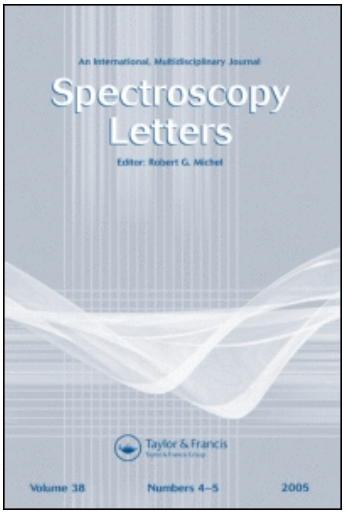

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NMR Study of Insect Adipokinetic Hormones

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NMR STUDY OF INSECT ADIPOKINETIC HORMONES

Keywords: AKH peptides, Mem-CC, [N7]-Mem-CC, Tem-HrTH, Del-CC.

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ABSTRACT

Mem-CC (pGlu-Leu-Asn-Tyr-Ser-Pro-Asp-Trp-NH_2), Tem-HrTH (pGlu-Leu-Asn-Phe-Ser-Pro-Asn-Trp-NH_2) and Del-CC (pGlu-Leu-Asn-Phe-Ser-Pro-Asn-Trp-Gly-Asn-NH_2) are adipokinetic hormones, isolated from the corpora cardiaca of different insect species. These hormones regulate energy metabolism during flight and so are intimately involved in an insect's mobility. In an attempt to measure the solution conformations of these peptides, their ^1H nmr spectra have been recorded in dimethylsulfoxide solution. The N7 analogue, [N7]-Mem-CC (pGlu-Leu-Asn-Tyr-Ser-Pro-Asn-Trp-NH_2) has also been studied. The solution dynamics of Tem-HrTH has been simulated in dmso and in a water droplet. These results have been correlated with measured coupling constants. The

molecule was found to have a β-turn at proline with asparagine (N7), which is postulated to be involved in receptor binding and/or activation, projecting outward from the turn.

INTRODUCTION

Insects form one of the largest groups of multi-cellular organisms inhabiting our planet. They are widely distributed and generally highly mobile. Important in this regard is their ability to fly. Insect flight metabolism is under hormonal control via the so-called AKH (adipokinetic hormones). These hormones regulate the hydrolysis of fat stores in the form of triacylglycerides to diacylglycerides and their subsequent transport to muscle cells. They are also involved in the regulation of carbohydrate and proline metabolism. More than 30 different AKH peptides have now been fully chemically characterised[1,2]. Distinguishing features of these hormones include that they are all octa-, nona- or decapeptides with blocked N- (pyroglutamate) and C-(amidate) termini, and that they display a high degree of homology.

Recently, the neurohormones Del-CC and Tem-HrTH have been isolated from the corpora cardiaca of the blister beetle species, *Decaptoma lunata* [3]. Previously, Tem-HrTH was isolated from the corpora cardiaca of the tenebrionid beetles, *Tenebrio molitor* and *Zophobas rugipes*[4] and of the cockroach *Polyphaga aegyptiaca*[5]. A related neuropeptide, Mem-CC has been isolated from the corpora cardiaca of various beetles of the large superfamily Scarabaeoideae, *Melolontha melolontha*, *Geotrupes stercorosus, Pachnoda marginata* and *Pachnoda sinuata*[2]. The primary sequences of these peptides are shown in Figure 1. Mem-CC is quite unique for this family in that it is one of the few family members with an acidic aspartate at position 7 and a tyrosine residue at position 4 instead of the typical phenylalanine.

Recently we have shown[6] with the gonadotropin releasing hormones, which are structurally quite similar to the AKH hormones, that there is a correlation between the preferred structure of the peptide in DMSO solution and

NEUROHORMONE	SEQUENCE
Tem-HrTH :	pE - L - N - **F4** - S - P - **N7** - W - NH_2
[N7]-Mem-CC:	pE - L - N - **Y4** - S - P - **N7** - W - NH_2
Mem-CC:	pE - L - N - **Y4** - S - P - **D7** - W - NH_2
Del-CC	pE - L - N - **F4** - S - P - **N7** - W - G - N - NH_2
Emp-AKH	pE - V - N - **F4** - T - P - **N7** - W - NH_2

FIG. 1. Primary sequence of some adipokinetic hormones.

its ability to bind to its receptor. Tsikaris *et al.*[7] have determined the mAbSRYD-bound structure of IASRYDQL in water and the free octapeptide structure in DMSO and found them to be very similar. Since AKH hormone receptors have not been isolated and are only poorly characterized[8], a study of the solution conformation of these peptides is of interest. CD studies indicate that the peptides do not have a preferred conformation in aqueous solution[9]. We have therefore used the success of Tsikaris[7,10-13] and others[14,15] to study these peptides in DMSO-d^6 solution using nmr and molecular dynamics. AKH peptides have been studied before with some success using nmr and computational techniques[16-18].

MATERIALS AND METHODS

Sample Preparation. — The neuropeptides were synthesised by Dr R. Kellner of Merck KGaA, Darmstadt, Germany. This was achieved using the standard protocol for solid phase peptide synthesis employing Fmoc-amino acid chemistry. The synthetic peptides were taken up in acetonitrile/water solution and purified using HPLC in the presence of 0.1 % TFA as solvent. The samples were then dried and weighed.

NMR experiments. — All NMR experiments were conducted at 298K on a Varian Unity 400 MHz spectrometer equipped with a 5mm inverse detection probe. The hydrophobic nature of the peptides rendered them poorly soluble in aqueous

solution, so all studies were conducted in DMSO-d_6 solution. The neuropeptides were dissolved in ~0.75ml of DMSO-d_6 (99.9 atom % D, Aldrich Chemical Company) yielding an approximately 4mM solution. Proton chemical shifts were referenced internally to DMSO-d_6 at 2.49ppm. No saturation of the residual water signal was employed in 2D spectral acquisition. All data processing and integration was conducted on a Sun data workstation employing VNMR software (Varian Associates). All phase sensitive 2D spectra were acquired according to the method of States *et al.*[19]. One dimensional ^{1}H spectra were recorded with a spectral width of 4284.9Hz and 32K data points with a digital resolution of 0.267Hz per point. 64 scans were acquired and exponential multiplication of the data, when processed, produced line broadening of 0.2Hz. Absolute value type COSY spectra[20,21] were recorded with the spectral width in both domains set to 4284.9Hz. 512 t_1 values of 32 transients each were recorded and zero filled to 2K x 1K. Phase sensitive TOCSY experiments[22,23] were recorded utilising a total MLEV spin lock of 80ms. 256 t_1 values of 32 scans were acquired. The data were processed by zero filling as a 2K x 1K matrix with application of sinebell and shifted sinebell window functions. Phase sensitive 2D NOESY spectra[24] were acquired with a mixing period of 200ms. 512 t_1 values of 32 transients were recorded and zero filled to 2K x 2K with the use of the sinebell and shifted sinebell window functions.

$^3J_{N\alpha}$ could be measured directly from the 1D spectra in most cases, however, where there was spectral overlap, J-resolved spectra were recorded.

Molecular modelling - Calculations were performed on a Silicon Graphics Indigo workstation using Molecular Simulation Incorporated (MSI)[25] software. InsightII was used as the visualization platform and a random structure of Tem-HrTH generated automatically using the Builder module. This was subjected to a short conjugate gradient minimization. Molecular dynamic calculations were performed using Discover3 at 298K using an *NVT* ensamble. Typically, dynamics were initialized for 1 psec and then dynamics resumed for a further 0.1-2 ns. To simulate the effect of DMSO, a dielectric constant of 4 was used.

For simulations in water, the peptide was soaked in a 17 Å sphere of equilibrated water (1500 water molecules). During the dynamics the rattle command was used to constrain the bond angles and lengths of the solvent molecules. Step lengths of 2 fs were used and structures archived every 2 ps.

$^3J_{NH}$ coupling constants were calculated using Bystrov's Karplus equation[26,27]:

$$^3J_{N\alpha} = \langle 6.4 \cos^2\theta - 1.4 \cos\theta + 1.9 \rangle \quad (2)$$

where $\theta = | \phi - 60° |$, ϕ is the peptide backbone dihedral angle and $\langle .... \rangle$ denotes averaging over a trajectory.

RESULTS

The ^{1}H NMR assignments for all four peptides were achieved using 2D COSY, TOCSY and NOESY spectra and the sequential assignment procedure of Koradi *et al.*[28]. In the assignment of MemCC, the spin systems L2, S5, pE1 and P6 were identified via their spin connectivity patterns. The ^{1}H resonances of leucine were easily assigned as result of the characteristic low frequency chemical shift of the δ methyl protons. Connectivity exists between these protons and the β and γ protons of the spin system was seen in the TOCSY spectrum (Figure 2). A poorly resolved triplet was observed at 4.99 ppm which is assigned to the hydroxyl group of serine. Connectivity was seen between this proton and the α and β protons of serine. P6 was identified from its connectivity to β, γ and δ protons and the absence of an amide backbone proton.

Sequential assignments in the NOESY spectra identify a $d_{\alpha N}$ connectivity between pE1 and L2. Additional sequential assignments in the NOESY spectra which assisted in clarifying the spin coupling networks include the $d_{\alpha N}$ connectivity between the α protons of L2 and the amide proton of N3. A $d_{\alpha N}$ connectivity between Y4 α_H and the serine amide proton was also seen. A final list of the chemical shifts and the ^{1}H NMR assignments are given in Table 1, while the $^3J_{N\alpha}$ coupling constants are given in Table 2.

Figure 3 shows the variation of torsion angle ϕ with time obtained from a dynamic simulation of Tem-HrTH in DMSO (dielectric constant of 4). Results

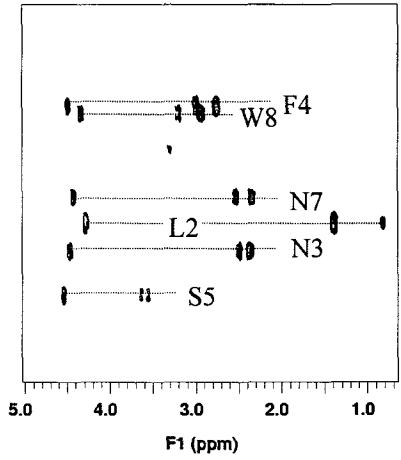

FIG. 2. Expanded region of the TOCSY spectrum of Tem-HrTH.

for a dynamics run of Tem-HrTH in a water droplet are shown in the form of a Ramachandran plot[29] of the ϕ and ψ torsion angles in Figure 4. The areas enclosed by the solid lined shapes represent regions in which no steric hindrance exists, while the areas enclosed by the dashed lines are regions in which steric clashing occurs but for which real molecular structures have been found. A probability distribution of the ϕ angles obtained from the water trajectory is given in Figure 5. Cluster analysis of this trajectory gave only one major family of structures. Three views of a low energy representative structure from this family are shown in Figure 6. Animation of the trajectory file showed that, after minimization and dynamics equilibration, while there was substantial movement of the peptide side chains, there was only limited motion in the backbone.

DISCUSSION

For all four peptides, residues 3 and 4, and residues 5 and 8 show inter-residue $d_{NN}(i,i+1)$ and $d_{NN}(i,i+3)$ nOe contacts which suggest the existence of some secondary structure at least for these last five residues. However, only

sequential $d_{\alpha N}$ inter-residue contacts are present for the first three residues, suggesting that these residues exists mainly in a random coil or "extended" form. These observations are consistent with CD studies on *Locusta migratoria* neuropeptides containing a proline residue where a β structure is proposed[9].

In simulation dynamic studies it is important to establish that the system has equilibrated before data collection is started. This is not always straight forward especially for peptides, which are intrinsically quite flexible. The most commonly used method is to monitor the kinetic and potential energy of the system with the assumption that equilibrium has been reached when these energies have stabilized. For simulations in vacuo or when a dielectric constant is used to mimic a solvent, equilibrium is generally established relatively quickly. However, if the peptide is soaked in an explicit solvent like water, the presence of the solvent molecules dampens the motion of the peptide making it take much longer to reach equilibrium and to sample conformational space. This is a double-edged sword because not only does the dynamics need to be run for longer, but each calculation step takes longer. It is for this reason that the affect of solvent in dynamic simulations is often modeled using a dielectric constant. In the present study, DMSO was modeled using a dielectric constant of 4. In these runs, the kinetic and potential energy stabilized within 100 fs and an equilibration time of 1 ps was used. Data were then collected for a further 2 ns. A plot of dihedral angle ϕ as a function of time (Figure 3) shows that there was considerable motion of the peptide backbone. As expected, this motion was more pronounced at the termini of the peptide and more restrained on either side of the proline residue. From this simulation, averages $^3J_{NH}$ coupling constants, were calculated and are compared with experimental values in Table 3 where the standard deviation in the simulated coupling constants reflects the variability of the dihedral angle. The agreement between the calculated and observed coupling constants is reasonable given the error in the measurements. However, it should be noted that there is quite a large (1.4 Hz) difference between the measured ϕ of Asn7 and that averaged from the water simulation. There is much better agreement with the DMSO simulation (0.3 Hz).

TABLE 1.
^{1}H Chemical Shifts (ppm) for Tem-HrTH,.Del-CC, Mem-CC and Mem-Anal in DMSO-d^6 Solution.

Residue	αH	NH	βCH$_2$	Other
		Tem-HrTH		
pE1	4.07	7.75	1.90,2.19	γCH$_2$ 2.03,2.07
L2	4.30	8.05	1.38,1.38	γCH 1.55
				δCH$_3$ 0.82
N3	4.45	8.12	2.48,2.35	
F4	4.48	7.81	2.75,2.99	
S5	4.55	8.22	3.55,3.65	OH 4.95
P6	4.30	-	1.93,1.69	γCH$_2$.1.89,1.76
				δCH$_2$ 3.58, 3.58
N7	4.40	8.01	2.52,2.35	
W8	4.35	7.83	3.20,2.90	NH 10.69
		Mem-CC		
<E1	4.03	7.73	1.94,2.20	γC<u>H$_2$</u> -2.05, 2.10
L2	4.30	8.07	1.42,1.42	γC<u>H</u>- 1.58,
				δCH$_3$ 0.82,0.86
N3	4.47	8.13	2.49,2.38	γN<u>H$_2$</u> - 6.8-7.6
Y4	4.41	7.69	2.85,2.67	O<u>H</u> - 9.1
S5	4.54	8.12	3.59, 3.59	O<u>H</u> - 4.99
P6	4.31	-	1.98, 1.74	γC<u>H$_2$</u>.1.75,1.78
				δC<u>H$_2$</u> -3.56
D7	4.45	8.03	2.65, 2.44	-
W8	4.35	7.63	3.28, 2.97	N<u>H</u> - 10.69

TABLE 1. Continued.

Residue	αH	NH	βCH$_2$	Other
			Del-CC	
PE1	4.03	7.75	1.88,2.21	γCH$_2$ 2.02,2.05
L2	4.29	8.05	1.40	γCH 1.57 δCH$_3$ 0.85, 0.83
N3	4.46	8.10	2.38,2.47	
F4	4.48	7.80	2.75,2.98	
S5	4.52	8.20	3.52,3.61	OH 4.58
P6	4.34		1.93,1.72	γCH$_2$ 1.89,1.76 δCH$_2$ 3.58
N7	4.50	8.00	2.39,2.53	
W8	4.41	7.97	2.90,3.15	NH 10.76
G9	3.57,3.73	8.27		
N10	4.49	7.93	2.98,3.18	
			Mem-Anal	
<E1	4.04	7.73	1.93, 2.20	γC<u>H$_2$</u> 2.07, 2.09
L2	4.29	8.06	1.42, 1.42	γC<u>H</u> 1.56 δCH$_3$ 0.86, 0.83
N3	4.47	8.13	2.49, 2.37	γN<u>H$_2$</u> 6.8-7.6
Y4	4.40	7.67	2.84, 2.67	O<u>H</u> 9.10, 2,6H 6.6 3,5H 6.95
S5	4.54	8.15	3.61, 3.53	O<u>H</u> 4.94
P6	4.30	-	1.95, 1.71	γC<u>H$_2$</u> 1.78 δC<u>H$_2$</u> 3.56
N7	4.43	7.98	2.53, 2.34	γNH$_2$ 6.8-7.6
W8	4.34	7.80	3.19, 2.94	N<u>H</u> 10.71, 4H 7.55 5H 6.97, 6H 70.4 7H 7.31, 2H 7.11

TABLE 2.

$^3J_{NH}$ coupling (Hz) as derived from ^1H and HOM2DJ spectra.

Residue	Mem-CC	Mem-Anal	Tem-HrTH	Del-CC
L2	8.34	8.10	8.10	8.39
N3	8.06	7.32	7.85	7.20
Y4	8.07	8.11	-	-
F4	-	-	8.11	7.99
S5	7.78	7.75	7.32	7.20
D7	7.64	-	-	-
N7	-	7.59	7.85	6.55
W8	8.34	8.11	7.88	7.89
G9	-	-	-	5.60
N10	-	-	-	8.39

Figure 4 is a Ramachandran plot of ϕ and φ for a 0.2ns simulation of Tem-HrTH in a water droplet. These results show that, in water, the peptide is less flexible, or its motion is more damped. The results are interesting in that they show that the terminal residues, leucine and tryptophan, fall in the ϕ,φ region of no steric clashing. Serine, and to a lesser extent phenylalanine, fall outside this region. Instead, their backbone dihedral angles fall in a region of the Ramachandran plot populated by known molecular structures but for which some steric hindrance exists. These structures must therefore be stabilized by some new interaction, most likely hydrogen bonding. It is also interesting to note that the ϕ,φ angles of Asn7 and Asn4 fall in two different regions of the plot. A similar plot is also found for the dynamic simulation in DMSO except that there is more scatter in the data points.

Figure 5 is a probability distribution of dihedral angle ϕ for Tem-HrTH in water. Again the results show that the two asparagine residues have different ϕ

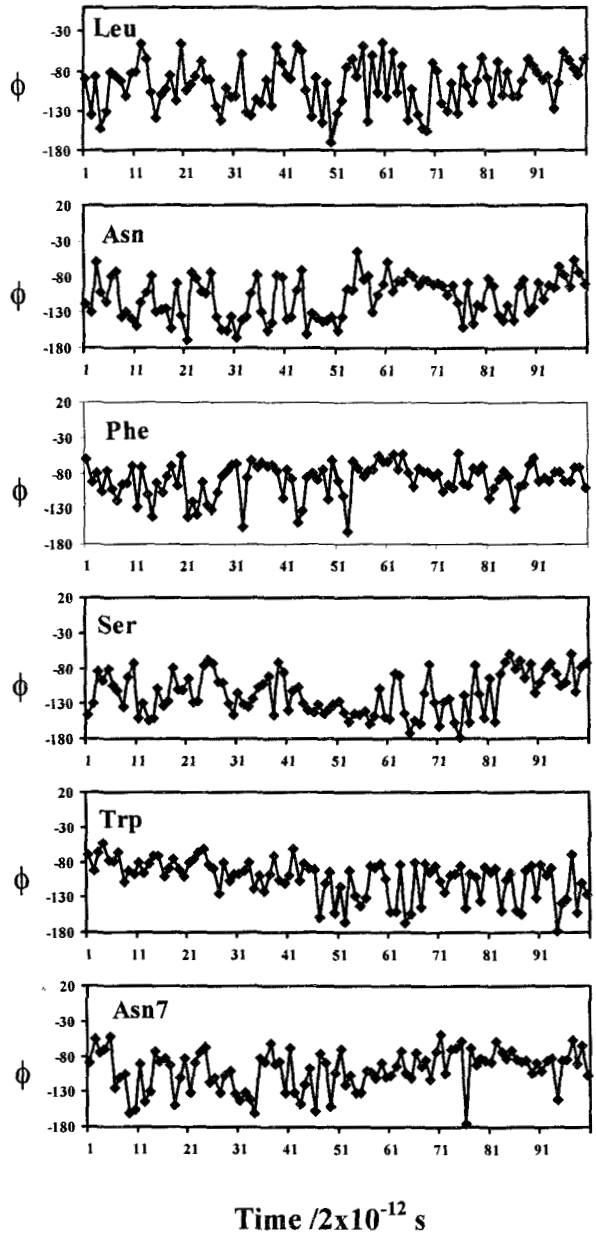

FIG. 3. Variation of dihedral angle ϕ during a 2 ns simulation of Tem-HrTH in DMSO.

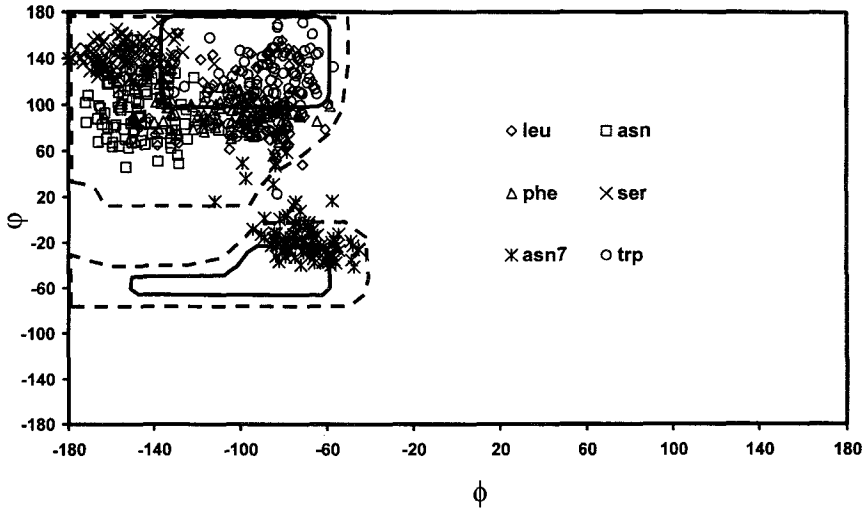

FIG. 4. Ramachandran plot of ϕ and φ dihedral angles measured during a 0.1 ns simulation of Tem-HrTH in water. The solid lines enclose regions of ϕ,φ space for which there is no steric hinderance, while the areas enclosed by dashed lines are areas within which real structures have been found.

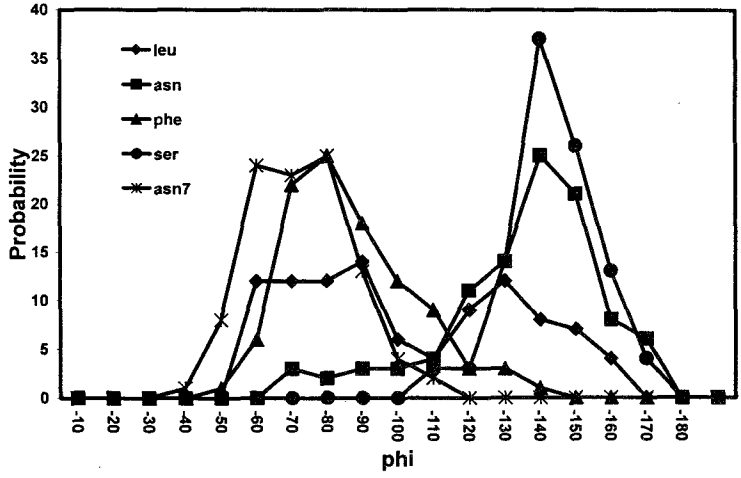

FIG. 5. Distribution of ϕ angles measured from 100 structures of Tem-HrTH collected during a 200 ps simulation in water.

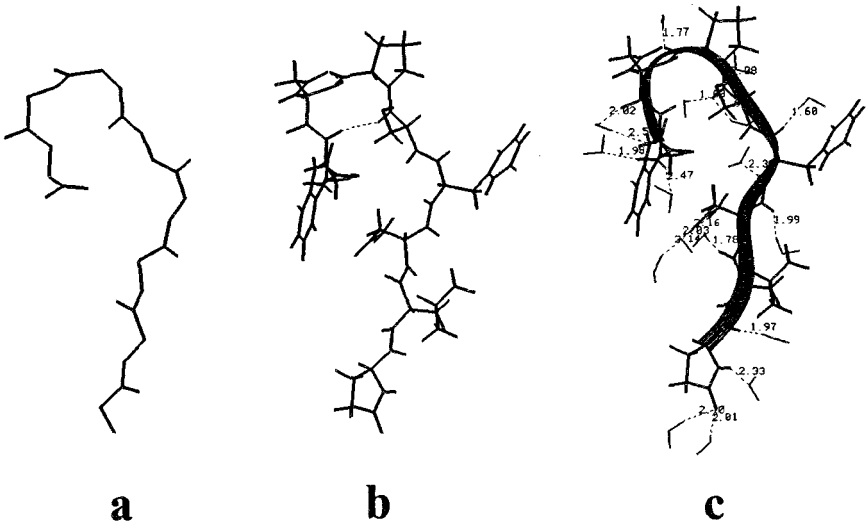

FIG. 6. Three views of the lowest energy structure obtained during a simulation of Tem-HrTH in water.

TABLE 3.

Homonuclear coupling constants $^3J_{NH}$ (Hz) for Tem-HrTH measured and calculated from dynamic simulations in water and DMSO. Standard deviations are given in parentheses.

	Leu	Asn	Phe	Ser	Asn7	Trp
exptl	8.10	7.85	8.11	7.32	7.85	7.85
water	7.73(1.59)	8.02(1.29)	7.65(1.45)	7.86(1.20)	6.40(1.62)	8.34(1.37)
DMSO	7.47(2.09)	7.99(1.50)	7.07(1.81)	8.06(1.48)	7.56(1.76)	7.78(1.43)

angles, Asn7 being centered around -70° while Asn3 is at -150°. Ser5, which is next to the proline residue, has a narrow distribution, while the terminal leucine has a very broad distribution.

Figure 6 shows three views of the lowest energy structure found in the water dynamic simulation. In Figure 6(b) a hydrogen bond is indicated between

Trp(NH) and Ser(CO). This hydrogen bond is transient, being continually formed and broken as the molecule moves. At the same time, new hydrogen bonds are formed and broken. In total, eight transient hydrogen bond contacts are seen. Four interactions involve Serine and 3 involve Tryptophan. Interactions between S5(CO) and W8(NH), S5(NH) and W8(CO), N7(NH) and S5(CO), and the ring proton of W8 and L2(CO) are all seen at different times during the dynamics simulation. At the same time, some 15 water molecules hydrogen bond to the peptide. In this particular view (Figure 6(c)), there are no bridging water molecules, but in other structures, inter-residue, bridging waters are seen. The participation of explicit solvent molecules in stabilizing the secondary structure of peptides highlights the need to include them in the simulation.

Wilmot and Thornton[30], in their study of β type turns, have defined a β turn as existing when the distance between the $C_\alpha(i)$ and C_α $(i+3)$ residue does not exceed 7Å. In addition, a turn is considered to be four residues long with the possibility of an $i,i+3$ {C=O (main chain) to NH} hydrogen bond. Using these criteria, the dominant conformation of Tem-HrTH has a β-turn between residues 4 – 8. This is not surprising as this peptide has the amino acid proline, which is known to favour the formation of β-turns of types I and II. In fact, Phe-Ser-Pro-Asn segment of Tem-HrTH, satisfies the inter-proton distances requirement $d_{\alpha N(1,4)}$ (3.1-4.2 Å) and $d_{NN(2,4)}$ (3.8 Å) for turn type I[31].

The existence of a β turn in the dominant conformers of Tem-HrTH is consistent with previous CD[9,32], nmr[16,17] and computational[18] studies on AKH peptides. This characteristic β structure at the C terminal end of the peptides has been postulated to be important in the interaction of the hormones with their putative receptors[17,32]. Sato *et al.*[32] have incorporated a β-turn in synthetic analogues of hypertrehalosemic hormones. Only those analogues which had a β type II turn were found to be biologically active. In contrast with previous studies, however, the present results provide no evidence for a β sheet[16] or P II[18] conformation as found for the first five residues of another AKH member, Emp-AKH. The first four residues conform to the definition of "extended structure" as

proposed by Siligardi and Drake[31] for small linear peptides and as denoted for single peptide chains whose ϕ,φ dihedral angles are located in the allowed β region of the Ramachandran plot[29]. Also, no evidence was found for an extended P_{II} conformation as recently proposed by Cusinato *et al.*[9] for *Locusta migratoria* AKH peptides.

It is interesting to note that, in the lowest energy structure, the Asn7 side chain of Tem-HrTH projects outwards from the β-turn. The importance of an asparagine residue at position 7 has been noted in structural studies on Emp-AKH, where it has been postulated to assist peptide-receptor interactions via its hydrophilic nature[31].

CONCLUSION

In summary, the nmr of four insect neuropeptides have been studied in DMSO-d_6 solution. For Tem-HrTH the results have been rationalised by molecular dynamics calculations performed in DMSO and water. The results indicate that Tem-HrTH is flexible in solution, but that the backbone has a β-turn between residues 4 – 8. In water, this structure is stabilised by transient inter-residue hydrogen bonding and by interactions with explicit water molecules.

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REFERENCES

1. Gäde G., Z. Naturforsch. 1996; 51c: 607.
2. Gäde G., Hoffman K.H. and Spring J.H., Physiol. Reviews. 1997; **77**: 963.
3. Gäde G., Phys. Entomol. 1995; **20**: 45.
4. Gäde G. and Rosinski G., Peptides 1990; **11**: 455.
5. Gäde G. and Kellner R., Gen. Comp. Endocrinol 1992; **86**: 119.
6. Maliekal J., Jackson G.E., Flanagan C. and Millar R.P., S. Afr. J. Chem. 1997; **50**: 217.

7. Tsikaris V., Mahn T., Sakarellos C., Tzinia A., Soterladou K and Sakarellos-Daitsiotis M, J. Chem. Soc. Perkin Trans. 1994; **2**: 821.

8. Ziegler R., Jasensky R. and Morimoto H., Regulatory Peptides 1995; **57**: 329.

9. Cusinato O., Drake A.F., Gäde G. and Goldsworthy G., Insect Biochem. Molec. Biol. 1998; **28**: 43.

10. Demetropoupos I., Tsibiris A., Tsikaris V., Sakarellos-Daitsiotis M. and Sakarellos C., J. Biomolec. Struct. Dynamics 1995; **12**: 755.

11. Oriewski P., Marraud M., Cung Mahn-Thong, Tsikaris V., Sakaellos-Daitsiotis M., Sakarellos C., Vatzaki E. and Tzartos S., Biopolymers (Peptide Science) 1996; **40**: 419.

12. Tsikaris V., Detsikas E., Sakarellos-Daitsiotis M., Sakarellos C., Vatzaki E., Tzartos S., Marraud M. and Cung M., Biopolymers 1993; **33**: 1123.

13. Petit M., Orlewski P., Tsikaris V., Sakarellos-Daitsiotis M., Sakarellos C., Konidou G., Soteriadou K., Marraud M. and Cung M., Eur. J. Biochem. 1998; **253**: 184.

14. Odell B., Hammond S., Osborne R. and Goosey M., J. Comput.-Aided Mol. Design 1996; **10**: 89.

15. Pispisa B., Palleschi A., Amato M., Segre A. and Venanzi M., Macromolecules 1997; **30**: 4905.

16. Zubrzycki I. and Gäde G. Biochem. Biophys. Res. Commun. 1994; **198**: 228.

17. Zubrzycki I. and Gäde G., Eur. J. Entomol. 1999; **96**: 337.

18. Zubrzycki I., Z. Naturforsch. (C) 1998; **53**: 857.

19. States D. J, Haberkorn R. A. and Ruben D. J., J. Magn. Reson. 1982; **48**: 286.

20. Aue W.P., Bartholdi E. and Ernst R.R., J. Chem. Phys 1976; **64**: 2229.

21. Bax A. and Freeman R., J. Magn. Reson. 1981; **44**: 542.

22. Braunschweiler I. and Ernst R.R, J. Magn. Reson. 1983; **53**: 521.

23. Bax A. and Davis D.G., J Magn Reson. 1985; **65**: 355.

24. Macura S. and Ernst R.R, Mol.Phys. 1980; **41**: 95.

25. InsightII 97.2, Molecular Simulations Inc., 1998, San Diego, USA.

26. Bystrov V.F., Prog. Nucl Magn. Reson. Spectrosc. 1976; **10**: 41.

27. Brooks B.R., Bruccoleri R.E., Olafson B.D., States D.J., Swaminathan S. and Karplus M., J. Comp. Chem. 1983; **4**: 187.

28. Koradi R., Billeter M. and Wüthrich K., J. Mol. Graphics. 1996; **14**: 51.

29. Ramachandran G. N. and Sasiesekharan V., Adv. Protein. Chem. 1968; **23**: 283.

30. Wilmot C. M. and Thornton J. M., J. Mol. Biol. 1988; **203**: 221.

31. Siligardi G. and Drake A.F., Biopolymers 1995; **37**: 281.

32. Sato M., Kahn M., Lin Z.L., Johnson M.E. and Hayes T., Bioorg. Med. Chem. Lett. 1993; **3**: 1277.

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