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Rapid communication

Fluorescence spectroscopy of small peptides interacting with microheterogeneous micelles

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ABSTRACT

Many peptides containing tryptophan have therapeutic uses and can be studied by their fluorescent properties. The biological activity of these peptides involves interactions with many cellular components and micelles can function as carriers inside organisms. We report results from the interaction of small peptides containing tryptophan with several microheterogeneous systems: sodium dodecyl sulphate (SDS) micelles; sodium dodecyl sulphate–poly(ethylene oxide) (SDS–PEO) aggregates; and neutral polymeric micelles. We observed that specific parameters, such as wavelength of maximum emission and fluorescence anisotropy, could be used to ascertain the occurrence of interactions. Affinity constants were determined from changes in the intensity of emission while structural modifications in rotameric conformations were verified from time-resolved measurements. Information about the location and diffusion of peptides in the microheterogeneous systems were obtained from tryptophan emission quenching experiments using N-alkylpyridinium ions. The results show the importance of electrostatic and hydrophobic effects, and of the ionization state of charged residues, in the presence of anionic and amphiphilic SDS in the microheterogeneous systems. Conformational stability of peptides is best preserved in the interaction with the neutral polymeric micelles.

Fluorescent properties of tryptophan (Trp) have been used to study peptides and are a useful tool to investigate their structural properties in the presence of aggregates which can function as carriers inside organisms (Romani et al., 2006; Romani and Ito, 2009). Microheterogeneous systems like amphiphilic micelles, polymer-surfactant aggregates and polymeric micelles have important technological implications for drug delivery, cosmetic preparation, and detergent action (De et al., 2005; Imamura and Konishi, 2006). In pharmaceutical formulations containing peptides, it is desirable to select surfactants which provide means to enhance the physical stability by preventing undesirable conformational changes and aggregation.

Polyethyleneoxide (PEO)-based surfactants are used in formulation with peptides (Sjögren et al., 2005). Representative of anionic surfactants, sodium dodecyl sulphate (SDS) micelles solubilize proteins and peptides. In aggregates with PEO, SDS micelle beads are supported along the polymer chain, with the surface protected from contact with water (Sen et al., 2002). Colloidal carriers such as polymeric micelles transport lipophilic substances, acting as a long-time circulation delivery system (Zhang et al., 2006). Polyethylene oxide (PEO) can be used as hydrophilic portion, due to ability to prevent opsonization and increase the circulation time (Owens and Peppas, 2006; Moghimi and Szebeni, 2003). The hydrophobic block polypropyleneoxide (PPO) has shown interesting results in pharmaceutical formulations (Scherlund et al., 2000; Mali et al., 2007) and the commercial copolymer F127 has been used in preparation of gels for the controllable delivery of hydrophilic and hydrophobic drugs.

In this paper we conduct a fluorescence study of small dipeptides containing tryptophan interacting with SDS micelles, SDS–PEO aggregates and LUTROL® F127 polymeric micelles. The peptides examined were: TrpX (X = Gly, Ala, Leu), XTrp (X = Leu) and acetyl-XTrp-NH_2 (X = Arg, Glu). Steady-state and time-resolved fluorescence experiments were performed to characterize the interaction and to verify if peptides in microheterogeneous systems maintain their structural stability. The location of Trp was investigated through quenching by alkylpyridinium halides, cationic surfactants containing the pyridinium moiety, with hydrophobicity and intramicellar mobility dependent on the size of the alkyl chain (Romani et al., 2001; Galán et al., 2005).

XTrp and TrpX dipeptides were purchased from Sigma–Aldrich and used as received. Acetylated peptides were synthesized as described (Marquezin et al., 2003). SDS (99%, Sigma, St. Louis) was purified by recrystalization from ethanol to avoid lack of

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Table 1
Association constants (K_b, M^{-1}) for peptides in interactions with the microheterogeneous systems.

	SDS	SDS/PEO	LUTROL® F127
TrpGly	51.3	37.2	27.1
TrpAla	46.0	30.3	26.4
TrpLeu	64.5	24.2	22.0
LeuTrp	38.4	27.5	24.4
AcArgTrpNH$_2$	111.3	81.6	36.5

reproducibility due to use of impure SDS. PEO (average molecular weight 8000 g/mol, Aldrich) and LUTROL®F127 (BASF) were used as received. N-ethylpyridinium (NEP$^+$) and N-hexylpyridinium (NHP$^+$) chlorides were prepared according to Romani et al. (2001). N-dodecylpyridinium chloride (NDP$^+$) was purchased from Aldrich and recrystallized from acetone.

Steady-state fluorescence measurements were performed on Hitachi F4500 or F-3010 spectrofluorimeters. Time-resolved fluorescence experiments were made in an apparatus based on the time-correlated single-photon counting technique (Romani et al., 2006).

In titration experiments, SDS and polymers were added in small aliquots to peptide solution at initial concentration 1×10^{-5} M (PBS 0.01 M, pH 7.4). In quenching experiments, aliquots of a peptide stock solution (1×10^{-3} M) were added to volumetric flasks containing, or SDS (0.05 M), or SDS (0.05 M)–PEO (2% weight), or LUTROL®F127 (2% weight). Solutions were stirred for 40 min to attain the partition equilibrium of peptides. Aliquots of concentrated solutions of quenchers (0.15 M) were added directly to the curvette, N-alkylpyridinium concentration was calculated based on molar absorptivity (4250 M^{-1} cm^{-1} at 258 nm).

We observed that maximum emission of dipeptides located near 360 nm in PBS and was blue shifted (3–15 nm) in microheterogeneous systems, indicating decreased polarity around the tryptophan residue. Steady-state anisotropies in aqueous medium are small and increase to above 0.020 in SDS and SDS–PEO, and to 0.015 in polymeric micelles. Anisotropy decays fitted to bi-exponential curves, with short rotational correlation time (<0.20 ns) due to the rotation of the indole ring, while long rotational correlation time (>1.0 ns) is ascribed to peptide overall tumbling. Rotational correlation times are higher in SDS compared to SDS–PEO. The smaller TrpGly has lowest rotational times and the larger acetyl-amide peptides presented correlation times around 4.5 ns in polymeric micelles.

Binding constants (K_b) were calculated from the fluorescence intensity data, using the relation between $\log[(I_0 - I_f)/(I_f - I_{inf})]$ and $\log[F]$, where I_0, I_f and I_{inf} are the fluorescence intensity in the absence, in a certain concentration $[F]$, and in saturating surfactant concentration, respectively (Aveline et al., 1995). Higher K_b values were obtained with SDS micelles, indicating the relevance of electrostatic interactions. In SDS–PEO the polymeric chains shield the micelle surface from contact with the bulk solution and the constants are lower than in SDS (Table 1). In neutral polymeric micelles, the absence of electrostatic interactions leads to the lowest values for K_b. Highest constants were for AcArgTrpNH$_2$, which interacts electrostatically with SDS, and has hydrophobic interaction with neutral polymeric micelles.

Emission decay profiles in every system were fitted to tri-exponential curves. Compared to PBS, long lifetime component in microheterogeneous systems raised significantly (Table 2). However, the corresponding pre-exponential factor in SDS and SDS–PEO drastically decreased to below 0.02, while the contribution from the short lifetime increased to more than 0.50. In the polymeric micelles, peptides have a decay profile similar to those in PBD, both in pre-exponential factors as in mean lifetime values. There is an identification of the lifetimes and corresponding Trp rotamers in proteins (Clayton and Sawyer, 1999; Pan and Barkley, 2004), and theoretical calculations (Goldman et al., 1995) showed that the long lifetime is associated to g^- rotamers. The presence of microheterogeneous micelles significantly decreases the pre-exponential factor corresponding to the long lifetime component, corresponding to a decrease in the contribution of g^- rotamers of the peptides. The effect is less pronounced in the peptides in polymeric micelles.

Table 2
Time-resolved fluorescence parameters for peptides (1.0×10^{-5} M) in PBS and microheterogeneous systems.

	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	α_1	α_2	α_3	$\langle\tau\rangle$ (ns)
PBS 0.01 M							
TrpX	7.4	1.85	0.39	0.35	0.42	0.24	5.95
XTrp	3.9	2.01	0.56	0.27	0.32	0.41	2.87
AcArgTrpNH$_2$	5.7	1.96	0.43	0.01	0.59	0.43	1.92
AcGluTrpNH$_2$	2.6	1.43	0.34	0.22	0.29	0.49	1.81
SDS 0.05 M							
TrpX	9.2	1.58	0.30	0.01	0.47	0.52	2.35
XTrp	5.88	1.49	0.21	0.03	0.42	0.55	2.14
AcArgTrpNH$_2$	6.72	1.54	0.30	0.02	0.36	0.62	2.11
AcGluTrpNH$_2$	9.91	1.69	0.26	0.01	0.42	0.57	2.34
SDS/PEO 2%							
TrpX	12.82	1.80	0.34	0.04	0.53	0.43	5.45
XTrp	13.24	2.04	0.44	0.04	0.41	0.55	5.33
AcArgTrpNH$_2$	13.50	2.27	0.47	0.05	0.44	0.51	6.11
AcGluTrpNH$_2$	14.04	2.07	0.56	0.06	0.46	0.48	6.53
LUTROL 2%							
TrpX	7.92	1.89	0.31	0.31	0.50	0.19	6.14
XTrp	5.57	2.31	0.40	0.11	0.49	0.41	3.17
AcArgTrpNH$_2$	9.28	1.93	0.27	0.02	0.54	0.44	2.59
AcGluTrpNH$_2$	5.76	1.82	0.28	0.02	0.46	0.52	2.06

The short chain NEP$^+$ ion is a mobile quencher, partially incorporated into SDS micelles, while the intermediate-sized alkyl chain NHP$^+$ ion is located at the interface. The NDP$^+$ ion has a chain long enough to incorporate the pyridinium into the aggregates (Gehlen and De Schryver, 1993). Extent of quenching was evaluated from Stern–Volmer constant (K_{SV}). In SDS micelles, the TrpX peptides locate near the micellar interface (higher K_{SV} values in the presence of NHP$^+$, Table 3), while LeuTrp was efficiently quenched by NEP$^+$ and should be located near the micellar surface. Electrostatic interactions are relevant for AcArgTrpNH$_2$ (highly quenched by NDP$^+$) and AcGluTrpNH$_2$ (lowest K_{SV} constant). In micelle-polymer aggregates the barrier to the approach of the alkylpyridinium ions results in decreased quenching efficiency (Table 3) and K_{SV} values parallels the association constants. In polymeric micelles the Stern–Volmer constants have lower values, following the same pattern as observed in binding constant values.

The fitting of decay profiles in the presence of quencher showed that the three lifetimes decreased. The collisional quenching constant (k_q) was calculated from $K_D = k_q \tau_o$, where the dynamic quenching constant (K_D) was obtained from Stern–Volmer plots

Table 3
Stern–Volmer (K_{SV}, M^{-1}) constants for the fluorescence quenching of peptides by alkylpyridinium ions, in microheterogeneous systems. Values are for the most efficient quencher, indicated in parenthesis.

	SDS 50 mM	SDS 50 mM + PEG 2%	LUTROL® 2%
TrpGly	123 ± 7 (NHP$^+$)	260 ± 20 (NDP$^+$)	232 ± 8 (NHP$^+$)
TrpAla	800 ± 7 (NHP$^+$)	350 ± 30 (NDP$^+$)	270 ± 20 (NEP$^+$)
TrpLeu	840 ± 50 (NHP$^+$)	370 ± 30 (NDP$^+$)	263 ± 4 (NHP$^+$)
LeuTrp	470 ± 40 (NEP$^+$)	380 ± 60 (NDP$^+$)	550 ± 7 (NHP$^+$)
AcArgTrpNH$_2$	450 ± 25 (NDP$^+$)	510 ± 30 (NDP$^+$)	234 ± 6 (NDP$^+$)
AcGluTrpNH$_2$	66 ± 2 (NEP$^+$)	110 ± 4 (NDP$^+$)	238 ± 9 (NDP$^+$)

Table 4
Bimolecular collisional rate constants (k_q, 10^8 M^{-1} s^{-1}) for tryptophan in dipeptides and alkylpyridinium ions. The quencher employed is indicated in parenthesis.

	SDS 50 mM	SDS 50 mM + PEG 2%	LUTROL® 2%
TrpGly	10.3 (NHP^+)	4.8 (NDP^+)	1.3 (NHP^+)
TrpAla	10.1 (NHP^+)	6.0 (NDP^+)	1.7 (NEP^+)
TrpLeu	5.7 (NHP^+)	4.2 (NDP^+)	–(NHP^+)
LeuTrp	4.8 (NEP^+)	4.2 (NDP^+)	4.1 (NHP^+)
$AcArgTrpNH_2$	5.1 (NDP^+)	3.1 (NDP^+)	2.9 (NDP^+)
$AcGluTrpNH_2$	12.0 (NEP^+)	5.5 (NDP^+)	2.7 (NDP^+)

of the average lifetimes and τ_o is the average lifetime in the absence of quencher. The alkylpyridinium ions were partitioned into the micelles and in calculations we used the local concentration of the aggregates. The values of k_q for the small peptides (Table 4), had the same order of magnitude as those observed with larger peptides and the quenching process is dependent on the affinity of the peptides for the micelles and on the partition of the quencher between the aqueous medium and the micelles.

Highest k_q values were observed in SDS micelles (Table 4), where peptides and alkylpyridinium quenchers have higher mobilities, particularly TrpGly and TrpAla in interaction with NHP^+. In SDS–PEO, NDP^+ is the most efficient quencher and polymers wrapping around the micelles restrict the diffusion of peptides and quenchers, decreasing k_q compared to pure SDS micelles. Diffusion is even more restricted in polymeric micelles, as seen by the lowest k_q values.

Concluding, the full set of fluorescence parameters demonstrates that the peptides interact with microheterogeneous systems. The association constants showed that electrostatic interactions increased the affinity of basic peptides for the negative charges in SDS and SDS–PEO aggregates, while in polymeric micelles the neutralization of terminal charges favored association driven by hydrophobic interactions. Data from time-resolved experiments demonstrated that the interaction proceeded with increase in fluorescence lifetimes, concomitant with modifications in pre-exponential factors. Thus, the distribution of peptide rotameric conformations changed, due to conformational changes induced by the interaction with the microheterogeneous systems. The extent of modifications is lower for interactions with polymeric micelles where the structural arrangements of the peptides are best preserved.

Quenching by alkylpyridinium ions showed that the TrpX peptides localize near the SDS micelles interface, while Trp in the acetyl-amide peptides has efficient contact with the long chain quencher. In SDS–PEO micelles, the peptides are protected from contact with water. In the polymeric micelles, zwitterionic peptides are in the aqueous interface of the external polymer layer, while acetyl-amide peptides locate in the hydrophobic core. Although the association constants with SDS micelles are higher, there are conformational changes which may affect the biological activity. In contrast, even if the association constants in the polymeric micelles are lower, the peptides are less mobile and their structural arrangements are best preserved.

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