A New Micelle-forming Peptide

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A new micelle-forming peptide TFA·Tyr-Gly-Phe-Ala-OBz (TFA = trifluoroacetic acid) is synthesised and evidence for its micelle formation obtained from UV–VIS, fluorescence spectroscopy, conductometry and pH techniques; the critical micelle concentration (cmc), standard free-energy change of micelle formation (ΔG°_{m}) and aggregation number of this peptide at 20 °C are 8×10^{-5} mol dm^{-3}, −28.8 kJ mol^{-1} and 11, respectively.

Many smaller peptides exhibit a wide variety of biological activities.[1] Recently, we have investigated[2,3] the micelle formation on various collagens in acetate and citrate buffer at acidic pH. Hydrodynamic and thermodynamic studies of their interaction with various surfactant micelles and urea have been made. Although β-casein[4] forms micelles, to the best of our knowledge not all peptides do so in aqueous solution. Here, we report the synthesis† and NMR spectroscopic characterisation of a new micelle-forming tetrapeptide TFA·Tyr-Gly-Phe-Ala-OBz (TFA = trifluoroacetic acid). Evidence for micelle formation of this peptide has been obtained from UV–VIS, fluorescence spectroscopic, pH and conductometric measurements.

The peptide was characterised by ^{1}H NMR (400 MHz). The structure of the tetrapeptide and its UV–VIS spectra are shown in Fig. 1 and 2, respectively. The specific conductance, pH, absorbance and fluorescence intensity were plotted as a function of concentration of peptide. The abrupt changes in the value of the initial slopes at a particular concentration were considered as the cmc[5] (Fig. 3). It should be mentioned that the fluorescence emissions were observed at 303 and 600 nm, respectively, when the peptide was excited at 274 nm. The cmc of the peptide obtained at both the emissions are the same. The cmc of the peptide obtained by various methods are in good agreement with each other and found to be 8×10^{-5} mol dm^{-3} at 20 °C.

However, for the determination of aggregation number[6] of the peptide, the Mg salt of 8-anilino 1-napthalene sulfonic acid (ANS) and *N*-cetyl pyridinium chloride (CPC) were used as external fluorescent probe and quencher, respectively. This technique assumes that the numbers of both probe and quencher molecules per micelle have Poisson distributions, which leads to the expression eqn. (1),[6] where I_0 and I

$$\ln (I_0/I) = \bar{N}\,[Q]/(C_s\text{-cmc}) \quad (1)$$

are the emitted light intensities with quencher concentations 0 and [Q], respectively, $\bar{N}$ is the mean peptide aggregation number and C_s is the total concentration of peptide; $\bar{N}$ is calculated from the slope of plot of ln (I_0/I) against [Q] (*cf.* Fig. 4). The probe ANS was used at a concentration small

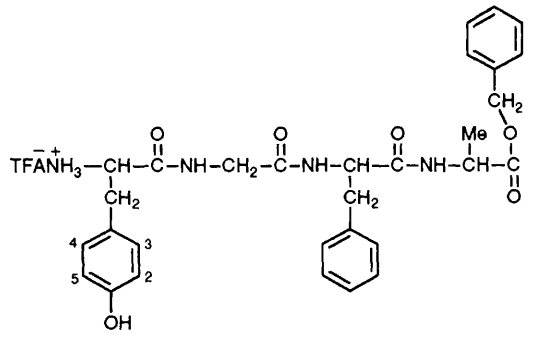

Fig. 1 Structure of TFA·Tyr-Gly-Phe-Ala-OBz peptide

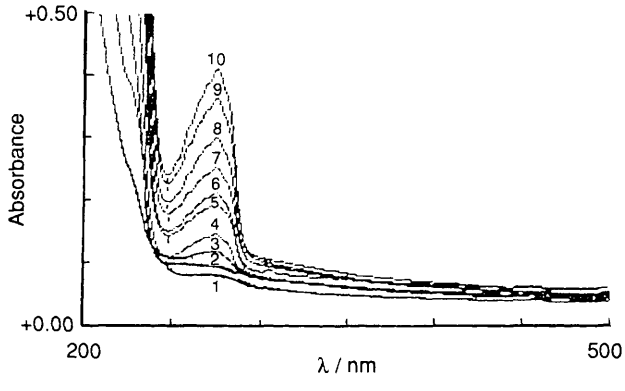

Fig. 2 UV–VIS spectra of the tetrapeptide in aqueous solution at 20 °C. Curve numbers 1–10; peptide concentrations = 1×10^{-5}, 2×10^{-5}, 4×10^{-5}, 6×10^{-5}, 8×10^{-5}, 10×10^{-5}, 12×10^{-5}, 16×10^{-5}, 20×10^{-5} and 24×10^{-5} mol dm^{-3}, respectively.

† *Synthesis and purification of TFA·Tyr-Gly-Phe-Ala-OBz*: Peptide was synthesised by solution-phase procedures and homogeneity was checked by TLC on silica gel. The structures of all the peptides were established by ^{1}H NMR (300 MHz or 90 MHz).

Boc-Phe-Ala-OBz **1**: Boc-Phe (2.65 g, 10 mmol) was dissolved in CH_2Cl_2 (15 ml) and cooled to 0 °C. Ala-OBz (1.79 g, 10 mmol) was added followed by dicyclohexylcarbodiimide (DCC) (2.16 g, 10 mmol) in CH_2Cl_2 (10 ml). The mixture was stirred at 0 °C for 4 h and at room temp. overnight. The dicyclohexylurea (DCU) was filtered off, and the filtrate washed (1 mol dm^{-3} HCl, 1 mol dm^{-3} $NaHCO_3$ and water) and dried (Na_2SO_4). Evaporation of solvent yielded **1** which was homogenous on TLC. Yield: 3.2 g (75%). (Boc = *tert*-butoxycarbonyl).

TFA·Phe-Ala-OBz **2**: The dipeptide **1** (2.56 g, 6 mmol) was dissolved in TFA (15 ml) and kept at room temp. for 3 h. The removal of Boc group was monitored by TLC. After evaporation of TFA, the residue was kept over KOH pellets under vacuum.

Boc-Tyr-Gly-OMe **3**: Boc-Tyr (2.81 g, 10 mmol) was suspended in dimethylformamide (DMF) (10 ml) and cooled to 0 °C. Gly-OCH_3·HCl (1.256 g, 10 mmol) with 1.4 ml of triethylamine (TEA) in 5 ml of DMF was added followed by DCC (2.16 g, 10 mmol) in 5 ml of DMF. The mixture was stirred at 0 °C for 4 h and at room temp. overnight. Yield: 2.59 g (70%).

Boc-Tyr-Gly-CO_2H **4**: The dipeptide **3** (2.305 g, 6.5 mmol) was dissolved in MeOH (10 ml) and cooled to 0 °C. 2 mol dm^{-3} NaOH (6.5 ml) was added dropwise over a period of 0.5 h. Stirring the mixture at room temp. for 1 h, the absence of **3** was confirmed by TLC, and acidified to pH 2.5 at 0 °C. The MeOH was evaporated under vacuum and the residue dissolved in $MeCO_2Et$ and filtered. The filtrate was dried (Na_2SO_4) and the dipeptide **4** was obtained as a gum. Yield: 1.87 g (80%).

Boc-Tyr-Gly-Phe-Ala-OBz **5**: The peptide **4** (1.78 g, 5 mmol) was taken in 8 ml of DMF and cooled to 0 °C. **2** (5 mmol) followed by TEA (0.7 ml), DCC (1.089 g, 10 mmol) and 0.675 g of HOBT (hydroxybenzotriazole) were added and stirred at 0 °C for 4 h. Stirring further at 4 °C for 24 h, the DCU was filtered and washed with $MeCO_2Et$. The DMF was evaporated under vacuum and the residue was dissolved in $MeCO_2Et$ and processed as in the case **1**. The tetrapeptide was purified over silica gel column with 10% MeOH in $CHCl_3$ as eluent. The peptide is further characterised by ^{1}H NMR (400 MHz). Yield: 2.29 g (60%).

TFA·Tyr-Gly-Phe-Ala-OBz **6**: The tetrapeptide **5** (1.91 g, 2.5 mmol) was dissolved in TFA (10 ml) and kept at room temp. for 3 h. The removal of Boc group was ensured by TLC. TFA was removed under vacuum and the TFA salt was precipitated by anhydrous diethyl ether. The TFA salt tetrapeptide was homogeneous on TLC. Yield: 1.56 g (80%).

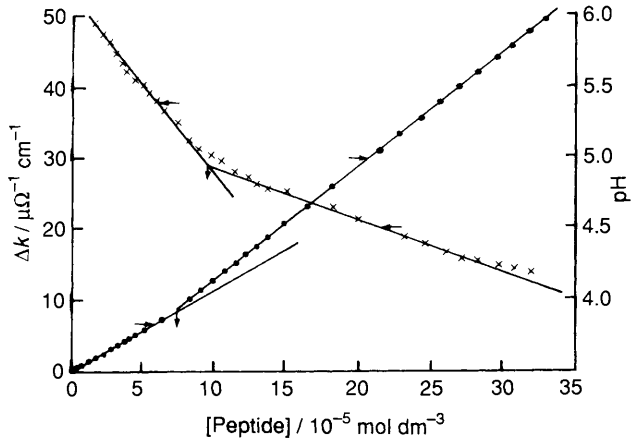

Fig. 3 Plot of difference specific conductance and pH *vs.* peptide concentration; at 20 °C.

enough to prevent excimer formation. The aggregation number of the peptide at 20 °C was found to be 11. Such a low aggregation number has been reported[7] recently for some micellar systems. Aggregation number of the peptide was also determined using pyrene as a probe and it was found to be 10 ± 1. Thus, the quenching kinetics with ANS probe and the validity of eqn. (1) have been established.

Using biphasic micellar model,[8] the standard free-energy change for micelle formation, ΔG^0_m of peptide has been calculated from eqn. (2) including $\bar{N}$ as a thermodynamic

$$\Delta G^0_m = RT \ln (\text{cmc}/\bar{N}) \quad (2)$$

variable. The ΔG^0_m value of peptide in aqueous solution at 20 °C was found to be −28.8 kJ mol^{-1}.

It is interesting to note that the specific conductance increases when peptide forms micelles (Fig. 3). The enhancement of specific conductivity in the post-micellar region of peptide is due to the increase in mobility of the peptide ion in aqueous solution. The result of conductivity is consistent with the low aggregation number found and suggests that the degree of dissociation of the micelles is very high *i.e.* little counter ion binding. The rate of decrease of pH with peptide concentration is larger below the cmc than above the cmc (Fig. 3). This suggests that above the cmc, although the small micelles have a high conductance, they could still be binding some protons. There is much evidence that the linear peptide hormone glucagon, His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr, readily aggregates[9] in solution at high concentrations and at acidic pH to form antiparallel β-conformations. Our results also suggest that the tetrapeptide TFA-Tyr-Gly-Phe-Ala-OBz forms micellar aggregates at a certain concentration at acidic pH. Further work regarding various temperature-dependent parameters,

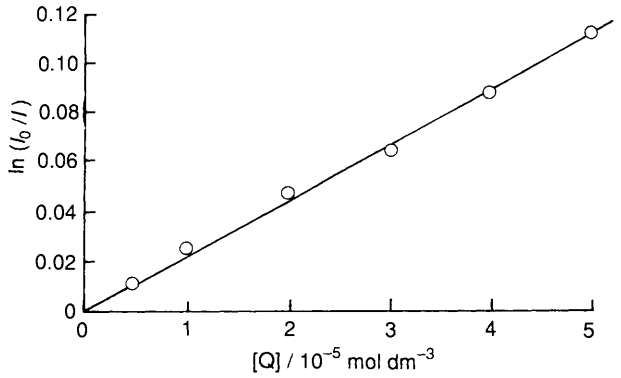

Fig. 4 Plot of ln (I_0/I) *vs.* quencher concentrations at 20 °C [ANS] = 1 × 10^{-5} mol dm^{-3} (fixed); [peptide] = 5 mmol dm^{-3} (fixed); λ_{ex} = 346 nm and λ_{em} = 387 nm

and detailed hydrogen-bonding conformations and thermodynamic studies will be reported elsewhere.

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