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A facile way to build up branched high functional polyaminoacids with tunable physicochemical and biological properties

Nicolò Mauro [a,*], Calogero Fiorica [a], Paola Varvarà [a], Giulia Di Prima [a], Gaetano Giammona [a,b]

[a] Laboratory of Biocompatible Polymers, Department of "Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche" (STEBICEF), University of Palermo, Via Archirafi, 32, 90123 Palermo, Italy
[b] Mediterranean Center for Human Advanced Biotechnologies (Med-Chab), Viale delle Scienze Ed. 18, 90128 Palermo, Italy

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ABSTRACT

Here, for the first time, branched polyaminoacids bearing α-amino acids as side functions, namely PAA-co-AA and PGA-co-AA, are prepared by heterophase ring opening of polysuccinimide (PSI) with L-arginine or glycine in aqueous environment and at controlled pH. The modulation of the pH of the reaction leads to high-molecular-weight copolymers with tunable functionalization and, as consequence, with tailor-made physicochemical properties. Furthermore, a branched polyaminoacid carrying a preformed bioactive peptide (L-trileucine) and L-arginine as side pendants, named PATA-co-AA, was synthesized via a similar pathway thus leading to complex biomimetic materials potentially exploitable in several biomedical fields. Acid-base titrations, circular dichroism studies and spectrofluorimetric analysis show that the physicochemical behavior of this class of bioinspired copolymers can be predicted considering the starting features of the selected building blocks, implying that a careful choice of functional amino acids or peptides provides good chance for obtaining macromolecule libraries with selected properties.

1. Introduction

Bioinspired synthetic polymers have gained considerable interest in material science being promising macromolecules which try to mimic nature and, as consequence, its "magic" biological properties [1–4].

A key feature of biological systems is complexity, that is usually exploited in nature to get to functional holistic properties, in which the behavior of highly hierarchical structures is more than the sum of the direct interactions between single components. Polymer chemistry, especially that committed in designing biomaterials of biomedical interest, should take into account this characteristic so as to yield to macromolecules endowed with advantageous biological behavior.

In recent years, the synthesis of carboxyl-substituted polyaspartic acid was proposed considering the ring opening of polysuccinimide (PSI), a polymer synthesized by polycondensation of aspartic acid widely used for the synthesis of highly biocompatible materials [5–12], with carboxyl-protected amino acids [13]. These polyaminoacids have an identical backbone to that of peptides, but the molecular architecture is characterized by high branching frequency and, therefore, they result in excellent water solubility and high accessibility of theirs functional groups. The use of natural building blocks,

* Corresponding author.
E-mail address: nicolo.mauro@unipa.it (N. Mauro).

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encompassing amino acids and related products, avoids the formation of toxic products after the enzymatic degradation of these peptidomimetics and provides functional derivatizations, making them useful as carriers in drug delivery systems. Because of these polymers are remarkably peptidomimetics most of them might potentially display inherent pharmacological activity as well. For instance, the functionalization of the main backbone with arginine, histidine or lysine may impart antibacterial and antiviral activity [14,15]. From the above consideration it is clearly evident that this class of branched peptidoyl-substituted polyaspartic acid are endowed with versatility unique in synthetic polymers, since they are incline to structure-tailoring for precise scopes.

However, a drawback of this synthetic strategy is that the functionalization with preformed bioactive peptides is precluded, since peptides are highly insoluble in organic solvents, thus limiting their use as active side chains in the synthesis of new smart materials. Moreover, as a rule several tedious synthetic steps and fine purification methods have been used to obtain such architectures, compromising the scalability of the entire industrial process [13,16,17].

In the present proof of concept work we explored a facile way to obtain branched polyaminoacids with a polyaspartic (PA) backbone bearing both amino acids and a peptide as side chains, in which the inter- and intra-chain hydrogen bonding in the backbone are dominated by the ones taking place between side chains. The pH-dependent degree of functionalization of PSI with L-arginine, glycine and L-trileucine was analyzed to assess the feasibility of the reaction. The influence of these pendants on the properties of the copolymers was also studied to show how a careful choice of the starting building blocks can potentially confer them self-assembling ability and specific biophysical behavior.

2. Materials and methods

2.1. Materials

Polysuccinimide was obtained as previously described [5]. L-arginine (98%), glycine (99%), L-trileucine (90%), N,N-diisopropylethylamine (99.5%), anhydrous N,N-dimethylformamide (DMF), anhydrous calcium chloride (97%), hydrochloric acid (37%) and pyrene (99%) were purchased from Sigma Aldrich ad used as received.

^{1}H and ^{13}C NMR APT spectra were recorded using a Bruker Avance II 300 spectrometer operating at 300.12 and 100. xx MHz respectively.

Size exclusion chromatography (SEC) traces were obtained using Tosho Bioscience TSK-Gel G4000 PWXL and G3000 PWXL columns connected to a Water 2410 refractive index detector. The mobile phase was a 0.1 M Tris buffer pH 8.10 ± 0.05 with 0.2 M sodium chloride. The flow rate was 0.6 mL min^{-1} and sample concentration 2.5 mg mL^{-1} and PEG standards (200–0.19 kDa, Polymer Laboratories Inc., USA) were used to set up calibration curve (R^2 = 0.9914).

Dispase II, Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin solution, glutamine solution and amphotericin B solution were purchased by Sigma Aldrich (Italy).

2.2. Synthesis of poly(argilylaspartamide-co-aspartic) acid (PAA-co-AA)

An aqueous solution of L-arginine (1.0000 g, 5.740 mmol) at pH 10.50 ± 0.05 was prepared dissolving it in ultrapure water (10 mL) and adjusting the pH with 1 M hydrochloric acid. Separately, polysuccinimide (PSI) (200 mg, 2.062 mmol) was dissolved in DMF (4 mL) and the resulting solution was added to the L-arginine solution dropwise under stirring. The reaction was maintained for 24 h at 22–28 °C using an ice bath and the pH was occasionally adjusted until to 10.5 ± 0.05 using 1 M sodium hydroxide. The crude product was then filtered and dialyzed against water using a test tube with 25 kDa nominal molecular weight cutoff (NMWC). After lyophilization a white powder was obtained. Yield 360 mg.

Relative average molecular weight: Mw = 66,000, PD = 1.84.

^{1}H NMR (D_2O, 300 MHz): δ_H 1.62 ($2H_{arg}$, br, $CHCH_2\underline{CH_2}$), 1.85 ($2H_{arg}$, m, $CH\underline{CH_2}CH_2$), 2.66 ($2H_{\beta\ aspartic\ acid}$, br, $NHCO\underline{CH_2}CH$), 2.72 ($2H_{amide}$, br, α and β CH_2; $2H_{\alpha\ aspartic\ acid}$, $\underline{CH_2}COO^-$), 3.15 ($2H_{arg}$, t, $\underline{CH_2}NHCN_2H_4$), 3.73 ($1H_{arg}$, t, $\underline{CH}COO^-$), 4.44 ($1H_{\beta\ aspartic\ acid}$, br, $NHCOCH_2\underline{CH}$), 4.62 ($1H_{\alpha\ aspartic\ acid}$, br, $\underline{CH}CH_2COOH$ and $1H_{aspartamide}$, α and β CH).

^{13}C NMR (D_2O, 100 MHz): δ_C 23.84 ($CHCH_2\underline{CH_2}$), 27.49 ($CH\underline{CH_2}CH_2$), 35.23 (α and β $CONH\underline{CH_2}$), 37.70 (α aspartic acid, $\underline{CH_2}COOH$), 38.48 (β aspartic acid, $NHCO\underline{CH_2}CH$), 40.42 ($\underline{CH_2}NHCN_2H_4$), 51.17 (α and β aspartamide, CH), 51.74 (α and β aspartic acid, CH), 54.20 (arg, $\underline{CH}CH_2CH_2$), 156.53 ($NH\underline{C}N_2H_4$), 171.50–174.00 ($\underline{C}ONH$), 174.40 ($\underline{C}ONHCOOH$), 175.50–177.50 ($\underline{C}OO^-$).

The same reaction was carried out at pH 10.00 ± 0.05, 9.5 ± 0.05, 9.00 ± 0.05, 8.5 ± 0.05. Results are reported in Fig. 4.

2.3. Synthesis of poly(glycylaspartamide-co-aspartic) acid (PGA-co-AA)

Glycine (777.7 mg, 10.36 mmol) was solubilized in ultrapure water (10 mL) and some drop of 1 M sodium hydroxide was carefully added until pH 10.50 ± 0.05 was reached. A solution of PSI (200 mg, 2.062 mmol) in DMF (4 mL) was so slowly added to that mixture, placed in an ice bath to maintain the temperature within the range of 22–28 °C, under vigorous stirring and the pH was kept at the starting value using 1 M sodium hydroxide for 24 h. After that, the reacting mixture was