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Physicochemical Properties of Mixed Micellar Aggregates Containing CCK Peptides and Gd Complexes Designed as Tumor Specific Contrast Agents in MRI†

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Abstract: New amphiphilic molecules containing a bioactive peptide or a claw moiety have been prepared in order to obtain mixed micelles as target-specific contrast agents in magnetic resonance imaging. The first molecule, $C_{18}H_{37}CONH(AdOO)_2$-G-CCK8 (C18CCK8), contains a C18 hydrophobic moiety bound to the C-terminal cholecystokinin octapeptide amide (CCK 26−33 or CCK8). The second amphiphilic compound, $C_{18}H_{37}CONHLys(DTPAGlu)CONH_2$ (C18DTPAGlu) or its gadolinium complex, (C18DTPAGlu-(Gd)), contains the same C18 hydrophobic moiety bound, through a lysine residue, to the DTPAGlu chelating agent. The mixed aggregates as well as the pure C18DTPAGlu aggregate, in the presence and absence of Gd, have been fully characterized by surface tension measurements, FT-PGSE-NMR, fluorescence quenching, and small-angle neutron scattering measurements. The structural characterization of the mixed aggregates C18DTPAGlu(Gd)−C18CCK8 indicates a spherical arrangement of the micelles with an external shell of ∼21 Å and an inner core of ∼20 Å. Both the DTPAGlu(Gd) complexes and the CCK8 peptides point toward the external surface. The measured values for relaxivity in saline medium at 20 MHz proton Larmor frequency and 25 °C are 18.7 mM^{-1} s^{-1}. These values show a large enhancement in comparison with the isolated DTPAGlu(Gd) complex.

Introduction

The more promising medical diagnostic imaging procedures currently in use are based on nuclear medicine (γ-scintigraphy and positron emission tomography, PET) and magnetic resonance imaging (MRI) techniques. The critical point for these techniques is that both require a different amount of reporter compounds (contrast agents) to be accumulated in the area of interest.[1,2] In fact, contrast agents help to discriminate between normal and pathological regions. The quantity of reporter compound to be accumulated in the area of interest strongly varies between these imaging techniques. While the very sensitive nuclear medicine techniques require very low tissue concentration (10^{-10} M) of radionuclide to give diagnostically significant, low resolved images, MRI gives very resolved images but, due to its very low sensitivity, needs higher concentration (10^{-4} M) of contrast agents such as paramagnetic Gd(III) complexes. To reach the required local concentration of the contrast agent, many carriers have been developed such as liposomes[3] and other microparticulates,[4] micelles,[5] dendrimers,[6] linear polymers,[7,8] proteins,[9] or peptides,[10] all of these derivatized with the metal complex of interest. Among these

† Abbreviations: AdOO, 8-amino-3,6-dioxaoctanoic acid; Boc, *tert*-butoxycarbonyl; tBu, *tert*-butyl; C12E5, polyoxyethylene-5-lauryl ether; CCK, cholecystokinin; CCK8, C-terminal octapeptide of cholecystokinin; CCK_A-R, CCK_B-R, cholecystokinin receptor types A and B; cmc, critical micellar concentration; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; DpyCl, dodecylpyridinium chloride; DTPAGlu, *N*,*N*-bis[2-[bis(carboxyethyl)amino]-ethyl]-L-glutamic acid; EDT, ethanedithiol; Fmoc, 9-fluorenylmethoxycarbonyl; FT-PGSE-NMR, Fourier transform pulsed gradient spin-echo nuclear magnetic resonance; GPCR, G-protein coupled receptor; HATU, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium; HOBt, 1-hydroxybenzotriazole; ICP, inductively coupled plasma; MRI, magnetic resonance imaging; Mtt, 4-methyltrityl; NMRD, nuclear magnetic relaxation dispersion; PyBop, benzotriazol-1-yl-oxytris(pyrrolidino)phosphonium; R_t, retention time; SANS, small-angle neutron scattering; TFA, trifluoroacetic acid; TIS, triisopropylsilane; TMS, tetramethylsilane.

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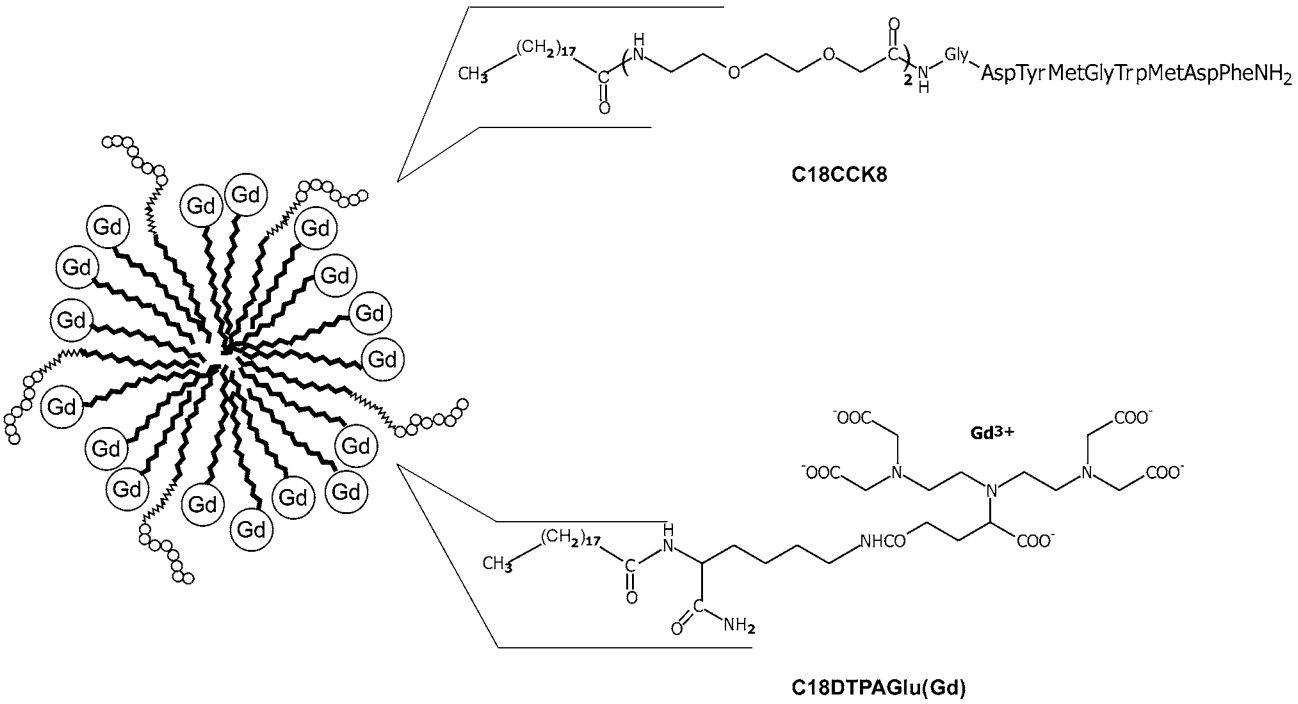

Figure 1. Schematic representation of mixed micellar aggregates.

carriers, micellar aggregates, formed by amphiphilic molecules and structurally constituted by a hydrophobic core and a hydrophilic shell, have recently drawn much attention owing to their easily controlled properties and good pharmacological characteristics.[11] For example, the self-assembling micellar organization of Gd(III)(DOTA) complex derivatized with a liphophilic tail allows obtaining a high relaxivity MRI contrast agent.[12]

There is an increasing interest in developing new contrast agents with enhanced properties. First, the new contrast agents should have high contrast activity of the metal complex. This is critical for MRI applications: high relaxivity of the paramagnetic Gd complexes allows reducing the very high concentration of the reporter compound to be realized in the area of interest. Second, the new contrast agents should be selective for a specific target: this property allows addressing the reporter compound only to the targets, such as membrane receptors overexpressed by cancer cells. Therefore, the total amount of the contrast agent to be injected for diagnostic analysis could be reduced: this result is very important in γ-scintigraphy in which radioactive isotopes are used.

This paper is concerned with the development of new contrast agents based on mixed micelles that fulfill both these properties. The basic idea behind the new contrast agents is that the two different monomers synthesized, one of which contains a bioactive molecule able to address the aggregates on the specific biological target and the other containing a chelating moiety able to form stable complexes with the metal of interest, and both presenting also a lipophilic tail, could self-assemble with each other in a mixed micelle. The hydrophobic chains allow the monomers to form mixed micelles, leaving the hydrophilic heads on the surface of the aggregates available for the appropriate task. In Figure 1 is reported a schematic representation of the mixed micellar structure formed in aqueous solution by the two monomers. The first monomer $C_{18}H_{37}CONH(AdOO)_2$-G-CCK8 (C18CCK8) contains a C18 hydrophobic moiety bound to the C-terminal cholecystokinin octapeptide amide (CCK 26−33 or CCK8).[13] Moreover, to ensure that the bioactive peptide remains on the external surface of the micelle when the aggregate is formed, two oxoethylene linkers and a glycine residue were introduced between the lipophilic tail and the CCK8 peptide. The choice of the CCK8 peptide is based on the knowledge that this peptide displays high affinity for both cholecystokinin receptors, CCK_A-R and CCK_B-R.[14] These receptors belong to the G-protein coupled receptors (GPCRs) superfamily and are localized in the cell membrane. Both CCK_A-R and CCK_B-R are very promising targets for specific contrast agents due to their overexpression in many tumors: CCK_A-R is overexpressed in pancreatic cancer and CCK_B-R is found in small cell lung cancer, colon, and gastric cancers, medullary thyroid carcinomas, astrocitomas, and stromal ovarian tumors.[15]

The second monomer, $C_{18}H_{37}CONHLys(DTPAGlu)CONH_2$ (C18DTPAGlu), or its gadolinium complex (C18DTPAGlu(Gd)), contains the same C18 hydrophobic moiety bound, through a lysine residue, to the DTPAGlu as chelating agent.[16] This monomer has been designed to keep the chelating agent on the external surface of the mixed micelle. The chelating moiety could complex radioactive metal isotopes such as ^{111}In(III) for application of the mixed micelles as reporter compounds in

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diagnostic γ-scintigraphy, or paramagnetic ions such as Gd(III) for application as contrast agent in MRI. A DTPAGlu−CCK8 conjugate has been recently used to give in vitro and in vivo very stable complexes with the radioactive isotope ^{111}In(III),[17] while Gd(III) complexes of DTPAGlu and of DTPAGlu−CCK8 conjugate have been completely characterized for both their stability and relaxometric properties.[18] The supramolecular aggregation of the C18DTPAGlu(Gd) complexes in the mixed micelle gives rise to a slow rotation and, consequently, increases the proton relaxivity with respect to that shown by monomeric Gd(III)−chelate complexes.[12] Finally, the presence in the micelles of two separate monomers gives the unique opportunity to tune the ratio between the two active components in order to find the right compromise between the number of bioactive peptides on the micellar surface to address the micelle on the target receptors and the number of the metal−chelate complexes able to give high contrast activity of the supramolecular adduct.

In this paper we present a complete physicochemical characterization of mixed micelles formed in aqueous solution by the two monomers C18CCK8 and C18DTPAGlu in the presence or in the absence of a nonionic surfactant (polyoxyethylene-5-lauryl ether, C12E5) used to stabilize the micelle formation. Moreover, the mixed micelles containing Gd(III) ions complexed by the chelating DTPAGlu agent have been studied for their relaxometric behavior in view of their use as CCK receptor specific contrast agents for MRI applications.

Materials and Methods

Materials. Protected N^{α}-Fmoc-amino acid derivatives, coupling reagents, and Rink amide MBHA resin were purchased from Calbiochem-Novabiochem (Laufelfingen, Switzerland). The Fmoc-8-amino-3,6-dioxaoctanoic acid (Fmoc-AdOO-OH) was purchased from Neosystem (Strasbourg, France). The DTPAGlu pentaester, *N*,*N*-bis[2-[bis[2-(1,1-dimethylethoxy)-2-oxoethyl]amino]ethyl]-L-glutamic acid 1-(1,1-dimethylethyl) ester,[16] was provided by Bracco Imaging SpA (Milan, Italy) and was used without further purification. For its synthesis refer to published methods.[16] C12E5 (stated purity >99%) was purchased from Sigma-Aldrich (Milwaukee, WI), as reagent grade, and it was used without further purification. All other chemicals were commercially available by Sigma-Aldrich, Fluka (Bucks, Switzerland), or LabScan (Stillorgan, Dublin, Ireland) and were used as received unless otherwise stated. The molar mass of the C12E5 surfactant was 406.60 g mol^{-1}. All solutions were prepared by weight using doubly distilled water. Samples to be measured by FT-PGSE-NMR and SANS techniques were prepared using heavy water (Sigma-Aldrich, purity >99.8%). The pH of all solutions was kept constant at 7.4.

Chemical Synthesis. Solid phase peptide synthesis was performed on a Shimadzu (Kyoto, Japan) Model SPPS-8 fully automated peptide synthesizer. Analytical RP-HPLCs were carried out on a Shimadzu 10A-LC using a Phenomenex C18 column (Torrance, CA), 4.6 × 250 mm, eluted with an H_2O/0.1% TFA (A) and CH_3CN/0.1% TFA (B). Two gradients from 60% to 80% B over 10 min at 1 mL/min flow rate and from 80% to 95% B over 15 min at 1 mL/min flow rate were used. Preparative RP-HPLCs were carried out on a Waters (Milford, MA) Model Delta Prep 4000 equipped with a UV lambda-Max Model 481 detector using a Vydac C18 column (Columbia, MD), 22 × 250 mm, eluted with an H_2O/0.1% TFA (A) and CH_3CN/0.1% TFA (B) linear gradient at 20 mL/min flow rate. UV−vis spectra were carried out by using an UV−vis Jasco (Easton, MD) Model 440 spectrophotometer with a path length of 1 cm. Mass spectra were carried out on a Maldi-tof Voyager-DE Perseptive Biosystem (Framingham, MA) apparatus using α-cyano-4-hydroxycinnamic acid as matrix and bovine insulin as internal reference. The monodimensional ^{1}H NMR in DMSO-d_6 spectrum was performed on a Varian (Palo Alto, CA) 400 MHz spectrometer.

$C_{18}H_{37}CONH(AdOO)_2$-G-CCK8 (C18CCK8). Peptide synthesis was carried out in the solid phase under standard conditions using Fmoc strategy. Rink amide MBHA resin (0.78 mmol/g, 0.1 mmol scale, 0.128 g) was used. The peptide chain was elongated by sequential coupling and Fmoc deprotection of the following Fmoc-amino acid derivatives: Fmoc-Phe-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp(Boc)-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Asp-(OtBu)-OH, Fmoc-Gly-OH, and two residues of Fmoc-AdOO-OH. All couplings were performed twice for 1 h, by using an excess of 4 equivalents (equiv) for the single amino acid derivative. The α-amino acids were activated in situ by the standard HOBt/PyBop/DIPEA procedure.[19] DMF was used as a solvent. Fmoc deprotections were obtained by 20% solution of piperidine in DMF. Only the two residues of Fmoc-AdOO-OH were added in a single coupling by using an excess of 2 equiv. When the peptide synthesis was complete, the resin was washed and the terminal Fmoc protection removed. To obtain the lipophilic monomer, 0.119 g (0.4 mmol) of nonaoctanoic acid were coupled on the linker peptide N-terminus by using 0.208 g (0.4 mmol) of PyBop, 0.0612 g (0.4 mmol) of HOBt, and 134 μL (0.8 mmol) of DIPEA in 2 mL of a mixture DMF/DCM = 1/1. Coupling time was 1 h under stirring at room temperature.

The yield for aliphatic acid coupling, monitored by the Kaiser test, was in the range 95−98%. For deprotection and cleavage, the fully protected resin was treated with TFA containing triisopropylsilane (2.0%), ethanedithiol (2.5%), and water (2.5%), and the free product precipitated at 0 °C by adding ethyl ether dropwise. Purification of the crude mixture was carried out by RP-HPLC, R_t = 21.9 min. Maldi-tof-MS confirms the product identity: $C_{18}H_{37}CONH(AdOO)_2$-G-CCK8 (MW = 1687), m/z = 1688.

$C_{18}H_{37}CONHLys(DTPAGlu)CONH_2$ (C18DTPAGlu). A sample of 624.79 mg (1.00 mmol) of Fmoc-Lys(Mtt)-OH activated by 1 equiv of PyBop and HOBt and 2 equiv of DIPEA in DMF was coupled to Rink amide MBHA resin (0.78 mmol/g, 0.250 mmol scale, 0.320 g), with stirring of the slurry suspension for 1 h. The solution was filtered and the resin washed with three portions of DMF and three portions of DCM. The Mtt protecting group was removed by treatment with 2.0 mL of DCM/TIS/TFA (94:5:1) mixture.[19] The treatment was repeated several times until the solution became colorless. The resin was washed by DMF and then the DTPAGlu pentaester chelating agent was linked, through its free carboxyl function, to the α-NH_2 of the lysine residue. This coupling step was performed using 2.0 equiv of DTPAGlu pentaester and HATU, and 4 equiv of DIPEA in DMF as solvent. The coupling time, compared with the classical solid phase peptide synthesis protocol, was increased up to 2 h, and the reaction was tested for completion by the Kaiser test. After removal of the Fmoc group by 20% piperidine in DMF, the coupling of nonaoctanoic acid was performed in a mixture DCM/DMF (1:1) in the previously described condition. For deprotection and cleavage, the fully protected fragment was treated with TFA containing TIS (2.0%) and water (2.5%). The crude product was precipitated at 0 °C, washed several times with small portions of water, and recrystallized from methanol and water. The product was characterized by ^{1}H NMR spectroscopy and Maldi-tof mass spectrometry.

^{1}H NMR (chemical shifts in δ, TMS as internal standard) = 4.18 (m, 1H, C*H* Lys α), 3.80 (overlapped, 1H, C*H*Glu α), 3.40 (overlapped, 8H, CH_2COOH) 3.02 (t, 2H, CH_2 Lys ϵ) 2.81 (m, 8H, N−CH_2), 2.22

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(t, 2H, CH_2CO), 2.14 (m, 2H, RCH_2CH_2CO), 1.87 (m, 1H, CH_2 Lys β), 1.76 (m, 1H, CH_2 Lys β), 1.65 (m, 1H, CH_2 Lys δ), 1.51 (m, 3H, CH_2 Lys γ,δ), 1.42 (t, 2H, RCH_2CH_2CO), 1.25 (m, 30H, CH_2 aliphatic), 0.90 (t, 3H, CH_3). Maldi-tof-MS confirms the product identity: $C_{18}H_{37}$-CONHLys(DTPAGlu)$CONH_2$ (MW = 870), *m*/*z* 871.

Preparation of Gadolinium Complex, C18DTPAGlu(Gd). The complexation has been carried out by adding light excess of the $GdCl_3$ to the aqueous solution of the C18DTPAGlu ligand at neutral pH and room temperature. The formation of Gd complex, C18DTPAGlu(Gd), was followed by measuring the solvent proton relaxation rate ($1/T_1$). The excess of uncomplexed Gd(III) ions, which yields a variation of the observed relaxation rate, was removed by centrifugation of the solution brought to pH 10; further relaxation rate measurements were made to check the complete Gd(III) ions removal.

Preparation of Solutions. All solutions were prepared by weighting, buffering the samples at pH 7.4 with 0.10 M phosphate and 34 mM NaCl. pH measurements were made by using the pH meter MeterLab PHM 220. The pH meter was calibrated with standards at pH 7 and pH 10. In most cases the samples to be measured were prepared from stock solutions. All solutions were stirred at room temperature until complete dissolution and then used without further treatment.

In C18DTPAGlu−C18CCK8−water and C18DTPAGlu−C18CCK8−C12E5−water solutions the imposed ratio between the solutes was as such to have an average of three peptide derivatives per micelle. This has been verified through the SANS measurements, as discussed below.

In C18DTPAGlu−C12E5−water and C18CCK8−C12E5−water ternary systems the imposed ratio between stoichiometric solute concentrations was 1:3, and 1:9, respectively.

Surface tension, fluorescence quenching, and SANS measurements were also performed on mixtures where C18DTPAGlu was replaced with the Gd complex C18DTPAGlu(Gd).

Surface Tension Measurements. The surface tension, γ, was measured with an accuracy of 0.1 dyn cm^{-1}, by the Du Nouy ring method, using a KSV Sigma 70 digital tensiometer, equipped with an automatic device to select the rising speed of the platinum ring and to set the time lag between two measurements. The temperature was kept constant at 25.00 ± 0.01 °C. The tensiometer was calibrated with water and acetone.[20]

Self-Diffusion Measurements. The self-diffusion coefficients were measured by the FT-PGSE-NMR technique at 25 °C.[21] A spectrometer operating in the ^1H mode at 80 MHz and equipped with a pulsed magnetic field gradient unit, made by Stelar (Mede, Pavia, Italy), was employed. The temperature was controlled within 0.1 °C through a variable-temperature controller, Model VTC-87. The expression for individual spin−echo peak amplitude for a given line is

$$A = A_0 \exp[-\gamma_{mg}{}^2 g^2 D \delta^2 (\Delta - \delta/3)] \quad (1)$$

where A_0 is a constant for a specific set of experimental conditions, γ_{mg} is the gyromagnetic ratio of the proton, D is the self-diffusion coefficient of the species responsible for the NMR signal, g is the magnitude of the applied gradient, and Δ and δ are spacing and duration of the gradient pulses, respectively. Echo delays were kept constant so that the relaxation effect must not be accounted for. Equation 1 was fitted by nonlinear least-squares routine to the attenuation of the echo amplitude sampled as a function of g.

Fluorescence Quenching. To evaluate the mean aggregation number of surfactants into the micelles, fluorescence quenching measurements were performed by a Jasco FP-750 (Easton, MD) spectrofluorimeter at 25 °C. The fluorescence probe pyrene and the quencher dodecylpyridinium chloride (DpyCl) have been used in all systems. The excitation wavelength was set at 335 nm, and fluorescence intensity was detected at 383 nm. This latter wavelength corresponds to the third out of five peaks of vibronic fluorescence that the pyrene spectrum exhibits. The aggregation numbers were measured in the assumption that the intramicellar "static" quenching between the probe and the quencher, both following Poisson distribution, is dominant. The pyrene concentration was 2×10^{-6} mol dm^{-3}, and the fluorescence intensity at 383 nm was monitored at decreasing amount of the DpyCl quencher starting from a molar concentration ratio R = [quencher]/[micelle] = [q]/[M] ~ 1.[22−24]

The fluorescence intensity in the presence of the quencher is given by

$$I = I_o \exp(-[q]/[M]) \quad (2)$$

where I_o is the fluorescence intensity in the absence of inhibitor. In the approximation of the phase separation model the following equation holds:

$$[M] = (C - \mathrm{cmc})/N_{agg} \quad (3)$$

where C is the concentration of the aggregating species. Hence, the aggregation number, N_{agg}, is obtained from the slope of $\ln(I/I_o)$ vs $[q]/(C - \mathrm{cmc})$.[25,26] Due to the very low concentration of the systems studied, in eq 3 molarity has been replaced by molality.

Water Proton Relaxation Measurements. The longitudinal water proton relaxation rates were measured on a Stelar Spinmaster (Mede, Pavia, Italy) spectrometer operating at 20 MHz, by means of the standard inversion−recovery technique (16 experiments, 2 scans). A typical 90° pulse width was 4 μs and the reproducibility of the T_1 data was ±0.5%. The temperature was kept at 25 °C with a Stelar VTC-91 air-flow heater equipped with a copper−constantan thermocouple (uncertainty ±0.1 °C). The proton $1/T_1$ NMRD profiles were measured over a continuum of magnetic field strength from 0.000 24 to 0.28 T (corresponding to 0.01−12 MHz proton Larmor frequency) on a Stelar Fast Field-Cycling relaxometer. This relaxometer works under complete computer control with an absolute uncertainty in $1/T_1$ of ±1%. Data points at 20 and 90 MHz were added to the experimental NMRD profiles and were recorded on the Stelar Spinmaster (20 MHz) and on a JEOL EX-90 (90 MHz) (Tokyo, Japan) spectrometer, respectively.

^{1}H Water Relaxation Rate. The relaxivity of a Gd(III) complex results from contributions arising mainly from water molecules in the inner and outer coordination spheres:

$$r_1 = r_1{}^{His} + r_1{}^{Hos} \quad (4)$$

$r_1{}^{His}$ refers to the contribution from the exchange of the water protons in the first coordination sphere of the paramagnetic metal ion:

$$r_1{}^{His} = \frac{n_w[C]}{55.6(T_{1M} + \tau_M)} \quad (5)$$

where n_w is the hydration number, [C] is the molar concentration of the paramagnetic chelate, T_{1M} is the longitudinal relaxation time of the inner sphere water protons, and τ_M is their residence lifetime.

The outer sphere term, $r_1{}^{Hos}$, describes the contribution from water molecules which diffuse around the paramagnetic complex (bulk solvent).

The complex theory that governs the relaxation process in a paramagnetic chelate is well described by the models developed by Solomon−Bloembergen−Morgan for what concerns the inner sphere contribution and by Hwang and Freed for the outer sphere one.[27]

Small-Angle Neutron Scattering. SANS measurements were performed at 25 °C on the KWS2 located at the Forschungszentrum

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Julich, Germany. Neutrons with an average wavelength of 7 Å and wavelength spread $\Delta\lambda/\lambda < 10\%$ were used. A two-dimensional array detector at two different sample-to-detector distances, 2 and 8 m, detected neutrons scattered from the sample. These configurations allowed collecting the scattered intensity in a range of moment transfer Q between 0.003 and 0.12 $Å^{-1}$. Samples were contained in 1 mm path length quartz cells, and measurement times ranged between 20 min and 2 h. The data were then corrected for background, empty cell, and solvent contribution, and then reduced to scattering cross section (in absolute units per centimeter), following the standard procedure.[28]

Method of Data Analysis. The general scattering intensity, containing information about shape, size, and interactions of monodisperse scattering bodies, is given by[29]

$$I(Q) = N_b \left(\sum_i b_i - V_b\rho_s\right)^2 P(Q)\, S(Q) + I_{inc} \tag{6}$$

where N_b is the number density of scattering bodies, V_b is the volume of each scattering particle, $\sum_i b_i$ is the sum of the scattering lengths over the atoms constituting the body, and ρ_s is the solvent scattering length density. $P(Q)$ and $S(Q)$ are the form factor and structure factor, respectively. I_{inc} is the incoherent scattering cross section.

The form factor contains information on the shape of the scattering objects, whereas the structure factor $S(Q)$ accounts for interparticle correlations and is normally important for concentrated or charged systems.

The structure of micelles present in the systems under study has been established by analyzing the scattering data through eq 6, imposing that the number density of scattering bodies and their volume are $N_b = (C - \text{cmc})L_a/N_{agg}$ and $V_b = N_{agg}V_m$, where C is the stoichiometric solute concentration, V_m is the volume of the free surfactant, N_{agg} is the aggregation number of the micelles, and L_a is Avogadro's constant.[29] The micelles were modeled as a spherical "core + shell", and hence the form factor $P(Q)$ is reduced to a sum of two Bessel first-order spherical functions:[29]

$$P(Q) = \left(f\frac{3j_1(u_1)}{u_1} + (1-f)\frac{3j_1(u_2)}{u_2}\right)^2 \tag{7}$$

with

$$u_1 = Qa \tag{8}$$

$$u_2 = Q(a+d) \tag{9}$$

$$f = \frac{V_f(\rho_1 - \rho_2)}{\sum_i (b_i - V_m\rho_s)} \tag{10}$$

$$j_1(x) = \frac{\sin x - x\cos x}{x^2} \tag{11}$$

V_f is the micelle core volume; a and d are the radius of the core and the thickness of the hydrophilic shell, respectively. ρ_1 and ρ_2 are the densities of scattering length of the core and shell.

The structure factor, $S(Q)$, can be calculated through the Hayter and Penfold theory.[30,31]

Results

Synthesis of Monomers. The peptide synthesis was performed by the solid phase approach using Fmoc/tBu chemistry. The CCK8 peptide was synthesized as previously described.[32] At the Asp N-terminal residue of the CCK8 peptide a glycine residue and two units of 8-amino-3,6-dioxaoctanoic acid were added directly on the solid phase. As a final step, the nonaoctanoic acid was bound to the N-terminal position of the peptide derivative. The cleavage of resin and the deprotection of the side chain functions were performed in a TFA/TIS/EDT/water mixture. The peptide derivative was isolated in good yields and purified by RP-HPLC. The analitycal data (Maldi-tof mass spectrum and RP-HPLC) confirm compound identity and purity. The two units of the hydrophilic linker increase the affinity for the polar solvent of the peptide moiety.

The amphiphilic chelating moiety C18DTPAGlu was also synthesized in the solid phase using a resin support. Fmoc-Lys-(Mtt)-OH was anchored to the resin and the side chain was selectively deprotected. Then, DTPAGlu pentaester, the chelating agent fully protected by *tert*-butyl (tBu) groups on its carboxyl functions with the exception of the carboxyl group on the side chain of L-glutamic acid, was linked, by a single coupling step, to the ϵ-NH_2 amino function of the lysine residue. Successively, the Fmoc protecting group was removed by the α-NH_2 amino function of the lysine residue and the nonaoctanoic acid was bound. After cleavage from the resin the product was characterized by ^{1}H NMR spectroscopy and Maldi-tof mass spectrometry.

Surface Tension. The surface tension was measured on C12E5−water, C18DTPAGlu−water, and C18CCK8−water binary systems; on C18DTPAGlu−C12E5−water, C18CCK8−C12E5−water, and C18DTPAGlu−C18CCK8−water ternary systems; and on C18DTPAGlu−C18CCK8−C12E5−water quaternary system. The surfactant concentration was raised well above the expected critical micellar concentration, cmc. Surface tension measurements were also performed on all systems once C18DTPAGlu was replaced by C18DTPAGlu(Gd).

All the above systems show a concentration dependence of the surface tension, γ, typical of systems containing amphiphilic molecules that form micelles. Addition of solute to pure water ($\gamma_o = 72.0$ dyn cm^{-1}) has the effect of decreasing γ to the cmc value, at which the surface tension curve changes its slope and approaches a constant value. However, there are significant differences among the systems studied, as inspection of Table 1 suggests. For the C18CCK8−water system the typical change in the slope of the γ vs ln m curve, of micellar systems, has not been observed.

Binary Systems: C18DTPAGlu−Water, C18CCK8−Water, and C12E5−Water. In Figure 2 the surface tension diagram of the C18DTPAGlu−water and C12E5−water binary systems along with C18DTPAGlu(Gd)−water system are reported. The cmc values detected for the C18DTPAGlu−water and C12E5−water systems are about the same, 5×10^{-5} and 4×10^{-5} mol kg^{-1} respectively, although the limit value of the surface tension for the two systems is quite different. This is to be assigned to the higher hydrophobic contribution of C12E5 to the surface composition with respect to the other compounds studied in this paper.

The behavior of C18DTPAGlu(Gd)−water is substantially similar to that of the corresponding system in the absence of

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