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pH-Dependent structuring and fluorescence properties of tryptophan-derived polyamidoamino acid (co)polymers

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The binding sites of proteins often contain tryptophan residues, whose fluorescent properties may be altered upon ligand binding. Conformational changes within the binding site can result in either fluorescence quenching or enhancement, which may be utilized to quantitatively investigate protein-ligand interactions [1,2]. Amphoteric fluorescent (L)-, (D)- and (D,L)-tryptophan-based polyamidoamino acid (PAAC) homopolymers and glycine- and arginine-L-tryptophan PAAC copolymers were synthesized by the polyaddition of N,N'-methylenebisacrylamide (MBA) with (L)-, (D)- and (D,L)-tryptophan and, respectively, glycine- and L-arginine-(L)-tryptophan mixtures. The polymerization reaction was run at 50 °C and $pH>10$ for 7 days under nitrogen atmosphere. All polymers were characterized by NMR spectrometry, UV-Visible spectroscopy, dynamic light scattering (DLS) and time-resolved fluorescence (TRF) spectroscopy. Solubility in aqueous solutions at different pH's were determined by measuring the transmittance at 450 nm. Homopolymers showed similar pH-dependent solubility patterns, with steep solubility drop at $pH<8$. Volumetric particle size distribution obtained by DLS analysis showed hydrodynamic radii (R_h) ≈ 100 nm and negligible changes with pH. TRF spectroscopy recorded for the (L) isomer was fitted by deconvolution curves with two lifetimes, namely, $\tau_1 = 0.90$ ns and $\tau_2 = 4.40$ ns, the latter showing a decrease in population in favour to the former, passing from pH 11 to 8. Quantum yield measured at 10^{-5} M repeating unit concentration decreased from 6 to 4 % from pH 11 to 8. Excitation and emission spectra showed maxima at 279 nm and 370 nm respectively, the latter showing a 10 nm red shift by decreasing pH from 11 to 8. PAAC copolymers were synthesized at the same experimental conditions of PAAC homopolymers. The solubility curves of copolymers in water showed complete solubility for tryptophan content up to 20% on a molar base. For tryptophan content up to 30 and 40%, transmittance dropped down respectively to 60% and 5% in the pH range 2-7. DLS measurements at different pH's showed average volume particle size 2.5 nm in the range considered. TRF spectroscopy proved that tryptophan-based copolymers are endowed with pH-dependent emission properties. Significant quantum yield was also detected at the lowest tryptophan content. In conclusion, tryptophan-based PAAC (co)polymers displayed flexibility for tuning of amphiphilicity, chirality and self-assembly properties.

References

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