Conformational Studies of the Emp-AKH Peptide Using Molecular and Langevin Dynamics Methods

Igor Zbigniew Zubrzycki

Biochemistry Laboratory, Institute of Medical Rehabilitation, Technical University of Opole, ul. Dzialkowa 4, 42–154 Opole, Poland

Z. Naturforsch. **53c,** 857–862 (1998); received April 29/June 12, 1998

Molecular Dynamics, Langevin Dynamics, Adipokinetic Hormones, Emp-AKH, 3D-Structure

The secondary structure of the member of the AKH/RPCH family has been studied by Molecular Dynamics and Langevin Dynamics methods. Molecular dynamics simulation were performed in vacuum, model aqueous solution and simulated membrane. Langevin dynamics simulation was performed using the friction factor γ equal to 2 ps^{-1}. Molecular dynamics as well as Langevin Dynamics simulation were conducted at 300 K. All minimum energy conformers have similar backbone structure characterised by the turn consisted out of 3 amino acids, Thr, Pro and Asn_7. Structures obtained from Molecular Dynamics simulation are characterised by the lack of hydrogen bonds whereas the structure obtained form Langevin Dynamics simulation is stabilised by the web of hydrogen bonds.

Introduction

Insects are the largest group of multicellular organisms inhabiting enormous area of our globe. One of the reason for such great spreading of insects is their ability to fly regulated by their metabolism. In this process triacylglycerols stored in body fat are metabolized to diacylglycerols which can be used as a fuel (Beenakkers *et al.,* 1985; Goldsworthy *et al.,* 1990a; Goldsworthy *et al.,* 1990b). This process is regulated through the use of AKH/RPCH (adipokinetic hormones/red pigment concentrating hormones) hormones. These hormones have been found in insects as well as in crustaceans. Until now, more than 30 sequences for AKH/RPCH hormones are known (Gäde *et al.*, 1997) with function and sequence homology. They are eight- to ten-mers, having amidated C-terminus and pyroglutamic acid at the N-terminus. All have highly conserved tryptophan residue at position eight and three hormones are so far known to have Phe at position 4 (Gäde *et al.*, 1997). With exception of three (Gäde 1990; 1992; Gäde *et al.*, 1997) all members of this family are uncharged. Recent studies suggest that these peptides interact with receptors located in the fat body (Ziegler *et al.,* 1995).

The Emp-AKH peptide, with the sequence pGlu-Val-Asn-Phe-Thr-Pro-Asn-TrpNH_2, from Praying mantis (Gäde, 1991), is synthesised and stored in neurosecretory organs (*corpora cardiaca*) as in other species of insects.

The use of structure prediction algorithms (Chou and Fasman, 1974; Stone *et al.,* 1978, Goldsworthy *et al.,* 1990a; Wheeler *et al.,* 1990) implies that this peptide should adopt a β-structure with a β-turn. So far direct structural studies (NMR, crystallography) had not been performed on this peptide or on any other member of its family in the environment mimicking physiological conditions. There have been performed NMR study (Zubrzycki and Gäde, 1994) on the Emp-AKH peptide in DMSO solution but the results obtained rather poorly refer to the real structure of this peptide. Recently circular dichroism CD (Cusinato *et al.,* 1998) study of the adipokinetic peptides has been performed giving some information concerning the structure of these peptides. Therefore, it is of a great interest what is the three dimensional detailed structure of these peptides. The present study employed molecular modelling techniques, molecular dynamic (MD) and Langevin Dynamics (LD) to survey the possible structural conformation of this peptide in different environ-

Reprint requests to Dr. Zubrzycki.
Fax: +48 77 556 080.
E-mail: izubrzycki@hotmail.com

0939–5075/98/0900–0857 $ 06.00

ments. MD simulation *in vacuo* was performed using the dielectric constant ε equal to 1, in membrane mimicking environment the dielectric constant was equal to 2, and in aqueous solution, dielectric constant was equal to 80 (Sewell *et al.*, 1995) following previously established methodology (Ilangovan and Ramamoorthy, 1998). For LD simulation friction factor γ was equal $2ps^{-1}$ allowing for the greatest isomerization rate during the dynamic run (Loncharis *et al.*, 1992).

Methods

Molecular Dynamics, Langevin Dynamics simulations and other computational procedures were performed on 100 MHz pentium Compatible computer using HyperChem 5.01 and ChemPlus programs. The linear model of the Emp-AKH peptide was built using intrinsic HyperChem procedure with φ, ψ and ω angles equal to 180°, corresponding to the fully extended structure. This conformation was subjected to the two step energy minimisation procedure. The first step consisted of Steepest Descend minimisation (100 steps) and the second of Polak-Ribiere conjugate gradient procedure run until maximum derivative was less than 0.0001 kcal/mol Å. The final structure was used further for Molecular Dynamics (MD) and Langevin Dynamics (LD) simulation.

MD simulation was performed under three different condition. *In vacuo* modelling was simulated by distance dependent dielectric constant ε equal to 1, membrane and aqueous environments were simulated by dielectric constants ε equal to 2 and 80, respectively. During all calculations ABMER force field was used.

The starting structure was equilibrated by running MD at 300K with a step of 1 fs. Data were collected from 20 ps run at 1 ps intervals along MD trajectory. These structures were minimised in two step fashion. The first step consisted of 100 cycles of steepest descent algorithm. The second of Polak-Ribiere algorithm run until the maximum derivative was smaller then 0.001 kcal/mol Å.

Langevin Dynamics was also performed at 300K, run time 20 ps with time step of 1 fs. The frictional coefficient γ was equal to 2 ps^{-1}. Twenty conformers were collected at 1 ps intervals along LD trajectory. All collected conformers were two step energy minimised as described above.

Results and Discussion

The energy of the starting extended linear structure of the Emp-AKH peptide was equal to 127811.5 kcal/mol Å. After initial energy minimisation, yielding the starting structure used further for unrestrained MD simulation and LD simulation, total energy was equal to 20.42 kcal/mol Å. The initial energy minimisation consisted of two steps as described in METHODS. The unrestrained molecular dynamics simulation was carried over 20 ps with 1 fs time step and 20 structures from each run, at 1 ps intervals, dielectric constant ε equal to 1, 2, and 80, were collected. These structures were energy minimised using the previously described two step procedure giving the set of 20 final structures from each simulation. The analysis of results obtained from *in vacuo* simulation shows the presence of two families of conformers. The first family is a group of conformers with the structure similar to the lowest energy conformer. This group consists of three structures and is characterised by a turn created by Thr, Pro, and Asn_7 residues. The second family is defined by much tighter turn. The average energy of the first group is on the order of 16 kcal/mol Å and the second of 20 kcal/mol Å. The RMS deviation for each family is equal to 3.4 Å and 4.0 Å. Fig. 1 shows the lowest energy conformer obtained from MD simulation, ε equal 1. The dihedral angles (φ, ψ, ω) were measured from the minimum energy conformer and are summarised in Table I. The analysis of the Table I shows that except pGlu residue all residues have negative φ values and positive ψ values. The

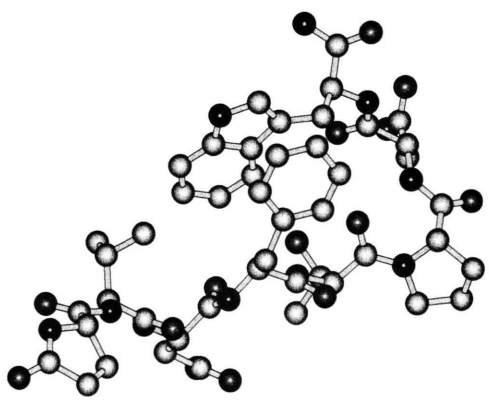

Fig. 1. Lowest energy conformer from *in vacuo* Molecular Dynamics simulation, ε equal 1.

Table I. Dihedral angles of the minimum energy conformer of Emp-AKH *in vacuo* obtained using molecular dynamics simulation.

Residue	φ	ψ	ω
pGlu	137.3	−172.8	177.9
Val	−157.0	160.3	175.1
Asn	−71.4	71.6	−177.4
Phe	−71.5	79.6	173.4
Thr	−62.0		179.2
Pro	−76.0	79.5	−178.3
Asn	−177.2	158.3	−166.5
$TrpNH_2$	−140.0		

analysis of Fig. 1 reveals the turn within the structure of the lowest energy conformer. However, turn angles cannot be classified as any of known β-turns. The analysis of this structure also shows that there are not any hydrogen bonding under given bond conditions i.e. the distance between the donor hydrogen and the acceptor is lower than 3.3 Å and angle made by covalent bonds to the donor and acceptor atoms is greater than 150 degrees.

In order to perform structural simulation in model membrane environment the dielectric constant ε was set to 2. It has been shown that such dielectric condition mimics well the membrane environment for peptides and proteins (Tobias *et al.*, 1993). The initial structure with the energy of 20.4 kcal/mol Å was subjected to the unrestrained MD at 300K over the period of 20 ps with time step of 1 fs. The molecular dynamics trajectory was sampled with 1 ps intervals giving 20 structures subjected to the two step energy minimisation procedure. The obtained minimum energy conformer had an energy of 13.43 kcal/mol Å. In membrane mimicking environment Emp-AKH peptide also forms structure having a turn. In this turn involved are Thr, Pro, and Asn_7 residues. It has been observed that the planes of aromatic rings of Phe and Trp create the angle of 90 degrees whereas the lowest energy conformer obtained "in vacuo" has aromatic rings of Phe and Trp in planar arrangement. The distance between them is on the order of 4 Å. Similar as for *in vacuo* simulation there are no hydrogen bonds. Twenty final structures can be divided into two main groups of conformers. These groups differ mainly in the turn area created by Thr, Pro, and Asn_7 residues. The lowest energy conformer is shown in Fig. 2 and its dihedral angles are summarised in Table II. The comparison of Tables I and II shows that there changes in ω angles whereas φ and ψ values are have nearly constant values. It can also be observed that all φ angles have negative values except pGlu residue and all ψ angles have positive values except pGlu residue. Similar observation was made for the conformer obtained for *in vacuo* MD simulation. The minimum energy conformer obtained in membrane mimicking environment has the energy lower of 1.09 kcal/mol Å than that obtained for *in vacuo* conditions.

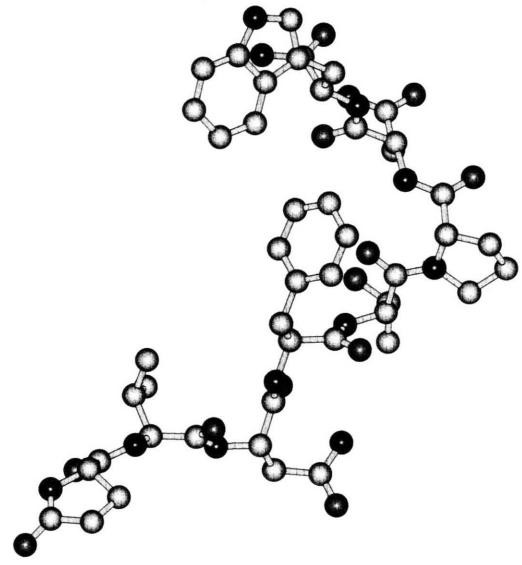

Fig. 2. Lowest energy conformer from molecular dynamics simulation with ε equal 2 (membrane mimicking simulation).

In order to study the solvent effect on the conformational behaviour of Emp-AKH peptide un-

Table II. Dihedral angles of the minimum energy conformer of Emp-AKH in model membrane obtained using molecular dynamics simulation.

Residue	φ	ψ	ω
pGlu	137.0	−176.2	178.8
Val	−153.8	152.3	174.9
Asn	−74.3	80.9	177.0
Phe	−68.4	104.2	163.8
Thr	−65.9		−176.7
Pro	−72.5	84.6	−179.0
Asn	−154.5	144.7	−179.9
$TrpNH_2$	−67.0		

restrained MD study using the dielectric constant ε equal to 80 was performed. The analysis of the lowest energy conformer, Fig. 3, shows that this structure also has a turn created by Thr, Pro, and Asn_7 residues. However, this turn is much tighter then those observed for the structures obtained for *in vacuo* and membrane mimicking environment simulations. Similar as for *in vacuo* simulation the aromatic rings of Phe, and Trp residues are in planar position with the average distance between them of 4 Å. Such alignment may stabilise the structure of this peptide since there are not observed any hydrogen bonds observed under these conditions. The dihedral angles of the lowest energy conformer are summarised in Table III. The analysis of the Table III shows that all φ dihedral angles adopt negative values except pGlu residue and all ψ angles have positive values except pGlu residue. The analysis of φ angles also show drastic decrease of its values and the increment of ψ values. The ω dihedral angles are generally on the same order as observed in Tables I and II.

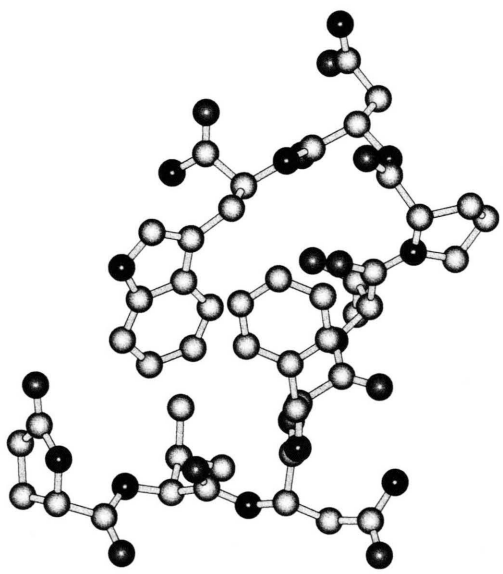

Fig. 3. Lowest energy conformer obtained from molecular dynamics simulation with ε equal to 80 (aqueous environment mimicking simulation).

The influence of solvent molecules can be simulated by the change of dielectric constant, introduction solvent molecules into dynamic simulation or by introduction of the factor simulating molecular collision. The last one can be solved by the use of Langevin Dynamics (LD) adding a frictional force γ to each atom at each time step of the run. The γ factor is a friction coefficient of the solvent and is expressed in ps^{-1}.

The initial structure obtained after two step energy minimisation was submitted to the unrestrained Langevin Dynamics with the γ factor equal to 2 ps^{-1}. It has been previously shown that this value maximises the isomerization rate. The dynamics was run for 20 ps at 300K with time step of 1 fs. Along the dynamics trajectory 20 conformers were collected with time interval of 1 ps and submitted to the two step energy minimisation. The lowest energy conformer was analysed in respect to its dihedral angles, carbon distances and hydrogen bonding. Dihedral angles are summarised in Table IV. The analysis of the Table IV shows that all φ angles adopt negative values except pGlu and Asn_3 residue and all ψ angles adopt positive values except pGlu residue. These results are similar to those observed in Tables I, II and III with the exception of Asn_3 residue having here positive value. The lowest energy conformer is shown in Fig. 4.

Table III. Dihedral angles of the minimum energy conformer of Emp-AKH in model aqueous solution obtained using molecular dynamics simulation.

Residue	φ	ψ	ω
pGlu	108.3	−57.0	177.0
Val	−68.9	161.7	177.9
Asn	−50.7	133.6	−172.6
Phe	−67.3	107.6	169.8
Thr	−78.6		176.7
Pro	−61.5	75.4	169.7
Asn	−82.4	150.6	−177.0
$TrpNH_2$	−75.8		

Table IV. Dihedral angles of the minimum energy conformer of Emp-AKH *in vacuo* obtained using Langevin dynamics simulation.

Residue	φ	ψ	ω
pGlu	130.5	−158.4	−169.5
Val	−76.4	51.6	−169.6
Asn	162.5	139.2	168.8
Phe	−80.8	92.4	172.0
Thr	−62.4		−174.2
Pro	−41.24	140.9	156.9
Asn	−103.2	147.5	177.1
$TrpNH_2$	−57.0		

Fig. 4. Lowest energy conformer from Langevin dynamics simulation with friction factor γ equal $2ps^{-1}$.

The analysis of this conformer reveals that it is stabilised by the web of hydrogen bonding between the following residues: pGlu C=0 and H_2N-Trp, pGlu side chain C=0 and HN-Thr, pGlu C=0 and H_2N-Asn_7, Trp C=0 and H_2N-Asn_7. It should be pointed out that hydrogen bonds are not present in conformers obtained by unrestrained molecular dynamics simulation. The orientation of aromatic rings of Phe and Trp are similar to that observed for conformer obtained from MD simulation using dielectric constant equal to 2 i.e. membrane mimicking environment.

Since, the analysis of intercarbon distances can be very useful for defining and predicting secondary structure of the peptide and for comparing the structures of peptide, all intercarbon distances from four final conformers were collected in Table V summarising all α_i, α_{i+1} and α_i, α_{i+2} distances.

The differences in the backbone arrangement between the final conformers is shown in Fig. 5. This comparison shows clearly that all conformers have the turn within its structure created by Thr, Pro, and Asn_7 residues. The first four residues of the molecule create a sort of "tail". The LD lowest energy conformer has pGlu residue turn into the pocket created by the amino acids taking part in turn.

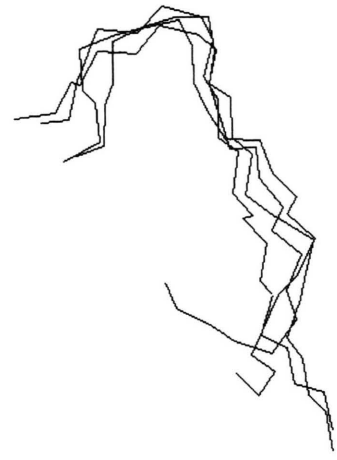

Fig. 5. Superposition of the lowest energy conformers obtained from molecular dynamics and Langevin dynamics simulations.

Generally there are great differences in positioning of aromatic ring of Phe and Trp between conformers. There is, however, always aromatic plane build out of aromatic rings of Thr and Trp residues.

Conclusions

Unrestrained molecular dynamics and Langevin dynamics were carried out to analyse conforma-

Table V. Comparison of intercarbon distances in A from the minimum energy conformers of the Emp-AKH peptide obtained using molecular dynamics simulations *in vacuo*, membrane, aqueous environment, and Langevin dynamics.

	in vacuo		Delectric constant ε=2		Dielectric constant ε=80		Langevin Dynamics	
	$C_{\alpha i,i+1}$	$C_i,C_{\alpha i+2}$	$C_{\alpha i,i+1}$	$C_i,C_{\alpha i+2}$	$C\alpha_{i,i+1}$	$C_i,C_{\alpha i+2}$	$C_{\alpha i,i+1}$	$C_i,C_{\alpha i+2}$
pGlu	3.83	7.12	3.82	7.11	3.83	6.72	3.83	4.99
Val	3.83	5.42	3.82	5.61	3.84	6.27	3.85	7.05
Asn	3.82	5.76	3.81	6.12	3.81	6.26	3.80	5.75
Phe	3.81	6.52	3.79	6.26	3.81	6.51	3.81	6.41
Thr	3.86	5.43	3.85	5.66	3.85	5.31	3.88	5.28
Pro	3.84	6.18	3.83	6.38	3.82	5.45	3.78	6.28
Asn	3.80		3.81		3.82		3.82	
$TrpNH_2$								

tional properties of Emp-AKH peptide in various environments. From the detailed MD studies in vacuo it can be seen that Emp-AKH peptide forms a structure having a turn created by Thr, Pro, and Asn_7 residues. Similar conformations were observed for membrane mimicking simulations and aqueous environment simulations. The first four residues create a structure which remain the extended structure. Overall MD simulations results are in agreement with the results reported by other researches (Cusinato *et al.*, 1998). The turn was also observed for Langevin Dynamics simulation using the friction factor γ equal to $2ps^{-1}$. The main differences are observed in the tail region where LD produced much more compact structure with pGlu pointing toward the pocket created by the turn and stabilised by hydrogen bonding.

Beenakkers A. M., Bloemen R. E., De Vlieger T. A., Van der Horst D. J. and Van Marrewijk W. J. (1985), Insect adipokinetic hormones. Peptides **6**(3), 437–444.

Chou P. Y. and Fasman G. D. (1974), Prediction of protein conformation. Biochemistry **13,** 222–245.

Cusinato O., Drake A. F., Gäde G. and Goldsworthy G. J. (1998), The molecular conformation of representative arthropod adipokinetic peptides determined by circular dichroism spectroscopy. Insect Biochem. Mol. Biol. **28**, 43–50.

Gäde G. (1990), The adipokinetic hormone red pigment-concentrating hormone peptide family-structures, interrelationships and functions. J. Insect Physiol. **36**, 1–12.

Gäde G (1991), The adipokinetic neuropeptide of Mantodea. Sequence elucidation and evolutionary relationships. Biol. Chem Hoppe Seyler **372**, 183–201.

Gäde G. (1992), The hormonal integration of insect flight metabolism. Zool. Jb. Physiol **96,** 211–225.

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

Goldsworthy G. J. (1983), The endocrine control of flight metabolism in locust. Adv. Insect Physiol. **17,** 149–204.

Goldsworthy G. J., Wheeler C. H., Cusinato O. and Wilmot C. M. (1990b), Adopokinetic hormones: structures and functions. Prog. Clin. Biol. Res. **342,** 28–34.

Ilangovan U. and Ramamoorthy A. (1998), Conformational studies of human islet amyloid peptide using molecular dynamics and simulated annealing methods. Biopolymers **45,** 9–20.

Loncharis R. J., Brooks B. R. and Pastor R. W. (1992), Langevin dynamics of peptides: the frictional dependance of isomerisation rates of N-acetylalanyl-N'-methylamide. Biopolymers **32**, 523–535.

Sewell J. C., Duve H., Thorpe A. and Altman J. A. (1995), Conformational preferences of the calliFMRFamides and their free-acid analogues. J. Biomol. Struc. Dynam. **13,** 181–199.

Stone J. V. Mordue W., Broomfield C. E. and Hardy P. M. (1978), Structure-activity relationships for the lipid-mobilising action of a series of hormone analogues. Eur. J. Biochem. **89,** 195–202.

Tobias D. J., Klein M. L. and Opella S. J. (1993), Molecular dynamics simulation of Pf1 coat protein. Biophys J. **64,** 670–675.

Wheeler C. H., Drake A. T., Wilmot C.M, Thornton J. M. and Goldsworthy G. J. (1990), Structures in the AKH family of neuropeptides. In: Insect Neurochemistry and Neurophysiopolgy (A. B. Borkovec and D. B. Gelman.Eds.). Humana Press, Clifton, New Jersey, pp 235–238.

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

Zubrzycki I. Z. and Gäde G. (1994) Conformational study on an insect neuropeptide of the AKH-RPCH family by combination of ^1H NMR and molecular mechanics. Biochem. Biophys. Res. Commun. **198,** 228–237.