CONFORMATIONAL CHANGE AND SELF ASSOCIATION OF MONOMERIC MELITTIN

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1. Introduction

Melittin is a well-known amphipathic peptide which acts as a direct lytic factor on biological membranes [1]. Its ability to induce leakage both on natural membranes and liposomes has been clearly demonstrated [2]. Melittin–phospholipid interactions have also been extensively studied [3–5], however few studies have been dealing with the structure of the peptide itself, in solution. It is known to behave as a tetramer [1,6]; it is also assumed from an early circular dichroism study [7] to be mainly in random coil. The possibility of conformational and aggregation changes induced by organic solvent or phospholipids was pointed out [8,9] but no definite experimental proof was presented on these problems.

Here, we report the existence of melittin as a monomer and give the parameters which govern the self-association of the peptide. We also present arguments indicating that the polymerization process involves important changes in the secondary structure of melittin. These findings allow a more coherent picture of the behaviour of the peptide in solution.

2. Materials and methods

Melittin (research grade) was purchased from Serva Feinbiochemica (Heidelberg). It was used either directly or after purification according to [10], no difference being detected. Gel filtrations on Sephadex G-50 with myoglobin, cytochrome *c*, insulins, carbonic anhydrase (products from Sigma Co.) and cardiotoxin II (a gift of Professor H. Rochat, Marseille) as standards were performed in 0.02 M Tris–acetate, pH 7.5 and 10.5, with 0.2 mM EDTA, with or without NaCl. The initial concentration of melittin was 1 mM, it was diluted ~10-fold during elution. Ionic strength experiments were performed on a Fica 55 MK II differential spectrofluorometer, or a Fica Spectropol I spectropolarimeter, at room temperature. NaCl aliquots were added to a solution of melittin (1.9–180 μM for fluorescence studies, and at 180 μM for polarimetric measurements) in 0.02 M Tris–HCl buffer (pH 7.5) containing 1 mM EDTA. For optical rotatory dispersions, buffers were filtered through Millipore filters in order to remove dust. These dispersions were analysed according to the Moffitt and Yang equation.

3. Results

3.1. *Intrinsic fluorescence*

In fig.1a the emission spectrum of diluted melittin at low ionic strength is reported. The maximum at 353 nm shows the Trp residue of the peptide totally exposed to the solvent. By increasing ionic strength, one can observe a shift towards 337 nm. The plot of this shift versus concentration of NaCl is reported in fig.1b, the half-effect depends on melittin concentration. Thus, the effect of ionic strength is a burying of the Trp residue in a less polar medium. Only two possibilities can account for such a result: a conformational change and/or an association of the peptide.

3.2. *Gel filtration*

In fig.2 the elution volumes are plotted of proteins of known molecular weight passed through a Sephadex G-50 column. The characteristic of the column was

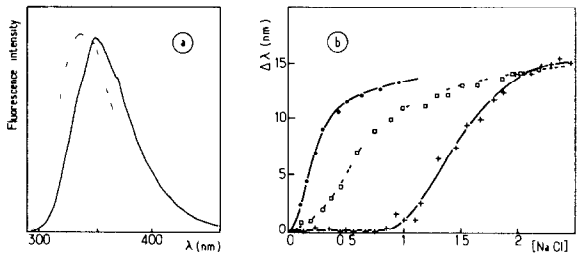

Fig 1 Trp fluorescence in melittin (a) Emission spectra of melittin, 20 μM at pH 7 5 (——) In low ionic strength 0 02 M Tris acetate, (- - -) after addition of 2 M NaCl (b) Shift of the maximum of emission versus NaCl concentration The maximum is measured as the wavelength of the mid-point at half-height of the spectrum Melittin conc 180 μM (-•-), 20 μM (-□-), 1 9 μM (- + -)

first determined at high ionic strength In these conditions at pH 7 5, melittin elutes with cytochrome *c*. Its app. mol. wt ~12 000, in agreement with previous data [1,6], indicates the existence of a tetramer But at low ionic strength, melittin elutes as a low molecular weight compound 3500 ± 500, very close to insulin B chain This indicates that it is probably in a monomeric state At low ionic strength by increasing

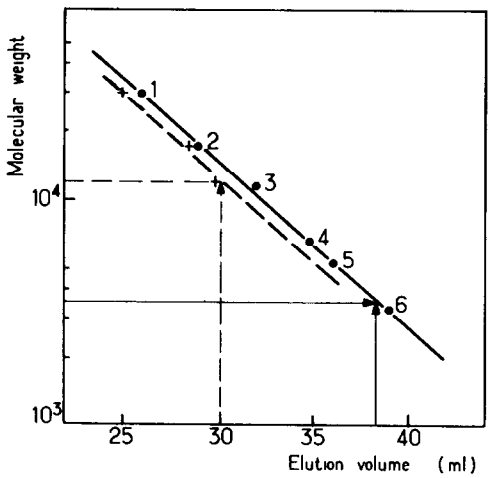

Fig 2 Molecular weight determination of melittin by gel filtration The column was Sephadex G-50 (diam 1 3 cm, length 30 cm), the eluant 0 02 M Tris acetate (pH 7 5), (——) without NaCl, (- - -) with 1 5 M NaCl Protein solutions (0 4 ml) were introduced, 0 5 ml fractions were collected, the protein content was monitored by A_{280} (1) Carbonic anhydrase, (2) myoglobin, (3) cytochrome *c*, (4) cardiotoxin, (5,6) insulin A and B chains

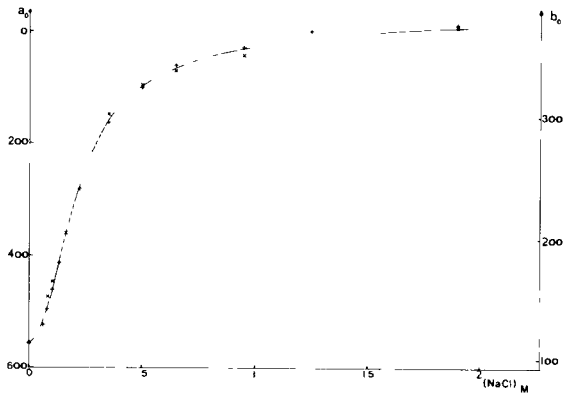

Fig 3 Modifications of Moffitt parameters with ionic strength, in 0 02 M Tris–HCl buffer (pH 7 5), 1 mM EDTA at 20°C Lightpath 0 1 cm melittin 180 μM (—x—) a_0, (— + —) b_0

to pH 10 5, which decreases the net positive charge of the peptide, melittin also aggregates and is eluted for smaller volumes

3 3 *Optical rotary dispersion*

The ORD spectrum of melittin changes with addition of NaCl The fit of experimental curves with Moffitt equation shows an important change in a_0 and b_0 values as plotted in fig 3 The two variations are quite similar, then a conformational change is ascertained Using the procedure in [11] the secondary structure of the peptide can be estimated, the results are summarized in table 1 By increasing NaCl concentration the conformation of melittin changes from a mainly random-coiled structure, with only 12% of α helix, to a mainly helical one, up to 65%, with a few residues in β pleated sheet and random coil

Table 1
Structure of melittin in monomeric and tetrameric states obtained from ORD, by the procedure in [11]

	α	β	Random
Melittin monomer	12	0	88
Melittin tetramer	65	15	20
From [7]	7		
From [8]	65	0	35
Predicted as in [17]	58	0	(42)

Comparison with literature values obtained by CD [7] or calculated by statistical method [8,17]

4. Discussion

Melittin does exist in solution in two states, differing both in secondary and quaternary structures. In low ionic strength and low concentration it behaves as a monomer; its fluorescence shows that Trp_{19} is totally exposed to the solvent. Then all studies done until now by this technique, which report a maximum of emission around 353 nm, were performed on the monomer, due to the low concentrations needed for such experiments [3,5].

On the contrary at high concentration and/or high ionic strength a monomer ⇆ tetramer equilibrium is observed. In that case Trp_{19} is buried probably in contact with hydrophobic residues of other protomers, this agrees with previous NMR results [12]. This hydrophobic burying on self-association has to be compared to another well-documented example of amphipathic peptide such as glucagon [13–15].

Our findings do not contradict previous data on molecular weight determinations. The reports in [6,16] were in experimental conditions, high melittin concentration and high ionic strength, in which we also showed that melittin behaves as a tetramer. However, melittin was shown [8] not to be eluted at the predicted position for the tetramer. The retention was described as a non-specific adsorption of hydrophobic residues on the gel. Given the conditions of pure water and ~1 mM melittin the results [8] could be better explained by dissociation of the tetramer, in the light of our experiments.

It was concluded from CD studies [7,8] that melittin is mainly random coil. Since in both cases melittin at moderate concentration was dissolved in pure water, the studies were with monomers. These results have to be compared to our values for monomeric melittin. As it can be seen on table 1 they are in very good agreement.

The increase of ordered configurations involving probably most of the residues is concomitant with self-association; this again compares well to what occurs for glucagon [13,15]. It must be noticed that the large amount of α helix obtained from ORD agrees with that predicted by teh procedure in [17] or that calculated by a slightly different method in [8] (cf. table 1).

In conclusion it has to be emphasized that melittin exists in two very different structural states. In any further studies it will be necessary to specify which one of the two states of melittin is present. These two species might account for the different behaviour of binding to membranes that we are now studying.

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