Colloids and Surfaces B: Biointerfaces 149 (2017) 196–205

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Factors affecting interactions between sulphonate-terminated dendrimers and proteins: A three case study

Estefanía González-García[a], Marek Maly[b], Francisco Javier de la Mata[c], Rafael Gómez[c], María Luisa Marina[a], María Concepción García[a,*]

[a] Departamento de Química Analítica, Química Física e Ingeniería Química, Universidad de Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares (Madrid), Spain
[b] Faculty of Science, J. E. Purkinje University, Usti nad Labem, Czechia
[c] Departamento de Química Orgánica y Química Inorgánica, Universidad de Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares (Madrid), Spain. CIBER-BBN, Spain

ARTICLE INFO

Article history:
Received 28 June 2016
Received in revised form 7 October 2016
Accepted 10 October 2016
Available online 11 October 2016

Keywords:
Sulphonate-terminated carbosilane dendrimers
Protein-dendrimer interaction
Fluorescence
Quenching
Computer modeling
Molecular dynamics

ABSTRACT

This work proposes a deep study on the interactions between sulphonate-terminated carbosilane dendrimers and proteins. Three different proteins with different molecular weights and isoelectric points were employed and different pHs, dendrimer concentrations and generations were tested. Variations in fluorescence intensity and emission wavelength were used as protein-dendrimer interaction probes. Interaction between dendrimers and proteins greatly depended on the protein itself and pH. Other important issues were the dendrimer concentration and generation. Protein-dendrimer interactions were favored under acidic working conditions when proteins were positively charged. Moreover, in general, high dendrimer generations promoted these interactions. Modeling of protein-dendrimer interactions allowed to understand the different behaviors observed for every protein.

1. Introduction

Dendrimers are synthetic macromolecules whose structure is constituted by layers, called generations, where functional groups or ligands with different biological activities can be introduced [1]. This structure lets differentiate four regions in dendrimers: the encapsulated core, which is surrounded by a singular microenvironment created by the flexible branches of the dendrimer; the own branches; the refuge formed by the cavities bordered by the branches; and the multivalent surface which delimits the macroscopic properties of the dendrimer [2]. This structure has allowed to use dendrimers for biomedical applications, both in the diagnosis and treatment of diseases as cancer, for genes and drugs delivery, molecular recognition, and, also, in the development of chemical sensors and enzymatic catalysis [1,2].

Interactions between proteins and poly(amidoamine) (PAMAM) dendrimers have been observed and explored [3–6]. Ottaviani *et al.* [3] studied the interaction of low and high-generations PAMAM dendrimers with selected amino acids (acidic, basic, neutral(zwitterionic)-polar, and low-polar) and proteins (acidic and basic isoelectric points). They observed that the binding of proteins and amino acids with dendrimers was predominantly promoted when both hydrophobic and electrostatic interactions were present. On the other hand, Nowacka *et al.* [4] studied the (end-group)-dependence of PAMAM dendrimers on the interaction with bovine insulin protein finding that the interaction was mainly electrostatic.

Carbosilane dendrimers present a more hydrophobic skeleton than further studied PAMAM dendrimers [7]. This skeleton, conformed by very strong Si-C bonds, makes them to have a high kinetic and thermodynamic stability [8]. Moreover, carbosilane dendrimers are more spherical than PAMAM and this fact make easier the synthesys of higher dendrimer generations without increasing the repulsion between end groups [9]. These characteristics make them very attractive for many applications involving the interaction with biomolecules. There are some works showing the interaction of proteins with carbosilane dendrimers. In 2007, Chonco *et al.* [10] studied the interaction between ammonium-terminated carbosilane dendrimers, phosphorothiate oligodeoxynucleotides (ODNs) and bovine serum albumin (BSA), observing that the dendrimer/ODN dendriplex stability depended on electrostatic interactions and that dendrimer protected ODN

* Corresponding author.
E-mail address: concepcion.garcia@uah.es (M.C. García).

http://dx.doi.org/10.1016/j.colsurfb.2016.10.020
0927-7765/

from BSA interaction. At the same time, Shcharbin et al. [11] studied the interaction between the same kind of dendrimers, short ODN and BSA, finding again that the dendriplex formation considerably decreased the interaction of ODNs with the protein. Later, Pedziwiatr *et al.* [12] studied the interaction between ammonium-terminated carbosilane dendrimers and BSA by fluorescence quenching. These publications confirmed the potential of this kind of dendrimers for drug delivery. Additionaly, Montealegre *et al.* [13] proposed the use of carboxylate-terminated carbosilane dendrimers as nanoadditive in electrokinetic chromatography (EKC) to improve protein separations. Moreover, high antiviral activity of carbosilane dendrimers was related to their capacity to strongly interact with some viral and/or cell proteins [14]. Nevertheless, no work has still characterized the interactions between sulphonate-terminated carbosilane dendrimers and different proteins. Protein-dendrimer interactions can be significantly different from one protein to another due to the heterogenic and complex nature of proteins that can present very different molecular weights, isoelectric points, structure, etc. For that reason, a comprehensive study of these interactions should involve the study and comparison of the interactions of dendrimers with different proteins and the characterization of these interactions. These studies are of great interest in order to find potential applications of carbosilane dendrimers, e.g. in protein sample preparation.

Protein sample preparation is a key step in the analysis of proteins from complex samples e.g. vegetable samples. Protein sample preparation mainly involves the extraction and purification/enrichment of proteins. Both steps are time-consuming and usually require the use of high amounts of solvents that make the process tedious, expensive, non-sustainable and not friendly with environment [15–17]. More suitable procedures are needed at this regard and new materials, as dendrimers, could be a key for the advancement in this area. In fact, dendrimers, in general, could play an important role since it has been demonstrated its interaction with proteins. These interactions could be the base for the designing of supports to specifically retain proteins from a matrix. This approach would be more sustainable and cheaper than usual methods.

The aim of this work was to explore the interactions between sulphonate-terminated carbosilane dendrimers and three different proteins and to identify the main factors affecting these interactions. Modeling of protein-dendrimer interactions with every protein at different pHs will enable to understand the singularities of interactions and the potential of this material in protein sample preparation.

2. Materials and methods

2.1. Chemicals and samples

All chemicals and reagents were of analytical grade. Water was daily obtained with a Milli-Q system from Milipore (Bedford, MA, USA). Tris(hydroxymethyl)aminomethane (Tris) and hydrochloric acid (HCl) were purchased from Merck (Darmstadt, Germany). Sodium hydroxide, trifluoroacetic acid (TFA), bovine serum albumin (BSA), lysozyme from chicken egg white and myoglobin from equine heart were acquired at Sigma-Aldrich (Saint Louis, MO, USA). Sulphonate-terminated carbosilane dendrimers were prepared according to a method described in literature [18]. Dendrimer structures from first to third generation are displayed in Fig. 1. Computer model for the second generation dendrimer is shown in Supporting information (Fig. S1).

2.2. Methods

All fluorescence measurements were performed in a spectrofluorometer RF-1501 (Shimadzu, Kioto, Japan). It was fixed a λ_{exc} of 279–281 nm and λ_{em} was measured from 290 to 400 nm. Dendrimer solutions at three different pHs (water (pH 6.5), 0.1% TFA (pH 1.8), and 5 mM Tris-HCl (pH 9.0)) were prepared. These solutions were added to three different standard proteins (BSA, lysozyme and myoglobin) at concentrations of 0.10, 0.35, and 0.30 μM, respectively. Blanks with the same concentration of dendrimer, in absence of protein, and at the three pHs were also measured and final fluorescence intensity of mixtures were obtained by subtracting blanks signals. Stern-Volmer equation was applied to analyze fluorescence quenching in proteins:

$$I_0/I = 1 + K_{SV}[Q]$$

where I_0 is the fluorescence intensity of proteins without dendrimer, I is the fluorescence intensity of proteins at the different dendrimer concentrations, K_{SV} is the Stern-Volmer constant and [Q] is the dendrimer concentration. K_{SV} corresponding to the interaction between proteins and dendrimers was presented when there was a linear relationship between I_0/I and the [Q].

All data were expressed as mean ± standard deviation of 4 measurements corresponding to two independent samples measured in duplicate.

2.3. Modeling of protein-dendrimer interactions

3D computer models of dendrimer structures were created using a dendrimer builder, as the implemented in the Materials Studio software package from BIOVIA (formerly Accelrys). GAFF force field (Generalized Amber Force Field) [19] was used for parameterization of dendrimers. AM1-BCC technique [20] was employed for calculation of dendrimer atoms partial charges. For this purpose, the Antechamber suite, part of the AMBER 14 software [21], was used. This tool was also employed for assigning force field atom types. QM calculations, needed for partial charges derivation, and calculation of missing force field parameters were done using GAMESS software [22]. Regarding proteins, the following experimentally determined (X-RAY difraction) protein structures were used: BSA (PDB: 4F5S) [23], lysozyme (PDB: 4RLM) [24], myoglobin (PDB: 3RGK) [25]. Furthermore, force field ff14SB was employed for simulations of all proteins.

On the other hand, the *pmemd.cuda* module from AMBER 14 package was employed for Molecular Dynamics simulations [26]. Moreover, the initial configurations of the protein/dendrimer complexes were created using UCSF Chimera software which was also used for final visualizations [27]. Please see Supporting information for more details.

3. Results and discussion

Interactions between sulphonate-terminated carbosilane dendrimers and proteins were monitored by measuring the intrinsic fluorescence intensity and emission wavelength of proteins. Fluorescence quenching studies are very powerful to explore the accessibility of fluorescence protein probes (mainly tryptophan (Trp) residues) to a quencher. Fluorescence measurements provide information on the molecular environment in the vicinity of fluorescence sites of the molecule [28]. Therefore, variations on fluorescence intensity and maximum emission wavelength could be due to a protein unfolding or to its binding to other molecules. In fact, changes on the fluorescence intensity (due to protein unfolding or binding to other molecules) are protein-dependent and could result in an increased or a decreased fluorescence intensity depend-