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Sustained and controlled release of lipophilic drugs from a self-assembling amphiphilic peptide hydrogel

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ABSTRACT

Materials which undergo self-assembly to form supramolecular structures can provide alternative strategies to drug loading problems in controlled release application. RADA 16 is a simple and versatile self-assembling peptide with a designed structure formed of two distinct surfaces, one hydrophilic and one hydrophobic that are positioned in such a well-ordered fashion allowing precise assembly into a predetermined organization. A "smart" architecture in nanostructures can represent a good opportunity to use RADA16 as a carrier system for hydrophobic drugs solving problems of drugs delivery. In this work, we have investigated the diffusion properties of Pindolol, Quinine and Timolol maleate from RADA16 in PBS and in BSS-PLUS at 37 °C. A sustained, controlled, reproducible and efficient drug release has been detected for all the systems, which allows to understand the dependence of release kinetics on the physicochemical characteristics of RADA16 structural and chemical properties of the selected drugs and the nature of solvents used. For the analysis various physicochemical characterization techniques were used in order to investigate the state of the peptide before and after the drugs were added. Not only does RADA16 optimise drug performance, but it can also provide a solution for drug delivery issues associated with lipophilic drugs.

1. Introduction

Hydrogels are cross-linked hydrophilic polymer networks that can absorb more than 100 times their dry weight in water, giving them physical characteristics similar to soft tissue (Gibas and Janik, 2010). Self-assembling peptide hydrogels are an important class of hydrogels, which are potentially excellent materials for various molecular controlled release applications (Nagai et al., 2006). Self-assembly is a spontaneous process by which several individual molecules are associated into a coherent and organized structure under thermodynamic equilibrium conditions by non-covalent interaction, such as ionic and hydrogen bonding (Zhang et al., 1993; Jun et al., 2004; Zhaoyang et al., 2008). In comparison with chemically synthesized polymer materials, self-assembling peptide hydrogels have numerous advantages, for example, (i) the peptides that construct the hydrogels can often be degraded in vivo, and the resulting products (amino acids) are nontoxic; (ii) the hydrogels are spontaneously formed without using harmful chemicals such as cross-linkers; (iii) the spontaneous process allows for an solution–gel transformation in vivo by injecting peptide solutions at specific locations, and it also enables a facile incorporation of cell-specific bioactive moieties into hydrogels; (iv) the peptide building blocks represent a variety of chemical groups that allow hydrogels to be easily modified with chemical and biological moieties; and (v) the hydrogel maintains a high water content, which may allow for the diffusion of a wide range of molecules (Zhang et al., 2002; Zhang, 2003; Huang et al., 2011). Peptide hydrogels have been demonstrated to be useful as controlled release devices (Nagai et al., 2006; Koutsopoulos et al., 2008). Depending on molecular design, many different hydrogels (e.g. P11-family (Aggeli et al., 2003; Carrick et al., 2007), MAX8 (Altunbas et al., 2011), Fmoc-FF with KGM (Jayawarna et al., 2009), EAK16 (Keyes et al., 2004) and RADA16 (Gelain et al., 2010)) have been constructed. For our study we have used RADA16 which has a high propensity to self-assemble into hydrogels with nanofibre structures containing ~99.5% w/v water ensuring the biodegradability (Arosio et al., 2012). RADA16, known as "molecular Lego" (Zhang, 2002), has two surfaces – one hydrophilic composed of alternating arginine (positive charge) and aspartic acid (negative charge), and one hydrophobic surface enabling formation of supra-molecular assemblies by a "lock and fit" model (Nune et al., 2013). Moreover, RADA16 contains a regular

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repeat of alternating hydrophobic and hydrophilic amino acids (Yokoi et al., 2005) forming a hydrogel with a large surface to volume ratio (Zhang, 2003). Alternation of hydrophobic and hydrophilic amino acids tends to promote β-strand secondary structure and two structural features that lead to stable nanofibre formation: (1) hypothesized hydrogen bonding between neighbouring peptide backbones, stabilizing a possible cross-β structural motif well-known to describe amyloid fibrils (Jonker et al., 2012; Eanes and Glenner, 1968) and (2) separation between hydrophobic and hydrophilic faces that are believed to form the core and surface of nanofibres, respectively (Yokoi et al., 2005). According to previous studies (Nagai et al., 2006; Koutsopoulous et al., 2008), RADA16 is an efficient delivery carrier but has not been used for hydrophobic drugs. Therefore, we hypothesize that this "smart" architecture in nanostructure would allow loading hydrophobic drugs and permitting a sustained and controlled release providing solution for delivery problems. Therefore, we hypothesized that this "smart" architecture may encapsulate small hydrophobic molecules between peptide chains, disrupting β-sheet formation to a more α-helix configuration but permitting a sustained and controlled release providing solutions for delivery problems. In order to investigate our hypothesis, we have explored the release profiles of Pindolol (P), Quinine (Q) and Timolol maleate (T) from RADA16 hydrogel. The drug release was investigated in PBS and BSS Plus solutions at 37 °C. The developed formulations were further characterized by atomic force microscopy (AFM), circular dichroism (CD) spectrometer and Fourier transform infrared spectroscopy (FT-IR). The molecules were chosen to have a range of partition coefficients (Log *P*) and acid dissociations constants (pK_a).

2. Materials and methods

2.1. Chemicals and reagents

The ac-$(RADA)_4$-$CONH_2$ peptide in 1% solution was obtained from BD Biosciences (Bedford, MA). Pindolol (Fig. 1a; Table 1(a)) is a nonselective β-blocker with partial β-adrenergic receptor agonist activity. Quinine (Fig. 1b; Table 1(b)) is a natural white crystalline alkaloid having antipyretic, antimalarial, analgesic and anti-inflammatory properties. Timolol maleate salt (Fig. 1c; Table 1(c)) is a non-selective beta-adrenergic receptor antagonist indicated for treating glaucoma, heart attacks and hypertension. All drugs were purchased from Sigma–Aldrich. Phosphate buffered saline (PBS) is an aqueous solution containing sodium chloride, sodium phosphate, and, in some formulations, potassium chloride and potassium phosphate. PBS solution was prepared using PBS buffer tablets (pH 7.4), and purchased from Sigma–Aldrich. BSS-Plus is a sterile intraocular irrigating solution with pH 7.4, which was purchased from Alcon – UK. It is a complex solution with sugar and salts and it copies the physiological conditions of eyes providing an interesting study for T, which is used in glaucoma therapy. Furthermore BSS-Plus is used in this study to investigate a possible novel interaction between peptide and selected drugs due the nature of the different solvent. BSS-Plus is formed from sodium chloride 7.44 mg, potassium chloride 0.395 mg, dibasic sodium phosphate 0.433 mg, sodium bicarbonate 2.19 mg, hydrochloric acid and/or sodium hydroxide, calcium chloride dihydrate 3.85 mg, magnesium chloride hexahydrate 5 mg, dextrose 23 mg, glutathione disulfide (oxidized glutathione), 4.6 mg in water for injection.

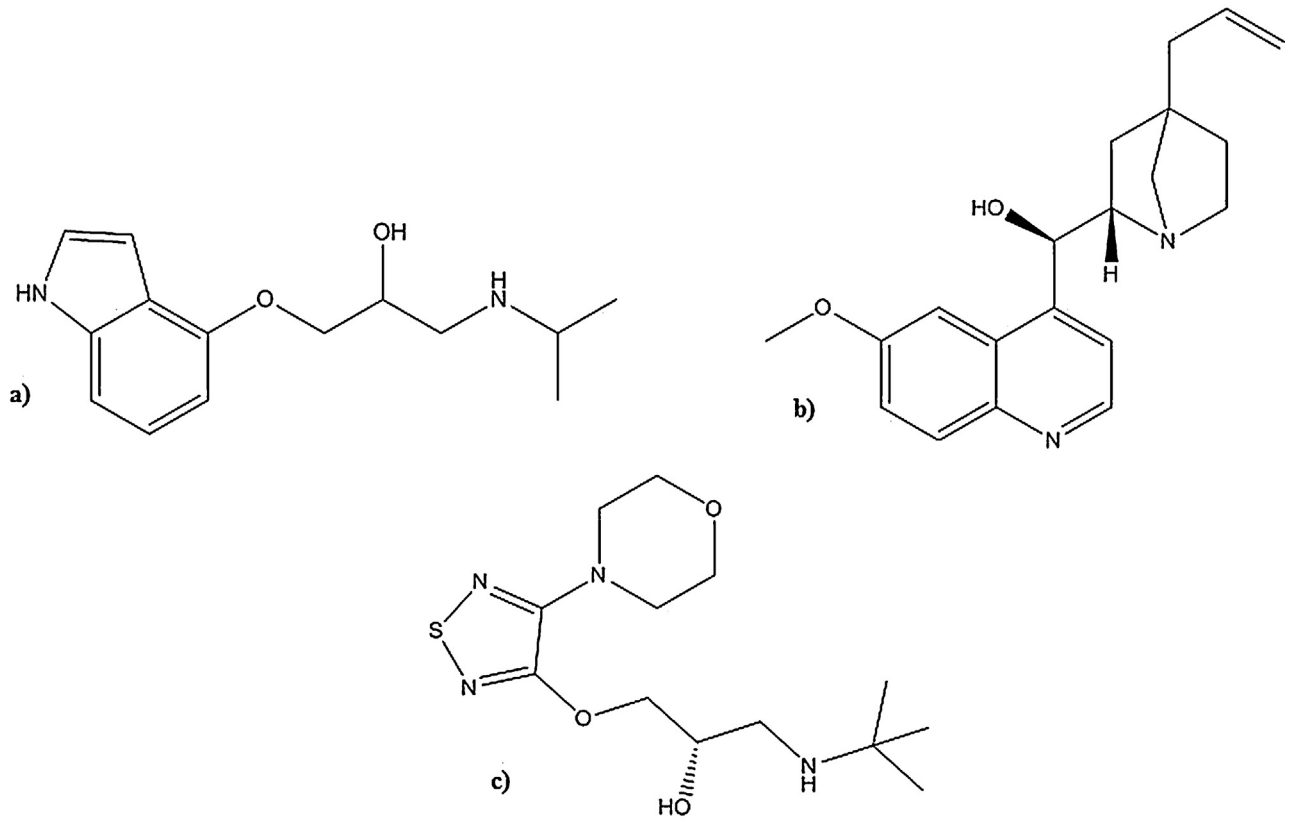

Fig. 1. Chemical structures of the compounds used. (a) Pindolol (P), (b) Quinine (Q), and (c) Timolol maleate (T).