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Dendrimer and Dendrimer-Conjugate Protein Complexes and Protein Coronas

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Abstract

Dendrimers and dendrimer conjugates are widely employed for biological applications such as bio-imaging and drug delivery. Understanding the interaction between dendrimers and their biological environment is key to evaluating the efficacy and safety of these materials. Proteins can form an adsorbed layer, termed a “protein corona”, on dendrimers in either a non-specific or specific fashion. A tight-binding, non-exchangeable corona is defined as a “hard” corona whereas a loosely bound, highly exchangeable corona is called a “soft” corona. Recent research indicates that small molecules conjugated to the polymer surface can induce protein structural change leading to tighter protein-dendrimer binding and further protein aggregation. This “triggered” corona formation on dendrimer and dendrimer conjugates is reviewed and discussed along with the existing hard/soft corona model. This review describes the triggered corona model in order to further the understanding of protein corona formation.

Keywords: dendrimer, PAMAM, protein, corona, aggregation, conjugate

Dendrimers and dendrimer conjugates are widely used in biomedical research in drug delivery, bio-imaging, and transport scaffolds.¹⁻³ The multiple functional group containing arms on the dendrimer surface provide a multivalent system that can be readily modified. The functional groups can be conjugated to a drug, targeting agent, and/or imaging dye, or they can be left unconjugated. The unconjugated arms still contribute to surface properties such as hydrophobicity, charge density, and potential for hydrogen bonding.⁴⁻⁸ Proteins can interact with the dendrimer surface in a specific fashion with conjugated ligands or in a non-specific fashion, or in a combination of both.⁹⁻¹² Proteins that are adsorbed onto the dendrimer surface form a colloidal layer called a "protein corona".¹⁴ This adsorption avidity is governed by the binding affinity between the protein and the dendrimer, either forming a loosely bound, rapidly exchanging "soft" corona or a tightly bound slow exchanging "hard" corona.^{15, 16} Proteins can also form a complex with the dendrimer conjugates in a more specific fashion, which is usually accompanied by protein structural change. This complex formation can also lead to a subsequent protein corona formation – defined as the "triggered" corona in this review (Fig. 1).¹⁰ This triggered corona formation involves two steps: tight protein binding and conformational change in the first step and subsequent apo-protein corona formation. Binding to the conjugated ligand can be quite tight and dissociation constants for the holoprotein as low as 2 nM have been reported.¹⁰ The triggered apoprotein corona can either be "soft" or "hard" since that is administered by the avidity between the apo- and holoproteins. Assessment of the formation of protein complex and corona upon interaction with dendrimer allows a better understanding of the dendrimer's biodistribution and ultimate biological fate.

1. Class of Dendrimers Used for Biological Applications

Since the first development of dendrimers in the 1980s,¹⁷⁻¹⁹ dendrimer and dendrimer conjugates have been widely applied in biological studies. Dendrimers have dramatically different surface properties depending on their structure and terminal group modifications, and this has substantial impact on the interaction with biomolecules such as nucleic acids, lipids, and proteins (Table 1). Dendrimers can be terminated by different functional groups to give either a positively-charged (amine), negatively charged (carboxyl), or a charge neutral surface (hydroxyl, acetamide) at physiological pH.²⁰⁻²² The terminal functional groups on the dendrimer surface facilitate direct conjugation to drugs, imaging agents, targeting agents, and to other nanomaterials such as polymers, iron oxide particles, and gold nanoparticles.²³ Dendrimers can be conjugated to one type of molecule or two several types of molecules leading to multi-functional materials.²⁴ After dendrimer conjugation, often with hydrophobic ligands, the change in surface properties alters protein interactions and this can dramatically impact protein complexation and corona formation. Understanding these changes is important for rational design of dendrimers to control biological half-life, uptake efficiency and transportation mechanism.

2. Protein Corona and Complex Formation on dendrimer and dendrimer conjugates and their implications

When dendrimers are administered intravenously, serum proteins are most likely the first molecules encountered in the biological environment. The proteins can form a corona on the dendrimer surface that is soft, hard, and/or triggered (Fig. 1).¹⁴ Serum proteins, such as albumin and globulin, can non-specifically adsorb onto the dendrimer surface.^{43, 44} Depending on the

exchange rate of the adsorbed protein, the corona can either be soft or hard^{16, 45, 46} For nanoparticles conjugated to a ligand, the serum binding proteins may experience a triggered conformational change and bind to the dendrimer surface more specifically and tightly.⁴⁷⁻⁵⁰

The soft/hard corona formation on dendrimer surface follows a passive interaction that includes non-specific van der Waals forces including electrostatic, hydrophobic, and hydrophilic interactions.⁴³ On the other hand, assembly of dendrimers or the disassembly of the already aggregated dendrimers also occurs upon the formation of protein coronas.⁵¹ Cationic dendrimers (e.g. PAMAM, PPI) are reported to have strong influence on aggregation state of peptides.⁵² The strength of the protein-peptide interaction is associated with the surface charge density of the dendrimer. It is reported that G6 and G7 PAMAM dendrimers showed most complement protein binding using gel electrophoresis.¹⁴ There are also efforts to reduce undesired corona formation. Acetylation and PEGylation of the dendrimer are common strategies to provide a “stealth” dendrimer surface.^{53, 54} Low degrees of PEGylation can increase dendrimers biocompatibility and reduce the immune response to the dendrimers.⁵³ However, non-specific protein corona formation does not necessarily lead to negative effects. For example, dendrimer functionalization on a gold surface can improve the protein-DNA interaction sensitivity in surface plasmon resonance imaging (SPRi).⁵⁵ In this case, the dendrimer coated surface significantly improved the specificity and sensitivity of SPRi by allowing discrimination between protein and DNA interactions.

Dendrimer and dendrimer conjugates can also trigger protein coronas in a more specific and active manner. After ligand modification on surface, the modified dendrimer can achieve desired bio-trafficking, bio-sensitivity, immune response, biocompatibility, and biological half-life.⁵⁶ Glycodendrimer decorated by sugar molecules on the arms bind to lectin, which significantly increases the bio-recognition and trafficking of the dendrimer to the target cells.⁵⁷

Ligand-conjugated dendrimers may also afford higher binding avidity towards the target proteins. For example, PAMAM dendrimers have been modified as a targeted drug delivery platform for anti-cancer drug methotrexate.⁵⁸ The primary amines on PAMAM surface were first partially acetylated then conjugated with folic acid as a targeting agent, methotrexate, and a dye. The multi-functional dendrimer was subsequently intravenously administered into mice. The conjugated methotrexate demonstrated significantly lower toxicity and higher efficacy compared to free methotrexate. Methotrexate and folic acid conjugated PAMAM dendrimer trigger the folic acid receptor corona in a two-step process with fused specific/non-specific mechanisms: first the ligand binds to the target protein leading to a conformational change; the holoprotein formed in the first step gives a favorable surface for interaction with free apoprotein leading to further aggregation onto the dendrimer-protein complex.^{10, 59} Dendrimers can also be used tandemly with other nanoparticles to optimize their biological response. Antibody conjugated dendrimer-encapsulated gold nanoparticles can afford enhanced sensitivity for protein detection due to protein amplifications on dendrimer surface.⁶⁰ The increased sensitivity utilized the high density of reactive arms on the dendrimer surface, leading to higher local concentration of antibody and subsequently target proteins.

3. Conclusions

The protein interactions of different dendrimer materials that are commonly used in biological applications are reviewed. Recent research has deepened the understanding of hard and soft protein coronas and also revealed a new type of “triggered” protein corona. The triggered corona formation is composed of three parts including a specific ligand binding, a protein conformational change, and the triggered corona formation on nanomaterial surface. The

addition of triggered corona formation^{10, 61} to the well-established soft/hard corona model⁶² gives a fuller scope to the types of dendrimer-protein interactions. This additional mechanism of protein corona formation allows a fuller understanding of dendrimer conjugates in biological environments.

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Table 1 Structures of some dendrimers commonly used in biological study

Types of Dendrimer	Generation 2 Structure	Applications
PAMAM poly(amidoamine)		Drug delivery ²⁵⁻²⁷ , Bioimaging ²⁸⁻³⁰ , Gene delivery ³¹⁻³³
Polyester	bis-MPA-COOH	Drug delivery ^{34, 35} , Bioimaging ³⁶
PPI poly(propyleneimine)		Drug delivery ³⁷ , Bioimaging ³⁸ , Gene delivery ³⁹
poly(L-lysine)		Drug delivery ⁴⁰ , Bioimaging ⁴¹ , Gene delivery ⁴²

Figure 1. Three types of protein corona formation on dendrimer surfaces are illustrated. The non-specific soft/hard corona formation and the triggered corona formation. The triggered protein corona model is a two-step process consisting of initial binding to a conjugated ligand followed by conformational change of the resulting holoprotein binding. In the second step, the structural change of the holoprotein increases binding to the remaining apoprotein present in solution.¹³

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Figure 1