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Nanoparticle corona for proteins: mechanisms of interaction between dendrimers and proteins

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ABSTRACT

Protein absorption at the surface of big nanoparticles and formation of 'protein corona' can completely change their biological properties. In contrast, we have studied the binding of small nanoparticles – dendrimers – to proteins and the formation of their 'nanoparticle corona'. Three different types of interactions were observed. (1) If proteins have rigid structure and active site buried deeply inside, the 'nanoparticle corona' is unaffected. (2) If proteins have a flexible structure and their active site is also buried deeply inside, the 'nanoparticle corona' affects protein structure, but not enzymatic activity. (3) The 'nanoparticle corona' changes both the structure and enzymatic activity of flexible proteins that have surface-based active centers. These differences are important in understanding interactions taking place at a bio-nanointerface.

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1. Introduction

The application of nanomaterials for use in human beings leads to interactions with biological systems and components of a body. Among them the nanoparticle-protein interactions are crucial in successful absorption, biodistribution, toxicity, efficiency of action and excretion of nanomaterials [1,2]. Proteins are absorbed at the surface of big nanoparticles due to electrostatic or van der Waal's forces, hydrophobic interactions or hydrogen bonds, forming a coating called a 'protein corona' [3,4]. This corona determines the biological properties of nanoparticles [1–5]. However, the nanoparticles have the different nature and have a range given by Critical Nanoscale Design Parameters that include size, shape, surface chemistry, flexibility, hydrophobicity, architecture and elemental composition [6]. Tomalia et al. [6,7] suggested dividing nanoparticles into 2 categories, the 'hard' and the 'soft'. 'Hard' nanoparticles include metal nanoparticles, fullerenes, carbon nanotubes and quantum dots. 'Soft' nanoparticles include liposomes, dendrimers, polymer micelles and polymeric nanocapsules [6,7]. These two classes will clearly have different interactions with proteins, based on significant differences in surface density determining their hydrophobicity and surface charge density [6,7]. The next important point is the size of nanoparticles [8,9]. On the one hand, toxic effects of quantum dots are determined by their size and surface charge [9], whilst on the other nanoparticles bearing the same charge, but having different size and shape, differ in their 'protein corona' [9]. We have estimated the interactions between 3 enzymes - alkaline phosphatase from *E. coli* (AP), lactate dehydrogenase from rabbit muscle (LDH) and human aspartate transaminase (AST) - and 2 'soft' nanoparticles of different size, cationic poly(amidoamine) dendrimers of 3rd and 4th generations (PAMAM g3 and PAMAM g4) with the size of 3 nm for PAMAM g3 and 4 nm for PAMAM g4 [6,7,10]). AP is a dimeric metalloenzyme, in which a 2-fold axis of symmetry relates two subunits that are 3.2 nm apart [11,12] with an ellipsoid shape of crystal structure of dimensions of 9.8 × 5.6 × 5.6 nm [PDB: pdb1aja.ent]. LDH comprises as five tetrameric isozymes composed of combinations of two different subunits [13]. The dimensions of rabbit muscle LDH

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(homo 4-mer) are 6.5 × 8.5 × 13.8 nm [18,19], [PDB: 3H3F.pdb]. AST is a dimeric protein, consisting of two identical subunits, each with ~400 amino acid residues with an Mr of ~45 kD, with an ellipsoid shape of 9.2 × 5 × 5 nm [14], [PDB: pdb1ARI.ent]. This means that in the case of these dendrimers, one can talk about a 'nanoparticle corona' on these proteins as opposed to a 'protein corona' on the dendrimers. For example, the cationic PAMAM g2, g4 and g6 form 'nanoparticle coronas' around serum albumins [15,16]. To explore the effect of 'nanoparticle corona' on structure, conformation and surface of these proteins, and to find relationships with their enzymatic activity has been our main aim.

2. Materials and methods

Alkaline phosphatase from *E.coli* (EC 3.1.3.1, AP), L-lactate dehydrogenase from rabbit muscle (EC 1.1.1.27, LDH), human aspartate transaminase (ERMAD457IFCC, AST), cationic poly(amidoamine) dendrimers of 3rd and 4th generations (PAMAM-NH2 g3 and PAMAM-NH2 g4), sodium phosphate buffer were purchased from Sigma–Aldrich (USA). 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. Circular dichroism

Measurements were made in 10 mmol/l sodium phosphate buffer (pH 7.4) at 25 °C. Proteins at different concentrations ([AST] = 0.2 μmol/l, [AP] = 0.9 μmol/l, [LDH] = 0.6 μmol/l) were incubated with corresponding aliquots of dendrimers (stock solutions in 10 mmol/l sodium phosphate buffer, pH 7.4) for 10 min at 25 °C. The CD spectra of proteins and their complexes with dendrimers were measured with a Jasco-815 spectropolarimeter (Jasco, Japan). Temperature dependencies of CD spectra of the proteins in the absence and presence of PAMAM g3 and g4 dendrimers at the molar ratio of 10:1 of dendrimer: protein were examined. Pure dendrimers under our conditions had no L-R absorption differences (i.e. were not chiral). All spectra were corrected using a baseline obtained with a dendrimer sample, and were smoothed using a binomial algorithm provided by Jasco. Scans were obtained from 320 to 200 nm at a rate of 50 nm min-1 with a bandwidth of 1 nm in quartz cuvettes, with a path-length of 0.2 cm.

2.2. Particle size and zeta potential

Samples intended for dynamic light scattering analyses were prepared in a 10 mmol/l sodium phosphate buffer (pH 7.4). Zeta potential experiments were carried out by phase analysis light-scattering using a Malvern Instruments Zeta-Sizer Nano S90 (Malvern, UK) at 25 °C. The electrophoretic mobility of the scattering samples was determined from the average of 6 cycles of an applied electric field in an electrophoretic disposable plastic cell. The zeta potential of complexes was determined from the electrophoretic mobility using the Smoluchowski approximation.

2.3. Fluorescence quenching

Proteins were dissolved in 10 mmol/l sodium phosphate buffer (pH 7.4) at 0.5 μmol/l. Increasing aliquots of a dendrimer were added to a protein from a stock solution in 10 mmol/l sodium phosphate buffer (pH 7.4). The fluorescence emission intensity (*F*) at λ = 350 nm was measured after excitation at 295 nm with a Perkin-Elmer LS-55 spectrofluorimeter (Perkin-Elmer, USA) at 25 °C. The emission slit width was kept at 5 nm and excitation was 5 nm. Fluorescence intensities were normalized as F/F_0, where F is the intensity of protein fluorescence in presence of a dendrimer and F_0 is the that of the pure protein. Under our conditions PAMAM g3 and g4 dendrimers had no fluorescence.

2.4. Transmission electron microscopy

Protein/dendrimer complexes were prepared at a dendrimer/protein molar ratio of 50:1 for AST, AP, LDH. The mixture was vortexed and incubated for 10 min at 25 °C. Ten micro liters of protein alone or dendrimer/protein mixture were placed on a 200 mesh copper grid with a carbon surface for 10 min and dried with filter paper. The sample was stained with 2% (m/v) uranyl acetate for 2 min and dried. Transmission electron microscopy images were taken with a JEOL–10 (Tokyo, Japan) transmission electron microscope.

2.5. Room temperature tryptophan phosphorescence

Phosphorescence measurements were made using a home-made system. The core of this system consisted of 800 MHz gated photon counters with 32 bit counter resolution and down to 250 ns time per channel (PMS-400A, Becker and Hickl GmbH, Germany). Emission was excited by UV xenon flash-lamp with 400 ns pulses of 7.8 μJ energy, light output stability of 1.9% p–p and repetition rate up to 100 Hz. Intensity of light decreases 1000 times after 30 μs (L9455-01, Hamamatsu, Japan). There were 2 modes of wavelength selection by monochromator or interference filters. Due to the low energy passing through the sample, acquisition was stopped at 100,000 sweeps. The system was calibrated with an aqueous solution of $TbCl_3$. The lifetime was 426.5 μs which agreed with published data (427 μs). The decay in room temperature protein phosphorescence was measured after excitation at 295 nm (tryptophan phosphorescence) at emission at 350 nm. O_2 was removed by applying a moderate vacuum and an inlet of ultrapure nitrogen. Purified nitrogen (0.1 ppm of O_2) was further purified by passing through an oxygen-trapping filter. This degassing procedure was accompanied by adding a 0.3 ml sample of 0.1 mol/l Na_2SO_3 as an O_2 scavenger (the final concentration of Na_2SO_3 in the sample was 0.01 mol/l) [20,33]. The sample was placed in a quartz cuvette, which was connected to the N_2/vacuum line by tubing. Five cycles of deoxygenating were performed. After deoxygenating, the cuvette was moved into the phosphorimeter, still attached to the tubing and was allowed to equilibrate to 25 °C before measurements. The background curve was first determined during measurement of a sample phosphorescence decay before its deoxygenating, which was subtracted from the phosphorescence decay of a sample after deoxygenating. All phosphorescence decays after subtraction of the background were analyzed in terms of a sum of exponential components by a non-linear least squares fitting algorithm, using the software provided by Edinburgh Analytical Instrumentation (Edinburgh Instruments, UK). τ average was calculated as $\tau = \alpha_1\tau_1 + \alpha_2\tau_2 + \alpha_3\tau_3$, α_i and τ_i - corresponding contribution and lifetime of RTTP decay [18–22].

2.6. Statistical analysis

The data are expressed as mean ± S.D. of 6 independent experiments. Significance of the data was assessed using the one-way analysis of variance with the post-hoc Newman-Keuls multiple comparisons test.

3. Results

Transmission electron microscopy of dendrimer/protein complexes for PAMAM g4 and the enzymes is given at Fig. 1. The presence of a dendrimer significantly changed their conformation, indicating formation of complexes. Unfortunately, drying samples

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