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Temperature Dependence of the Melittin Folding Equilibrium Studied by Means of Fluorescence Excitation Spectra

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The temperature dependence of fluorescence excitation spectra is investigated. This gives information on melittin folding equilibrium complementary to that obtained from fluorescence emission studies. A fit of the excitation spectra of melittin with three Gaussian functions gives good results. It suggests that in melittin a reversal of L_a and L_b electronic states of tryptophane takes place. The separate L_a and L_b bands exhibit a temperature dependence of the width and maximum.

KEY WORDS: Melittin; excitation spectrum; folding equilibrium.

INTRODUCTION

Melittin is a natural occurring peptide consisting of 26 amino acid residues, the main component of bee venom. It contains a single intrinsic fluorophore, tryptophane [1]. When fully folded it forms symmetrical tetramers [2,3].

The folding process of melittin can be divided into two distinct processes: α-helix formation and tetramerization of single melittin molecules. However, these processes are dependent on each other and under steady-state conditions are thought to occur simultaneously [4].

The melittin folding equilibrium depends on many variables and we are particularly interested in its temperature dependence, since we want to use the system also for dynamical studies using a temperature-jump set-up. So far, the temperature dependence of the folding equilibrium has been studied by means of a number of different spectroscopic techniques: NMR [5], circular dichroism [4,6], fluorescence [7], and fluorescence polarization [8]. In this note we report a complementary approach by measuring the temperature dependence of fluorescence excitation spectra of melittin.

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EXPERIMENTAL

Melittin (85% pure, Sigma-Aldrich) was used as received. Fluorescence and fluorescence excitation spectra were recorded with Perkin-Elmer LSB-50 Fluorimeter equipped with a Peltier PT-1 thermostat.

RESULTS AND DISCUSSION

We have recorded both fluorescence emission and fluorescence excitation spectra at different temperatures for 120 μM melittin in phosphate buffer (20 and 150 mM), pH 7.2, with and without 1 M NaCl. According to the literature the sample with high salt concentration contains mostly fully folded tetramers [9]. The salt-free sample is reported as monomeric melittin, however, with a certain α-helical content [6,9].

For the tetrameric melittin sample we have observed marked, reversible changes with temperature both in fluorescence emission and fluorescence excitation spectra. A pronounced shift of the fluorescence emission maximum to the red (Figs. 1 and 2) is consistent with the data reported by Demchenko *et al*. for an analogous solution of melittin in Tris buffer [7]. The shift indicates heat-induced unfolding of melittin in this solution. Part of the shift is due to the so-called Red Edge Effect (REE [10]), but obviously there is also a reversible chemical process occurring in the

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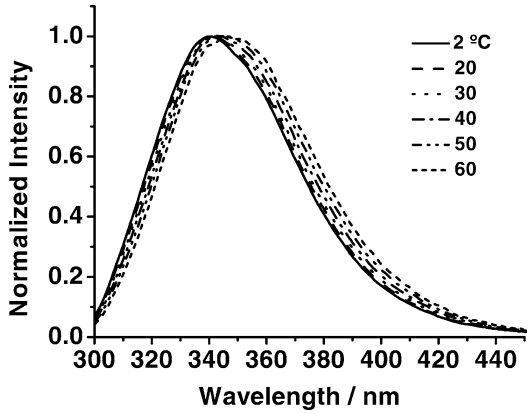

Fig. 1. Normalized fluorescence spectra of tetrameric melittin (120 μM, 20 mM phosphate buffer, pH 7.2, 1M NaCl) in different temperatures. Excitation at 280 nm.

solution, because the overall shift is too large to be fully assigned to the REE. Both NMR [5] and gel-filtration [7] studies exclude deaggregation and suggest heat-induced loosening of the tetrameric structure. Based on fluorescence emission spectra it is difficult to say whether cold-denaturation occurs (reported for melittin by a few authors [4,6,8]), since a small shift to the red with decrease of temperature may be obscured by the REE resulting in a overall shift to the blue.

Fortunately, the changes observed with temperature in the excitation spectra of melittin are not obscured by this effect. For the excitation spectra of tetrameric melittin (emission monitored at 340 nm) we have observed a temperature dependence for the ratio of the intensity of the bands at 228 nm and 282 nm, which is not

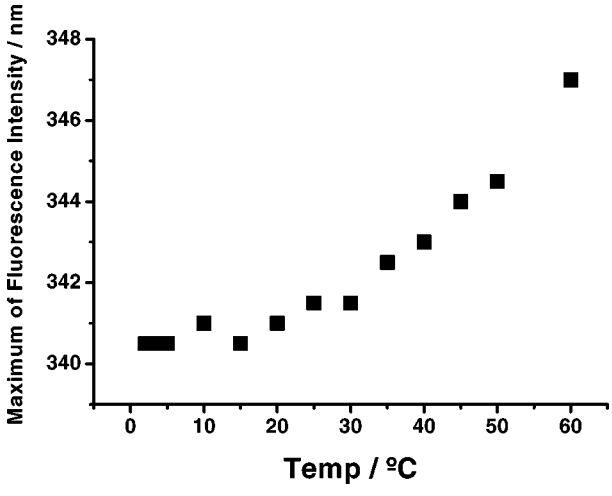

Fig. 2. Maximum of fluorescence band versus temperature for tetrameric melittin (120 μM, 20 mM phosphate buffer, pH 7.2, 1M NaCl).

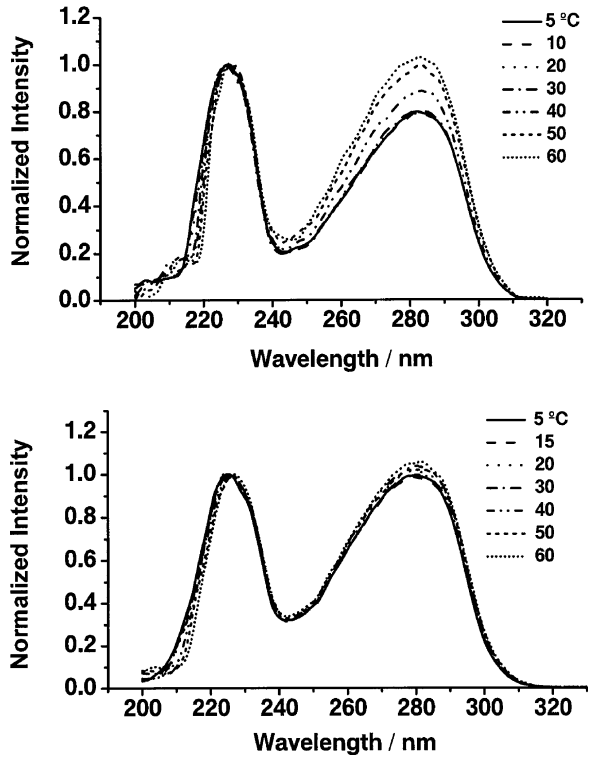

Fig. 3. Normalized fluorescence excitation spectra for tetrameric (120 μM, 150 mM phosphate buffer, pH 7.2, 1M NaCl; upper panel) and monomeric (120 μM, 20 mM phosphate buffer, pH 7.2, no salt; lower panel) melittin in different temperatures. Emission at 345 nm.

present in the spectra of the monomeric sample (Figs. 3 and 4). This dependence exhibits a tendency similar to the temperature-dependent shift of the fluorescence maximum of tetrameric melittin (see Fig. 2). The value of the ratio at high temperatures for the tetrameric sample approaches the value characteristic for monomeric sample (approximately 1, see Fig. 4). This is also consistent with the change in fluorescence emission maxima for monomeric and tetrameric melittin. The change in fluorescence maximum is usually explained by assuming that the tryptophane is in a more polar environment for the unfolded protein. Our results indicate, that, even if at high temperatures the sample is still tetrameric [5], the tryptophane fluorophore experiences an environment very similar to that in monomeric, unfolded, melittin, so the loosening of the tetramer must be considerable.

The dependence of the excitation spectra of tetrameric melittin on temperature strongly suggests that there is no cold-denaturation under the conditions studied here: a reversal of the trend in the ratio of excitation maxima is not observed below 20°C. Probably, the high salt concentration in the solution effectively hinders this

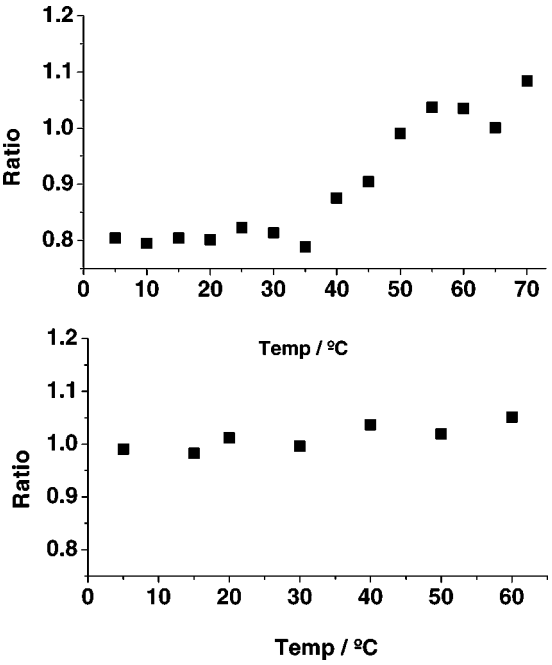

Fig. 4. Ratio of the intensity of fluorescence excitation bands versus temperature for tetrameric (upper panel) and monomeric (lower panel) melittin. The value of intensity at the maximum of each band (228 nm and 282 nm) was used to create the plot.

process, which is usually much weaker than heat-denaturation [11].

The excitation spectra were fitted with the Origin 7.0 Multi-Peaks Fit Program. The best fit was obtained using three Gaussian functions (see Fig. 5). The band at 227 nm can be fitted very well with one Gaussian and corresponds to a higher excited state (S_2) of tryptophane. The results of the fit of the band at 282 nm are in good agreement with the literature: they can be assigned to the L_a (270 nm, broader) and L_b (287nm, narrower) absorption bands of tryptophane [12]. The fit results suggest that in melittin a reversal of these states takes place similar to tryptohane in water. The separate L_a and L_b bands also exhibit a temperature dependence of the width and maximum, and differences between monomer and tetramer. A more extensive experimental and theoretical analysis is still in progress.

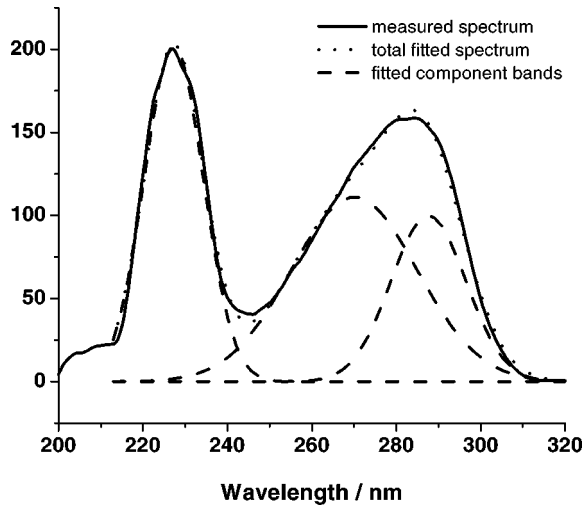

Fig. 5. Results of the multi-Gaussian fit for the excitation spectrum of tetrameric melittin recorded in 20°C. The values of the maxima obtained for the three component bands = (227.3 ± 0.05) nm, (270 ± 2) nm and (287.6 ± 0.3) nm and the correlation coefficient $R^2 = 0.995$.

CONCLUSIONS

We have observed temperature-induced changes in the fluorescence excitation spectra of tetrameric melittin. The temperature dependence exhibited by the excitation spectra provides additional information, complementary to the fluorescence emission spectra data, regarding the melittin folding equilibrium.

Currently we have no good explanation of the behavior of the excitation spectrum as a function of temperature. The change is correlated with the structural changes of the melittin. The usual changes due to changing environment are shifts in the absorption/emission maxima and enhanced quenching upon exposure to the solvent environment. Neither of these can explain the observed effect, which appears to be related to changes in the (relative) oscillator strength of the L_a and L_b transitions.

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