Colloids and Surfaces B: Biointerfaces 155 (2017) 11–16

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Binding of poly(amidoamine), carbosilane, phosphorus and hybrid dendrimers to thrombin—Constants and mechanisms

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ARTICLE INFO

Article history:
Received 24 February 2017
Received in revised form 28 March 2017
Accepted 29 March 2017
Available online 31 March 2017

Keywords:
Dendrimer
Thrombin
Interactions
Mechanisms

ABSTRACT

Thrombin is an essential part of the blood coagulation system; it is a serine protease that converts soluble fibrinogen into insoluble strands of fibrin, and catalyzes many other coagulation-related reactions. Absorption at its surface of small nanoparticles can completely change the biological properties of thrombin. We have analyzed the influence on thrombin of 3 different kinds of small nanoparticles: dendrimers (phosphorus-based, carbosilane based and polyamidoamine) and 2 hybrid systems containing carbosilane, viologen and phosphorus dendritic scaffolds in one single molecule, bearing different flexibility, size and surface charge. There was significant alteration in the rigidity of the rigid dendrimers in contrast to flexible dendrimers. These differences in their action are important in understanding interactions taking place at a bio-nanointerface.

1. Introduction

Dendrimers are a new class of nanotechnological polymers with well-defined molecular structures suitable for targeting, microarray systems, catalysis and drug delivery systems. These approaches are based on the injection of dendrimer-based drugs or agents into the blood stream [1–6]. Thrombin is an essential part of the blood coagulation system; it is a serine protease that converts soluble fibrinogen into insoluble strands of fibrin, as well as catalyzing many other coagulation-related reactions [7]. To date, some experiments have been made on the interaction between proteins and dendrimers that have indicated the role of the protein structure in these interactions [8–10]. Small nanoparticles can also form a 'nanoparticle corona' with proteins, with at least 3 different types of interactions seen depending on the nature of the protein. First, this corona has no effect on proteins with rigid structures and active sites buried deep within them. Second, it affects the structure of flexible proteins without changing their enzyme activity in cases where the active site is deeply buried. Third, it changes both structure and enzyme activity of flexible proteins that have surface-based active centers [8–10].

To explore the role of nanoparticle flexibility in the 'dendrimer-protein' interactions, we obtained circular dichroism spectra of thrombin in the presence of nanoparticles and their effect on thrombin fluorescence. Circular dichroism shows the secondary structure of proteins, whereas fluorescence quenching monitors changes in protein conformation after addition of nanoparticles [8–10].

2. Materials and methods

Human thrombin, cationic poly(amidoamine) dendrimers of 3rd and 4th generations (PAMAM-NH2 g3 and PAMAM-NH2 g4),

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http://dx.doi.org/10.1016/j.colsurfb.2017.03.053
0927-7765/

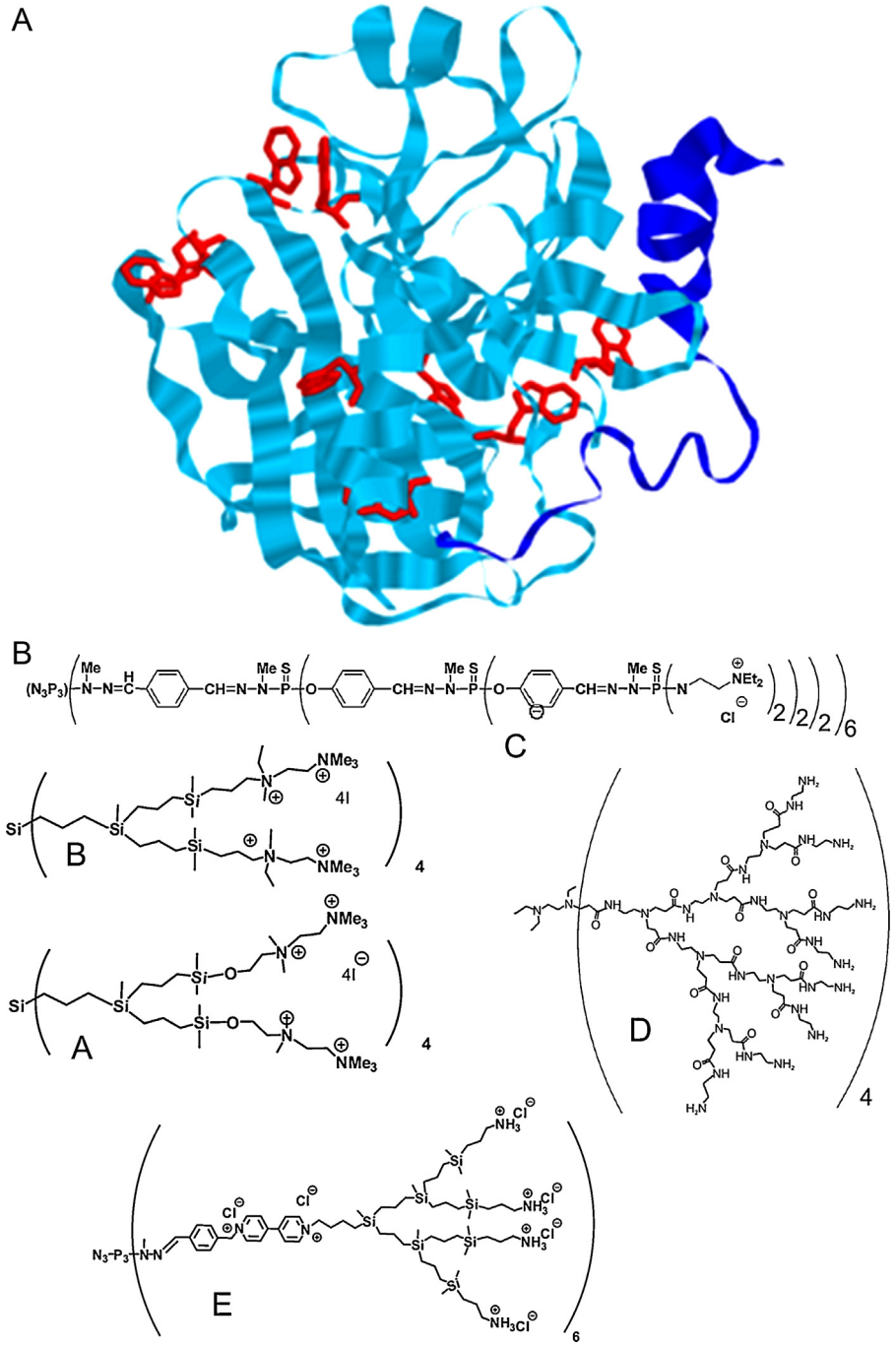

Fig. 1. Structure of α-thrombin from human blood (small subunit – 36 AA – is marked as dark blue, big subunit – 259 AA – is marked as blue, 9 tryptophanyls are marked as red). Dendrimers: A – CBD-OS (2G-NN16), B – CBD-CS, C – phosphorous CPD g3, D – PAMAM g3, E – hybrid SMT2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and sodium phosphate buffer were purchased from Sigma-Aldrich (USA). Other dendrimers had previously been synthesized by us and are described in detail in the following reerences [1–3,11–14]. The buffer was passed through a 0.22-μm filter to remove trace particles. Complexes for the experiments were prepared in PBS by mixing samples at different molar ratios for 15 min in a Vortex mixer.

2.1. Transmission electron microscopy

A copper grid of 200 grid mesh precoated with carbon was glow discharged. A sample in solution was dropped on to the grids, followed by absorbing the excess liquid with filter paper before they were air-dried under a heat lamp. They were stained with 2% uranyl acetate to enhance the contrast between the aggregates and the background before being examined in a Jeol JEM-1010 (Japan) electron microscope.

2.2. Circular dichroism

Measurements were made in 10 mmol/l sodium phosphate buffer (pH 7.4) at 25 °C. Thrombin at different concentrations was incubated with corresponding aliquots of dendrimers (stock solutions in 10 mmol/l sodium phosphate buffer, pH 7.4) for 10 min