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Conformational changes in melittin upon complexation with an anionic melittin analog

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Melittin and its Glu-(7,21,22,23,24) analog upon mixing in equimolar concentrations form a hybrid oligomer with significant helical structure, in conditions in which each peptide separately adopts a largely disordered structure. The hybrid exhibits both cold- and heat-induced denaturations similar to the phenomena exhibited by proteins. The hybrid also retains significant residual structure at higher temperature, similar to the 'molten globular state' that has been suggested for proteins. Melittin, at concentrations in which it forms helical tetramers, also exhibits these phenomena and may be used as a model for protein-denaturation studies.

Melittin; Anionic melittin analog; Circular dichroism; Conformation; Melting profile; Cold-denaturation

1. INTRODUCTION

The amphipathic bee venom lytic peptide melittin consists of 26 amino acid residues with 6 positive and no negative charges: H_3N^+G-I-G-A-V-L-K^+-V-L-T-T-G-L-P-A-L-I-S-W-I-K^+-R^+-K^+-R^+-Q-Q-NH_2 [1,2]. This peptide is an excellent model for the study of protein folding since it lacks organized or stable secondary structure at submillimolar concentrations in pure water, yet becomes predominantly helical and tetrameric at high pH, or high ionic strength and high peptide concentration [3–6]. Also it adopts a helical conformation in membranes and surfactants [7], and in the presence of the anionic polymers poly(L-Glu) and poly(D-Glu) [8]. The anionic melittin analog, E-MLT, with the sequence H_3N^+G-I-G-A-V-L-E^--V-L-T-T-G-L-P-A-L-I-S-W-I-E^--E^--E^--E^--Q-Q-NH_2, wherein all the Lys and Arg residues were replaced by Glu residues, has a net charge of −4. This analog adopts a largely disordered structure and, unlike melittin, does not show a concentration-dependent conformational change in the range of 30 μM to 150 μM in 20 mM phosphate, pH 7.2.

In the present paper we report, based on circular dichroic data, that mixing of melittin and E-MLT results in the formation of a hybrid with significant helical content at a condition in which each peptide adopts a largely disordered structure. We also show from a melting profile that the hybrid exhibits both cold-induced and heat-induced denaturation similar to those of the globular proteins such as myoglobin, apomyoglobin and staphylococcal nuclease [9–11]. Melittin at a concentration of 120 μM also shows a similar melting profile. From this study we provide evidence that a small peptide like melittin, as an environment-dependent, associated four-chain aggregate, can be used as a model for protein folding and denaturation studies.

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2. MATERIALS AND METHODS

Melittin (Sigma) was purified twice by gel filtration using Sephadex G-50 with 20 mM acetic acid as the eluent. E-MLT was prepared by solid-phase peptide synthesis as follows: Starting from *p*-methylbenzhydrylamine resin, peptide chain was elongated on a peptide synthesizer, Model 430a (Applied Biosystems Inc., Foster City, CA) employing a double coupling protocol of the system software version 1.4. After the peptide chain assemblage, the peptide resin was treated with anhydrous hydrogen fluoride containing 15% 1,4-butanedithiol and 5% *p*-cresol at 0°C for 90 min. The crude material was purified on a reversed-phase HPLC using acetonitrile gradient in 0.1% trifluoroacetic acid. Amino acid composition (expected values are given in parentheses following observed values): T, 1.82(2); S, 0.84(1); E, 7.16(7); P, 1.02(1); G, 2.94(3); A, 2.03(2); V, 2.01(2); I, 2.91(3); L, 4.0(4); W, 0.95(1). Mass spectrometric data showed $(MH)^+$=2794.3 (2794.7). The peptide concentration was determined from the absorption spectrum and an ε_{280} value of 5600 $M^{-1}\cdot cm^{-1}$.

All CD spectra were obtained using a Jasco-500 spectropolarimeter at room temperature (25°C) using a silica cell of 1 or 2 mm path length. Sample solutions were made in 20 mM phosphate buffer, pH 7.2. Thermal effects on conformation were monitored by recording $[\theta]_{222}$ with temperature on an X-Y recorder. Temperature was ramped with a Neslab ETP-3 programmer at about 1°C per min.

3. RESULTS AND DISCUSSION

The circular dichroic spectra of melittin, E-MLT and the 1:1 mixture of E-MLT and melittin in 20 mM phosphate buffer, pH 7.2 at 25°C are shown in Fig. 1. At 30

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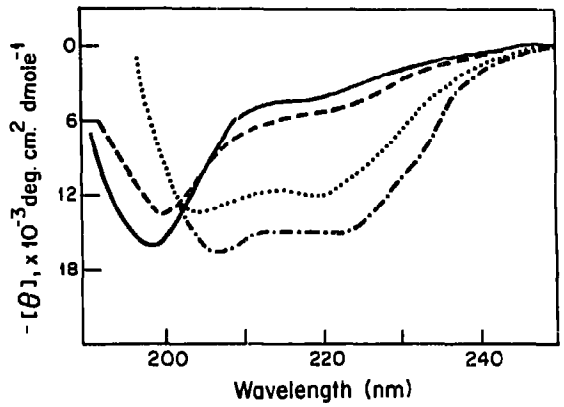

Fig. 1. Comparison of the CD spectra of melittin (----) and E-MLT (—) in 20 mM phosphate buffer, pH 7.2, at a concentration of 30 μM of the peptide with the CD spectra of the hybrid at a total concentration of 30 μM) (....) and at 65 μM (·····). The spectra were measured at 25°C.

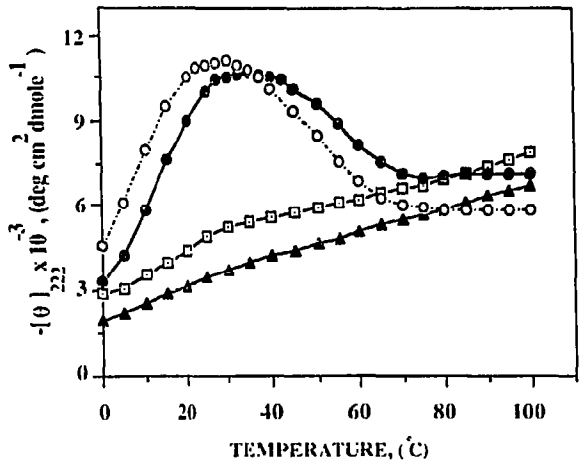

Fig. 2. Ellipticity of the melittin (□), E-MLT (△), and the hybrid (●) at 222 nm vs. temperature. Peptide concentration 30 μM; buffer 20 mM phosphate, pH 7.2. Melittin at 120 μM (○) is also shown for comparison.

μM concentration, both melittin and E-MLT have little order, with $[\theta]_{222}$=−4800 deg·cm^2·dmol^{-1}, and −3000 deg·cm^2·dmol^{-1}, respectively. The hybrid formed between equimolar amounts of melittin and E-MLT at a total monomer concentration of 30 μM, on the other hand, shows significant helical content with $[\theta]_{222}$=−10 600 deg·cm^2·dmol^{-1}. At a total monomer concentration of 65 μM, the hybrid was slightly turbid. Nonetheless, the spectrum shows the characteristics of a helix with $[\theta]_{222}$ = −15 000 deg·cm^2·dmol^{-1}. The formation of the hybrid results, most likely, from hydrophobic interactions, as suggested in the association of melittin monomers [3], and from the reduction of charge repulsion, and perhaps from stabilization by way of interactions between opposite charges.

At 120 μM concentration, melittin is a partially helical tetramer with a $[\theta]_{222}$ = −11 200 deg·cm^2·dmol^{-1}; however, at this concentration, E-MLT still exists as a largely disordered chain with an ellipticity of −3000 deg·cm^2·dmol^{-1} at 222 nm, suggesting that the negatively charged carboxylate groups of E-MLT render it more resistant to self-association than do the ammonium groups of melittin. Such resistance may arise from the characteristic property of the carboxylate groups, from the destabilization caused by the dipole interaction between the carboxylate groups and the C- terminus, or from the closeness of the negative charges of E-MLT to the backbone compared to the positive charges of melittin [12,13]. We made a series of melittin derivatives in which the amino groups of melittin were acylated with ω-aminoacyl residues (from glycyl through 7-aminoheptanoyl), thereby retaining the positive charges, but shifting them progressively away from the backbone. The results (to be published in detail) show that the ellipticity increases with the length of the ω- aminoacyl group.

The thermal unfolding for melittin and E-MLT was completely reversible. Fig. 2 shows the $[\theta]_{222}$ vs. temperature profiles for melittin, E-MLT and the hybrid. At 30 μM concentration, melittin shows continuous increase in ellipticity at 222 nm from −2900 to −7900 deg·cm^2·dmol^{-1} as the temperature changes from 0 to 100°C. E-MLT also behaves like melittin with the increase in ellipticity at $[\theta]_{222}$ ranging from −2000 to −6700 deg·cm^2·dmol^{-1}. But E-MLT is less helical at all temperatures than melittin, indicating that the negative charges make E-MLT different from melittin in some of the physical properties. The continuous increase in ellipticity with temperature exhibited by both melittin and E-MLT suggests that there may be a *temperature-induced association* in melittin and E-MLT as opposed to temperature-induced denaturation that is commonly observed in proteins.

The complex partially forms a helical hybrid which is most stable around 35°C with $[\theta]_{222}$ = −10 700 deg·cm^2·dmol^{-1} and less stable at the extremes of the temperature range, with $[\theta]_{222}$ = −3300 deg·cm^2·dmol^{-1} at 0°C and −7100 deg·cm^2·dmol^{-1} at 100°C. The hybrid exhibits a sigmoidal melting curve with T_m=55°C. It is largely random at 0°C but has significant residual structure at 100°C. The greater order at high temperature appears to result from hydrophobic forces [14,15]. The partially structured hybrid at 100°C may have a portion of the original native conformation which may be similar to the 'molten globular state' observed in proteins [16–18]. This state has been termed to represent stable intermediates which have the compact globularity of the molecule with native-like secondary structures but unfolded tertiary structure. The melting temperature profile of E-MLT and the complex seem to converge at higher temperatures. At present, the state of the melting profile above 100°C is unknown.

Figure 2 also shows the melting profile for 120 μM melittin which forms a partially helical structure with ellipticity at room temperature similar to that of the 30 μM hybrid. Melittin at 120 μM shows significant helical content around 30°C with $[\theta]_{222}$ = −11 100 deg·cm^2·dmol^{-1}, which decreases to −4600 deg·cm^2·dmol^{-1} at 0°C and −5800 deg·cm^2·dmol^{-1} at 100°C, and exhibits a sigmoidal type of melting curve with a T_m of 50°C. The general behavior of melittin at 120 μM is akin to the hybrid having significant structure at high temperature. However, compared to the hybrid it is less random at 0°C, and more random at high temperatures. From the T_m values obtained for the hybrid at 30 μM, and melittin at 120 μM, the hybrid is more stable to an extent of 5°C. This stability may arise from the reduction of charge repulsion in the hybrid in comparison to melittin.

The ellipticity of melittin (120 μM) at 80–100°C is flat, while the ellipticity of 30 μM melittin (monomer at low temperature) continues to rise and is, indeed, more negative than the former. Thus 30 and 120 μM melittin are in different states at 100°C. Melittin, 120 μM, at 100°C may not be monomeric. Melittin, 30 μM, may be monomeric or oligomeric at 100°C, a point not decided.

Short peptides like melittin can be useful models, not only for polypeptide–polypeptide interactions, but also for protein-denaturation studies, since our results indicate that melittin exhibits cold- and heat-induced denaturations with significant structure at higher temperatures.

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