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ARTICLE

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Molecular dynamics simulation of α-melanocyte stimulating hormone in a water-membrane model interface

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Abstract The conformation of the tridecapeptide α-melanocyte stimulating hormone in the presence of a double water-membrane interface was studied by molecular dynamics simulation, using the computational package THOR. In this program the solvent is represented by a continuous medium with dielectric constant ε, and the interface between different media is simulated by a surface of discontinuity of the dielectric constant. The electrostatic image method was used to write down the terms, added to the force field, that describe the polarisation effects induced in the interface by the atomic charges. The program was further improved by the introduction of a second surface, parallel to the first one, to mimic the membrane. A conformational search using the software Prelude was employed to find an initial geometry for the peptide in water. The molecular dynamics simulation performed during 10 ns showed that the peptide structure is flexible in water, without stabilisation of any preferential conformation. In the presence of the model membrane, the peptide moved to the medium representing the interior of the membrane. Inside the low dielectric constant medium, the structure of the peptide showed a turn in the central sequence of amino acids and a packed conformation remained stabilised during more than 7.0 ns of simulation.

Key words Melanotropic peptides · Peptide-lipid interaction · Dielectric discontinuity · Molecular dynamics · Electrostatic image method

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Introduction

α-Melanocyte stimulating hormone (α-MSH) is a hormone peptide involved in several physiological and neurological processes. It has long been known as the relevant hormone regulating skin pigmentation (Sawyer et al. 1980; Al-Obeidi et al. 1989a, b). More recently, evidence was found of its action also as a neurotransmitter or neuromodulator in learning, memory and attention (Jegou et al. 1993). It is a tridecapeptide having the amino acid sequence Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2.

Studies relating chemical structure and biological activity of the hormone have been performed, looking for analogues of α-MSH having higher potency and prolonged activity (Sawyer et al. 1980; Hruby et al. 1983; Al-Obeidi et al. 1989a, b). It was established that the central 6–9 tetrapeptide, His-Phe-Arg-Trp, is essential for the action of the hormone as a pigmenting agent (Hruby et al. 1984). Exploration of the characteristics of that central region have been conducted in the development of the superpotent agonist [Nle4, D-Phe7]α-MSH (Sawyer et al. 1980). Its superpotency was attributed to a β-turn conformation stabilised by a D-Phe7 residue and a possible Glu5-Arg8 or Glu5-Lys11 salt bridge (Sugg et al. 1988; Hruby et al. 1988, 1993; Al-Obeidi et al. 1989c). The importance of this β-turn on the biological activity of melanotropins was verified in cyclic analogues of α-MSH containing S-S and lactam bridges (Hruby et al. 1993). Structural features of the lateral chains of the central 6–9 sequence also would be of importance. It was suggested that enhanced biological activity could be observed in those analogues having, in one surface of the peptide, the residues His, Phe and Trp and, in the other surface, the residue Arg (Sugg et al. 1988; Hruby et al. 1993).

Receptors for α-MSH have been cloned and identified as pertaining to the superfamily of receptors coupled to the G-protein (Chhajlani and Wikberg 1992; Mountjoy et al. 1992; Grantz et al. 1994) and some important amino acids for the receptor-ligant interaction were

identified in the extra cellular loops of the receptor in human melanoma (Chhajlani et al. 1996). On the other hand, there are several reports indicating that the native hormone and some potent analogues show affinity for lipid membranes (Ito et al. 1993; Biaggi et al. 1996, 1997; Macêdo et al. 1996). There is a correlation between an increase in pigmenting activity and the strength of the interaction with model membranes, and, in particular, the results of fluorescence (Ito et al. 1993) and circular dichroism (Biaggi et al. 1997) spectroscopies suggest structural differences for the peptides in water and in the lipid phase of vesicles, with a possible stabilisation of the β-turn in the central region of the peptide. This would be consistent with the hypothesis of an active role for the lipid phase in the interaction of the hormone with its receptor in the biological membrane.

The experimental results about the structure of α-MSH are not conclusive and there are not yet reports about the conformation of the peptide in lipid-water interfaces. We developed a program package named THOR, based on the Gromos force field, for molecular optimisation and dynamics simulation (Moret et al. 1998). The solvent is not simulated explicitly, but is represented by a continuous medium with a characteristic value for the dielectric constant. We introduced in the program THOR the simulation of a water-membrane interface by a dielectric discontinuity and used the method of electrostatic images to describe the polarisation induced by the atomic charges in the surface of the discontinuity. The method has been applied with success to simulate the hydrophobic, hydrophilic and amphiphilic behaviour of some peptides (tetra-aspartic acid, tetra-lysine, β-endorphin fragment and signal sequence peptide), in the presence of a water-membrane interface model (Arêas et al. 1995; Pascutti 1996).

In this work we present an improvement to the method, with the introduction of a second surface of discontinuity, parallel to the first one, achieving a more realistic representation of the membrane separating two aqueous compartments. It was possible then to simulate the behaviour of the hormone peptide α-MSH in the presence of a water-membrane double interface and to analyse its conformational dynamics in such a heterogeneous medium during times as long as 7.4 ns.

Methods

Membrane simulation

The program package named THOR is based on the Gromos force field and parameters (van Gunsteren and Berendson 1987). The electrostatic term of the potential energy function contains the interaction between the atomic charges in a medium characterised by the dielectric constant ε. Values of 80 and 2 were assumed for the aqueous medium and the lipid environment, respectively. Based on the electrostatic image method, we introduced electrostatic terms describing corrections due to the presence of a dielectric discontinuity that mimics the water-lipid interface. These terms are dependent on the interacting charges, their positions with respect to the interface, and the medium in which they are immersed. Given a pair of charges, the terms are different when both charges are in water, or when both are in the lipid medium, or when one charge is in water while the other is in the lipid phase (Arêas et al. 1995; Pascutti 1996; Pascutti et al. Journal of Computational Chemistry, in press).

The direct interactions with the solvent are not considered here, so that each charge will not feel the reactive field generated by its own image, thus avoiding the divergence in the electrostatic potential energy at the interface. Each charge feels the field generated by the other charges and the polarisation due to their images, excluding the first and second chemically bound neighbours. In this way, we renormalised the intramolecular electrostatic interactions under the effect of the polarisation of the water-lipid interface.

To simulate the membrane, which has a finite width, we used two parallel surfaces of dielectric discontinuity, separated by a distance of 30 Å, the value estimated as representative of the hydrophobic core of biological membranes. The contributions from each interface were summed and included in the total potential of the molecule. The electrostatic image method was employed, taking into account only the charge image located nearest to each discontinuity surface. When a charge was positioned between the two discontinuity surfaces, the image of its image was neglected. As the dimensions of α-MSH are smaller than 30 Å, to neglect all but the first image would be equivalent to the introduction of a cut-off radius of 30 Å in the potential of the charge image in the space between the interfaces. For the charges located outside the discontinuity surfaces, when taking into account only the images from the nearest surface, we are neglecting contributions from the second surface, which are located more than 30 Å apart.

Conformational search

In order to start the dynamics simulation from a conformation of the peptide that was not an arbitrary one, we made a conformational search using the software Prelude (Rooman et al. 1991), to look for the lowest energy conformations of the molecule. In Prelude, the conformation of the peptide backbone is described as a combination of seven conformational states based on the Ramachandran's steric contour diagram and a mean field is derived from an statistical treatment of databases obtained from known protein structures. The dihedral angles (ϕ, ψ) of the conformational states named A, C, B, P, G, E and O are, respectively, (−65, −40), (−89, −1), (−117, 142), (−69, 140), (78, 20), (103, −176) and (−82, 133). The O state is defined for conformations where the backbone dihedral angle ω is zero. For the others, that angle is 180°. The method takes into account

the statistics of conformational states of individual residues and peptides. The probabilities for a given residue to adopt the seven conformational states are then converted in mean field potentials (Sippl 1990; Rooman et al. 1991). Thus one component of the force field due to local interactions is obtained and used to predict low energy conformations in a given amino acid sequence, considering the influence of each residue along the chain.

The method of Rooman gives only peptide backbone conformations. To have the position of the lateral residues, we fixed the backbone in the proposed lowest energy conformation with an extra harmonic potential, leaving the side chains in random conformation. Then we applied an annealing procedure using the THOR molecular dynamics program, heating gradually the molecule during 30 ps, until 900 K, and cooling it slowly during 150 ps. The structure was then reoptimised and velocities were given to each atom to simulate the temperature of 10 K. In 150 ps the temperature was raised to 300 K, adjusting a reference temperature. After 250 ps dynamics at 300 K, the restrictions that maintained the backbone in the conformation obtained from Prelude were removed. To equilibrate the system, the dynamics was followed by 100 ps, giving a total of 500 ps of simulation time, after which data were collected. The dielectric constant used was 80.

Water-membrane simulations

Starting from the equilibrated conformation, we applied 10 ns dynamics with $\varepsilon = 80$, obtaining the structure for the peptide in water. Then the model membrane was introduced, simulated by the two interfaces of dielectric discontinuity and the dynamics followed by another 7.4 ns. Data of the coordinates at the end of the first 10 ns in $\varepsilon = 80$ were used to draw the energy profile of the potential across the membrane acting on the conformation of the peptide. During the total dynamics we registered the variations in energy, backbone dihedral angles ϕ and ψ, and the trajectory of the centre of mass of the peptide. Structures were visualised from the coordinates obtained in several instants of the dynamics using RasMol, a molecular visualisation program.

Another dynamics simulation was performed, starting with the peptide already positioned in the interior of the membrane. In this case the dynamics was initialised using the same equilibrated conformation that we used to start the dynamics in water, and the peptide conformation was followed during 6.0 ns.

Results and discussions

Initial structure for the dynamics

In the Rooman's method the conformational energy, given in arbitrary units, is related to the probability of occurrence of that conformation in a databank. Peptides adopting a well-defined conformation in aqueous solution present an energy gap separating the lowest energy conformation and other predicted conformations. On the other hand, in flexible peptides there are several conformations that are close in energy (Rooman et al. 1991). Table 1 shows the 50 lowest energy conformations obtained for α-MSH. Predictions for Val13 are missing because it is in the C-terminal, which was excluded by the method. The energy values of the α-MSH conformations increase continually, without any energy gap, suggesting that the peptide is flexible in aqueous

Table 1 Lowest energy conformations for α-MSH, using Rooman's method

Conformation	Amino acid sequence SYSMEHFRWGKPV	Energy (arbitrary units)
1	CBAAACACCGPP	−1.87
2	CBAAACABCGPP	−1.86
3	CBAAACGCCGPP	−1.82
4	CBAAACGBCGPP	−1.80
5	CBAACCACCGPP	−1.79
6	CBAAACAACGPP	−1.78
7	CBAACCABCGPP	−1.78
8	CBAAACACCEPP	−1.74
9	CBAACCGCCGPP	−1.74
10	CBAAACABCEPP	−1.73
11	CBAAACGACGPP	−1.72
12	CBAACCGBCGPP	−1.72
13	CBAAACBBCGPP	−1.70
14	CBBAACACCGPP	−1.70
15	BBAAACACCGPP	−1.69
16	CBAAACGCCEPP	−1.69
17	CBBAACABCGPP	−1.68
18	BBAAACABCGPP	−1.68
19	CBAAACGBCEPP	−1.67
20	CBAACCACCEPP	−1.66
21	CBAAACAACEPP	−1.65
22	CBAACCABCEPP	−1.65
23	CBAACCGACGPP	−1.65
24	CBAAAAACCGPP	−1.65
25	CBBAACGCCGPP	−1.64
26	BBAAACGCCGPP	−1.64
27	CBAAACABCGBP	−1.63
28	CBAAAAABCGPP	−1.63
29	CBAAACBACGPP	−1.62
30	CBAACCBBCGPP	−1.62
31	BBAAACGBCGPP	−1.62
32	CBBACCACCGPP	−1.62
33	BBAACCACCGPP	−1.61
34	CBAACCGCCEPP	−1.61
35	CBBAACAACGPP	−1.61
36	CBBACCABCGPP	−1.60
37	BBAAACAACGPP	−1.60
38	BBAACCABCGPP	−1.60
39	CBAAACGACEPP	−1.60
40	CBAACCGBCEPP	−1.59
41	CBAAACGCCGBP	−1.59
42	CBAAAAGCCGPP	−1.59
43	CBAAACBCCEPP	−1.59
44	CBAAACGBCGBP	−1.58
45	PBAAACACCGPP	−1.58
46	CBAAACCCCGPP	−1.58
47	CBAAAAGBCGPP	−1.58
48	CBAAACBBCEPP	−1.57
49	CBAACCAACEPP	−1.57
50	CBAACCACCGBP	−1.57

medium. However, we can notice the generalised occurrence of certain individual conformational states for some of the residues. Thus, Tyr2 has the B conformational states, which lies in the β-sheet region of the Ramachandran diagram; Ser3 and Met4 have the A state, which is in the α-helix region; His6 and Trp9 have the C state, in the helix-3_{10} region; Lys11 and Pro12 have the P state, in the poly-proline helix region; Ser1 is frequently in the C state; Glu5 is frequently in the A and C states; Phe7 and Arg8 have no preferential conformational states, indicating that they are in a flexible region of the backbone; and finally, Gly10 appears in the G and E states, which are frequently visited by this residue that has an H-atom as a side chain.

Starting from the lowest energy conformation for the peptide backbone, the side chain residues were introduced and the molecule was submitted to the annealing procedure using the THOR program, allowing for a total of 500 ps dynamics for the structure equilibration in a medium with $\varepsilon = 80$. After this initial procedure the backbone structure was practically coincident with the lowest energy conformation of Table 1. The charged residues Arg8 and Lys11 were exposed in the surface of the peptide, on opposite sides of the chain. The acidic residue Glu5 was also exposed in the same side as Lys11, without the formation of a salt bridge. The less polar central residues His6, Phe7 and Trp9 were less exposed to the solvent, and the overall residues distribution (Fig. 1) were as expected for peptide side chains in a polar medium.

To examine the stability of this conformation in water, we performed a dynamics simulation for a time as long as 10 ns in $\varepsilon = 80$ and at 300 K. The result was that the starting conformation was not stable, consistent with the continuous energy variation for the conformations listed in Table 1. There was no stabilisation of any conformation, and the initial packed conformation evolved to an extended one (Fig. 2). The initial helix opened in the first nanoseconds, originating a loop (Fig. 2e) that moved to the region of the residues 7–10 (Fig. 2f). This behaviour is in agreement with the conformational instability of peptides in water. Besides, in the simulation the intramolecular hydrogen bonds were weakened by the high dielectric constant of the medium.

Potential across the membrane

The membrane was introduced placing the dielectric discontinuity surfaces perpendicular to the X coordinate axis, at the positions 50 Å and 80 Å (Fig. 3a), assuming $\varepsilon = 2$ for the medium internal to the surfaces and $\varepsilon = 80$ for the external medium. A potential energy profile for the peptide across the membrane (Fig. 3a) was calculated using the extended conformation obtained at the end of the dynamics in water. The coordinate of the centre of mass of the molecule was displaced in 0.1 Å intervals between 30 Å and 100 Å, and at each point the potential energy of the molecule was calculated. The result was a symmetrical energy profile in the central region of the membrane, the $\varepsilon = 2$ medium, where the overall minimum of the potential energy is located. The potential energy profile is not symmetrical near to the interfaces, where some local minima appear. This asymmetry may be due to the fact that the molecule was displaced across the membrane without changing its orientation.

The atomic coordinates and velocities of the extended configuration were recovered to follow the movement of the centre of mass during the dynamics in the presence of the double interface. Starting from rest at the X position equal to 32 Å, the centre of mass began to move towards the membrane. The peptide was not trapped by the local minima at the first interface, moving to the position corresponding to the central minimum. After that, the centre of mass acquired an oscillatory movement, centred in the middle of the double interface (Fig. 3b).

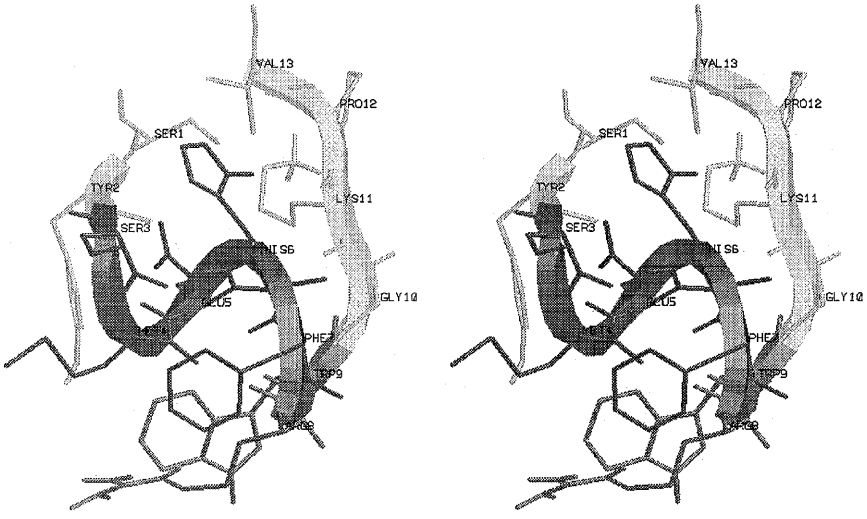

Fig. 1 Stereo-image for the thermalised lowest energy conformation of α-MSH, according to Rooman's method

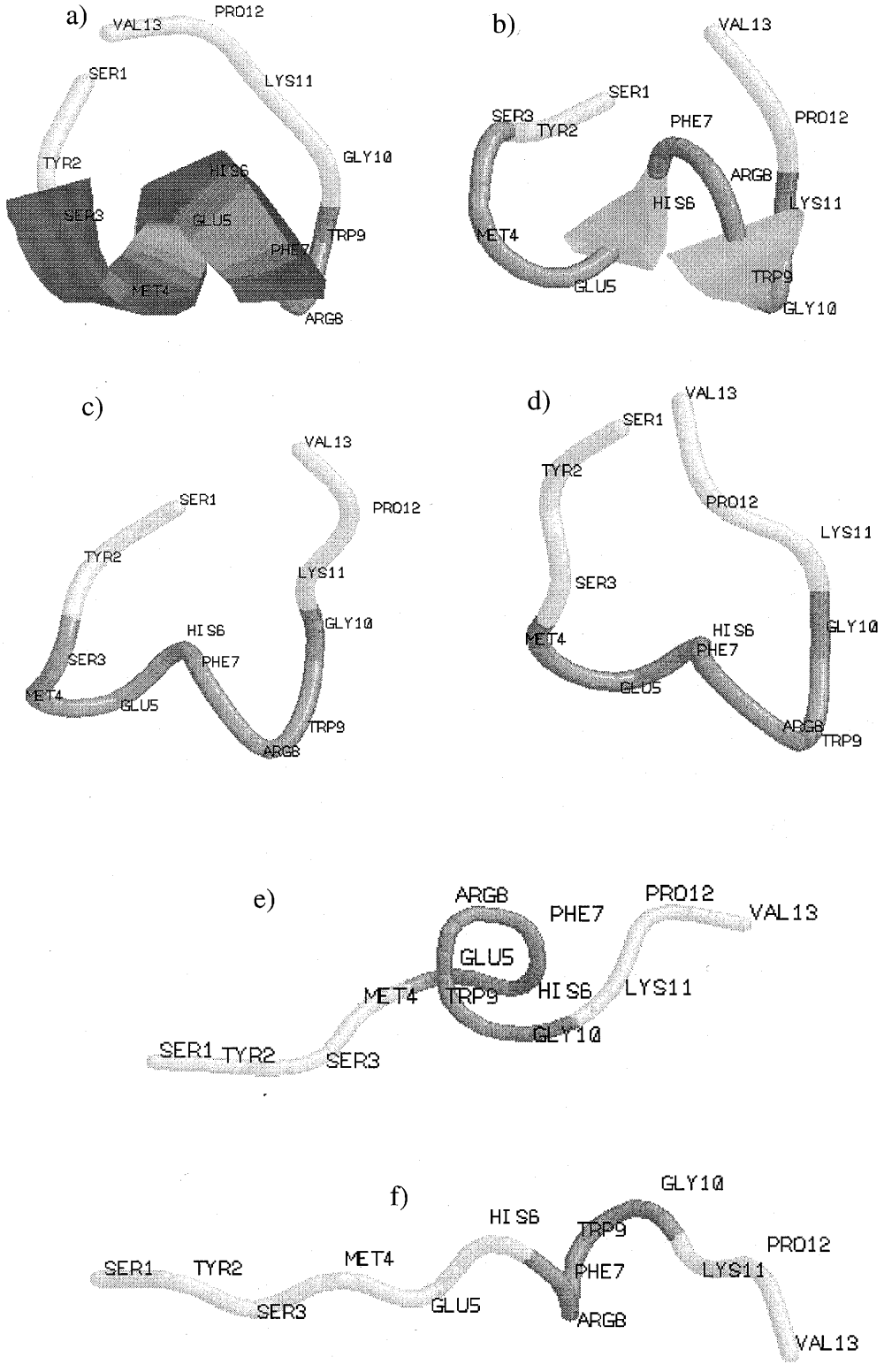

Fig. 2 a–f Conformations for the peptide backbone of α-MSH obtained from molecular dynamics simulations in a polar solvent. Figures obtained from atomic coordinates, registered at 2 ns intervals: **a** $t = 0$, **b** $t = 2$, **c** $t = 4$, **d** $t = 6$, **e** $t = 8$, **f** $t = 10$ ns

Dynamics in water and in the membrane

The potential, kinetic and total energies were monitored during the entire dynamics (10 ns in the aqueous medium and 7.4 ns in the presence of the double interface). The kinetic energy remained constant owing to the coupling with a thermal reservoir (Areas et al. 1995), and the decrease in the potential energy owing to the entry into the membrane caused the decrease in the total energy (Fig. 4).

We also monitored all the dihedral angles ϕ and ψ of the peptide during the dynamics (Fig. 5). The residue

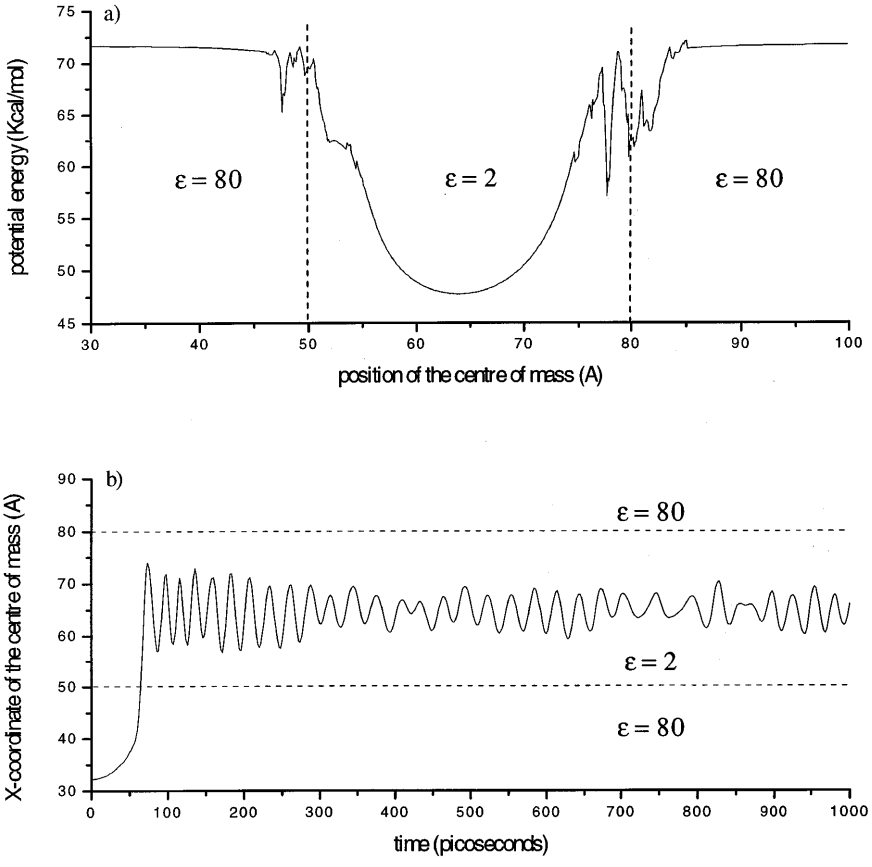

Fig. 3 a Potential energy profile for α-MSH in the membrane. **b** Trajectory of the centre of mass of α-MSH in the presence of the membrane

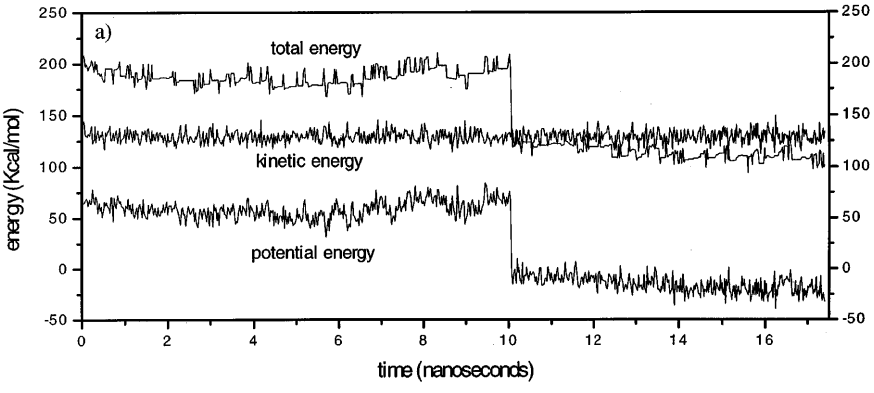

Fig. 4 Time evolution of the potential, kinetic and total energy for α-MSH in a polar medium (before 10 ns) and in the presence of the membrane (after 10 ns)

Tyr2 started in the β-region of the Ramachandran plot and visited the helices region in water (continuum with $\varepsilon = 80$). After the passage to the membrane (continuum with $\varepsilon = 2$), it remained for 1 ns in an unfavourable region (around 60, −60), ending the dynamics in the helix region. Ser3 left the is α-helix region in water and returned to it after the peptide went inside the membrane. Met4 remained mainly in the α-helix, going to the β for short periods. Glu5 left the α-helix conformation at 8 ns, going to the β-region and stabilising there in the membrane. Dihedral angles for His6 were practically the same as obtained in Rooman's search. Phe7 and Arg8, which did not have a preferential conformation with the Rooman's method, remained in helix region most of the time in water. In the membrane, they stabilised in the α- and β-regions, respectively. Trp9 practically stabilised in the α-helix in the membrane while Gly10 did not stabilise in water, going to the β-form in the hydrophobic medium ($\varepsilon = 2$). Lys11 was in the predicted conformation and did not change it in the membrane. Pro12 changed to the helix after the peptide went to the membrane.

A conformational search for α-MSH using a matrix algorithm has been previously performed (Jacchieri and Ito 1995). Lowest energy conformations for the Trp9 side chain rotamers *gauche*$^-$ and *gauche*$^+$ showed a helix turn in the region of residues 3–7, as is also present in the minimised structure obtained here (Fig. 1 and Table 1, first conformation). It was proposed that this confor-

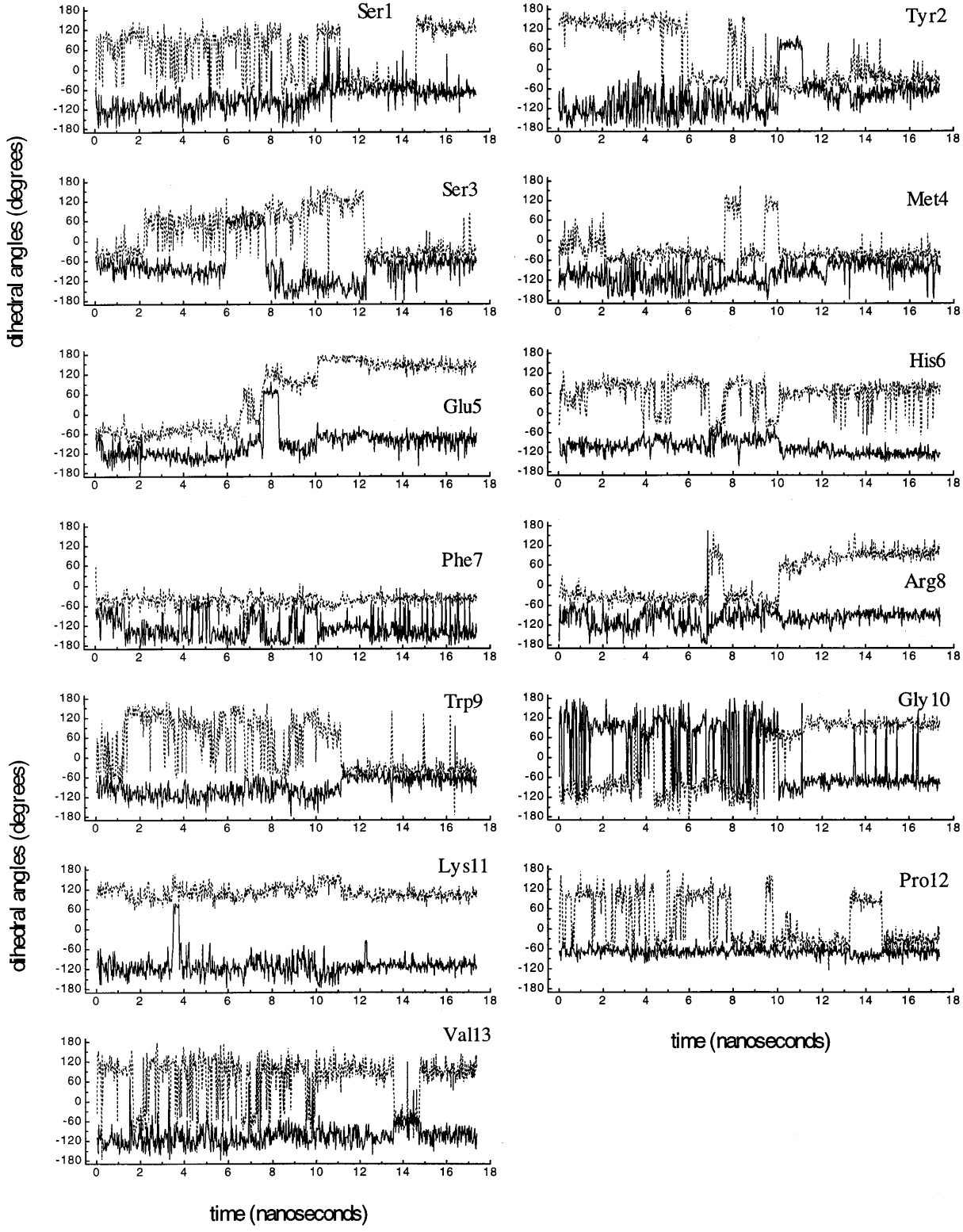

Fig. 5 Time evolution of dihedral angles ϕ and ψ of α-MSH for the dynamics starting in aqueous medium

mation was predominant in aqueous medium and that another low energy conformation containing a turn in the region of residues 6–9 would be favoured for the interaction with lipids (Jacchieri and Ito 1995). The present results show that the behaviour of the molecule is more complex. The initial minimised structure changed in water while the molecule started to move in the direction of the medium with a low dielectric constant.

The monitoring of the dihedral angles shows the conformational stabilisation of the backbone inside the membrane. Residues Ser3, Met4 and His6 stabilised in

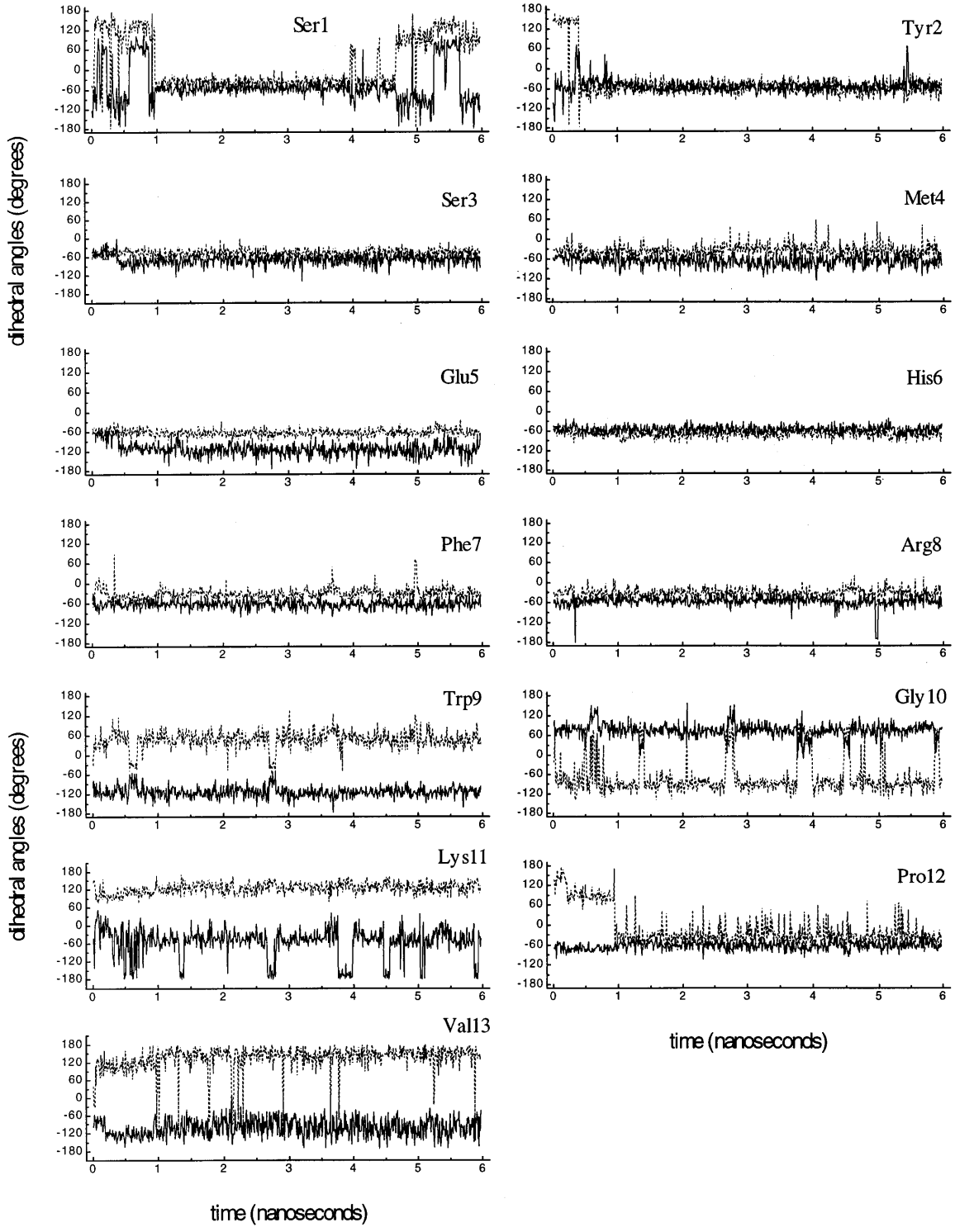

Fig. 6 Time evolution of dihedral angles ϕ and ψ of α-MSH for the dynamics starting in the membrane

the conformations listed as more frequent in Table 1 and Tyr2, Glu5 and Trp9 finished in conformations different from those predicted by Rooman's method. This method is based on the databank from proteins, where the majority of the amino acid residues are packed in their hydrophobic interior, and was created based on the existence of local packing before the global structure stabilisation. If the local interactions are important for the previous stabilisation of secondary structure, peptides with the same amino acid sequence would have stable conformations in water, presenting a gap in energy between the preferred conformation and others. This is not the case for α-MSH, for there are no energy gaps among the conformations listed in Table 1 and the

dynamics in $\varepsilon = 80$ showed that the peptide can assume conformations different from those predicted as the more stable.

It is interesting to notice the results obtained when the dynamics started with the peptide already in the medium that mimics the membrane interior. The same conformation obtained from Rooman's method was subjected to 6 ns dynamics in a low dielectric constant medium. In this case, the monitoring of the dihedral angles (Fig. 6) showed that there were no conformational changes in residues 3–11, keeping stable a helix between Tyr2 and Arg8, indicating that packed conformations can be stabilised in the non-polar medium.

Conclusions

In living organisms the α-MSH peptide is exposed to the extracellular polar solvent prior to the interaction with its specific receptor in the melanocytes. From the results of the simulation, it may be said that the peptide does not adopt a single conformation in water, and that the helicoidal conformation predicted by statistics of a similar sequence in proteins is not stabilised in aqueous medium. In the presence of the double interface of dielectric discontinuity that mimics the membrane, the peptide showed preference for the less polar medium.

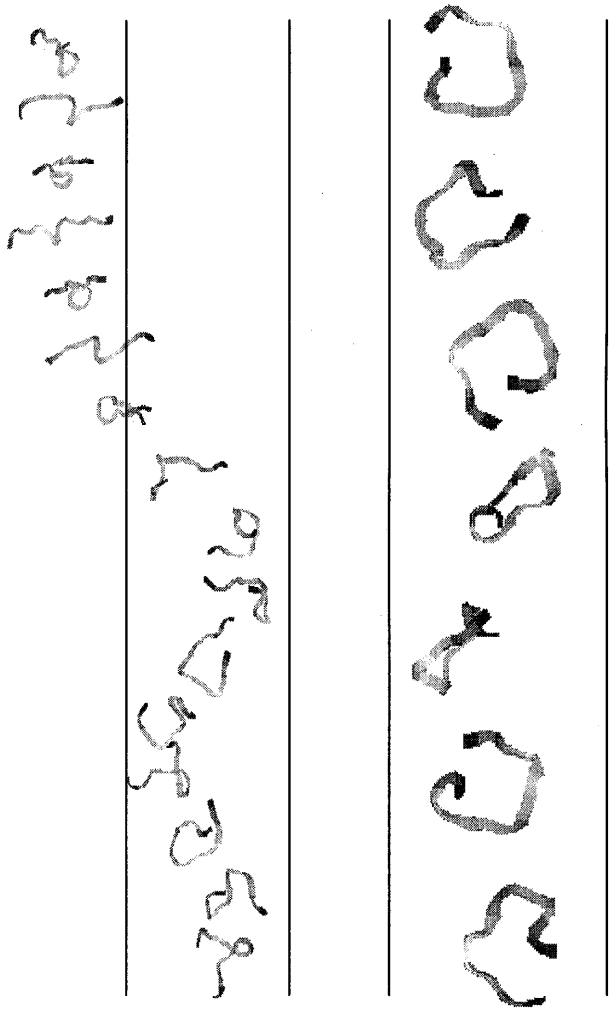

Fig. 7 Representation of the peptide backbone of α-MSH: **a** migrating to the interior of the membrane (coordinates generated in 2 ps intervals); **b** after conformational stabilisation in the membrane (coordinates generated in 50 ps intervals). The *lines* are at the water-membrane interfaces

The passage of the hormone to the interior of the membrane is shown by the sequence of images in Fig. 7a, generated from the atomic coordinates of the atoms in the peptide taken at 2 ps intervals. One can visualise the peptide in an extended conformation, rotating and moving in the aqueous phase towards the membrane. When it goes into the membrane the changes in the dihedral angles of Arg8 lead to a turn in the backbone structure and the peptide evolves to the stabilised conformation, while its centre of mass oscillates around the mid point between the two interfaces. Images generated at the end of the dynamics in the low polarity medium (Fig. 7b, with 50 ps interval between the images) show the stabilisation of the conformation inside the membrane.

The amino acid sequence of the peptide suggests an amphiphilic character for the peptide, while experimental results from fluorescence studies indicate that the tryptophan residue from α-MSH and analogues goes into the lipid phase of vesicles and micelles (Ito et al. 1993; Macêdo et al. 1996). Our simulations show the preference of the peptide for the lipid phase, directed by a decrease in potential energy of the peptide in the low dielectric constant medium. We are not simulating specific interactions with water or lipid molecules, nor including hydrophobic effects, and the decrease in the potential energy is probably stressing more the role of salt bridges in the stabilisation of the peptide structure than expressing effects like changes in energy due to transfer of individual side chain residues from water to alkane solvents.

A more detailed view of the conformations resulting from the dynamics in polar and non-polar environments gives emphasis to the salt bridges between the charged residues in the hormone (Fig. 8). During the 10 ns dynamics in a polar medium, the extended structure precludes the formation of salt bridges (Fig. 8a). However, inside the non-polar medium, the low dielectric constant allows a higher electrostatic attraction, and favours the stabilisation of the salt bridges (Fig. 8c) involving residues Glu5, Arg8 and Lys11.

It is interesting to notice that the β-turn of the peptide in Arg8 generates a hydrophilic core containing the charged residues Glu5, Arg8 and Lys11, surrounded by a hydrophobic surface comprising the residues His6, Phe7 and Trp9. Thus, the central 6–9 region, the melanotropic minimal message sequence, stabilises in a defined conformation. These results are in agreement with the β-turn and the side chain structural features proposed as necessary for biological activity of the native hormone, and for the synthesis of superpotent and

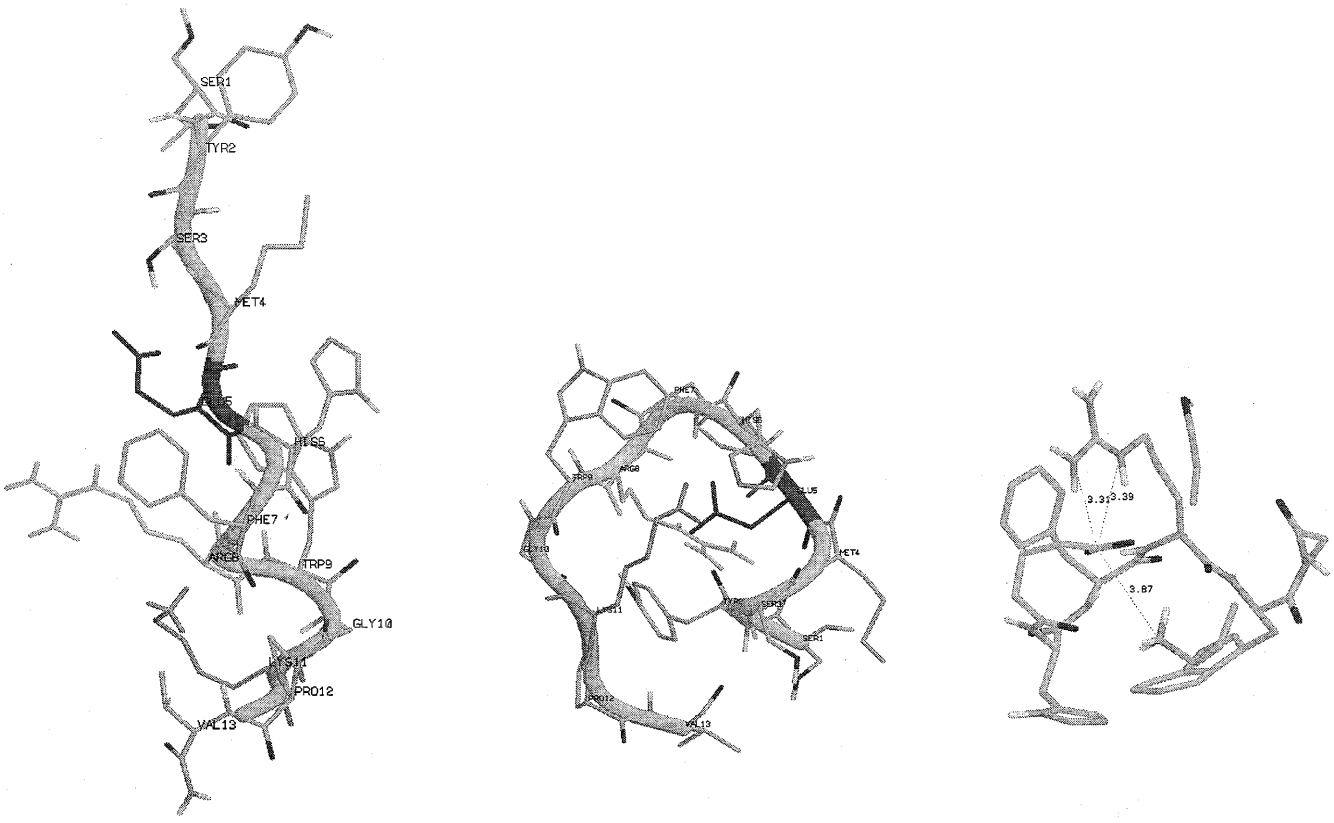

Fig. 8 Representation of the α-MSH molecule: **a** after 10 ns in polar solvent; **b** after 7.4 ns in the interior of the membrane; **c** detail of the hydrophilic core formed in the membrane by residues $Glu5^{-}$, $Arg8^{+}$ and $Lys11^{+}$ and distances (in Å) between some pairs of atoms

superprolongued analogues (Sugg et al. 1988; Hruby et al. 1988, 1993; Al-Obeidi et al. 1989c).

The simulation result showing the conformation change that accompanies the insertion of α-MSH into the non-polar medium is consistent with the observed changes in the fluorescence lifetime parameters of Trp9, measured in water and in the presence of lipid vesicles, that could be related to structural modifications in the hormone (Ito et al. 1993). It is also consistent with the possible role of hydrophobic regions involved in the hormone-receptor interaction, and the observed correlation between an increase in biological activity and the depth of penetration of the analogue into model membranes (Macêdo et al. 1996; Biaggi et al. 1997). The result of our simulation suggest that a conformation that would be biologically active is attained by the native hormone after its migration from a pure polar environment (water) to a heterogeneous non-polar environment comprising the lipid phase and the membrane receptor.

The method that we have developed for the simulation of the peptide in the presence of a double water-membrane interface, with the limitations inherent to a simplified model, has the advantage of allowing the analysis of conformational dynamics during times as long as 16 ns. The result obtained show a picture show a picture that is qualitatively consistent with known experimental results for the behaviour of the α-MSH molecule.

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