CONFORMATIONAL ANALYSIS OF MELITTIN USING THE RESIDUAL REPRESENTATION

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1. Introduction

The conformational analysis of polypeptide chains, for which the closing of the S–S bridges requires some special arrangement of the molecule, usually provides a small number of possible conformations. In [1], we described the use of the residual representation to search for the most probable conformations of apamin, a short polypeptide neurotoxin from bee venom, with two intramolecular S–S bridges.

When such constraints do not exist, the number of starting conformations must be larger and the number of final possible conformations too. Here we intend to use the same residual representation to search for the most probable conformations of melittin, another peptide from bee venom which displays haemolytic activity. Melittin is a polypeptide chain of 26 amino acids, without any S–S bridge, the N-terminal part of which is a cluster of mainly hydrophobic residues (1–20), followed by a strongly basic C-terminal portion (21–26) [2]:

NH_2–Gly–Ile–Gly–Ala–Val–Leu–Lys–Val–Leu–Thr–Thr–Gly–Leu–Pro–Ala–Leu–Ile–Ser–Trp–Ile–Lys–Arg–Lys–Arg–Gln–Gln–$CONH_2$

2. Materials and methods

A residual representation derived from the model in [3] with 2 spheres/residue [1] was used. With this representation, a protein conformation is completely defined by the set of the torsion angles α_i between the couples of residual spheres. The sidechain–solvent interactions are described in the same way as we had proposed.

The folding of a completely extended chain cannot be a continuous process; it is impossible to reach a final correct conformation by the simple energy minimisation of a single set of initial torsion angles (for instance all the residues in extended conformations $\alpha = 220°$). Thus, from a completely extended chain it is impossible to recover a real helical form through energy minimisation [3].

Generally the mathematical minimisation of the complete energy function leads to different final conformations depending on the initial values given to the torsion angles. In the residual representation, each residue displays two most probable conformations: the extended form $\alpha = 220°$ and the helical form $\alpha = 40°$. To pass from one conformation to the other it is necessary to jump over an energy barrier. To study all the possible conformations of a polypeptide chain it would be necessary to assign each residue both possible conformations. For a protein of n residues the number of starting sets would be 2^n. It is possible to reduce drastically this huge number, if in a first step we consider only the stable secondary structures.

The residual representation was defined to deal with large proteins and provided a good description of long range sidechain–sidechain interactions. However, as short or medium range interactions are responsible for the formation of β turns and α helices the residual representation is required to give satisfactory results even at the level of the tetrapeptides.

In the residual representation, the conformation of a tetrapeptide is defined by 3 torsion angles. If we assign to each of them both possible values 40° and

220°, there are 8 possible conformations for a tetrapeptide. If E_i is the value of the energy potential function corresponding to one conformation, the probability of existence of this conformation is:

$$P(\alpha_{i-1}, \alpha_i, \alpha_{i+1}) = \frac{\exp(-E_i/RT)}{\sum_j^8 \exp(-E_j/RT)}$$

As the tetrapeptide is included in a greater polypeptide chain, it would be necessary to introduce the influence of adjacent tetrapeptides. The study of secondary structures through the tetrapeptide analysis finally provides a set of possible starting conformations defined by the greatest probalities:

$$\prod_2^{n-1} P(\alpha_{i-1}, \alpha_i, \alpha_{i+1})$$

This way of forecasting secondary structures may be compared to the prediction scheme in [4] for it is based on the study of short or medium range interactions between the side chains.

Here we have used the torsional potentials defined in [3].

3. Results and discussion

From the tetrapeptide analysis, the secondary structure of the melittin was found to be of the following type:

$$220°-(40°)_5-220°-\begin{pmatrix}220° \\ \text{or} \\ 40°\end{pmatrix}-(220°)_4-(40°)_{4 \text{ or } 6}-220°$$

that is to say two α helices 1–8 and 13–17 (or 13–19) parted by a random coiled portion. Such a result is in agreement with predictions:

(i) Using a modified Chou-Fasman prediction scheme [5,6] (2–11 and 15–21 helices); or

(ii) Experimental spectroscopic measurements [6,7].

Then using energy minimisation we searched for the stable conformations corresponding to different starting sets with α helices of variable lengths. As extended conformations cannot be completely discarded [6], we also studied completely extended molecules (which could be a folding simulation according to [3]), and a β-sheet structure. These different minimisations are reported in table 1 and the 5 most probable conformations shown in fig.1.

Table 1
Different conformations of melittin and their corresponding minimum energy (in kcal/mol)

Conformation	$E_{tot.}$	Side chain–side chain interactions	Main chain–main chain interactions	Side chain–solvent interactions
(a) Enveloped 15–20 α helix	−40.30	0.43	−8.51	−32.20
(b) 1–8 and 14–18 α helices	−40.27	−1.24	−7.11	−31.92
(c) 1–8 α helix, (11–14)–(17–20) β sheet	−38.91	−1.13	−8.30	−29.52
(d) (4–8)–(11–15) β sheet	−36.84	−0.04	−8.12	−28.68
(e) 1–8 and 12–18 α helices	−36.70	−1.71	−7.11	−27.88
[6] [a](2–11)–(15–21) α helices	−33.85	−1.19	−8.26	−24.36
1–8 and 13–20 α helices	−33.69	−0.91	−6.72	−26.06
1–8 and 13–18 α helices	−33.65	−1.14	−7.63	−24.88
1–8 and 12–20 α helices	−31.42	−1.11	−6.03	−24.28
Extended with 8–9 turn	−30.09	0.26	−6.93	−24.42
13–20 α helix	−26.87	−0.53	−5.30	−21.04
Extended (folding)	−21.10	−0.05	−3.43	−17.62

[a] Chou-Fasman prediction [6]

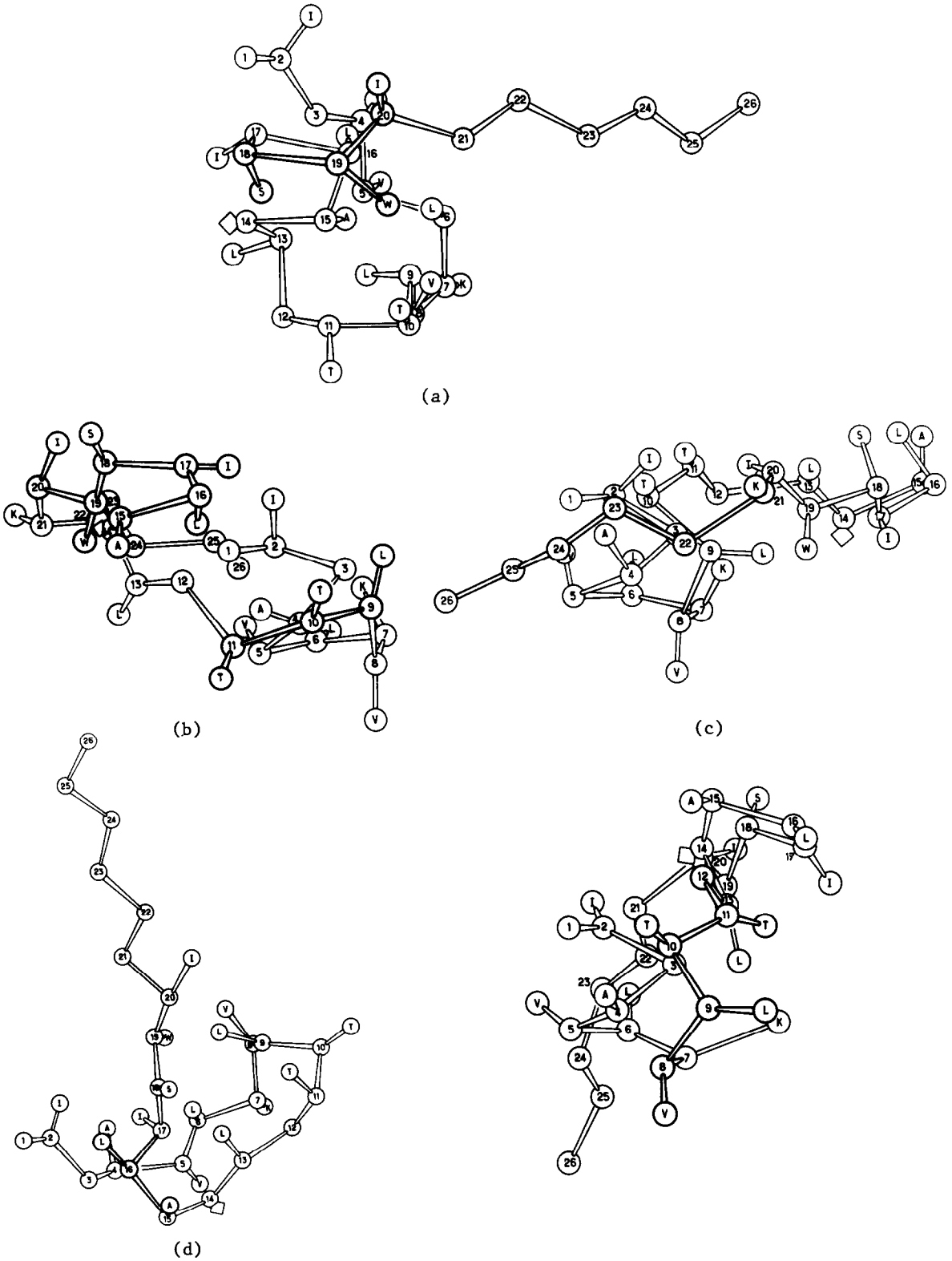

Fig.1. The most probable conformations of melittin. The single letter notation for amino acid (Eur. J. Biochem. 5 (1968) 151–153) is indicated in the corresponding sidechain.

Table 2
Torsional angles which define the (a) and (b) conformations

1–13	(a)	(b)	13–25	(a)	(b)
α_{1-2}	250.05	205.01	α_{13-14}	89.37	333.71
α_{2-3}	180.37	37.18	α_{14-15}	84.79	87.36
α_{3-4}	208.37	56.72	α_{15-16}	31.77	31.46
α_{4-5}	253.40	26.12	α_{16-17}	52.76	3.36
α_{5-6}	166.29	34.55	α_{17-18}	13.44	241.23
α_{6-7}	281.59	5.42	α_{18-19}	30.14	196.98
α_{7-8}	338.23	257.95	α_{19-20}	207.41	179.30
α_{8-9}	208.77	145.16	α_{20-21}	173.20	307.26
α_{9-10}	273.22	312.28	α_{21-22}	207.71	237.39
α_{10-11}	21.94	265.91	α_{22-23}	179.97	232.02
α_{11-12}	279.37	140.99	α_{23-24}	197.35	280.72
α_{12-13}	309.80	189.66	α_{24-25}	184.17	189.11

Table 1 displays a great variety of conformations with 3 most probable conformations and other ones which may be considered as intermediate conformations.

The most probable (a) and (b) conformations which are defined by the torsional angles reported in table 2 have 23% and 50% of α helix, respectively. Perhaps this result may be significantly compared with the experimental amount of α helix in the monomeric form of melittin [12%) and in the tetrameric form (65%) [7].

The Trp_{19} residue is reported to be exposed to the solvent in the monomeric form of melittin, and buried in the tetrameric form [7,8]. This burying was explained by contact with hydrophobic residues of other protomers. Our study suggests another explanation. We computed the % of burying of Trp_{19} expressed as the ratio of:

$$\Delta S_{calc.}/\Delta S_{max}$$

where ΔS_{max} is the maximum of accessible surface of Trp_{19} (always considered as a mean sphere);

and $\Delta S_{calc.}$ is the calculated buried surface of the spherical side chain of Trp_{19} in the different conformations.

It is equal to 45% in form (a) and to 72% in form (b).

The fitting of our results with experimental observations could allow us to consider that the (a) conformation is the major component of the random coil conformation which is described for the monomeric form of melittin, whereas the (b) conformation appears in the tetrameric form and at the level of lipid membranes. These assumptions are in good agreement with the observations in [6] although the (b) conformation is somehow different from the model which was then proposed; both α helices are shorter and their hydrophobic areas are packed together (Ile_2, Ala_4, Ala_5, with Leu_{16} and Ile_{17}). These interactions ensure a rather compact conformation and may provide another explanation for the total absence if the lytic properties by the melittin 8–26 peptide.

As one face of the molecule of melittin is hydrophobic and the other hydrophilic, the 'wedge' effect proposed in [6] remains possible, with a different orientation of the molecule.

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