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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.

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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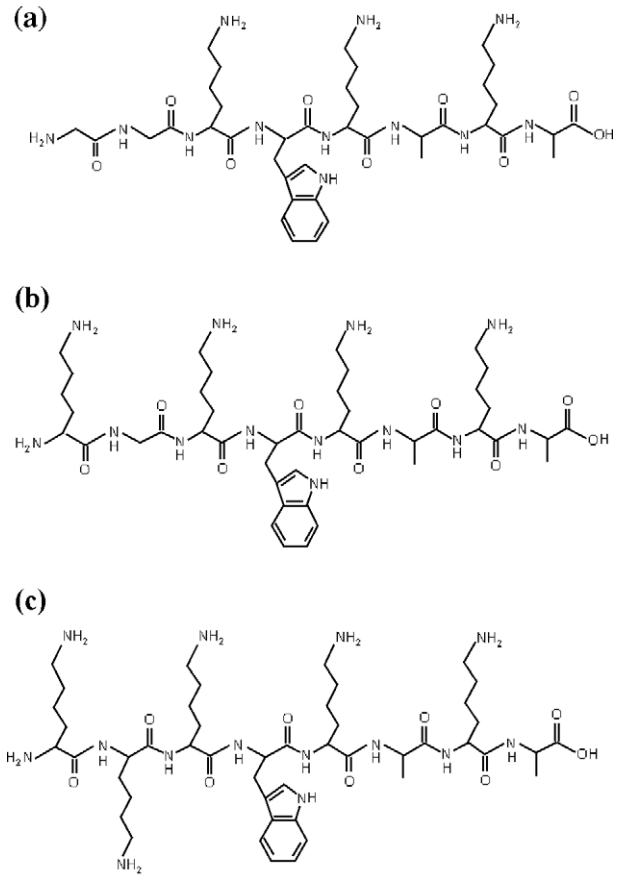

Fig. 1. Structures of the peptides (a) peptide 1 (GGKWKAKA), (b) peptide 2 (KGKWKAKA), (c) peptide 3 (KKKWKAKA).

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

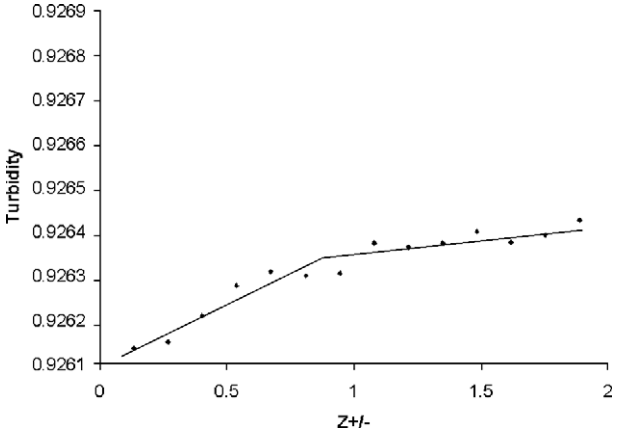

Fig. 2. Variation of the turbidity as a function of the charge ratio ($Z_{+/-}$) of cationic peptide 3 (KKKWKAKA) and polyanionic polymer (PAA). Turbidity measurements, reported as $(100-\%T)/100$, where T is the transmittance, were carried out at wavelength 420 nm. A break point is observed near $Z_{+/-}=1$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

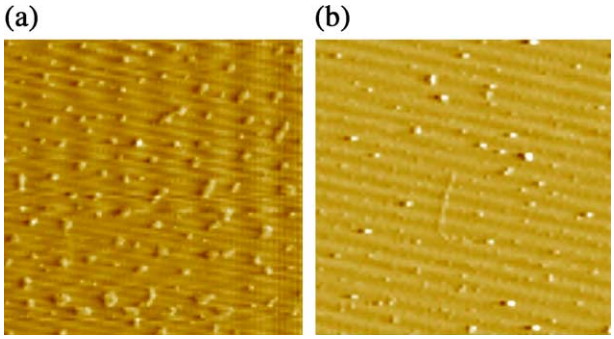

Fig. 3. AFM images of complexes of polyanion (PAA) and cationic peptide 1 (GGKWKAKA) at (a) $Z_{+/-}=1$ and (b) $Z_{+/-}=10$. Scan range=4.0×4.0 μm. Particles of size 120–200 nm and some elongated structures in the size range of 250–350 nm are seen at $Z_{+/-}=1$. More or less spherical particles of size in the range of 75–100 nm are seen at $Z_{+/-}=10$. Height of particles is observed in the range of 10–20 nm.

was deposited on a freshly cut piece of mica (1×1 cm) and allowed to adsorb for 2 min at room temperature. Imaging was carried out under dried condition in air. 250 μm long cantilevers with a resonance frequency of about 41 kHz were used for imaging. Minimal image processing (flattening only) was carried out in the presented images.

3. Results and discussion

Three different types of peptides were chosen i.e. peptide 1 (GGKWKAKA), peptide 2 (KGKWKAKA), and peptide 3 (KKKWKAKA) having three, four and five lysine residues which introduce cationic nature in solution, respectively. Chemical structures of all the peptides are presented in Fig. 1. These peptides were designed to contain different numbers of lysine residues in order to gain insight into the mechanism of interaction between the cationic peptides and anionic polymer, PAA. Turbidity measurements were done to analyze such type of interactions by using UV–Vis Spectrophotometer at wavelength 420 nm. In 1×10^{-3} M (in acrylate unit) solution of PAA different amount of cationic peptide solutions was added to obtain solutions of different charge ratio, $Z_{+/-}$, and turbidity of the solution was measured at wavelength 420 nm. Fig. 2 shows a representative curve of variation of turbidity of the peptide 3 versus $Z_{+/-}$. Turbidity of the solution increased on increment in $Z_{+/-}$, indicating that the cationic peptides were interacting with the negatively charged polymer, PAA.

Peptide–polymer solutions in different charge ratios i.e.; 1, 2, 4, 10 and 20 were prepared and AFM studies were done to characterize the morphology of the complexes. As a control, polymer without peptide was also observed through AFM but no particle formation was found (figure not shown). At lower $Z_{+/-}=1$, the complexes formed between the positively charged peptide and the polyanionic polymer were observed in the nanometer size in the range of 120–200 nm and some elongated structures with length in the range of about 250–300 nm as presented in Fig. 3(a). Similar morphological characteristics were observed at $Z_{+/-}=2$ and 4 (figure not shown). Particles of bigger size and elongated structures at low $Z_{+/-}$ could have been formed due to less number of peptide molecules being available for electrostatically interacting or complexing with the polymer. The extent of complexation increased at higher charge ratio. More or less spherical particles of size 75 to 100 nm were found at intermediate $Z_{+/-}=10$ as shown in Fig. 3(b). However, fibrils forming interconnected structures were observed for all the three peptides at $Z_{+/-}=20$, but as the number of positive charges increases in the peptides (1–3) complexes formed from cationic peptides and anionic polymer show lesser aggregation, which is depicted in Fig. 4(a)–(c). This type of aggregation could be inferred due to nearly electroneutral complex formation between peptide and polymer. Thus complex formation depends on the ratio of the concentration of positive units of peptides to the concentration of negative charges present in the polymer. Height ranges of nanoparticles were observed in the range of 10–20 nm at all charge ratios for all peptides irrespective of the positive charges present in the peptide.

4. Conclusions

We found elongated particle formation of nanometer size due to self assembling of cationic peptides on interaction with the oppositely charged polymer of poly(acrylic acid) at lower $Z_{+/-}$ but spherical particles were observed at higher $Z_{+/-}$ value. Size and shape of the kinds of nanoparticles depend on the ratio of cationic and anionic units present in the system. Coulombic interactions, hydrophobicity of the polymer–molecule pair,

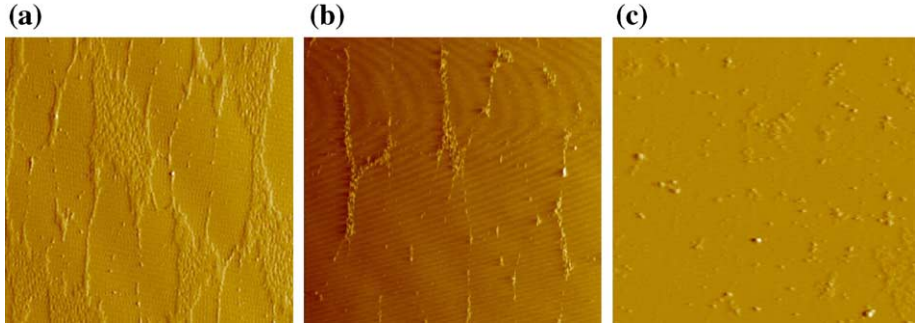

Fig. 4. AFM images of complexes of polyanion (PAA) and cationic peptides (a) peptide 1(GGKWKAKA), (b) peptide 2 (KAKWKAKA) and (c) peptide 3 (KKKWKAKA) at $Z_{+/-}=20$. Scan range=15.0×15.0 μm. Fibrils forming interconnected structures are observed for all the three peptides but with the increment of positive charge in the peptides (1–3) complexes formed with the PAA showing lesser aggregation as shown in the figure from (a) to (c).

and the conformational features of the polymer are the factors that contribute to such type of association. The studies for the enhancement of antinociception effect of chimeric peptide using such type of nanoparticle particles containing cationic peptides obtained by this methodology as delivery system, providing better metabolic stability and bioavailability to the peptides, are underway in our laboratory.

Acknowledgements

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References

[1] R.D. Egleton, S.A. Mitchell, J.D. Huber, J. Janders, D. Stropova, D. Polt, H.I. Yamamura, V.J. Hruby, T.P. Davis, Improved bioavailability to the brain of glycosylated Met Enkephalin analogs, Brain Res. 881 (2000) 37–46.

[2] C.L. Gentry, R.D. Egleton, T. Gillespie, T.J. Abbruscato, H.B. Bechowski, V.J. Hruby, T.P. Davis, The effect of halogenation on blood–brain barrier permeability of a novel peptide drug, Peptides 20 (1999) 1229–1238.

[3] T. So, H.O. Ito, M. Hirata, T. Ueda, T. Imoto, Extended blood half-life of monomethoxypolyethylene glycol-conjugated hen lysozyme is a key parameter controlling immunological tolerogenicity, Cell. Mol. Life Sci. 55 (1999) 1187–1194.

[4] Y. Tsutsumi, M. Onda, S. Nagata, B. Lee, R.J. Kreitman, I. Pastan, Site-specific chemical modification with polyethylene glycol of recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) improves antitumor activity and reduces animal toxicity and immunogenicity, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 8548–8553.

[5] R. Duccan, Drug–polymer conjugates: potential for improved chemotherapy, Anti-Cancer Drugs 3 (1992) 175–210.

[6] Y. Mu, H. Kamada, Y. Kaneda, Y. Yamamoto, H. Koidara, S. Tsunoda, Y. Tsutsumi, M. Maeda, K. Kawasaki, M. Nomizu, Y. Yamada, T. Mayumi, Bioconjugation of laminin peptide YIGSR with poly (stysene co-maleic acid) increases its metastatic effect on lung metastasis of B16-BL6 melanoma cells, Biochem. Biophys. Res. Commun. 255 (1999) 75–79.

[7] E. Bigon, E. Boarato, A. Bruni, A. Leon, G. Toffano, Pharmacological effects of phosphatidylserine liposomes: regulation of gylcolysis and energy level in brain, Br. J. Pharmacol. 66 (1979) 167–174.

[8] C. Vauthier, C. Dubernet, E. Fattal, H. Pinto-Alphandry, P. Couvreur, Poly(alkylcyanoacrylates) as biodegradable materials for biomedical applications, Adv. Drug Deliv. Rev. 55 (2003) 519–548.

[9] P. Couvreur, G. Barratt, E. Fattal, P. Legrand, C. Vauthier, Nanocapsule technology: a review, Crit. Rev. Drug Deliv. Syst. 19 (2002) 99–134.

[10] E. Fattal, C. Vauthier, Encyclopedia of Pharmaceutical Technology, Marcel Dekker, New York, 2002, pp. 1874–1892.

[11] G. Barratt, G. Couarraze, P. Couvreur, C. Dubernet, E. Fattal, R. Gref, D. Labarre, P. Legrand, G. Ponchel, C. Vauthier, Polymeric Biomaterials, 2nd edn., Marcel Dekker, New York, 2001, pp. 753–782.

[12] C.K. Nisha, V.S. Manorama, J.N. Kizhakkedathu, S. Maiti, Water-soluble complexes from random copolymer and oppositely charged surfactant: 2. Complexes of poly(ethylene glycol)-based cationic random copolymer and bile salts, Langmuir 20 (2004) 8468–8475.

[13] G. Coppi, V. Iannuccelli, M.T. Bernabei, R. Cameroni, Alginate microparticles for enzyme peroral administration, Int. J. Pharm. 242 (2002) 263–266.

[14] S. Rossi, G. Sandri, F. Ferrari, M.C. Bonferoni, C. Caramella, Buccal delivery of acyclovir from films based on chitosan and polyacrylic acid, Pharm. Dev. Technol. 8 (2003) 199–208.

[15] B.S. Lele, A.S. Hoffman, Insoluble ionic complexes of polyacrylic acid with a cationic drug for use as a mucoadhesive, ophthalmic drug delivery system, J. Biomater. Sci., Polym. Ed. 11 (2000) 1319–1331.

[16] S. Gupta, S. Pasha, Y.K. Gupta, D.K. Bhardwaj, Chimeric peptide of Met-enkephalin and FMRFa induces antinociception and attenuates development of tolerance to morphine antinociception, Peptides 20 (1999) 471–478.