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Geometric Structure of a Molecule of Epitalon Tetrapeptide: A Molecular-Dynamics Simulation

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Abstract — Variation of the geometric parameters of a molecule of Epitalon tetrapeptide (Ala–Glu–Asp–Gly) over a period of 1500 ps was simulated by the method of molecular dynamics using AMBER force field. The structure of the molecule is stabilized by two salt bridges formed by the *N*-terminal nitrogen atom and oxygen atoms of Asp and Glu side chains. The biological effect of Epitalon was attributed to formation of salt or hydrogen bonds involving one or several ionizable functional groups of the molecules.
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Epiphysis (pineal gland) plays an important role in the control of aging and other physiological functions of a living body by secretion of peptide and hormonal factors [1]. A complex of polypeptides isolated from epiphysis, epitalamine, prolongs animals' life and retards aging and tumor formation [2, 3]. Epitalamine also stimulates endogenic secretion of melatonin indole hormone, which participates in the control of biological rhythms and strongly affects the activity of endocrine, nervous, and immune systems [4]. Synthetic tetrapeptide Epitalon (Ala–Glu–Asp–Gly) designed at the Laboratory of Peptide Chemistry, St. Petersburg Institute of Bioregulation and Gerontology, North-West Division, Russian Academy of Medical Sciences, exhibits a higher biological activity compared to epitalamine [5–9].

The goal of this study is examination of the structure and conformational features of the Epitalon molecule by the method of molecular dynamics. All the computations were performed using the HYPERCHEM program package [10]. The Epitalon molecule was considered in the zwitter-ionic form in the water surrounding. The peptide molecule was placed in a $37.5 \times 22.5 \times 13.5$ Å rectangular box containing 347 solvent molecules. Cyclic boundary conditions were imposed on the peptide–water system. The solvent was considered within the framework of the TIP3P model [11]. The optimization of the geometry and determination of the trajectory of evolution of the tetrapeptide molecule were performed by the method of molecular mechanics using the AMBER force field [12]. The computation procedure is described in more detail elsewhere [13, 14].

The trajectory of evolution of the solvated Epitalon molecule was traced for a period of 1500 ps. The integration step was 0.5 fs. For the first 30 ps, we simulated heating of the system to 300 K followed by equilibration. The equilibrium was considered to be attained within 300 ps, after which the statistical data were analyzed. For the time interval between 300 and 1500 ps, the geometric parameters of system were saved (without interruption of the dynamics) at 10-ps intervals and optimized by the method of molecular mechanics. As a result, 121 conformations were obtained.

Since the simulation of the dynamics of the Epitalon molecule and optimization of its geometry were performed in the solvation environment, the energetically most favorable conformation was chosen on the basis of the energy of solvated, rather than gaseous, peptide molecules. The lowest-energy conformer among those we obtained does not necessarily correspond to the global minimum, but the goal of this study was to examine the dynamics of the evolution of the geometric parameters in time, rather than the structure of the molecule in the global minimum.

The steric structure of the lowest-energy conformation of the Epitalon molecule among the conformations we obtained and the accepted designations of the geometric parameters are given in Fig. 1. The designations and counting of the torsion angles correspond

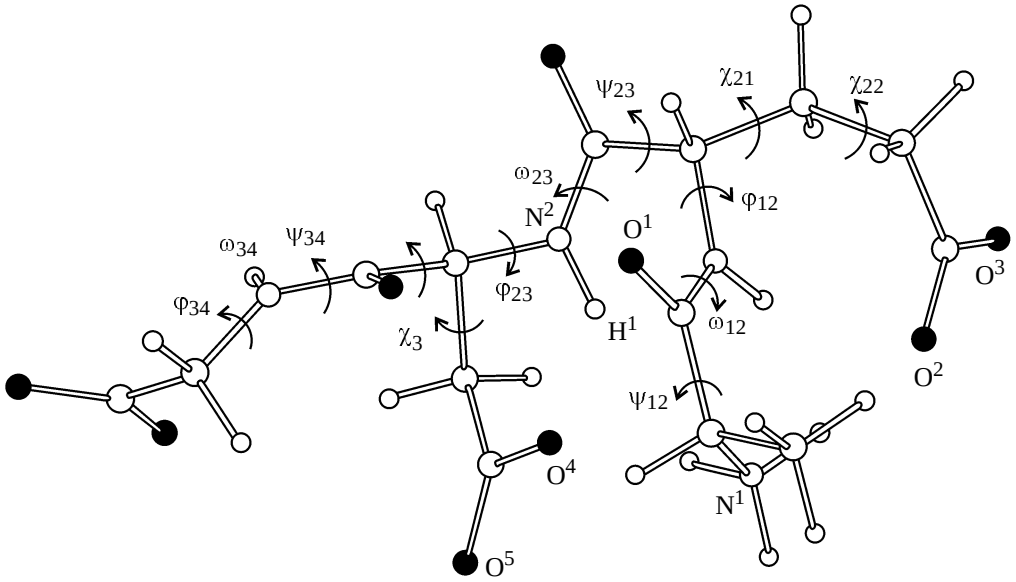

Fig. 1. Steric structure of the lowest-energy conformer (among those we obtained) of the Epitalon molecule and designations of atoms and dihedral angles.

to the accepted nomenclature [15]. In the molecule there are two intramolecular salt bridges: those between the N^1 atom of the N end of the peptide and the O^4 atom of the carboxy group of Asp and between the N^1 atom and O^2 atom of the carboxy group of Glu. The bridge involving Asp is apparently more favorable energetically, because it is present in all the conformations obtained, whereas the second bridge is formed in the course of molecular dynamics. In particular, in the 30 energetically most favorable conformations among those we obtained, the N^1–O^4 distance is within 2.68–2.77 Å, and the N^1–O^2 distance, within 2.66–3.96 Å. Below are the dihedral angles and selected interatomic distances in the lowest-energy conformer: ψ_{12} –13.5°, ω_{12} 179.7°, φ_{12} –97.8°, ψ_{23} –7.9°, ω_{23} –164.0°, φ_{23} –79.7°, ψ_{34} –179.2°, ω_{34} –135.9°, φ_{34} –156.5°, χ_{21} –42.1°, χ_{22} –91.0°, χ_3 –59.8°, N^1–O^2 2.71, N^1–O^3 4.54, N^1–O^4 2.74, N^1–O^5 4.31 Å.

Since the HYPERCHEM program allows saving of structural characteristics of the system at a definite step (25 fs in our case), it becomes possible to trace the evolution of the geometric parameters of the Epitalon molecule in the course of the dynamics simulation. We considered the evolution of virtually all the dihedral angles involving no hydrogen atoms and of certain interatomic distances during the time interval 300–1500 s.

Figure 2 shows how some dihedral angles and interatomic distances vary with the simulation time. Several conformational transitions that occurred in the monitored time interval can be distinguished. On the 535th picosecond, a number of geometric parameters of the peptide molecule abruptly change. The most pronounced changes are observed with the dihedral angles φ_{23} and ψ_{23}: from –139.5° ± 15.8° in the interval 519–535 ps to –69.3° ± 14.4° in the interval 535–665 ps and from 32.6° ± 11.3° in the interval 526–535 ps to –31.1° ± 10.4° in the interval 535–1020 ps, respectively. The dihedral angle ψ_{12}, equal to –48.4° ± 10.5° in the interval 300–535 ps, becomes –71.0° ± 12.7° in the interval 535–553 ps. The angle φ_{12}, fairly stable in the interval 300–535 ps (–98.4° ± 9.8°), starts to sharply increase in the interval 535–565 ps. Thus, this transition can be characterized as a turn of the Ala–Glu fragment relative to the Asp–Gly fragment in combination with a turn of Ala relative to Glu. As a consequence, the N^1–O^2, N^1–O^3, and N^1–O^5 interatomic distances change: from 4.96 ± 0.31, 4.70 ± 0.15, and 3.31 ± 0.27 Å in the interval 300–535 ps to 5.63 ± 0.26, 4.95 ± 0.20, and 2.93 ± 0.19 Å in the interval 535–553 ps, respectively. It should be noted that the angles φ_{23} and ψ_{23} experience appreciable fluctuations before the transition at 535 ps. In particular, the angle φ_{23} is –150.5° ± 9.8° in the interval 300–510 ps and –126.1° ± 11.9° in the interval 510–519 ps; ψ_{23}, –45.8° ± 10.3° in the interval 300–510 ps, 10.0° ± 13.3° in the interval 510–519 ps, 42.2° ± 7.7° in the interval 519–521 ps, and 0.3° ± 9.4° in the interval 521–526 ps. The angles φ_{23} and ψ_{23} are so unstable that in some time intervals the rms deviation exceeds the mean value.

The next change in the conformation of the Epi-

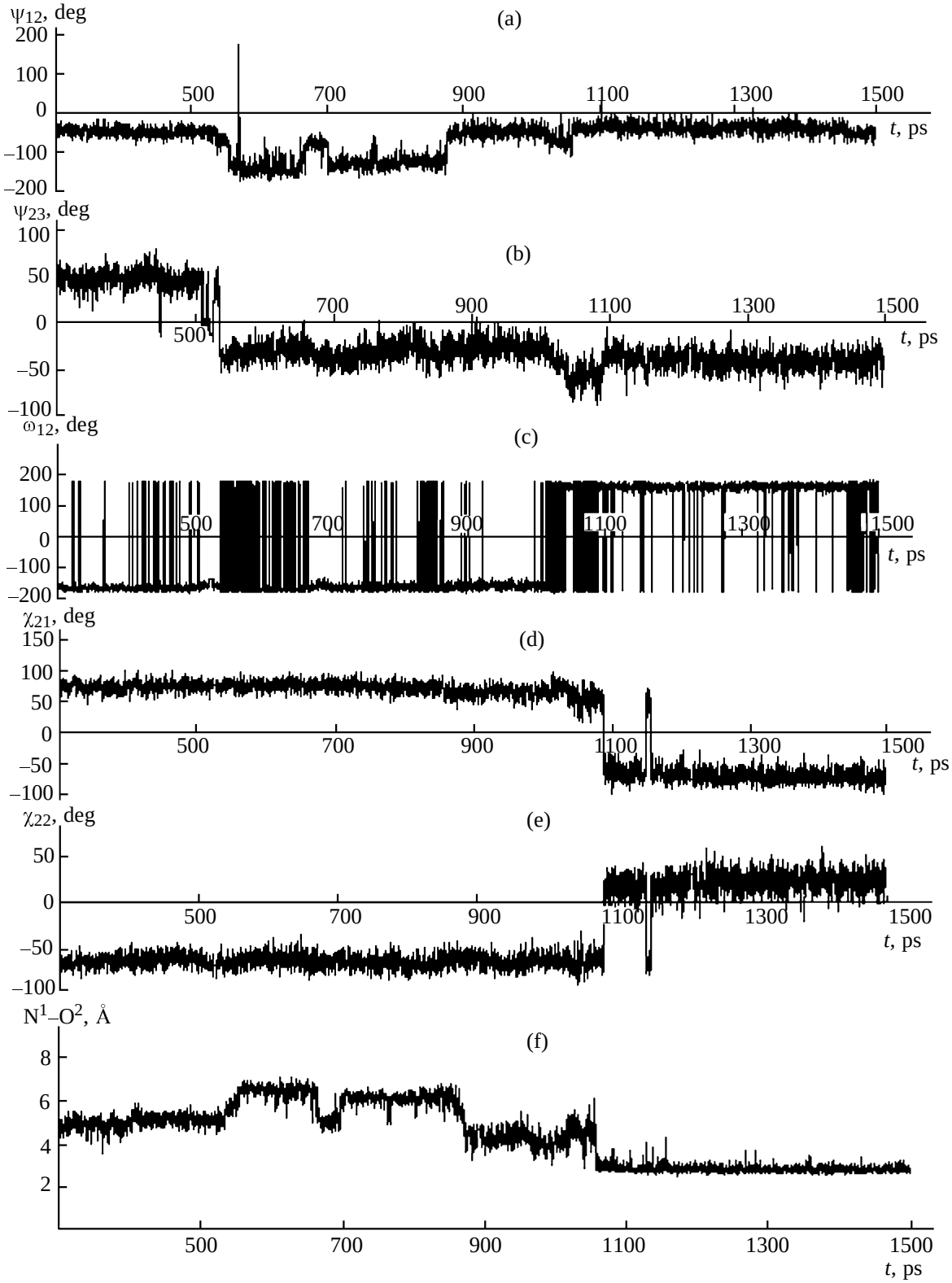

Fig. 2. Changes in the structural parameters of the Epitalon molecule in time (*t*, ps) in the course of molecular-dynamics simulation: (a–c) dihedral angles ψ_{12}, ψ_{23}, and ω_{23} of the molecular skeleton; (d, e) dihedral angles χ_{21} and $\chi_2 2$ of the side Glu chain; and (f) N^1–O^2 distance.

talon molecule occurs at an instant of 553 ps and is characterized by a change in ψ_{12} (from $-71.0° \pm 12.7°$ in the interval 535–553 ps to $-137.0 \pm 25.5°$ in the interval 553–665 ps), which corresponds to a turn of Ala relative to the remaining part of the molecule. This turn causes an increase in the N^1–O^2 and N^1–O^3 distances from 5.63 ± 0.26 and 4.95 ± 0.20 Å in the interval 535–553 ps to 6.39 ± 0.28 and 5.44 ± 0.22 Å in the interval 553–665 ps, respectively. The N^1–O^5 distance also increases: from 2.93 ± 0.19 Å in the interval 535–553 ps to 3.08 ± 0.27 Å in the interval 553–558 ps and to 3.74 ± 0.29 Å in the interval 558–697 ps. On the other hand, the conformation of the peptide after 553 ps is stabilized by the N^2–$H^1 \cdots O^1$ hydrogen bond: The distance between these atoms decreases from 3.62 ± 0.24 Å in the interval 300–553 ps to 2.95 ± 0.20 Å in the interval 553–806 ps. Apparently, the formation of a hydrogen bond compensates an increase in the energy of the Epitalon conformation, caused by an increase in the distance between the positively charged N end of the molecule and the carboxylate anions.

The next transition occurring at an instant of 665 ps involves mutual approach of the N end of the molecule and the Glu carboxy group, with the preservation of the N^2–$H^1 \cdots O^1$ bond in Ala owing to a turn around the CH–CO bond in Ala. The angle ψ_{12} changes from $-137.0° \pm 25.5°$ in the interval 553–665 ps to $-79.1° \pm 11.9°$ in the interval 665–697 ps. The angle φ_{23} somewhat increases in the absolute value: from $-69.3 \pm 14.4°$ in the interval 553–665 ps to $-97.4° \pm 15.4°$ in the interval 665–1020 ps. As a result of these turns, the N^1–O^2 and N^1–O^3 distances decrease from 6.39 ± 0.28 and 5.44 ± 0.22 Å in the interval 553–665 ps to 5.03 ± 0.28 and 4.61 ± 0.27 Å in the interval 665–697 ps, respectively.

The transition occurring at an instant of 697 ps returns the molecule to the conformation in which it occurred in the interval 553–665 ps, at the expense of a change in ψ_{12} from $-79.1° \pm 11.9°$ in the interval 665–697 ps to $-130.3° \pm 13.4°$ in the interval 697–872 ps. The N^1–O^2 and N^1–O^3 distances increase from 5.03 ± 0.28 and 4.61 ± 0.27 Å in the interval 665–697 ps to 6.06 ± 0.28 and 5.50 ± 0.23 Å in the interval 697–872 ps, respectively. The N^1–O^5 distance increases from 3.74 ± 0.29 Å in the interval 558–697 ps to 4.47 ± 0.19 Å in the interval 697–872 ps. In the interval 759–768 ps, the molecule returns for a short time to the conformation in which it occurred in the interval from 665 to 697 ps, with the corresponding changes in the angle ψ_{12} and distances N^1–O2 and N^1–O^3. An instant of 806 ps is characterized by a small increase in the dihedral angle φ_{12} in the absolute value (from $-41.8° \pm 12.2°$ in the interval 553–806 ps to $-60.1° \pm 11.4°$ in the interval 806–1020 ps), which, however, leads to weakening of the $N^2 \cdots O^1$ hydrogen bond: The N^2–O^1 distance increases from 2.95 ± 0.20 Å in the interval 553–806 ps to 3.23 ± 0.21 Å in the interval 806–1089 ps. Before that, for a period of 2 ps (804–806 ps), the N end of the molecule and the carboxy group of the side Glu chain become closer to each other by ~0.4 Å.

The next transition also involves a change in the dihedral angle ψ_{12}: from $-130.3° \pm 13.4°$ in the interval 553–872 ps to $-48.8° \pm 13.8°$ in the interval 872–1020 ps. Correspondingly, the N end of the molecule changes the spatial orientation relative to the carboxy groups of Glu and Asp, which is manifested in shortening of the N^1–O^2, N^1–O^3, and N^1–O^5 distances from 6.06 ± 0.28, 5.50 ± 0.23, and 4.47 ± 0.19 Å in the interval 697–872 ps to 4.18 ± 0.36, 4.69 ± 0.20, and 3.93 ± 0.20 Å in the interval 872–1020 ps.

At an instant of 1020 ps, the Ala–Glu fragment rotates relaitve to the Asp–Gly fragment, with a simultaneous rotation of Ala relative to Glu. The most pronounced change is experienced by the dihedral angle ψ_{23}: from $-31.1° \pm 10.4°$ in the interval 535–1020 ps to $56.9° \pm 12.2°$ in the interval 1030–1090 ps. The other angles also change: ψ_{12}, from $-48.8° \pm 13.8°$ in the interval 872–1020 ps to $-70.7° \pm 17.6°$ in the interval 1020–1055 ps; φ_{12}, from $-60.1° \pm 11.4°$ in the interval 806–1020 ps to $-46.9° \pm 11.7°$ in the interval 1020–1055 ps; and φ_{23}, from $-97.4° \pm 15.4°$ in the interval 665–1020 ps to $-60.2° \pm 12.1°$ in the interval 1020–1500 ps. These changes lead to an increase in the N^1–O^2 and N^1–O^5 distances from 4.18 ± 0.36 and 3.93 ± 0.20 Å in the interval 872–1020 ps to 4.72 ± 0.30 and 4.27 ± 0.22 Å in the interval 1020–1040 ps, respectively.

During the interval 1020–1055 ps, significant changes in the peptide conformation occur, but these changes are difficult to describe quantitatively. Certain dihedral angles, e.g., ω_{23}, φ_{34}, and ψ_{34}, are about 180° and vary in the range from –150° to 150°. Appreciable changes in these geometric parameters are revealed most easily by visual examination of the plots of these parameters vs. time. Figure 2c shows that, in the interval 1020–1055 ps, initially negative ω_{23} becomes positive. Similar changes occur with ψ_{34}. On the contrary, φ_{34} becomes negative. The fluctuations of these parameters are very large. This is manifested in large rms deivations of the distances N^1–O^2, N^1–O^3, and N^1–O^5. These distances in the interval 1020–1055 ps are 4.68 ± 0.45, 5.95 ± 0.38, and 3.86 ± 0.44 Å, respectively. The N^1–O^2 distance in some cases reaches ~3.5 Å, which practically corresponds to the formation of a salt bridge between these atoms.

The second salt bridge in the Epitalon molecule (between the N^1 atom of the N end of the peptide and the O^2 atom of the carboxy group of the side chain of Glu) can be considered as completely formed after the turn of Ala relative to the Glu–Asp–Gly fragment at an instant of 1055 ps. The angle φ_{12} changes from $-46.9° \pm 11.7°$ in the interval 1020–1055 ps to $-27.5° \pm 17.5°$ in the interval 1055–1090 ps; ψ_{12}, from $-70.7° \pm 17.6°$ in the interval 1020–1055 ps to $-38.2° \pm 12.9°$ in the interval 1055–1451 ps. For clearness, below we give several specific values of the N^1–O^2 distance: 1054.5 ps, 4.84 Å; 1055 ps, 4.03 Å; and 1055.5 ps, 3.34 Å. Starting from an instant of 1057 ps, the N^1–O^2, N^1–O^3, and N^1–O^5 distances are stabilized and in the interval 1057–1500 ps amount to 2.77 ± 0.16, 4.65 ± 0.17, and 4.52 ± 0.20 Å, respectively. The formation of the second bridge gives rise to a strain in the backbone and side chains of the peptide molecule and causes a turn of the Asp carboxy group relative to the CH–COO^- bond and a corresponding increase in the N^1–O^5 distance. The strain is lifted in the course of the subsequent conformational transitions.

At an instant of 1090 ps, the conformation of the Glu side chain changes. The dihedral angles $\chi_2 1$ and χ_{22}, equal in the interval 300–1090 ps to $63.6° \pm 7.2°$ and $-71.0° \pm 10.3°$, respectively, in the interval 1090–1151 ps are equal to $15.6° \pm 12.3°$ and $-65.3° \pm 15.8°$. This fact is a direct consequence of the formation of a salt bridge between the N^1 and O^2 atoms. In the interval 1151–1158 ps, the Glu side chain returns to the conformation in which it occurred in the period from 300 to 1090 ps: χ_{21} $-65.4° \pm 6.9°$, χ_{22} $-51.4° \pm 13.1°$, but at an instant of 1158 ps it finally takes the new conformation, and the angles χ_{21} and χ_{22} in the interval 1158–1500 ps are $22.7° \pm 11.3°$ and $-71.1° \pm 10.4°$, respectively.

The last conformation transition observed in the interval 300–1500 ps occurs at an instant of 1457 ps. At this instant, the N^2–$H^1 \cdots O^1$ hydrogen bond is formed owing to a change in the dihedral angle φ_{12}, which is equal to $-58.1° \pm 16.7°$ in the interval 1090–1457 ps and $-36.4° \pm 15.1°$ in the interval 1457–1500 ps. The N^2–O^1 distance is 3.46 ± 0.27 Å in the interval 1090–1457 ps and 2.88 ± 0.16 Å in the interval 1457–1500 ps. Changes in the Gly skeleton also occur: The initially positive dihedral angles ψ_{34} and ω_{34} become negative, and the initially negative angle φ_{34} becomes positive.

Certain geometric parameters of the Epitalon molecules vary insignificantly throughout the simulation interval. The N^1–O^4 distance is 2.76 ± 0.15 Å, and the dihedral angle χ_3, $-61.6° \pm 8.8°$. This fact indicates that, by an instant of 300 ps, the N^1–O^4 bridge has already formed. The dihedral angle ω_{12} is about 180° (from –160° to 150°).

Thus, the conformation of the Epitalon molecule is stabilized by two salt bridges N^1–O^2 and N^1–O^4, formed by the *N*-terminal N^1 atom and O^2 and O^4 atoms of the carboxy groups of the Glu and Asp side chains, respectively, and also by the hydrogen bond N^2–$H^1 \cdots O^1$. The presence of salt bridges somewhat restricts the conformation freedom of the molecule, and this is primarily manifested in the essentially constant value of the dihedral angle χ_3 describing the mobility of the Asp side chain. However, there are 8–10 covalent bonds between the N and O atoms forming the bridges; therefore, the peptide molecule remains fairly flexible. The spatial orientation of Gly is apparently governed by the electrostatic repulsion of the carboxy groups of Asp and C end of the molecule, bearing the negative charge; therefore, the dihedral angles ψ_{34}, ω_{34}, and φ_{34} are fairly close to 180°, which ensures maximal spatial separation of these groups.

Since Epitalon is a drug, it is appropriate to make some assumptions concerning possible mechanism of its biological effect. The Epitalon molecule exhibits hydrophilic properties owing to the ionizable carboxy groups of the C end of the molecule and of Asp and Glu side chains, and also to the *N*-terminal amino group, which hinders the direct transport of Epitalon through the hydrophobic bilipid layer of a cell membrane. Apparently, the effect of Epitalon involves its binding with a certain protein structure incorporated in a cell membrane, e.g., with one of the kinds of membrane receptors or ion channels. The interaction may occur via formation of salt or hydrogen bonds involving one or several charged functional groups in the Epitalon molecule.

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