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Branched amphiphilic peptide capsules: Cellular uptake and retention of encapsulated solutes☆

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ABSTRACT

Branched amphiphilic peptide capsules (BAPCs) are peptide nano-spheres comprised of equimolar proportions of two branched peptide sequences bis(FLIVI)-K-KKKK and bis(FLIVIGSII)-K-KKKK that self-assemble to form bi-layer delimited capsules. In two recent publications we described the lipid analogous characteristics of our BAPCs, examined their initial assembly, mode of fusion, solute encapsulation, and resizing and delineated their capability to be maintained at a specific size by storing them at 4 °C. In this report we describe the stability, size limitations of encapsulation, cellular localization, retention and, bio-distribution of the BAPCs in vivo. The ability of our constructs to retain alpha particle emitting radionuclides without any apparent leakage and their persistence in the peri-nuclear region of the cell for extended periods of time, coupled with their ease of preparation and potential tune-ability, makes them attractive as biocompatible carriers for targeted cancer therapy using particle emitting radioisotopes. This article is part of a Special Issue entitled: Interfacially Active Peptides and Proteins. Guest Editors: William C. Wimley and Kalina Hristova.

1. Introduction

There is a great deal of interest in the area of nanoparticle-mediated therapies. Nano-carrier mediated targeted cellular therapy is a rapidly growing area of research for the treatment of malignant and infectious diseases. Particle emitting radioisotopes complemented with a targeting moiety are being recognized as some of the most promising cytotoxic candidates for the treatment of cancerous tumors. Nano-particles enjoy distinct advantages in the delivery of drug payloads. Their nanosizes enable them to be directly injected into systemic circulation [1,2] and afford them longer circulating times [3,4]. Furthermore, the circulating time can be increased by the surface modification of nanoparticles with hydrophilic moieties such as polyethylene glycol [5–7], and nanoparticles composed of biodegradable polymers can be tuned to release their drug payload in a controlled fashion; either by micelle dissociation, polymer degradation, diffusion or in combination [8–10]. Mechanisms of nanoparticle internalization into cells are influenced by their physiochemical properties. Biocompatible nanocomposites such as lipid based carriers (liposomes and micelles); polymeric vesicles designed from amphiphilic block co-polymers [11] such as polyethylene glycol–polylactic acid (PEG–PLA) and PEG–polycaprolactone (PEG–PCL) [12]; nanocapsules [13,14], bola-amphiphiles (amphiphilic molecules possessing two polar heads on both sides of an aliphatic chain) such as aminoundecyltriethoxysilane (AUT) [15,16]; and carbon nanotubes [17] have been studied for their efficacy as delivery systems.

Liposomes are preferred over other delivery systems due to their ability to encapsulate both hydrophobic and hydrophilic contents. They can also be modified with respect to their fatty acid and head group composition, and surface alterations to modulate drug release and target affinity. Some of the issues associated with liposomes such as degradation by hydrolysis, oxidation, sedimentation, aggregation, or fusion during storage are being addressed with the development of niosomes [18] and proniosomes [19,20], however further testing is needed to fully establish safety and efficacy.

The selection of any nanoparticle for a specific pharmacological use is contingent on its mechanism of cellular uptake and intracellular trafficking [21]. In addition, concerns relating to the successful encapsulation of cargo, stability, specificity, bio-reactivity, biodegradability and toxicity are also relevant. The ability to release their contents is not necessarily a requirement for certain cargos. In the case of targeted alpha particle therapy (TAT) – a treatment modality for metastatic cancer and infectious diseases – the advantageous properties of ^{225}Ac [22] are partially offset by its systemic toxicity [23] due to the potential accumulation of its daughter nuclides in off-target sites. Utilization of

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alpha-emitters requires containment systems that allow the high-energy alpha particles to penetrate target tissues while retaining the radionuclide and its daughter isotopes. This poses a considerable challenge since the energy (5 to 8 MeV/^{225}Ac α-particle) released is sufficient to disrupt the integrity of most traditional nano-carriers. This property has hampered the development of ^{225}Ac as a viable radio-therapeutic agent [24,25]. The current use of chelating agents for ^{225}Ac radioimmunotherapy has been challenging as a consequence of the poorly defined coordination chemistry of Ac(III), owing to the lack of stable isotopes to enable routine chemical analysis [26]. Chelators like EDTA, DTPA, DOTA and PEPA [27,28] have been used to complex with ^{225}Ac with varying degrees of success. On the other hand the potential of the otherwise promising ^{225}Ac-HEHA macrocyclic complex in radiotherapy [29] has been marred by instability, due to the coordinated ^{225}Ac radionuclide decaying into its daughter isotopes [26].

Efforts to develop bifunctional chelators capable of stably binding ^{225}Ac to antibodies as well as competently containing resulting daughter nuclides at target sites, have not been successful. This has forced the development of sterically stabilized pegylated liposomes [30] and stable pegylated phosphatidylcholine–cholesterol liposomes [31] for radioimmunotherapeutic applications despite the inherent instability and retention based limitations associated with traditional liposomal systems. Moreover, novel liposomal carriers such as MUVELs (Multivesicular liposomes) – involving the passive entrapment of small vesicles into large liposomes – have been designed to enhance the targeting capabilities and the retention of alpha particle emitting daughters of ^{225}Ac, in an effort to better utilize their positive cytotoxic potential [32]. All of this liposome directed encapsulation techniques are however lengthy and tedious; [30–32] and involve considerable preparation times that include complex formation of ^{225}Ac with a chelate, annealing procedures, extended waiting periods, extrusions and centrifugation; apart from addressing various issues to counter physiochemical problems such as possible oxidation due to alpha emissions [33,34] and beta [35] and gamma [36] radiation. The work presented herein presents an alternative and flexible means of radionuclide encapsulation that is easy to perform and generates stable in vivo constructs.

Peptide based nano-assemblies show promise as nano-delivery vehicles for the safe, targeted transport of drugs to specific tissues and organs, with minimal off target accumulation [37] by overcoming some of the problems associated with traditional lipid and viral based delivery systems. BAPCs (Branched Amphiphilic Peptide Capsules) are a new class of self-assembling peptide nano-capsular spheres [38,39] formed during the cooperative association of a mixture of two (15–23 residue) poly-cationic branched amphiphilic peptides (Fig. 1). The hydrophobic core sequences are derived from an internal fragment of CaIVS3, the human dihydropyridine sensitive L-type calcium channel segment [40]. The ability of the BAPCs to form bilayer-delimited spheres (Fig. 2) capable of trapping solutes is a consequence of the unique characteristics of its constituent peptides — bis(FLIVI)-K-K$_4$ and bis(FLIVIGSII)-K-K$_4$, which reversibly transition from an alpha helical conformation in 2,2,2-Trifluoroethanol, to a beta sheet in water [38,39]. The branch point lysine in the sequence orients the two peptide segments at ~90° angle, mimicking the geometry of diacyl phospholipids. Coarse grain molecular dynamic simulations, [38] consistent with S/TEM analysis, indicate the presence of a single capsular bilayer (3–4 nm) comparable to that of a phospholipid system, which is below the discerning resolution of electron microscopy.

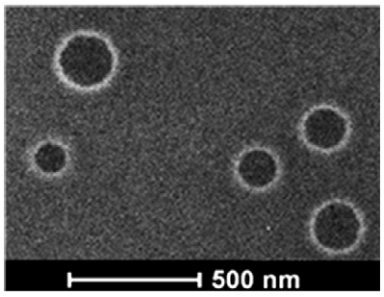

Fig. 2. S/TEM image of BAPCs. They containing 30% Hg label and imaged at 2 h post hydration w/ negative glow discharge and uranyl acetate staining prepared as previously described [39].

Recently, we described how the flexible BAPCs possess many of the properties of phospholipid vesicles, such as fusion, solute encapsulation and an ability to be resized by membrane extrusion through polycarbonate filters with defined pore sizes [39]. We also demonstrated several biophysical characteristics including, their mode of assembly, high thermodynamic stability, and their kinetics of fusion. The BAPCs can – like their lipid counterparts – be both resized, and maintained there by placing them at 4 °C. The versatility of these peptides to self-assemble enables us to tag individual monomers with ligands and molecular markers for a variety of analytical and functional assays, making these constructs particularly suited as biocompatible vehicles for the targeted delivery of cargo into the cells. In this report, we study the stability, cellular uptake, load capacity, retention within biological environments for extended periods of time, tolerance to a radionuclide load, biodistribution and capacity to maintain their structural integrity even when subjected to alpha particle emissions.

2. Materials and methods

2.1. Peptide synthesis

2.1.1. Synthesis of bis(FLIVI)-K-K$_4$ and bis(FLIVIGSII)-K-K$_4$ variants

Peptides were synthesized using solid phase peptide chemistry on 4-(2,4-dimethoxyphenyl-Fmoc-aminomethyl) phenoxyacetyl-norleucyl-cross-linked ethoxylate acrylate resin [41] (Peptides International Inc.; Louisville, Kentucky) on a 0.1 mmol scale using Fmoc (N-(9-fluorenyl) methoxycarbonyl)/tert-butyl chemistry on an ABI Model 431 peptide synthesizer (Applied Biosystems; Foster City, CA). This resin yields the carboxyamide at the C-terminus upon cleavage. The Fmoc amino acids were obtained from Anaspec, Inc. (Fremont, CA). The branch point was introduced by incorporating N$^{\alpha,\varepsilon}$ di-Fmoc-L-lysine in the fifth position from the C-terminus. Deprotection of the two Fmoc protecting groups leads to the generation of two reactive sites that allow for the generation of the bifurcated peptide branch point. This enables the simultaneous addition of either of the hydrophobic tail segments, FLIVI and FLIVIGSII to the common hydrophilic oligo-lysine segment by the stepwise addition of Fmoc amino acids [42]. The N-termini

Fig. 1. Bilayer forming branched amphiphilic peptide sequences.