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Delta-sleep inducing peptide entrapment in the charged macroporous matrices

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ABSTRACT

Various biomolecules, for example proteins, peptides etc., entrapped in polymer matrices, impact interactions between matrix and cells, including stimulation of cell adhesion and proliferation. Delta-sleep inducing peptide (DSIP) possesses numerous beneficial properties, including its abilities in burn treatment and neuronal protection. DSIP entrapment in two macroporous polymer matrices based on copolymer of dimethylaminoethyl methacrylate and methylen-bis-acrylamide (Co-DMAEMA-MBAA) and copolymer of acrylic acid and methylen-bis-acrylamide (Co-AA-MBAA) has been studied. Quite 100% of DSIP has been entrapped into positively charged Co-DMAEMA-MBAA matrix, while the quantity of DSIP adsorbed on negatively charged Co-AA-MBAA was only 2–6%. DSIP release from Co-DMAEMA-MBAA was observed in saline solutions (0.9% NaCl and PBS) while there was no DSIP release in water or 25% ethanol, thus ionic strength was a reason of this process.

1. Introduction

The design of novel biomaterials for tissue engineering and wound healing is one of the important challenges in medicine. As well known, matrices for tissue engineering should provide an optimal microenvironment, in order to support cell adhesion, three-dimension growth, migration and production of extracellular matrix [1,2].

Polymer matrices loaded with various therapeutics can be considered as "depots" for various medicines, for example antibiotics [3] or anesthetics [4], which can be released in a controlled manner. Various bioactive substances, such as peptides, enzymes, growth factors, DNA, or oligonucleotides used as therapeutic agents are protected by matrix against rapid destruction by peptidases or nucleases [5,6].

In the current study DSIP has been chosen as a model peptide due to its neuroprotective and wound healing properties. Due to its indirect antioxidant activity, DSIP was proposed for burn treatment of experimental animals [7]. Moreover, this neuropeptide possesses many other beneficial properties, for example, stress protective, adaptogenic, antiepileptic activities [8–10]. However, DSIP molecule is digested by plasma peptidases in a human body in a few minutes [11]. Thus, DSIP protection against peptidases and prolongation of its lifetime in the body is of great importance.

Preparation of hydrogel from polymer frozen aqueous solutions allows designing macroporous systems with developed surface, which provides optimal interaction of polymer surface with entrapped peptide.

Various compounds, for example, proteins, peptides etc., can be entrapped into polymer matrices for improvement of their properties [12–14]. These compounds are bound with matrix surface covalently or by electrostatic, hydrogen and Van-der-Vaals bonds. Evidently, proteins, peptides and other compounds could be entrapped into these matrices by adsorption due to interaction of oppositely charged functional groups of a compound to be entrapped and a matrix [15].

Since there are two negatively charged s (Asp and Glu) and an N-terminal aromatic amino acid residue (Trp) with amino group as a donor of H^+ in DSIP structure, we used two oppositely charged porous hydrogels, namely based on 1) dimethylaminoethyl methacrylate and methylen-bis-acrylamide (Co-DMAEMA-MBAA); 2) copolymer of acrylic acid and methylen-bis-acrylamide (Co-AA-MBAA) as matrices for DSIP entrapment. Both these polymer materials are widely used for biomedical applications. For example, positively charged poly-DMAEMA and its copolymers are used as restorative and prosthetic materials in tissue engineering, in particular for bone reparation [16,17] or in stomatology [18,19]. They are also widely employed in drug and gene delivery systems [20–26]. Moreover, poly(DMAEMA) has also antibacterial activity and mucoadhesive properties [24]. Negatively charged poly(AA) shows less mechanical stability and has to be

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copolymerized with other monomers. AA was copolymerized with Pluronics and was used for solubilization of hydrophobic drug [27,28], preparation of nanoparticles with entrapped peptides [29] and for fabrication of composite restorative system in stomatology [30].

The aim of the study was to design macroporous polymer matrices with entrapped DSIP and to study its release using various in vitro models.

2. Experimental part

DSIP (pI 4.7, 849 Da, TrpAlaGlyGlyAspAlaSerGlyGlu) was synthesized as previously reported [31]. PBS (Paneko, Russia), ethanol 96% (Glavspirt, Russia), NaCl, potassium peroxysulfate (PPS), 2-dimethylaminoethyl methacrylate (DMAEMA), acrylic acid (AA), N,N′-methylen-bis-acrylamide (MBAA) were purchased from Sigma, USA. Acetonitrile (Biosolve B.V., Netherlands), trifluoroacetic acid (TFA) (Aldrich, USA) and MilliQ purified water (Sartorius, Germany) were used in this study.

2.1. Matrices preparation and characterization

Two charged polymer matrices have been prepared from the monomer solutions with final monomer concentration 10% in the case of Co-AA-MBAA and 8% for Co-DMAEMA-MBAA. The molar ratio of ionic monomer and cross-linking agent (MBAA) was 96:4, and the concentration of polymerization initiator was 2% of total monomer weight [32].

2.1.1. Synthesis of the matrix from copolymer of acrylic acid and methylen-bis-acrylamide (Co-AA-MBAA)

For synthesis of this matrix, 0.525 g (6.94 mM) AA was dissolved in 8 ml of distilled water, and then 0.0683 g (0.44 mM) MBAA was added. Dissolved oxygen was removed from the obtained solution by vacuum degassing and using an argon flow. The solution was cooled to 0 °C, then 0.56 ml (20 mg/ml) PPS and 0.28 ml (100 mg/ml) ascorbic acid were added. The mixture was incubated at −15 °C for 18 h, and then it was thawed out, purified from unbound molecules by boiling in water (3 h, 3 times) and finally lyophilized.

2.1.2. Synthesis of the matrix from copolymer of dimethylaminoethyl methacrylate and methylen-bis-acrylamide (Co-DMAEMA-MBAA)

To synthesize the matrix, 0.467 g (3.18 mM) DMAEMA was dissolved in 7.8 ml distilled water, and 0.031 g (0.2 mM) MBAA was added. All next procedures were carried out as described previously except only 53 ml (20 mg/ml) of PPS was added.

2.1.3. Hydrogel structure characterization

Scanning electron microscopy (SEM) runs were carried out at magnifications of 100×, 300× and 1000× on a scanning microscope equipped with a WinEDS system at an accelerating voltage of 15 kV and electron ray current 1 × 10 150–10 A (JSM U3, Japan).

2.2. Study of Co-DMAEMA-MBAA matrix swelling

Swelling of the prepared hydrogels was studied by gravimetric method. The hydrogel samples were weighed on an analytical balance (Shimadzu, Japan), and then placed either in distilled water or in various solutions within a pH range of 4–12 in the case of study of gels osmotic stability, and incubated for 1 h. The samples of macroporous hydrogels were wringed out between several layers of filter paper to the constant weight, and thus all free water was removed from the hydrogel pores. Weight of the swollen hydrogel was measured by analytic balance again. Testing of each hydrogel sample was carried out at least five times, and then an average weight of the swollen hydrogel was calculated. Swelling was estimated using the equation presented below.

The value of equilibrium swelling was determined using a following Eq. (1):

$$S = \frac{m_w - m_d}{m_d} \cdot \frac{1}{\rho} \quad (1)$$

where

m_w weight of the swollen hydrogel;
m_d weight of the dry hydrogel;
ρ density of water or the buffer solution.

2.3. The entrapment of DSIP in Co-AA-MBAA and Co-DMAEMA-MBAA matrices

The samples of porous polymer matrices (6–20 mg) were incubated in 1 ml of aqueous DSIP solution (0.1 mg/ml) with gentle shaking (Vibrax® (Ika, Germany)) at room temperature for 16 h. After incubation the matrices were taken from solutions, frozen and lyophilized. DSIP residue content in solutions was determined by HPLC.

HPLC was carried out in an isocratic mode using a system gold high performance liquid chromatograph (Beckman, USA), Gemini column 5u C18 110A (250 × 4.6 mm) (Phenomenex, USA) at 222 nm. An injection sample was 20 μl. The mobile phase contained 0.07% TFA in a 13% acetonitrile aqueous solution, and a flow rate was 1 ml/min. DSIP concentration was evaluated as a square of the peak on the chromatogram, the sample of DSIP solution (1 mg/ml) has been taken for comparison [33].

2.4. Study of DSIP storage in Co-DMAEMA-MBAA matrices at room temperature

Dry Co-DMAEMA-MBAA macroporous matrix samples loaded with DSIP were stored in open tubes at room temperature for 1.5 months. The peptide stability was then studied by HPLC as described above. To determine the accumulation of water from the air in Co-DMAEMA-MBAA matrix samples, they were weighed after lyophilization and then the dried matrices were kept in open tubes at room temperature for 24 h. Then the matrices were weighted again to calculate water content in the matrix samples. When any additional peak appeared in the chromatogram, it was a signal of any DSIP structure change (supporting data).

2.5. Study of DSIP release

In vitro DSIP release was studied by HPLC. The polymer matrices containing DSIP were incubated in 0.5–1 ml water, 25% ethanol in water, 0.9% NaCl or PBS (pH 7.4) with gentle shaking (Vibrax-VXR, Ika, Germany). The peptide release from Co-DMAEMA-MBAA matrix was studied in two release models. A static model [34] was characterized by sampling 5–10% of supernatant volume with a replacement with an equivalent volume of saline solutions (PBS or 0.9% NaCl) after 0.5, 1, 3, 6, 9, 12, 24 hs of incubation. DSIP release kinetics has been expressed in reduced concentrations of peptide (peptide quantity/ml of the diluted supernatant). A dynamic model was characterized by a total medium replacement every 2 h, as described earlier [35].

2.6. Statistical analysis

Each experimental sample was evaluated in triplicate. The results were expressed as mean ± SEM.