DOES DIMERIC MELITTIN OCCUR IN AQUEOUS SOLUTIONS?

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ABSTRACT Melittin, a peptide from bee venom, is known to undergo a monomer / tetramer conversion in aqueous solutions. We have studied the possible participation of dimers in the association equilibrium of melittin by sedimentation equilibrium experiments in the analytical ultracentrifuge and subsequent mathematical analysis of the concentration distributions obtained. It was found that the dimeric state is not significantly populated, the contribution of dimer to the total peptide weight probably being below 0.5%.

INTRODUCTION

Melittin is an amphipathic 26-residue peptide from bee venom (mol wt 2,840). It has been intensely studied, both with respect to its biological effects and to its physicochemical properties. The latter studies include numerous investigations on its self-association (e.g., 1–8).

In aqueous solutions, the occurrence of both monomers and tetramers of melittin has been established. The melittin monomer was found to be the predominant form at acid pH (3). It was also found to occur at neutral or alkaline pH if peptide and salt concentration were kept low. On the other hand, at high peptide and salt concentration the peptide was found to exist as a tetramer in that pH range (1–8). The tetramer is a dimer of dimers, at least in melittin crystals (9). In addition, it was shown that the two forms of melittin coexist at intermediate peptide and salt concentrations, and that they are linked to each other in an association equilibrium (1–8). The same behavior was found after insertion of the peptide into planar lipid bilayers (10). Surprisingly, the question whether or not the association equilibrium includes significant populations of melittin dimers has not yet been addressed. We have therefore looked at this problem by sedimentation equilibrium experiments in the analytical ultracentrifuge.

MATERIALS AND METHODS

Melittin ("reinst") was purchased from Serva (Heidelberg, Federal Republic of Germany). 20 mg of the peptide was dissolved in 2 ml 1 mM HCl (where it is predominantly monomeric [3]) and subjected to gel filtration on a column of Sephadex LH-20 (Pharmacia, Uppsala, Sweden) (60 × 2 cm) run in the same solvent. The peak fractions were then titrated to pH 7–8 and brought to 300 mM NaCl, 10 mM Tris-HCl (pH 8) by addition of appropriate buffer stock solutions. Peptide concentration was determined photometrically, using a value of $A^{1\%}_{1cm}$ (280 nm) = 19.7 (5).

Sedimentation equilibrium experiments in the analytical ultracentrifuge were performed in a Spinco model E ultracentrifuge equipped with an ultraviolet scanning system (Beckman Instruments, Inc., Palo Alto, CA). An An-G rotor was used in combination with standard Epon double sector cells. Sample volume per cell was 0.12 ml, rotor speed 40,000 rpm and rotor temperature 10°C. The cells were scanned at 280 nm. Sedimentation equilibrium was attained after ~30 h. The concentration vs. radius diagrams obtained were evaluated by fitting to the experimental concentration vs. radius ($c[r]$) data, by least-squares techniques, equations corresponding to a monomer/tetramer or a monomer/dimer/tetramer model of self-association, respectively (11). In the calculations, the partial specific volume $\bar{\nu}$ of the peptide was assumed to be 0.782 ml/g (12) and independent of local pressure in the pressure range occurring in our studies (1–40 bar) (8, 13).

RESULTS

As judged from published data under the conditions used in our experiments, monomers and tetramers of melittin should coexist in solution (1, 2). This is confirmed by the values of the weight average molecular weight $M_w(r)$ found by us that, in the same cell, varied between ~1.2 M_1 and 2.5 M_1. The average M_w value of melittin in the cells was thus close to that of the dimer, which should offer optimum conditions to discriminate between the two models of interest. A set of experimental $c(r)$ data and the results of the mathematical analysis according to a monomer/tetramer model are shown in Fig. 1. Both of them are representative of the outcome from three different centrifuge runs (altogether 12 cells, with initial protein concentrations between 100 and 400 μg/ml). As can be seen from the figure, fully satisfactory fits to the experimental data were obtained, the differences $c_e(r) - c_f(r)$ between experimental and fitted concentration data not exceeding the uncertainty of the c_e values. The fitted curves were unique and did not depend on the starting values used in the calculations. The fits obtained by use of a monomer/

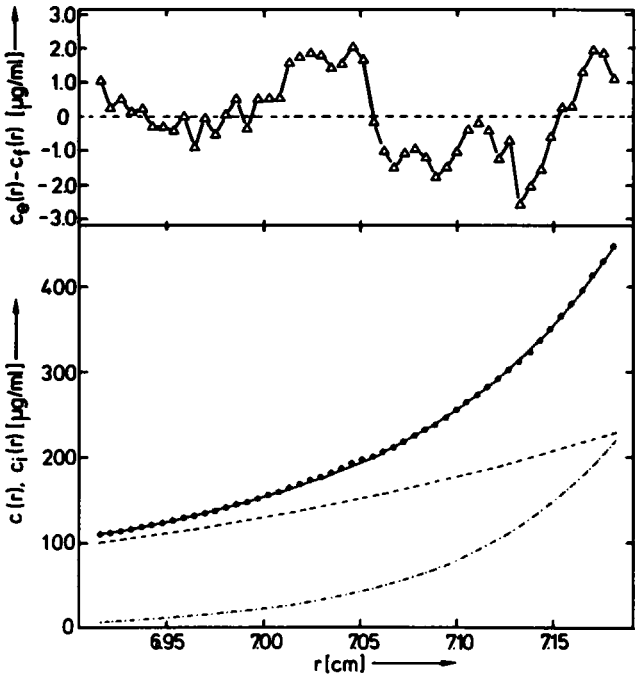

FIGURE 1 (*Bottom*): Experimental $c(r)$ distribution for a melittin solution of initial concentration 185 µg/ml (●), and fitted curves obtained by use of a monomer/tetramer model of self-association for total protein concentration (—) and for the contributions of monomer (---) and tetramer (···). The latter curves correspond to an equilibrium constant K_{14} of 81 l^3/g^3 ($1.85 \cdot 10^{12}$ M^{-3}). (*Top*): Differences $c_e(r) - c_i(r)$ between the experimental and fitted $c(r)$ data. The uncertainty of the experimental data was approximately ±2.5 µg/ml (without considering baseline errors).

dimer/tetramer model were not unique but fell into two classes, depending on the starting values chosen. One class, which led to lower values of the root mean square deviation, was characterized by negative dimer concentrations and was thus physically meaningless. Fits of the second class yielded positive dimer concentrations. However, the dimer populations at maximum amounted to ~0.5% and in most cases to <0.1% of the total local melittin concentration, whereas the calculated local monomer and tetramer concentrations were virtually identical to those obtained from a monomer/tetramer model.

Our sedimentation equilibrium experiments could not be of the meniscus depletion type, which inevitably will lead to some uncertainty in the baseline of the $c(r)$ diagrams. We have therefore performed additional calculations in which the position of the baseline was shifted by reasonable constant values. The results obtained by this procedure did not differ significantly from those described above.

DISCUSSION

It is clear that the process of the formation of melittin tetramers from monomers will have to include dimers as intermediates of the monomer-tetramer association. However, the results of the present paper show that there is no significant population of dimers: (*a*) when the $c(r)$ distributions obtained from the equilibrium sedimentation experiments were analyzed according to a monomer/dimer/tetramer model of self-association, the maximum contributions of dimer to the distributions consistently did not exceed 0.5% (wt/wt) and in most cases 0.1% (wt/wt) of the total local peptide concentration. These figures exclude the presence of significant amounts of dimer, even though the relative uncertainty of each of the figures is certainly quite large. (*b*) In accord with this conclusion, the experimental data could be fitted fully satisfactorily by a monomer/tetramer model of self-association, the differences between experimental and fitted concentration values being within the uncertainty of the experimental data. Thus, with respect to the populations of the different oligomers present, the self-association of melittin in aqueous solutions is sufficiently well characterized by a monomer/tetramer model.

It seems worthwhile to add that we have obtained entirely different results by applying the approach used in this paper to the self-association of two other proteins, band 3 protein from human erythrocyte membranes (14–16) and glucose dehydrogenase from *Bacillus megaterium* (17). With both systems, the experimental $c(r)$ data cannot be fitted satisfactorily by a monomer/tetramer model, whereas fits of excellent quality are obtained by use of a monomer/tetramer scheme (maximum dimer content calculated being 35–60% and 20–40%, respectively).

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