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Nanoparticle formation from poly(acrylic acid) and oppositely charged peptides

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Abstract

Cationic peptides self assemble upon interacting with sodium salt of oppositely charged polymer, poly(acrylic acid), PAA, giving rise to water-soluble nanoparticles at very low concentration (0.1 mM of PAA). The morphology of these kinds of nanoparticles is mainly governed by the composition of the complexes, which can be expressed as $Z_{+/-}$, i.e., the ratio of positively charged units to the concentration of anionic units of the polymers present in the system. In the present study, at lower $Z_{+/-}$, the particles are elongated in shape but adopt spherical shape of 75–100 nm in diameter at higher $Z_{+/-}$ values. We propose that the nanoparticles containing cationic peptides obtained by this methodology can serve as delivery system to enhance the antinociception effect of the chimeric peptide with previously administered doses.
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Keywords: Sodium salt of polyacrylic acid (PAA); Cationic peptides; Turbidity measurement; Atomic force microscopy; Nanoparticles

1. Introduction

Bioavailability of a large number of drugs, particularly peptides and proteins to the desired target has always been a problem due to their poor metabolic stability. To rectify this problem various approaches have been adopted including chemical modifications like glycosylation [1], halogenation [2], conjugation with macromolecular polymers [3–6] etc. and delivery with liposomes [7]. Unfortunately, none of these approaches resulted in handsome increment in stability and bioavailability of the drugs. Application of nanoparticles as drug delivery system seems to be a better method of efficient drug targeting. Amongst different kinds of nanoparticles that have been applied for such purposes, polymeric nanoparticles [8] pose as delivery systems, which are not only used for efficient targeting purposes [9–11], but are also biodegradable and biocompatible.

Recently it has been demonstrated that nanoparticles can be obtained on interaction between charged polymers and oppositely charged molecules. Such association depends on many factors including Coulombic interactions, hydrophobicity of the polymer–molecule pair, and the conformational features of the polymer. One special class of such systems is the complexes formed from polyions of opposite charges. The solution behavior of these complexes strongly depends on their composition. Electroneutral complexes that contain equivalent amounts of polyion units and monomers are water-insoluble. Nonstoichiometric complexes containing an excess of one of the components are generally soluble in water. Since these complexes are capable of forming aggregates of nanometer size, they have been termed as polyion complex (PIC) micelles or block ionomer complexes (BICs) [12].

In this study, we were interested to examine the ability of interaction of cationic peptides with an anionic polymer polyacrylic acid (PAA) and the morphology of the complexes. PAA polymer was found as a good protectant additive to preserve bioactivity of L-lactate dehydrogenase (LDH) as it interacts with alginate microparticles [13] and it was also used for buccal [14] and ophthalmic drug delivery systems [15]. We designed and synthesized three peptides viz.; peptide 1 (GGKWKAKA), peptide 2 (KGKWKAKA), peptide 3 (KKKWKAKA), containing different number of lysine residues, which impart positive charge to the peptides in aqueous solution so that they can bind electrostatically to the PAA polymer. All the three peptides electrostatically interacted with the PAA polymer, which was determined by turbidity measurements using UV–Vis Spectrophotometer. Atomic

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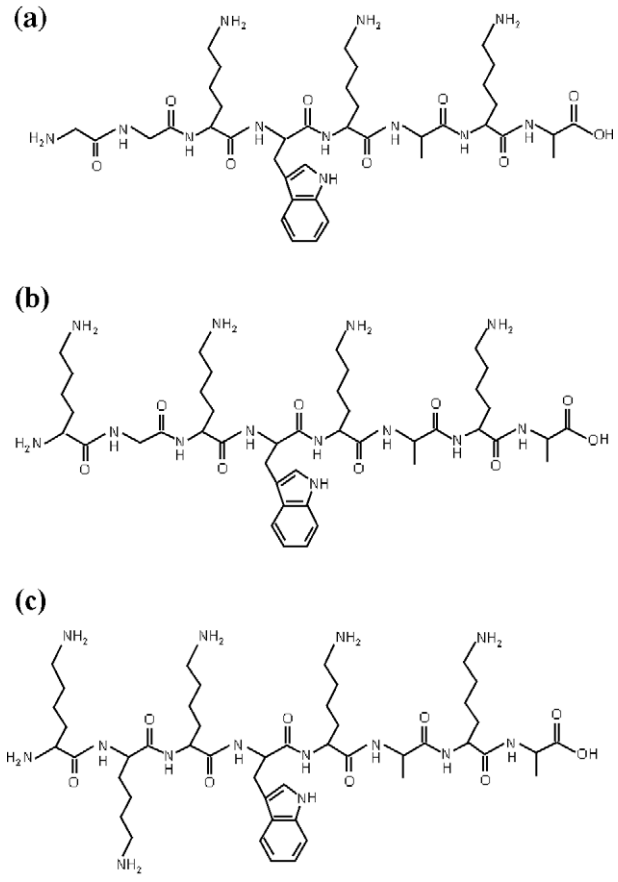

Fig. 1. Structures of the peptides (a) peptide 1 (GGKWKAKA), (b) peptide 2 (KGKWKAKA), (c) peptide 3 (KKKWKAKA).

force microscopic images showed that the morphology of the complexes depends on the relative amount of peptides and polymer in the complexes. The complexes of lower charge ratio (cationic units in the peptide vs anionic units in the polymer) were elongated in nature, became spherical nanoparticles at higher charge ratio with the sizes in the range of 75 to 100 nm. We propose that the nanoparticle particles containing cationic peptides obtained by this methodology can serve as delivery system to enhance the antinociception effect of the chimeric peptide (YGGFMKKKFMRFa) [16] with previously administered doses and its antinociceptive effect can be observed even with lower doses providing better bioavailability and metabolic stability with this delivery system.

2. Experimental section

2.1. Chemicals

Sodium salt of polyacrylic acid (PAA, Mw=10,000), *N*, *N*′-diisopropyl carbodiimide (DIPCI) and trifluoroacetic acid was obtained from Sigma-Aldrich Co. All Fmoc-amino acids and 1-hydroxybenzotriazole (HOBt) were purchased from Nova Biochem (Switzerland). Acetonitrile was obtained from Merck Ltd. (India). Wang resin was obtained from Advanced Chemtech (USA). 2, 5-dihydroxybenzoic acid was supplied by Bruker Daltonics (Germany).

2.2. Sample preparation

1.0×10^{-3} M solution of PAA was prepared. Solutions of peptides 1, 2 and 3 were prepared taking constant w/v ratio as 1 mg/mL. All solutions were prepared in triple distilled water having pH ≈ 6.8–7.0.

2.3. Methods

2.3.1. Peptide synthesis

Peptides 1,2 and 3 were synthesised by the solid phase method using automated peptide synthesizer (Advanced chemtech), using the standard chemistry of flourenylmethoxy carbonyl (Fmoc) aminoacids and 1-hydroxybenzotriazole (HOBt)/*N*, *N*′-diisopropyl carbodiimide (DIPCI) activation method on a Wang resin. The peptides were purified by semi-preparative reverse phase HPLC (Waters) with a 40 min linear gradient from 10% to 50% acetonitrile containing 0.05% trifluoroacetic acid in water. The mass analysis of the peptides was carried out in linear positive ion mode using MALDI-Tof-Tof (Bruker Daltonics Flex Analysis) using 2,5 dihydroxybenzoic acid as the matrix. The correct peptide sequences synthesised were confirmed by automated peptide sequencing (Procise 491 Applied Biosystems).

2.3.2. Turbidity measurements

Turbidity measurements, reported as $(100-\%T)/100$, where T is the transmittance were carried out to determine interaction between PAA and cationic peptides (1, 2 and 3) using Cary 400 (Varian) UV–visible spectrophotometer equipped with a thermostat cell holder at wavelength 420 nm. PAA solution was used as reference.

2.3.3. Atomic force microscopy measurement

All AFM images were procured using PicoSPM equipment (Molecular Imaging, Tempe, AZ, USA) using AAC (Acoustic Alternating Current) mode. 2 μL of peptide–polymer solution

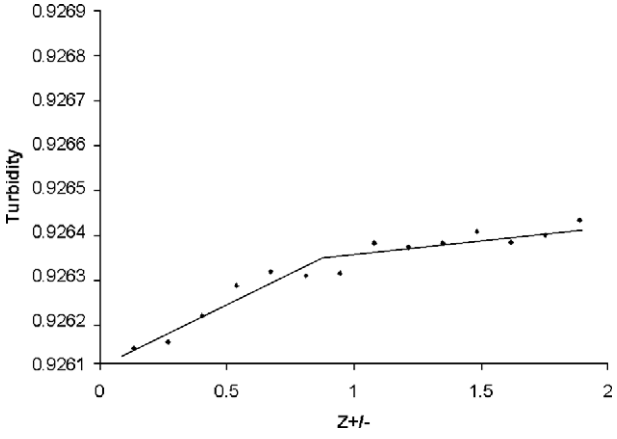

Fig. 2. Variation of the turbidity as a function of the charge ratio ($Z_{+/-}$) of cationic peptide 3 (KKKWKAKA) and polyanionic polymer (PAA). Turbidity measurements, reported as $(100-\%T)/100$, where T is the transmittance, were carried out at wavelength 420 nm. A break point is observed near $Z_{+/-}=1$ which shows rise in turbidity indicating peptide and polymer are forming complexes.

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