Molecular Dynamics Study on an Adipokinetic Hormone Peptide in Aqueous Solution

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Lom-AKH-I is a member of the adipokinetic hormone/red pigment concentrating hormone (AKH/RPCH) family of peptides found in flying insects. A molecular dynamics simulation at room temperature (293 K) in water has been performed to survey the folding path of the Lom-AKH-I peptide in water and to establish the secondary structure of Lom-AKH-I. The obtained results indicate the presence of an undefined extended conformation.

Introduction

The ability to fly is extremely well developed in insects allowing them to spread over vast areas. Being able to interfere with the flight ability would make possible to control their populations. Flight is one of the most energetically demanding processes and is regulated by the family of adipokinetic hormones through mobilization of diacylglycerols from triacylglycerols stores in the fat body used as fuels during the flight. Adipokinetic hormones belong to the large family of peptides found in insects and crustaceans and there are over 20 sequences identified so far. They are 8 to 10 residues long with a pyroglutamyl residue at the N-terminus and amidated C-terminus. The two aromatic amino acids are at position 4 (Phe, mostly, and Tyr) and 8 (Trp) are predominant but molecules with 3 aromatic amino acids are also known (Gäde, 1996; Gäde and Auerswald, 1998; Gäde *et al.,* 1997). Generally, they are uncharged and only three contain negatively charged Asp residue at position 7 (Gäde *et al.,* 1997). The outlined structural similarities are based on the interdependency of hormone and specific receptor in the fat body (Ziegler *et al.,* 1995). The changes altering the secondary structure prevent the binding of the hormone to the receptor. In the locust, one of the hormones regulating flight is Lom-AKH-I peptide with the sequence given below:

$_{P}$Glu–Leu–Asn–Phe–Thr–Pro–Asn–Trp–Gly–ThrNH$_2$.

A preliminary structural study indicates the presence of a β-turn conformation. It has also been shown that in non-aqueous solutions they adopt an ordered conformation. CD spectra collected in water solution at room temperature are defined by a positive a peak at ~217 nm and a negative one at ~200 nm. These features could be the indication for the presence of the left-handed polyproline II helix (P_{II}) conformation (Cusinato *et al.,* 1997), dominant conformation for linear oligopeptides in aqueous solutions.

The presented study employs a molecular dynamics simulation technique to survey the folding path of the Lom-AKH-I peptide in water solution.

Methods

Molecular modeling simulations were performed on an OCTANE SGI computer (Silicon Graphics Inc.) using Xplor 3.8 program (Brunger, 1992). The extended linear structure of the peptide was built based on the Charmm22 topology and parameters file (MacKerell *et al.,* 1998). pGlu residue was built using the topology and parameter file form HIC-Up; Uppsala Software Factory (http://alpha2.bmc.uu.se/usf). For the solvent simulation a non-polarizable water model having 3 interaction sites (TIP3P) was used (Jorgensen *et al.,* 1983). The extended structure was immersed into the equilibrated box of water with the dimensions of the box equal to 6.4 × 4.1 × 4.1 nm and the distance between solute and solvent of 0.23 nm. The immersed structure was 500 steps energy minimized using the Powell energy minimization algorithm (Powell, 1964). 400 ps molecular dynamics (MD) simulation was performed on minimized structure.

The temperature (293 K) was maintained by coupling to an external bath (Berendsen *et al.,* 1984) with friction parameter (Fbet) equal to 10. The SHAKE algorithm, with the accuracy of 10^{-5},

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was used to constrain the lengths of bonds involving hydrogen atoms (Ryckaert *et al.*, 1977). All motions were integrated using the Verlet algorithm. The time step was equal to 2 fs. The cutoff parameter was set to 1.3 nm. The consecutive conformers were collected at 10 ps intervals during the whole simulation period. The temperature and the total energy values were elucidated at every 10 steps of the simulation process.

Results and Disscusion

Total energy (ETOT) and the temperature changes were monitored continuously and are shown in Fig. 1. The radical change in the energy curve can be observed at the beginning of the simulation. It stabilizes at around 15 ps and keeps constant until the end of simulation. The temperature curve was stable during the whole simulation process with the temperature oscillating around 293 K, as intended. MD trajectory was sampled in 10 ps intervals in order to fully analyze the conformational space.

Root mean square deviation (RMSD) from the starting structure (minimized structure), the average structure (the average of 20 structures from last 200 ps of simulation) as well as the radius of gyration changes are shown in Fig. 2A–C. The analysis of Fig. 2A reveals that the RMSD of backbone atoms as well as all heavy atoms grows during the simulation time indicating undergoing structural changes. However, the changes are rather small since the RMSD value does not cross a 0.2 nm threshold for backbone atoms. RMSD differences from the average structure for backbone atoms and all heavy atoms, Fig 2B, indicate

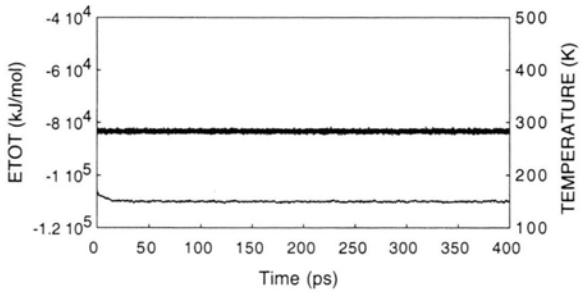

Fig. 1. The changes in the temperature and the negative total energy (ETOT), the sum of positive kinetic energy and negative potential energy, during molecular dynamics (MD) simulation.

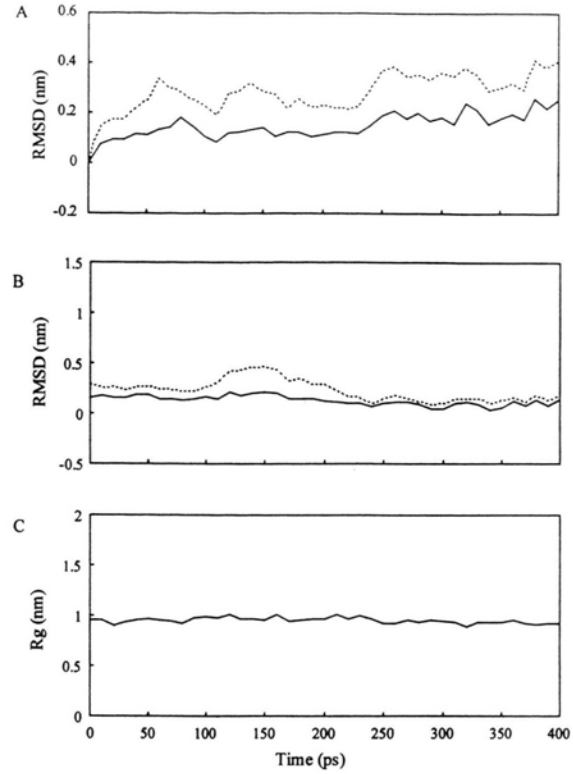

Fig. 2. A) RMSD from the starting structure; B) from the average structure: ——— all backbone atoms, – – – – all heavy atoms. C) changes in radius of gyration (Rg).

the lack of distinctive structural changes occurring during the simulation period with the exemption for the period between 100 and 175 ps. The changes in radius of gyration (Fig. 2C) indicate that the volume of the peptide is rather constant during the whole simulation period. The starting and the average of 20 final conformers, the average structure, are shown in Fig. 3A and B, respectively.

Summarizing the obtained results it has to be stressed that conformational changes occurring in the Lom-AKH-I peptide structure during simulation in water solution at room temperature are rather small and oscillate around the extended conformation. It has been previously suggested, basing on CD spectrum analysis, that Lom-AKH-I peptide adopts the left-handed polyproline II helix conformation (P_{II}) in water solution at room temperature. In order to evaluate this hypothesis the

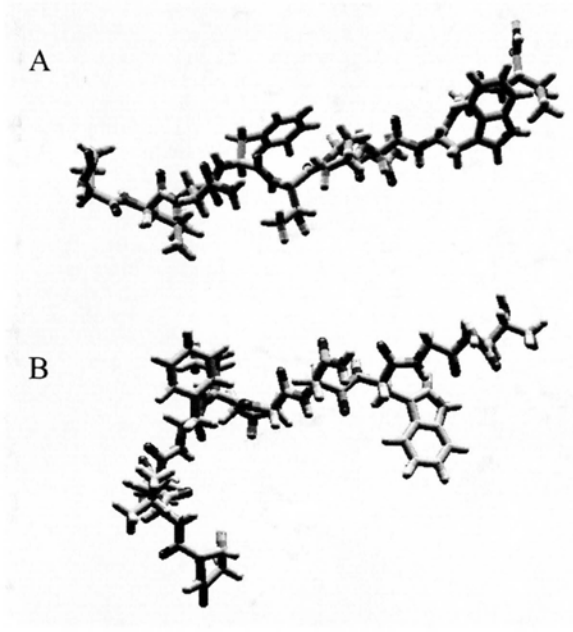

Fig 3. A) The staring conformer obtained by 500 steps of Powell minimization, B) the average structure of last 20 conformers.

short description of P_{II} conformational features is given below.

P_{II} structure has been found in large number of proteins. It deviates slightly form the theoretical helix and the length of P_{II} segments is shorter than α-helices, due to lack of stabilizing effect of intramolecular hydrogen bonds. The side chains are exposed to the solvent (more than in other secondary structures). Pro is the dominant amino acid. However, other amino acids such as Gln, Ser, Arg, and Ala have also been found in the P_{II} region. The P_{II} helix followed by a beta-turn has been described as a novel supersecondary structure and is characterized by φ and ψ dihedral angles of −75° and 145° (Adzhubei and Sternberg, 1994). The angles formed by the intersection of two planes in space are defined by $C_i-N_{i+1}-C\alpha_{i+1}/N_{i+1}-C\alpha_{i+1}-CO_{i+1}$ and $N_i-C\alpha_i-CO_i/C\alpha_i-CO_i-N_{i+1}$ atoms for the φ and ψ angles, respectively. The analysis of the distribution of φ and ψ angles during MD simulation indicates the presence of an extended structure with side chains exposed to the solvent environment Fig. 3B. However, referring to the description of P_{II} conformation given above, there is no indication of its presence during the simulation period. Thus, the model proposed by the previous researchers should rather be modified to the extended structure followed by a turn than those proposed *i.e.* P_{II} conformation followed by type-II β-turn (Rose *et al.,* 1985). It has to be stressed that further structural studies on the Lom-AKH-I peptide and other members of AKH/RPCH family by means of spectroscopic as well as simulation techniques are necessary to elucidate the "real" solution structure.

Adzhubei, A. A. and Sternberg, M. J. E. (1994), Conservation of polyproline-II helices in homologous proteins – implication for structure prediction by model-building. Protein Sci. **3**, 2395–2410.

Berendsen, H. J. C., Postma, J. P. M., van Gunsteren, W. F., DiNola, A. and Haak, J. R. (1984), Molecular dynamics with coupling to an external bath. J. Chem. Phys. **81**, 3684–3690.

Brünger, A. T. (1992), X-PLOR, Version 3.1:A System for X-ray Crystallography and NMR. The Howard Hughes Medical Institute and Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT.

Cusinato, O., Drake, A. F., Gäde, G. and Goldsworthy, G. J. (1997), The molecular conformation of representative arthropod adipokinetic peptides determined by circular dichroism spectroscopy. Insect Biochem. Mol. Biol. **28**, 43–50.

Gäde, G. (1996), The revolution in insect neuropeptides illustrated by the adipokinetic hormone/red pigment-concentrating hormone family of peptides. Z. Naturforsch. **51c**, 607–617.

Gäde, G. and Auerswald, L. (1998), Insect neuropeptides regulating substrate mobilisation. South Afr. J. Zool. **33**(2), 65–70.

Gäde, G., Hoffmann, K. H. and Spring, J. H. (1997), Hormonal regulation in insects: Facts, gaps, and future directions. Physiol. Rev. **77**, 963–1032.

Jorgensen, W. L., Chandrasekhar, J., Madura, J. D., Impey, R. W. and Klein, M. L. (1983), Comparison of simple potential for simulating liquid water. J. Chem. Phys. **79**, 926–935.

MacKerell, J., A. D., Bashford, D., Bellott, M., Dunbrack, J., R. L., Evanseck, J. D., Field, M. J., Fischer, S., Gao, J., Guo, H., Ha, S., Joseph-McCarthy, D., Kuchnir, L., Kuczera, K., Lau, F. T. K., Mattos, C.,

Ngo, T., Nguyen, D. T., Prodhom, B., Reiher, I., W. E., Roux, B., Schlenkrich, M., Smith, J. C., Stote, R., Straub, J., Watanabe, M., Wiorkiewicz-Kuczera, J., Yin, J. and Karplus, M. (1998), All-atom empirical potential for molecular modeling and dynamics studies of proteins. J.Phys.Chem. (B) **102**, 3586–3616.

Powell, M. J. D. (1964), An efficient method for finding the minimum of a function of several variables without calculating derivatives. Comp. J. **7**, 155–162.

Rose, G. D., Gierasch, L. M. and Smith, J. A. (1985), Turns in peptides and proteins. Adv. Prot. Chem., 1–109.

Ryckaert, J. P., Ciccotti, G. and Berendsen, H. J. C. (1977), Numerical integration of the cartesian equations of motion of a system with constraints: Molecular dynamics of n-alkanes. J. Comp. Phys. **23**, 327–341.

Ziegler, R., Jasensky, R. D. and Morimoto, H. (1995), Characterization of the adipokinetic hormone receptor from the fat body of Manduca sexta. Regul. Pept. **57**, 329–338.