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Anomalous Temperature Dependence of Peptide Films at Air–Water Interface

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Abstract—The tetrapeptide derivative Tyr-Gly-Phe-Ala-OBz (**1**) forms monolayers as confirmed by compressibility studies carried out at various temperatures. Peptide **1** monolayer exhibits an anomalous structural transition at 40 °C as evidenced by π–A isotherms recorded at different temperatures. The structural transition is also observed in aqueous solution of trifluoroacetate of peptide **1** as evidenced by fluorescence and Raman scattering intensity measurements.

The design of molecular subunits that self-assemble into defined structures like micelles, microemulsions, monolayers, bilayers and vesicles are of current interest.[1,2] Earlier results have demonstrated the formation of two- and three-dimensional networks of assemblies of oligopeptides namely nanotubles,[3] membranes[4] and micelles.[5] A key to controlling the type of aggregate lies in manipulating the energetics involved in self-association. The strong and directional nature of hydrogen bonds had led to the design of various kinds of self-assembling systems. However microscopic details regarding other non-covalent interactions that operate in the self-assembly of these oligopeptides are not been dealt yet. It has been previously shown that the trifluoroacetate salt of tetrapeptide Tyr-Gly-Phe-Ala-OBz (**1**) forms an organised aggregate in aqueous solution and undergo a sharp structural transition at 40 °C.[5c] Similar anomalous thermal behaviour in self-assembled systems has been observed by the other investigations in past years.[6] The aim of the present work is to investigate the peculiar temperature discontinuity for monolayer of peptide **1** at the air-water interface around a structural transition temperature namely 40 °C.

The tetrapeptide was synthesised using DCC/HOBT method. The synthesis, characterization and its micellar behaviour is published elsewhere.[7] The water used for the subphase was triply distilled from the deionized water. Dichloromethane was used as the spreading solvent. A rectangular trough with a Wilhemy balance was employed for the Langmuir–Blodgett (LB) film fabrications. The films were compressed at a constant rate of about 10 Å^2 mol^{-1} min^{-1}. The monolayers were rested for 5 min prior to compression at all temperatures.

The study of surface pressure molecular area (π–A) isotherms for peptide **1** obtained at various temperatures (22–55 °C) (Fig. 1) can explain the interactions between film and subphase molecules. From Figure 1(a), it is seen that all the isotherms of peptide 1 are of liquid expanded type indicating the fairly rigid nature of the film. The value of the molecular area of the monolayer (A_c) can be directly evaluated from the intersection of the x-axis with the tangent line of the isotherm, on the onset of condensed phase of the film. The surface entropy ΔS_s of a spread monolayer can be calculated at a constant area A, with the following formula.

$$\Delta S_s = A(\partial\pi/\partial T)_A \quad (1)$$

From eq (1), we can evaluate ΔS_s (J mol^{-1} K^{-1}) above and below 40 °C, for the fixed value of A. The ΔS_s value can be obtained from the plot of π versus T at a fixed A for all temperatures. At A = 30 Å^2/molecule, above 40 °C ΔS_s is negative (−48.0) and is positive (+25.3) below 40 °C for the peptide monolayers.

The structural transition of the trifluoroacetate of the peptide **1** aggregate in aqueous solution was also determined by using 8-anilino-l-naphthalene sulfonic acid (ANS)[8] as fluorescence probe, peptide tyrosine fluorescence and Raman scattering intensity measurement at various temperatures. The plot of intensity against

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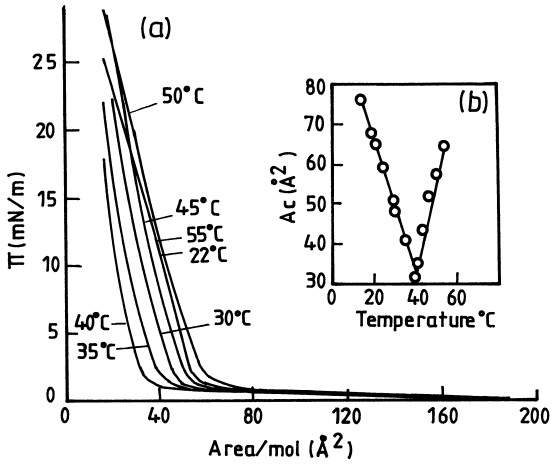

Figure 1. (a) Surface pressure–molecular area (π–A) isotherms of Tyr-Gly-Phe-Ala-OBz (**1**) at various temperatures; (b) Plot of A_c against temperature for peptide **1**.

temperature shows the occurrence of transition ~40 °C by a sudden inflection in the initial slope of this plot (Fig. 2) similar to that observed in the peptide films. The unaltered structural transition temperature in the film and micelle suggests that molecular interactions involved in these aggregates are not very different in nature.

The formation of water–amphiphile complexes strengthens the polar group penetration into the aqueous subphase, and also changes the interfacial water structure.[9–12] It is known that surface film introduces a strong ordering effect on water molecules, which are entrapped in the intermolecular space in the monolayers. In the 22–40 °C region (first process), the molecular area in the compressed state (A_c) decreases with increase in the temperature (Fig. 1(b)), indicating the extrusion of water molecules into the bulk phase. This results in the positive entropy change observed in the first process. At 40 °C it attains the minimum value of 32.5 Å^2 and the peptide molecules are tightly packed in the monolayer, and the intermolecular interactions between them are stronger.

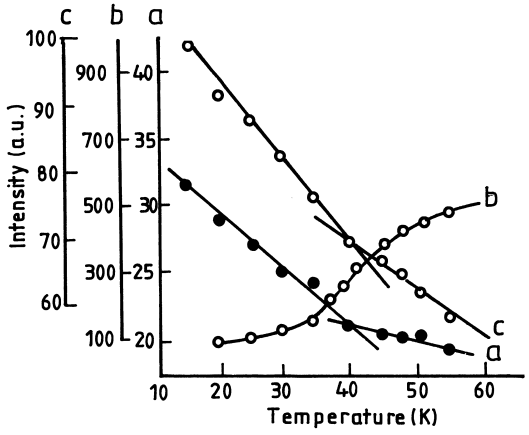

Figure 2. Determination of structural transition of trifluoroacetate of peptide (**1**) in aqueous solution: (a) Raman scatter method $\lambda_{ex}=345$ nm; $\lambda_{em}=396$ nm; (b) ANS fluorescence method (ANS) $=2\times10^{-6}$ M $\lambda_{ex}=345$ nm; $\lambda_{em}=461$ nm; (c) tyrosine fluorescence method $\lambda_{ex}=274$ nm; $\lambda_{em}=309$ nm.

Above 40 °C (second process), the molecular area increases indicating a structural transition leading to an assembly in which molecular area increases on increasing the temperature attributing to thermal agitation and entanglement of amphiphiles resulting in the formation of intermolecular hydrogen bonding which will explain the negative entropy effect. It should be noted that at below 40 °C the ANS molecules binds to the region containing water molecules indicated by the lower intensity when compared to the region above 40 °C. After attaining 40 °C all the water molecules associated with the peptide molecules are extruded and the environment experienced by the ANS molecules are more hydrophobic resulting in the increased fluorescence of ANS (Fig. 2(b)).

The fluorescence of tyrosine can be quenched by the presence of charged amino group.[13] However after attaining the structural transition temperature the rate of quenching is decreased indicating that the distance between the charged α-amino group and the neighboring tyrosine side chain increases. This interpretation agrees with the proposed entanglement in the monolayer above 40 °C. The nature of the hydrogen bonding among the monomers in the aggregated state is reported by our group using ^{1}H NMR of amide protons.[5c] In the first process, the $\Delta\delta/\Delta T$ value for alanine, glycine and phenylalanine amide protons are -2.3×10^{-3}, -3×10^{-3} and 2.8×10^{-3} ppm °C^{-1}, respectively, indicating the breakage of hydrogen bond formation. This may be due to the extrusion of water molecules from the peptide aggregates resulting in breakage of peptide–water hydrogen bond. As breakage of hydrogen bonds leads to upfield proton resonance which results in negative temperature coefficients. Above 40 °C, as proposed now, the amphiphiles seemingly entanglements with intermolecular hydrogen bond formation. Increase in the temperature (above 40 °C) results in more amide protons participating in hydrogen bonding which shifts the amide protons to low field with positive temperature coefficients.

π–A isotherms of simple amphiphilic molecules at the air–water interface usually show that A_c increases with the temperature, because the stronger thermal agitation produces repulsion between the hydrophobic chains. However there are some cases[6,12,14–23] where the opposite trend was reported, that is A_c decreases when T increases, and the loss of film molecules through evaporation or solubilisation cannot be invoked as the only explanation of such an effect. This unexpected behaviour has already been related to the squeezing out of some water molecules from the coordinating shells that surround the polar head groups of the amphiphile. The proposed structure of peptide monolayers at the air–water interface is shown in the Fig. 3.

The present study reveals that the structural transition involved in monolayer films of peptide **1** is similar to that observed in aggregates in solution. These results also support that the second process (above 40 °C) is more ordered than the first process (40 °C).

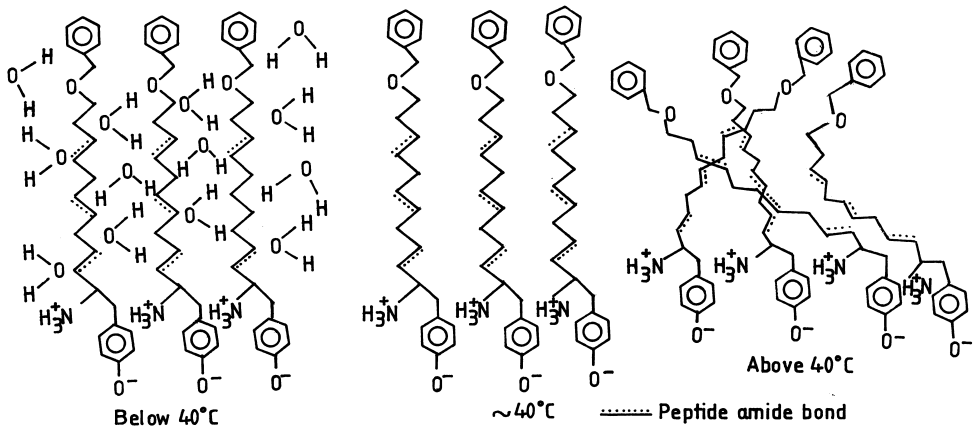

Figure 3. Proposed structure of peptide monolayer at the air–water interface at different temperature regions.

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