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Complexation of HIV derived peptides with carbosilane dendrimers

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ABSTRACT

Dendrimers have been proposed as new carriers for selected HIV-1 peptides. This paper reports on the complexation behaviour of the three HIV-derived-peptides: Gp160, NH-EIDNYTNTIYTLLEE-COOH; P24, NH-DTINEEAAEW-COOH and Nef, NHGMDDPEREVLEWRFDSRLAF-COOH with second generation cationic carbosilane dendrimers (CBD) branched with carbon—silicon bonds (CBD-CS) or oxygen—silicon bonds (CBD-OS). Studies on the formation of complexes between HIV peptides and CBDs by fluorescence polarization, zeta-potential, electrophoresis and transmission electron microscopy have shown that both studied dendrimers form complexes with HIV peptides. At a molar ratio of (2.5–3):1 (dendrimer:peptide), the complexes formed were in the size range of 180–275 nm and with significant positive surface charge. The results suggest that interactions between dendrimers and HIV peptides have electrostatic nature due to the negative charge of peptides backbone and positive charge of dendrimer functional groups. Dendriplex stability depended on the type of studied dendrimers. Time of peptides release from the complexes ranged from 1 (CBD-OS) to ~36 (CBD-CS) h. Basing on the obtained results, we propose that the water-soluble cationic carbosilane dendrimers can be considered for delivery of HIV peptides to dendritic cells.

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1. Introduction

Dendritic cells (DC) loaded with viral peptides are a potential form of immunotherapy of human immunodeficiency virus type HIV-1 infection [1]. The application of DCs as a vaccine adjuvant represents a promising approach to improving deteriorated immune function in HIV-1-infected individuals [2]. Until now, a wide range of antigens has been used to stimulate DCs, but the principal complicating aspect of antigenic choice is the large degree of genetic diversity of HIV. In view of those different strategies were used to determine the best peptides to use into immunotherapeutic model. DCs are potentially great immunotherapy vector, but a choice of the way to load them with antigens is critical. So far, none of preceding approaches seem responding to all points for the development of a safe and easy-to-product immunotherapy [1].

Recently, synthetic molecules have been shown to be highly efficient to transduce multiple types of cells [3]. Different types of systems as carriers for drugs or biomacromolecules have been used including liposomes, nanoparticles, polimeric micelles, nanogels or dendrimers [4–11]. In this context, dendrimers emerge as an alternative approach for HIV peptide delivery to DCs. The cationic dendrimers can form complexes with biomolecules, and are widely studied as carriers for the transfection of cells with DNA and siRNA [12–16]. Cationic dendrimers interact efficiently with biomolecules, forming complexes by bonding to the surface groups [17–19]. Among the cationic dendrimers the carbosilane dendrimers (CBD) are of special interest [20–22]. CBD are branched structures made of either carbon—silicon bonds (CBD-CS) (Fig. 1, left panel), which are stable in water or oxygen—silicon bonds (CBD-OS) (Fig. 1, right panel), which are slowly hydrolyzed in aqueous solutions. These dendrimers can serve as carriers of siRNA providing its time-dependent release between 4 and 24 h [23], can form stable complexes with nucleic acids [24] and protect them from binding to proteins [25]. For drug administration into the bloodstream it is also important to protect DNA/RNA from sequestration and degradation [21–25]. Second generation of CBD show good toxicity profiles

Abbreviations: CBD, carbosilane dendrimers; CBD-CS, carbosilane dendrimers with carbon—silicon bonds; CBD-OS, carbosilane dendrimers with oxygen—silicon bonds; DC, dendritic cells; DLS, dynamic light scattering; HIV, human immunodeficiency virus; TEM, transmission electron microscopy.

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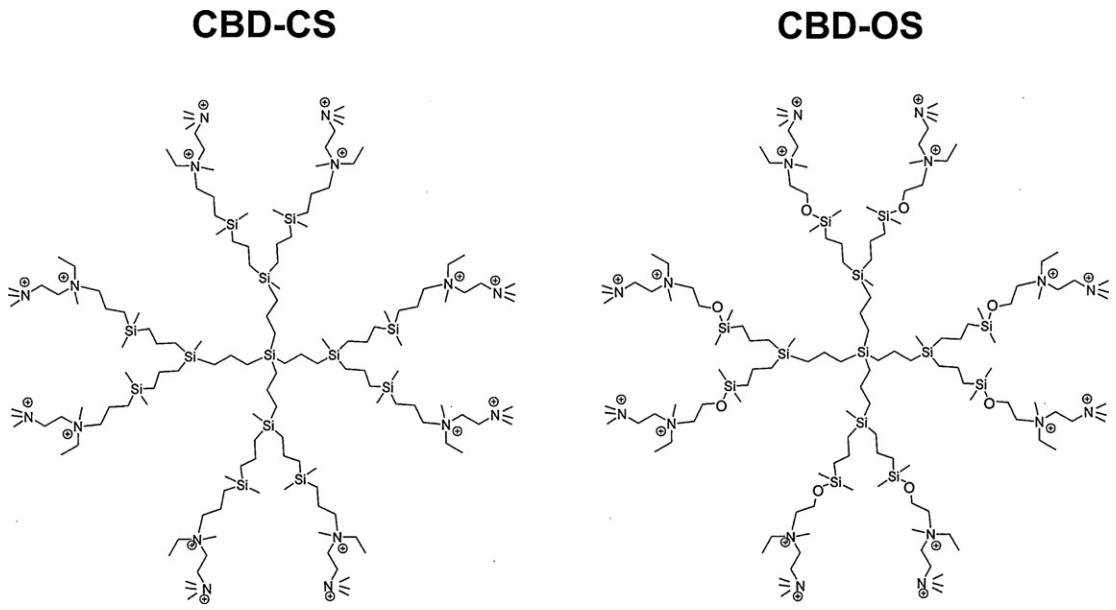

Fig. 1. Molecular structure of the carbosilane dendrimers 2nd generation. With Si—C bonds-CBD-CS (BDBR0011); with Si—O bonds-CBD-OS (NN16).

in primary cell cultures and erythrocytes up to concentrations of 5 μM [26]. Recently, we have reported the ability of carbosilane dendrimers to transport biomolecules as HIV-derived peptides into human monocites derived-DCs [27]. It was also shown that peptides can be protected by dendrimers from binding to proteins. In this work we evaluated a second generation of CBD-CS and CBD-OS as possible carriers for HIV derived peptides. The results of this study can be helpful in creating the new strategy of immunotherapy of HIV-1 infection using the DCs loaded with synthetic HIV derived peptides.

2. Experimental

2.1. HIV-derived peptides

Three different HIV-derived peptides unlabeled or fluorescein-labeled were synthesized in Eurogentec Company (Belgium). Peptide derived from envelope Gp160 sequence, HIV-HXB2 location Gp160 (634e648): NH-EIDNYTNTIYTLLEE-COOH, length 15 amino-acids, charged (−4); peptide derived from Gag-P24 sequence, HIV-HXB2 location P24 (71e80): NH-DTINEEAAEW-COOH, length 10 amino-acids, charged (−4) and peptide derived from Nef sequence, HIV-HXB2 location Nef (172–191): NHGMDDPEREVLEWRFDSRLAF-COOH, length 20 amino-acids, charged (−3). Since these peptides were only part of proteins, names of complete proteins were used to distinguish peptides used in experiments as Gp160, P24 and Nef, peptides.

2.2. Carbosilane dendrimers and reagents

We used the second generation of CBD with carbon—silicon bonds (CBD-CS), which are water-stable and that with oxygen—silicon bonds (CBD-OS), which are slowly hydrolyzed in aqueous solutions. CBDs were synthesized in the Departamento de Quimica Inorganica, Universidad de Alcalá, Spain as described elsewhere [23,26,28]. The structure of CBD-CS: $C_{128}H_{316}I_{16}N_{16}O_8Si_{13}{}^{+16}$ (Mw = 4603.56 g/mol) and CBD-OS: $C_{144}H_{348}I_{16}N_{16}Si_{13}{}^{+16}$ (Mw = 4 699.99 g/mol) are presented in Fig. 1, left and right panels, respectively. All other reagents used were of analytical grade and purchased from Sigma–Aldrich Chemical Company.

2.3. Formation of peptide–dendrimer complex

Complexes were formed in Na-phosphate buffer, pH 7.4, by addition of dendrimers into peptides solutions at a different (dendrimer/peptide) molar ratio. The mixture was vortexed and incubated for 10 min at room temperature (approx. 22 °C).

2.4. Peptide–dendrimer complexes time-stability

The stability and time of degradation of dendriplexes was measured using fluorescence polarization of fluorescein-labeled peptides incubated with dendrimers at a (dendrimer/peptide) molar ratio 3:1. Dendriplexes were prepared and the fluorescence polarization was measured as a function of time. The time-dependent destruction of a complexes leads to the decrease of fluorescence polarization degree of labeled peptides [29,30].

2.5. Fluorescence polarization

Samples were prepared by incubation of 0.5 μM peptide labeled with fluorescein with various concentrations of dendrimers in Na-phosphate buffer 10 mmol/L, pH 7.4 at a room temperature (approx. 22 °C), and the increase in fluorescence polarization was measured using a PerkinElmer LS-50B spectrofluorimeter (UK). Polarization was monitored at 521 nm (with a bandwidth of 3 nm) with excitation at 494 nm (with a bandwidth of 5 nm). Polarization is expressed as:

$$P = \frac{I_V - GI_H}{I_V + GI_H}$$

where I_V and I_H are the vertically and horizontally polarized emission intensities, respectively, when vertically polarized light is used to excite the sample. The G factor (measured experimentally) takes into account instrumental effects and can be expressed as:

$$G = \frac{I_{HV}}{I_{HH}}$$

where I_{HV} and I_{HH} are the vertically and horizontally polarized emission intensities, respectively, when horizontally polarized light is used to excite the sample [31]. The polarization of each

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