid residues are not involved in intramolecular interactions. In the charged form, on the contrary, the majority of hydrogen bonds involve the atoms of pendant chains. Among 16 functional groups containing nitrogen or oxygen atoms and potentially capable of intermolecular interactions, only the hydroxy groups of Ser^2 and Ser^{19}, guanidine group of Arg^6, and imidazole ring of His^{27} remain free. Additional stabilization of the conformation of the charged form is provided by two intramolecular salt bonds arising between the *N*-terminal amino group and C-terminal carboxy group, and also between the carboxy group of Glu^{14} and guanidine group of Arg^{15}.

This fact shows that the neutral form of NP-4 is

Table 2. Lengths of hydrogen bonds present in the neutral and charged forms of the NP-5 molecule, according to AM1 calculations

Neutral form			Charged form		
bond	$r(O^1–H)$, Å	$r(O^1–X^2)$, Å	bond	$r(O^1–H)$, Å	$r(O^1–X^2)$, Å
$CO(Ile^{22})–NH(Gly^{24})$	2.11	2.91	$CO(His^{27})–NH^*(His^{27})$	2.01	2.84
$CO(Gly^{24})–NH(Thr^{28})$	2.11	2.99	$CO(Gly^{24})–NH(His^{27})$	2.03	2.97
$CO(Ser^{17})–OH^*(Ser^{17})$	2.12	2.79	$NH(Gly^{11})–CO(Glu^{14})$	2.03	2.98
$CO(Thr^{21})–OH^*(Thr^{21})$	2.12	2.80	$CO(Thr^{21})–OH^*(Thr^{21})$	2.11	2.74
$CO(Arg^{26})–NH^*(His^{27})$	2.12	2.98	$NH(Cys^{10})–COOH^*(Glu^{14})$	2.11	2.96
$CO(Thr^{28})–OH^*(Thr^{28})$	2.15	2.79	$NH(Leu^{9})–COOH^*(Glu^{14})$	2.13	3.01
$NH(Ser^{19})–CO(Thr^{21})$	2.16	3.08	$CO(Glu^{14})–NH(Ala^{16})$	2.14	2.95
$CO(Thr^{28})–NH(Cys^{30})$	2.17	2.99	$CO(Thr^{28})–OH^*(Thr^{28})$	2.15	2.75
$CO(His^{27})–NH(Ile^{29})$	2.19	3.01	$CO(Gly^{24})–NH(Thr^{28})$	2.16	2.98
$CO(Ala^{16})–NH(Asn^{23})$	2.19	3.17	$OH^*(Ser^{19})–NH(Cys^{20})$	2.17	2.86
			$CO(Cys^5)–NH_2^*(Arg^{32})$	2.17	3.07
			$OH^*(Ser^{19})–OH^*(Thr^{21})$	2.18	2.87
			$CO(Gly^{11})–NH_2^*(Arg^{15})$	2.18	2.92
			$CO(Ser^{12})–NH_2^*(Arg^{15})$	2.19	2.94
			$NH(Ser^{19})–CO(Thr^{21})$	2.19	3.13
			$CO(Cys^3)–NH(Arg^{33})$	2.20	3.07

less conformationally rigid than the charged form, since the hydrogen bonds present in the neutral form stabilize only the conformation of the peptide skeleton, whereas the arrangement of the pendant chains is mainly limited by the steric factors.

The NP-5 molecule was also examined in the neutral and charged (total charge +5) forms, but full optimization of the geometries of both forms of NP-5 was performed only by the AM1 method, since, as noted above, the MNDO method inadequately considers hydrogen bonds. The NP-5 molecule (Val–Phe–Cys–Thr–Cys–Arg–Gly–Phe–Leu–Cys–Gly–Ser–Gly–Glu–Arg–Ala–Ser–Gly–Ser–Cys–Thr–Ile–Asn–Gly–Val–Arg–His–Thr–Leu–Cys–Cys–Arg–Arg) is stabilized by three intramolecular disulfide bridges, and its primary structure largely resembles that of NP-4. The NP-5 molecule is somewhat more hydrophobic, as it contains Phe^2, Gly^7, Leu^9, and Ser^{12} instead of Ser^2, Arg^7, Ser^9, and Phe^{12} present in the respective positions in NP-4.

The steric structures of the neutral and charged forms of the NP-5 molecule are shown in Figs. 2a and 2b. The molecular volume of the system is 3500 ± 100 Å^3, and the linear dimensions of the molecule are $35 \times 38 \times 21$ Å; the hydrophilic groups are oriented outwards, and the hydrophobic groups, inwards. The similarity of the amino acid sequences in NP-4 and NP-5 and of the molecular dimensions could suggest the stereochemical similarity of these molecules; however, actually their steric structures differ essentially.

The characteristics of hydrogen bonds in both forms of NP-5 are given in Table 2. In the neutral form of NP-5, the hydrogen bonds mainly involve the atoms of the peptide skeleton, except the hydroxyl oxygen atoms of the pendant chains of Ser^{17}, Thr^{21}, and Thr^{28} and the nitrogen atom of the imidazole ring of His^{27}. A structural feature of the neutral form of NP-5 is the β-pin consisting of three amino acid residues $Val^{25}–Arg^{26}–His^{27}$ and stabilized by the hydrogen bonds $CO(Gly^{24})–NH(Thr^{28})$ and $CO(Arg^{26})–NH(His^{27})$. The same β-pin is present in the charged form of NP-5, but it is stabilized by more hydrogen bonds: $CO(Gly^{24})–NH(Thr^{28})$, $CO(Gly^{24})–NH(His^{27})$, $CO(Val^{25})–NH(Arg^{26})$, and $CO(His^{27})–NH^*(His^{27})$.

The intramolecular salt bonds between the carboxy group of Glu^{14} and guanidine fragment of Arg^6, and also between the C-terminal carboxy group and pendant chains of Arg^{32} and Arg^{33} make the conformation of the charged form of NP-5 rigid. The molecular structure in the regions of formation of the salt bonds is additionally stabilized by the hydrogen bonds: $NH(Gly^{11})–CO(Glu^{14})$, $NH(Leu^9)–COOH^*(Glu^{14})$, $NH(Cys^{10})–COOH^*(Glu^{14})$, $CO(Glu^{14})–NH(Ala^{16})$, $CO(Cys^5)–NH_2^*(Arg^{32})$, and $CO(Cys^3)–NH(Arg^{33})$. The intramolecular disulfide bridges, salt bridges, and the branched system of hydrogen bonds make the NP-4 and NP-5 molecules conformationally rigid,

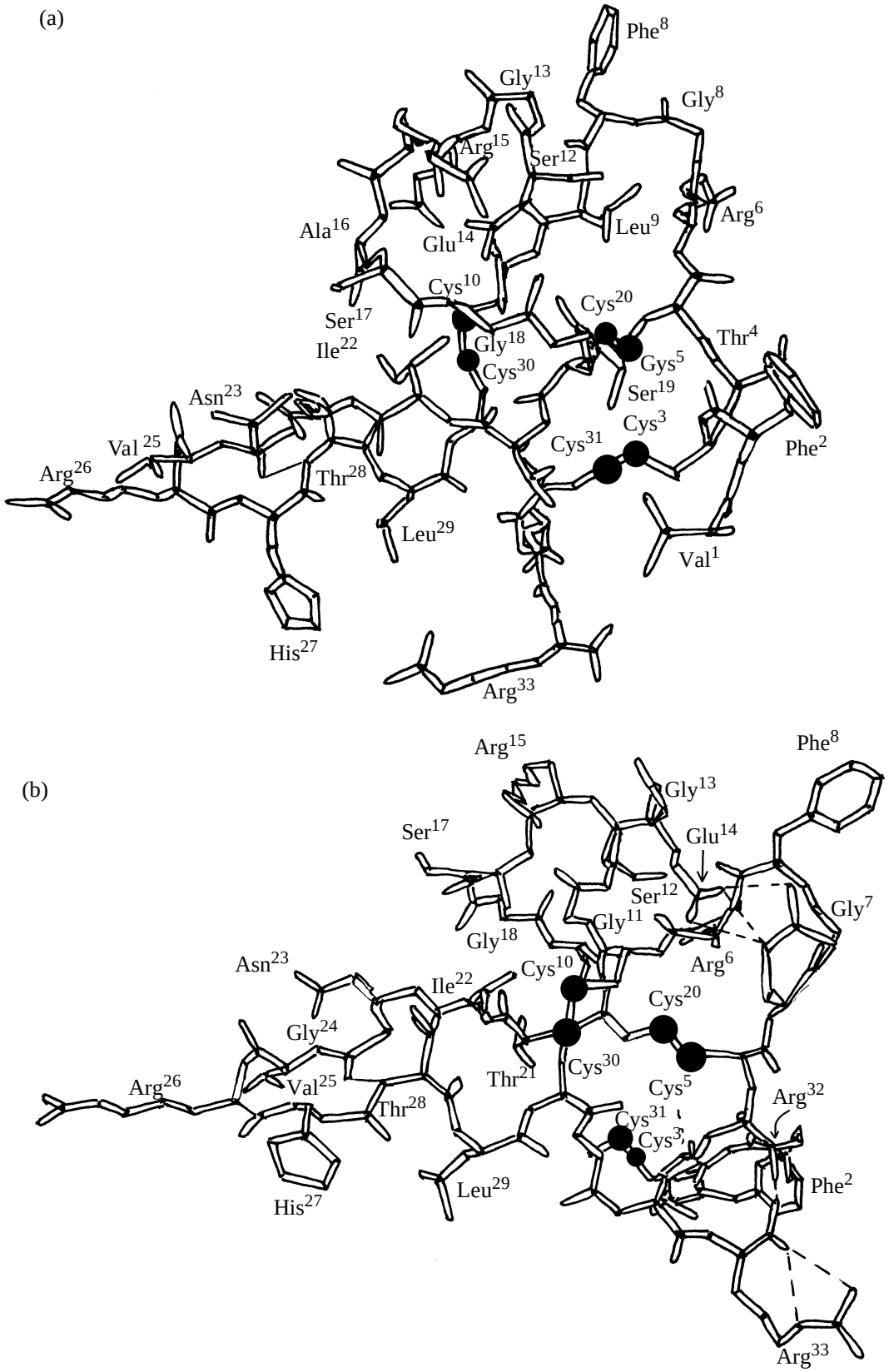

Fig. 2. Steric structure of the (a) neutral and (b) charged forms of the defensin NP-5 molecule, according to AM1 calculations. The solid line denotes the CO(Gly^{24})–NH(Thr^{28}) hydrogen bond stabilizing the β-pin Val^{25}–Arg^{26}–His^{27}; the other designations are the same as in Fig. 1.

which is consistent with the experimental data that the defensin molecules are resistant to destructive peptidases [2].

Using the experimental method of the local fixation of potential [21] on neurons of rat spinal ganglia, we showed that the possible ligand–receptor binding of the NP-4 molecule occurs in a 1 : 1 stoichiometric ratio. This fact suggests not only the existence of a specific defensin receptor, as indicated by extremely low K_D, 80 nM, but also the presence of a single binding center. This means that defensins are capable not only to accomplish their direct function of a "perforator" of the microorganism membrane but also to act as agonists modulating the transfer of nervous pulses to the central nervous system.

It was suggested previously [14, 15] that the ligand–receptor binding of the defensin NP-1 molecule, also occurring in a 1 : 1 ratio, is most probably provided by formation of a hydrogen bond involving the carboxy group of the pendant chain of Glu^{14}. The mechanisms of interaction of NP-4 and NP-1 with receptors can be expected to be similar. According to the quantum-chemical calculations, the following groups of NP-4 are sterically accessible and are not involved in the intramolecular interactions: in the neutral form, the carboxy group of the pendant chain of Glu^{14}, hydroxy groups of the pendant chains of Thr^4, Ser^{17}, Thr^{21}, and Thr^{28}, and the guanidine groups of all the arginyl residues; in the charged form, the hydroxy groups of Ser^2 and Ser^{19}, the guanidine group of Arg^6, and the imidazole ring of His^{27}. It is reasonable to assume that the structurally related defensins NP-4 and NP-1 have the same mechanism of the ligand–receptor binding. Hence, the most probable pathway of formation of the ligand–receptor complex is formation of a hydrogen bond with the carboxy group of Glu^{14}. However, participation in the hydrogen bonding of other functional groups, e.g., the hydroxy groups of the pendant chains of Ser or Thr, cannot be fully ruled out also. There are good grounds to believe that the NP-5 molecule has a similar mechanism of the membrane reception.

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