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A nano-bio interfacial protein corona on silica nanoparticle

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ABSTRACT

Nano-bio interaction takes the crucial role in bio-application of nanoparticles. The systematic mapping of interfacial proteins remains the big challenge as low level of proteins within interface regions and lack of appropriate technology. Here, a facile proteomic strategy was developed to characterize the interfacial protein corona (noted as IPC) that has strong interactions with silica nanoparticle, via the combination of the vigorous elution with high concentration sodium dodecyl sulfate (SDS) and the pre-isolation of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The trace level IPCs for silica nanoparticle were thus qualitatively and quantitatively identified. Bioinformatics analyses revealed the intrinsic compositions, relevance and potential regularity addressing the strong interactions between IPC and nanoparticle. This strategy in determining IPCs is opening an avenue to give a deep insight to understand the interaction between proteins and not only nanoparticles but also other bulk materials.
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1. Introduction

Nanoparticles are widely applied in biological processes including drug delivery, diagnosis, imaging etc. [1–6] The widespread applications of nanoparticles have increased the exposure opportunity of human body to nanoparticles. Once nanoparticles enter into body fluidic systems, biomolecules will interact with nanoparticle to form the bio-corona such as the protein corona (PC) on nanoparticles [7]. The PC could shield and/or alter the inherent surface properties of nanoparticles, and thus confer nanoparticles the new identities which are responsible for subsequent biological behaviors such as cellular uptake and immune responses etc. [8,9] Also, the different functionalization of nanoparticles with ligands could cause protein corona variation which could cause the consequent changed biological behaviors of nanoparticles such as active targeting, blood circulation time and organ distribution etc. [10,11] The nano-bio interactions comprise complex kinetic and thermodynamic exchanges among proteins and nanoparticles in a biological environment. To date, a variety of techniques have been applied to resolve the nano-bio interactions, including isothermal titration calorimetry (ITC), circular dichroism (CD), fluorescence correlation spectroscopy, and computer simulations etc. [12,13] Indeed, the interactions between nanoparticles and some common serum/plasma proteins have been widely investigated to uncover the interfacial binding behaviors between corona and nanoparticles [14,15]. The synchrotron radiation-based techniques revealed that at least 12 Au-S bonds formed on the interface for bovine serum albumin adsorption on CTAB-coated gold nanorods [16]. And two binding modes were observed for the lysozyme adsorbing on nano-silica surface during protein-nanoparticle complexation by ITC analysis [17]. These techniques definitely enhanced the understanding on nano-bio interaction including protein conformation, affinity, adsorption thermodynamic properties and attraction forces etc. at the interface, but mainly limited to individual proteins.

To probe the complex nano-bio interactions at a molecular and "omic" level, proteomic techniques are becoming a powerful tool with great ability in providing qualitative and quantitative information for proteins involved in nano-bio interaction [18,19]. Thus, the specific corona effector that might influence nanoparticle interaction with cell could be spotted and further validated [15,20]. A comparative proteomic method could present a significant variability in protein fingerprints, and map a correlation between protein identities and the variables [21,22]. Up to date, proteomic studies mainly focused on the global identification of proteins such as soft/hard protein corona [23,24] on nanomaterials, the interfacial proteins between the corona phases and nanoparticles that could directly represent the interaction of a nanoparticle towards

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proteins are limited and not yet reported for complex biological environment.

In this work, a simple proteomic approach was developed to systematically characterize the interfacial proteins that retained on silica nanoparticle strongly due to the higher affinity with silica, by coupling a vigorous elution and a subsequent sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) pre-separation of the retained interfacial proteins for nanoliquid chromatography tandem mass spectrometry (nanoLC–MS/MS) analysis. A class of low level interfacial proteins, referred to as "interfacial protein corona" (IPC), which has been long ignored in protein corona studies due to the difficulty in protein recovery from nanoparticles was well identified by this approach. Bioinformatics analyses of the IPC were fully performed to explore the intrinsic compositions, relevance and potential regularity to address the strong interactions of IPC proteins with silica nanoparticle. The formation of IPC regarding the physicochemical properties such as the particle size and curvature of silica nanoparticles as well as the concentrations of serum samples was thus explored. The facile approach for the IPC is promising to provide new insights into the understanding of the protein corona formation taking place at the bio-nanoparticle interface.

2. Materials and methods

2.1. Synthesis and characterization of silica nanoparticles

Silica nanoparticles with sizes of 60 nm (denoted as SiO_2_60) were synthesized via a modified St$_{ö}$ber process [25]. A well-mixed solution of 40 ml anhydrous ethanol and 1.25 ml ammonia solution (25%) was first prepared and stirred at 40 °C for 10 min. Then 0.5 ml tetraethyl orthosilicate was added dropwise. After that, 2.1 ml deionized water was added, and the solution was stirred for another 12 h. The resulting nanoparticles were collected by centrifuging the solution, washed with ethanol for several times and dried under vacuum at 60 °C overnight. Silica nanoparticles with sizes of 100 nm, 380 nm and 850 nm (denoted as SiO_2_100, SiO_2_380 and SiO_2_850, respectively) were kindly presented by others.

Transmission electron microscopy (TEM) measurements were carried out on a JEM-2000 EX (JEOL) microscope operated at 120 kV. Dynamic light scattering (DLS) and zeta potential measurements were made on a Zetasizer Nano Series (Malvern). The nitrogen sorption/desorption isotherms were measured at 77 K using an Quadrasorb SI adsorptometer (Quantachrome Instruments).

2.2. The formation of hard protein corona

Prior to serum exposure, silica nanoparticles were first suspended homogenously in phosphate-buffered saline (PBS) at a concentration of 1 mg ml^{-1}. Subsequently, an aliquot containing 2 mg of nanoparticles was incubated with 10% bovine serum (BS) at 37 °C for 1 h with shaking. After that, the nanoparticle-protein complex was isolated via centrifugation and washed 3 times with PBS to ensure that the loosely-bound proteins were removed from the solution.

2.3. Interfacial protein corona by vigorous eluent treatments

The obtained hard protein corona (HPC) was further treated by several eluents to obtain interfacial protein corona (IPC). In particular, the nanoparticle-HPC complex was incubated with various eluting solutions, including 2%, 5%, 8%, 10% of SDS (w/v), 80% ACN/0.1% TFA (v/v) and 1 M of KCl, at room temperature for 0.5 h with shaking, after which the nanoparticle-IPC complex was pelleted via centrifugation. The desorption process was repeated for twice. Combinations of 5% SDS and either 80% ACN/0.1% TFA or 1 M of KCl were also used for sequential elution of the nanoparticle-HPC complex to obtain the corresponding IPC.

2.4. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining

Then the HPC and IPC were further visualized and analyzed by SDS-PAGE. Particularly, the pellets were resuspended in PBS or NH_4HCO_3 solution and an equal volume sample was mixed with SDS-PAGE loading buffer, after which the mixture was treated at 100 °C for 6 min to denature the proteins thoroughly. After centrifugation, the supernatant was loaded in 12% polyacrylamide gel and separated by SDS-PAGE. The protein patterns of HPC and IPC were visualized after Coomassie blue staining and subsequent destaining process. For IPC, silver staining was further performed to visualize the minute trace of proteins. Silver staining was operated as instructions without changes. The protein bands of interest were cut and performed in-gel digestion as previously described by Andrej et al. [26]. The gel densitometry was performed using ImageJ (1.48 version).

2.5. On-beads digestion of HPC

The nanoparticle-HPC complexe was resuspended in a solution containing 8 M urea and 100 mM NH_4HCO_3 to denature the proteins, then reduced and alkylated with DTT (20 mM, 1 h at 56 °C) and IAA (40 mM, 30 min in the dark at room temperature), respectively. Next, the solution was diluted with 100 mM NH_4HCO_3 to 1.6 M urea, and the proteins were digested with trypsin (4 ug) at 37 °C overnight with shaking. Finally, the digestion solution was adjusted to pH 2–3 with a 10% TFA solution and desalted with C18 SPE columns. The samples were stored at −20 °C until RPLC–MS/MS analysis.

2.6. RPLC–MS/MS analysis

The LC–MS/MS analyses were performed on a LTQ-Orbitrap Elite mass spectrometer (Thermo Scientific, USA) equipped with a Dionex UltiMate 3000 RSLCnano system (Thermo Scientific, USA) for separation. The lyophilized samples were re-dissolved in 0.1% TFA and an equal sample amount was taken for each analysis. The injected digest was enriched on a C18 trap column (5 cm × 200 um i.d.) and the peptides were separated on a reverse phase (RP) C18 analytical column (15 cm × 100 um i.d.) packed with C18 AQ beads (5 um, 120 Å pore size, Sunchrom). The flow rate was set at ∼400 nl min^{-1}. The binary separation gradient was employed as followed: from 5% to 28% buffer B (80% ACN/0.1% FA) for 60 min, from 28% to 45% buffer B for 15 min, from 45% to 95% buffer B for 1 min.

The mass spectrometry was operated in positive ion data dependent (DDA) mode. The scan range for full MS was set from m/z 200–1600. CID fragmentation was performed for twenty of the most abundant precursors with the following parameters: minimum intensity 500, isolation width 2, normalized collision energy 35, dynamic exclusion was enabled (repeat count 1, repeat duration 30, exclusion duration 90).

2.7. Database searching

All the MS/MS raw files were first converted to MGF files using MSconvert and then submitted to an in-house Mascot server (version 2.3.0) against a UniProt bovine database (24148 sequences). Search parameters specified: enzyme, trypsin; peptide mass tolerance, 20 ppm; fragment mass tolerance, 0.8 Da; fixed modification, carbamidomethyl (C); variable modification, oxidation (M); all peptide identifications were filtered to only accept expect value of 0.05

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