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Human Plasma Protein Corona of A β Amyloid and Its Impact on IAPP Cross-Seeding

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Abstract

Alzheimer's disease (AD) is the most severe form of neurological disorder, characterized by the presence of extracellular amyloid- β (A β) plaques and intracellular tau tangles. For decades, therapeutic strategies against the pathological symptoms of AD have often relied on the delivery of monoclonal antibodies to target specifically A β amyloid or oligomers, largely to no avail. A β can be traced in the brain as well as in cerebrospinal fluid and the circulation, giving rise to abundant opportunities to interact with their environmental proteins. Using liquid chromatography tandem-mass spectrometry, here we identified for the first time the protein coronae of the two major amyloid forms of A β – A β _{1–42} and A β _{1–40} – exposed to human blood plasma. Out of the proteins identified in all groups, 58 proteins were unique to the A β _{1–42} samples and 31 proteins unique to the A β _{1–40} samples. Both fibrillar coronae consisted of proteins significant in complement activation, inflammation and protein metabolic pathways involved in the pathology of AD. Structure-wise, the coronal proteins often possessed multi domains of high flexibility to maximize their association with the amyloid fibrils. The protein corona hindered recognition of A β _{1–42} fibrils by their structurally specific antibodies, and accelerated the aggregation but not β -cell toxicity of human islet amyloid polypeptide (IAPP), the peptide associated with type 2 diabetes. This study highlights the importance of understanding the structural, functional and pathological

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Conflict of Interest

The authors declare no conflict of interest.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Unique proteins in the A β fibrillar coronae (Table S1). ThT kinetic assay of A β (Fig. S1). TEM imaging of A β coronae (Fig. S2).

Most abundant coronal proteins vs. molecular weight (Fig. S3). Physicochemical properties of unique proteins in A β coronae (Figs. S4&S5). Structures of top-10 abundant coronal proteins (Fig. S6).

implications of the amyloid protein corona for the development of therapeutics against AD and a range of amyloid diseases.

Graphical Abstract

The protein corona impedes the recognition of antibody for A β fibrils, and promotes IAPP fibrillization but not toxicity through cross seeding.

Keywords

A β ; amyloid; protein corona; Alzheimer's disease; cross seeding

1. INTRODUCTION

Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative disorder clinically characterized by memory loss and cognitive decline in the elderly.¹ The extracellular amyloid- β (A β) plaque formation in hippocampus and neocortex is considered a pathogenic hallmark of the disease.² The amyloid hypothesis explains the progression of AD with an increase in the level of A β peptides due to their imbalance of production and clearance, which consequently induces A β plaque formation accompanied by inflammation and neurodegeneration.^{3, 4}

A β is formed due to the proteolytic cleavage of amyloid precursor protein (APP) expressed in the brain as well as in peripheral organs and tissues.⁵ Among the A β isoforms, A β_{1-40} is the most abundant (~80–90%), followed by A β_{1-42} (~5–10%).⁶ A β_{1-42} tends to aggregate much faster and is more neurotoxic than A β_{1-40} , due to their differences in structure and hydrophobicity.⁷ The fibrillization of A β is a nucleation-dependent process, consisting of the production of toxic oligomeric intermediates and protofibrils as well as their elongated polymorphic amyloid fibrils. The amyloid fibrils possess a distinct cross- β backbone, while displaying semiflexible filamentous structures a few nanometers in diameter and hundreds of nanometers to micrometers in length.^{8, 9}

It is known in the field of nanomedicine that, when introduced into a biological milieu, the surface of a nanoparticle spontaneously acquires a “protein corona”.¹⁰ The binding of proteins to nanoparticles is mediated by electrostatic and hydrophobic interactions as well as hydrogen bonding.^{11, 12} The protein corona dictates the biocompatibility, cellular uptake and clearance of the nanoparticles in biological systems.^{13–17} The protein amyloid fibrils are essentially nanostructures of amphiphilicity,¹⁸ which can also acquire a protein corona *in vivo* to elicit different biological responses such as inflammation and metabolic signaling.¹⁹ Fibrillar human islet amyloid polypeptide (IAPP), for example, collected a corona of unique proteins, which are key elements of the cellular machinery, including the proteasomal system, membrane adhesion factors and signal transduction pathways.²⁰ Indeed, A β peptides have shown association with other amyloid proteins, membrane proteins and human blood proteins such as serum albumin, apolipoproteins and serum amyloid P,^{21, 22} with serum albumin and metal ions minimizing the aggregation propensity of amyloid peptides.^{23, 24} Furthermore, amyloid plaques in the brain are known to be associated with proteins involved in cell adhesion and inflammatory responses.²⁵

While A β plaques are usually identified in the AD brain post mortem, the peptide itself has been found in cerebrospinal fluid and the circulation.^{5, 26} Recent clinical evidence suggests A β _{1–42} can cross-seed human islet amyloid polypeptide (IAPP), a 37-residue peptide hormone implicated in β -cell destruction in type 2 diabetes (T2D), pointing to a link between AD and T2D in accelerating both pathologies.^{27–29} Indeed, A β _{1–42} and IAPP show 50% similarity in structure and have been found to co-localize in amyloid plaques, blood vessels and cerebrospinal fluid.³⁰ It has also been shown that A β _{1–42} efficiently cross seeded IAPP monomers *in vitro*, utilizing the association of their highly similar U-shaped β sheets.³¹

In the present study, A β _{1–42} and A β _{1–40} fibrils were incubated with human plasma proteins to render amyloid protein-coronae, which were then isolated through centrifugal spin capture and the coronal proteins were identified using liquid chromatography tandem-mass spectrometry (LC-MS/MS). The pathogenic associations and structural characteristics of the coronal proteins were examined by protein network analysis and molecular docking. Towards understanding the impact of the protein corona, an immunohistochemical assay confirmed the compromised recognition of A β _{1–42} fibrils by their structurally specific antibodies, while the effect of the A β _{1–42} protein corona on IAPP amyloidosis was assessed by a thioflavin T (ThT) kinetics assay, transmission electron microscopy (TEM) and a cell viability assay (Scheme). These findings pointed to significant roles of the protein corona in interfering with structure-based anti-amyloidosis strategies and in the cross-seeding of amyloid proteins associated with different pathologies.

2. MATERIALS AND METHODS

Human amyloid beta fibril formation

Lyophilized human amyloid beta A β _{1–42} (42 residues, DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA, MW=4514.1 Da; purity: 95% by HPLC) and A β _{1–40} (40 residues, DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV, MW=4329.9 Da; purity:

95% by HPLC) as well as human islet amyloid polypeptide IAPP (37 residues, *KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY*, MW=3904.5 Da; purity: 95% by HPLC) were purchased from AnaSpec. 1.0% NH₄OH was used for initial solubilization of the peptide monomers, which were subsequently diluted in MilliQ water (pH 6.8). Monomeric concentrations were confirmed by a nanophotometer (NanoDrop), with stock solutions kept at 200 μ M and all further dilutions done with MilliQ water unless otherwise specified. Complete conversion of monomers and intermediate aggregation species to mature amyloid fibrils was ensured through fibrillization for >1 week at 37 °C. For protein corona preparation, stocks were diluted using 1 $\times$ phosphate-buffered saline (PBS).

Collection and processing of human plasma

Fresh human blood was collected in sodium heparin vacuettes (Greiner Bio-One) from a healthy volunteer, after obtaining informed consent in accordance with the University of Melbourne Human Ethics Approval 1443420 and the Australian National Health and Medical Research Council Statement on Ethical Conduct in Human Research. Plasma samples were prepared by centrifugation of the blood (900 g, 15 min, without brake) and collection of the top fraction. Centrifugation was repeated twice to ensure the removal of any remaining cells, and the clear plasma was transferred to fresh vials and stored at –80 °C prior to experimental use.

ThT kinetic assay

The fluorescent dye Thioflavin T (ThT) was utilized as a probe to monitor amyloid fibril formation. Upon binding to the surface grooves of amyloid fibrils, ThT emits strong fluorescence at 482 nm. The ThT kinetics assay was conducted with 50 μ M monomeric A β solution combined with 50 μ M ThT dye in a 96-well plate (black/clear bottom, Costar), and ThT fluorescence was recorded every 12 h at 37 °C over 10 days in order for the aggregation saturation phase to be reached. For cross-seeding experiments, 50 μ M of monomeric IAPP was mixed with 5% A β ₄₂ seeds pre-incubated with human plasma, in addition to 50 μ M ThT dye. Measurements were carried out using a PerkinElmer EnSight HH33400 plate reader (excitation/emission: 440/485 nm) with Kaleido 1.2 software utilized for data storage. Three key kinetics parameters, i.e., the lag time, fibrillization rate constant (*k*), and time to reach 50% fibrillization (*t*_{1/2}) were calculated from the sigmodal fibrillization curve.³² The assay was performed in triplicate and average spectra of the measurements were presented, with error represented through standard deviation of mean.

Transmission electron microscopy

Carbon-coated copper grids (400 mesh, formvar film, ProSciTech) were glow-discharged for 15 s prior to deposition of samples (5 μ L). After 70 s of absorption, excess sample was drawn off using filter paper and grids washed 1 $\times$ with 10 μ L MilliQ water. For negative staining, grids were exposed to 5 μ L of 1% uranyl acetate (UA) for 30 s, with excess stain drawn off as previously and grids allowed to air-dry. The samples were imaged on a Tecnai G2 F20 transmission electron microscope (FEI, 200 kV) with UltraScan 1000 P 2k CCD camera (Gatan) and Gatan Digital Micrograph 3.9.5 software utilized for the acquisition and processing of images. ImageJ (FIJI) was used to extract features such as fibril length and thickness.

Corona formation and isolation

Human plasma, pre-filtered through a 0.45 μm filter, was mixed with a 300 μL aliquot of A β fibrils at a mass ratio of 1:5 and incubated for 2 h at 37 °C with shaking. 1 $\times$ PBS buffer was added to Vivaspins 2 (1,000 kDa) spin columns followed by centrifugation at 6,000 g for 10 min for initial equilibration. Then, the preincubated fibril samples were thoroughly mixed and loaded into the equilibrated spin columns and centrifuged at 6,000 g for 10 min. Supernatants were collected and made up to 300 μL with fresh 1 $\times$ PBS buffer, mixed well and spun down as previously described. The rinse step was repeated twice to remove unbound proteins, with the amyloid-protein corona complex retained in the upper portion of the filtration unit.

Proteolytic digestion of amyloid protein corona

Coronal proteins were isolated from captured amyloid-corona complexes at the spin column filter interface via a 'soft' proteolytic digestion protocol. In brief, reduction of coronal protein disulphides was undertaken over 1 h at room temperature using DL-1,4-Dithiothreitol (DTT, Sigma) prepared in 50 mM ammonium bicarbonate buffer (pH 8.5) at a final concentration of 10 mM. Proteins were subsequently alkylated using iodoacetamide (IAA, Sigma) in 50 mM ammonium bicarbonate buffer, and incubated in dark at room temperature for 15 min with gentle agitation. Reduced and alkylated proteins were then digested for 16 h at 37 °C using 2 μg of sequencing grade trypsin (Promega) to a final concentration ratio of 1:100 for trypsin:coronal proteins. Elution of digested proteins from the spin column filter was achieved through centrifugation at 6,000 g for 5 min.

Liquid chromatography tandem-mass spectrometry (LC-MS/MS)

A Dionex UltiMate 3000 RSLCnano system equipped with a Dionex UltiMate 3000 RS autosampler was utilized for LC-MS/MS analysis of tryptically-digested coronal proteins. Samples were loaded onto an Acclaim PepMap RSLC analytical column (75 $\mu\text{m} \times 50 \text{ cm}$, nanoViper, C18, 2 μm , 100Å; Thermo Scientific) via an Acclaim PepMap 100 trap column (100 $\mu\text{m} \times 2 \text{ cm}$, nanoViper, C18, 5 μm , 100Å; Thermo Scientific). Peptides were then separated through increasing concentrations of buffer B (80% acetonitrile / 0.1% formic acid) for 158 min, with an Orbitrap Fusion Tribrid mass spectrometer (Thermo Scientific) utilized for analysis, operated in data-dependent acquisition mode using in-house, LFQ-optimized parameters.

Protein identification and label-free quantification

Peaks version X software (Bioinformatics Solutions) was utilized to interrogate LC-MS/MS data against the human proteome (UniProt v_05112018). Parameters for Peaks X searches were as follows: precursor mass tolerance at 10 ppm; cysteine (Cys) carbamidomethylation was set as a fixed modification; variable modifications were considered for oxidation (Met) and deamidation (N&Q); lastly, enzyme digestion was set to unspecific, allowing a maximum number of two missed cleavages. The false discovery rate (FDR) for peptide identifications was set to 1% via the target-decoy approach, with a 6 min time retention window utilized for matches between runs. Only unmodified peptides were used for quantification.

Protein filtering method

Experiments and controls were all in triplicate and only proteins detected in at least two experiments were considered for analysis. Coronal protein abundance in each sample type was represented by LFQ intensity, and was compared against the plasma control background to quantitate significant coronal proteins. The top abundant coronal proteins were identified for further analysis through comparison of mean log intensities to the plasma control proteome. Statistical significance was assessed through Student's t-test performed using Prism software (GraphPad), with a p-value of <0.05 considered statistically significant.

Protein informatics and network analysis

STRING (v10, string-DB.org)³³ was used for online protein network analysis, with protein uniprot IDs considered for multiple protein entries. The whole genome background of *Homo sapiens* was selected and different interaction sources mined via a very high confidence interaction score (0.9). Further clustering of the network into 4 different nodes, based on the closest-related GO terms, was facilitated with K-means analysis. Protein Fasta sequences³⁴ were extracted by Uniport, with the physicochemical properties of the proteins (Molecular Weight, MW; grand average of hydropathy; GRAVY, isoelectric point; pI) identified with the ProtParam tool from <https://web.expasy.org/>.³⁵ All plots were reported either directly from Prism (GraphPad) or Excel (Microsoft).

Structural analysis of A β fibrils and plasma coronal proteins

Fibril model of A β were constructed based on replicating the cryo-EM (Protein databank, PDB; 5OQV for A β ₁₋₄₂) and solid-state NMR (PDB: 2LMN for A β ₁₋₄₂) protofibril structures, incorporating a left-handed twist between consecutive β -sheets of A β species. 3D structural information concerning plasma proteins was extracted from a PDB search in the following order: whole sequence, close homologues (e.g. human), sub-sequences. PDB IDs for top-10 common and unique plasma proteins identified in A β ₁₋₄₂ and A β ₁₋₄₀ fibril coronae are listed in Tables 1&2, respectively. Plasma protein net charges were estimated by their total quantities of basic and acidic amino acids under physiological conditions; i.e., Arg and Lys = +1; Asp and Glu = -1, and His was assigned as neutral. Binding structures between a protein and the amyloid fibril in Fig. 5b were approximated by aligning the two molecules with a maximum contact.²⁰

Immunohistochemistry of A β corona

A drop (50 μ L, 25 μ M) of fibrillized A β ₁₋₄₂ or A β ₁₋₄₂ with their protein corona was placed on a glass slide and allowed to air-dry. Samples were then fixed with 5% paraformaldehyde and washed with 1 $\times$ PBS (pH 7.4) and MilliQ water for 5 min. Once dried, a drop (50 μ L, 2 μ g mL⁻¹) of primary antibody (anti-A β ₄₂, mouse monoclonal, AnaSpec, AS-55922) was added to each sample and incubated at 4 °C overnight. Unbound antibodies were then removed via a 1 $\times$ PBS (pH 7.4) wash. The sections were then incubated with a drop (50 μ L, 2 μ g mL⁻¹) of secondary antibody (goat anti-mouse HiLyte™ Fluor 488-labeled, AnaSpec, AS-61057-05-H488) for 6 h at room temperature. Unbound secondary antibodies were removed as previously described. The slides were then dried and imaged with a fluorescence microscope (Nikon Ti-Eclipse).

Cell culture and viability assay

A viability assay was performed utilizing an insulin-producing pancreatic β -cell line (β TC-6; ATCC), cultured in complete Dulbecco's modified Eagle's medium (DMEM) (Sigma) supplemented with 15% fetal bovine serum (FBS) (Sigma). In brief: 70 μ L poly-L-lysine (Sigma, 0.01%) was applied to a 96 well plate (black/clear bottom, Costar) and incubated at 37 °C for 30 min. Poly-L-lysine was then removed and the wells washed three times in 1 $\times$ Dulbecco's PBS (DPBS) (Sigma). Cells were added to the wells at a density of ~ 50,000 cells/well and incubated at 37 °C, 5% CO₂ for 48 h to reach ~ 80% confluency. Old medium was replaced with fresh medium containing 1 μ M propidium iodide (PI) (Invitrogen) and incubated for 30 min in the dark at 37 °C, 5% CO₂. Samples pertaining to 25 μ M IAPP monomers, IAPP monomers with 5% A β ₁₋₄₂ seeds and IAPP monomers with 5% A β ₁₋₄₂ seeds pre-incubated with plasma proteins were then added to the wells. Measurements were conducted in triplicate (5 reads/well) within a live cell chamber (37 °C, 5% CO₂) on an Operetta Imaging System (PerkinElmer; microscope objective: 20 $\times$ PlanApo, numerical aperture: 0.7) after 15 h of treatment. The percentage of PI-positive cells, as an indicator of cell death, within the total cell count was determined by the built-in bright-field mapping function of Harmony High-Content Imaging and Analysis software (PerkinElmer). Untreated cells were recorded as controls.

3. RESULTS AND DISCUSSION

A β fibrillization kinetics

The fibrillization of A β ₁₋₄₂ and A β ₁₋₄₀ was monitored by a ThT fluorescence kinetic assay (Fig. S1). Parallel measurements for 50 μ M A β ₁₋₄₂ and A β ₁₋₄₀ were performed. By analyzing the A β fibrillization processes as a function of relative fluorescence, we determined that A β ₁₋₄₂ possessed a lag time of 47.1 $\pm$ 5.5 h, followed by a fibrillization rate constant k of 0.087 $\pm$ 0.019 h⁻¹ and a reaction half-time $t_{1/2}$ of 70.9 h to convert half of the monomers into fibrils. In comparison, A β ₁₋₄₀ fibrillization displayed an extended lag time of 72.9 $\pm$ 40.0 h, followed by a much slower fibrillization rate constant k of 0.021 $\pm$ 0.009 h⁻¹ and a prolonged reaction half-time $t_{1/2}$ of 178.2 h. It was also observed that the mean fluorescence intensity was 4-fold lower for A β ₁₋₄₀, indicating significantly less cross- β content associated with A β ₁₋₄₀ than with the more amyloidogenic A β ₁₋₄₂.

Characterization of A β fibril-coronae

The formation of mature amyloid fibrils by A β ₁₋₄₂ and A β ₁₋₄₀ was confirmed by TEM imaging. Both A β peptides displayed straight, unbranched filaments of 1~10 μ m in length. The average fibril diameter for the fibrils was determined to be 9.0 $\pm$ 1.7 nm for A β ₁₋₄₂ and 8.5 $\pm$ 1.7 nm for A β ₁₋₄₀, respectively (Fig. 1e&1f). 2-week old A β ₁₋₄₂ and A β ₁₋₄₀ fibrils were then incubated with human plasma, and TEM imaging revealed that both types of amyloid fibrils formed hard coronae within a few hours of incubation (Fig. 1c&1d). The average diameters of both types of fibrils widened to 17.5 $\pm$ 3.0 nm upon corona formation (Fig. 1f&1f). Distinct patterns were observed on both fibrillar-corona isolates due to their binding with lipoproteins, among other types of proteins (Fig. S2).

Analysis of A β fibril protein coronae

The protein corona formed on human amyloid peptides was previously observed for human IAPP fibrils (associated with T2D) in fetal bovine serum (FBS) using mass spectrometric approaches.²⁰ Here we delineated the proteins identified in the coronae of fibrillar A β ₁₋₄₂ and A β ₁₋₄₀. The hard coronae were isolated using the centrifugal capture method,²⁰ and to identify the A β -associated proteins we employed proteolytic digestion to extract the peptide mixtures and analyzed them using LC-MS/MS. All resulted peptides were identified by comparing them with the originating proteins using PEAKS proteomics data analysis software.

A total of 200 and 173 proteins were detected in at least 2 out of the replicates for A β ₁₋₄₂ and A β ₁₋₄₀, respectively, out of which 142 identified proteins were common in both groups. Compared to plasma control, 58 proteins were unique to the A β ₁₋₄₂ samples and 31 proteins were unique to the A β ₁₋₄₀ samples (Fig. 2a). Out of the proteins identified in all groups, 19 proteins were only found in the A β ₁₋₄₂ samples and 9 proteins in the A β ₁₋₄₀ samples (Table S1). The significant proteins were selected by the protein filtering method, by taking into consideration of the higher intensity and peptide count, and by comparing their relative abundance with control plasma samples. The top-10 proteins identified in both A β ₁₋₄₂ and A β ₁₋₄₀ fibril samples were listed (Table 1) and the top-70 coronal proteins associated with both A β ₁₋₄₂ and A β ₁₋₄₀ fibrils were selected based on the filtering method for further analysis (Fig. 2b). There were no significant differences in the abundance of similar coronal proteins for the two different types of A β fibrils. Not surprisingly, the top enriched protein in both A β fibril coronae was human serum albumin, the most abundant protein constituent of the human plasma. Other top coronal proteins isolated were complement C3, serotransferrin, haptoglobin, alpha-2-macroglobulin, vitamin D-binding protein, hemopexin, complement C4-B, complement C4-A and plasminogen. The significant proteins enriched in fibrillar coronae, such as apolipoproteins, albumin and complement proteins, have been repeatedly identified for the coronae of synthetic nanoparticles.^{17, 36} The top unique coronal proteins identified for the A β ₁₋₄₂ and A β ₁₋₄₀ fibrils are listed in Tables 2a&2b. There was no significant relationship between the unique proteins identified and A β fibril composition, and no clear trends were observed for the unique proteins in the amyloid coronae, underlining the non-specific nature of binding between globular plasma proteins and amyloid fibrils.

The top coronal proteins tended to be neutral in charge with varying molecular weight (Fig. S3). The A β amyloid-associated proteins had a wide range of hydropathy scores (<0 for more hydrophilic proteins, >0 for more hydrophobic proteins), with most of the proteins having a hydropathy score below zero (Fig. 3a). Despite wide variations of molecular mass (Fig. S3) and hydrophilic GRAVY score (Fig. 3a) of the top enriched proteins, weak anti-correlations existed with respect to their abundance. In contrary, the pI values of the fibril-associated proteins were mostly neutral or slightly acidic, with exceptions like serum amyloid A-4 protein (SAA4), platelet basic protein (CXCL7) and complement C1q subcomponent subunit B (C1QB), which had pI >7 (Fig. 3b). There was no discernible correlation between the pI values of these proteins and their amyloid coronae abundance.

The unique proteins identified for the A β _{1–42} and A β _{1–40} fibrils appeared to have wide variations in their physicochemical properties. The A β _{1–42} unique coronal proteins had GRAVY scores ranging from –1 to 0 with an exception of TM212, which had a high GRAVY score of 0.6 (Fig. S4a). The pI values for the A β _{1–42} unique coronal proteins were between 4.3 and 9.5, where most proteins were basic or acidic in nature and only a few proteins were in the neutral pI range (Fig. S4b). For the unique coronal proteins of A β _{1–40} fibrils, their range of GRAVY score was from –1.1 to 0.35 (Fig. S5a), while their range of pI values was between 4.3 and 9.5 (Fig. S5b).

Protein network analysis of identified proteins

Our findings suggest that amyloid fibrillar coronae interacted with a large number of human plasma proteins. The identified proteins were analyzed by String-DB online resources to identify the molecular/biological actions and enriched disease-related subnetwork associations across the corona protein network. This extensive computational analysis revealed significant topological characteristics of the detected proteins and their functional roles in different associated pathways. The network analysis showed 4 major protein groups, based on K-means clustering nodes derived from the interaction records assembled from KEGG and Gene Ontology (Fig. 4). The most significant proteins identified in the corona formation were mainly involved in complement activation (19 proteins), protein metabolism and transport mechanisms (19 proteins), acute phase/inflammation/blood coagulation (14 proteins), plasma lipoproteins (11 proteins), immune response/immunoglobulin (7 proteins), which are involved in the biological processes implicated in AD (Fig. 2c).

One of the most abundant proteins in the plasma, serum albumin, displayed a chaperone-like inhibitory effect on amyloid fibrillization in the brain interstitium²³ and albumin-A β complexes were associated with decreased A β removal from the brain to blood.³⁷ Moreover, plasma unsaturated fatty acids promoted binding of serum albumin to A β _{1–42} to enable clearance of the peptide.³⁸ Similar to the nanoparticle protein corona, a large number of closely-interacting complement proteins, inflammatory proteins and immunoglobulin chains were identified in the amyloid fibril coronae (Fig. 2c). In nanoparticle protein corona, activation of complement pathways and their interactions with immunoglobulins contributed to the clearance of nanoparticles from the host systems.³⁹ Complement activation and interactions with inflammatory proteins in the brain promoted plaque clearance through different opsonization or inflammatory pathways.^{40–43} A major fraction of the coronal proteins was identified to be plasma lipoproteins (e.g. apolipoprotein E, apolipoprotein B-100, and apolipoprotein A) interacting with vital plasma proteins like alpha-2-macroglobulin, serum albumin and complement proteins. The apolipoproteins identified in the coronal samples affected cognitive functions,⁴⁴ regulation of A β deposition⁴⁵ and the pathology of AD.⁴⁶ Proteins identified in metabolic process and inflammatory pathways like alpha-2-macroglobulin, haptoglobin, prothrombin, serotransferrin and hemopexin were implicated in neuropathogenic pathways and may be used for developing blood-based AD markers.^{47–50}

Structural analysis of A β fibrils and plasma coronal proteins

A β _{1–42} and A β _{1–40} fibrils have similar charge distributions on their surfaces (Fig. 5a), exposing both positively and negatively charged residues to their environments. Due to the similar surface physicochemical properties of the two types of fibrils, the protein coronae also possessed similar plasma protein compositions (Fig. 2). The structural information of top-10 proteins common in both amyloid coronae (Table 1) and also of those unique to A β _{1–42} (Table 2a) and A β _{1–40} (Table 2b) amyloids was obtained by searching the PDB with available structures or close homologs. Interestingly, those proteins unique to either A β _{1–42} or A β _{1–40} amyloids were mostly immune-related proteins, possessing an immunoglobulin-like topology with β -sandwich structures. Structural analysis indicated the top proteins enriched in both A β _{1–42} and A β _{1–40} amyloid coronae possessed diverse secondary structures, including all- α , all- β , and α/β protein topologies (Fig. S3). Majority of the abundant proteins were multi-domain proteins possessing conformational flexibility, which were able to make a large number of contacts with amyloid fibrils (e.g., alpha-2-macroglobulin in Fig. 5b) to elicit strong binding. This observation is consistent with the weak anti-correlations of the abundance of top-70 enriched proteins with their molecular mass (Fig. S2) and GRAVY scores (Fig. 3a), suggesting that larger molecules with higher hydrophobicity tend to be more enriched in the coronae. Together with the nil correlation between protein net charge and their abundance (Fig. 3b), our results suggest that the binding of plasma proteins with A β amyloids was mainly driven by non-specific hydrophobic interactions instead of electrostatics. In contrast, electrostatic interactions played an important role in the formation of IAPP amyloid coronae, since the amyloid peptide contained positively charged residues.^{19, 20}

Effect of corona formation on A β fibril detection

The A β _{1–42} plaques found in brain and circulation systems are enriched by functional plasma and cellular proteins.^{21, 22, 51} Here the effects of human plasma coronae on A β _{1–42} fibrils were assessed by immunohistochemistry (IHC), using A β _{1–42} fibril specific primary antibody and fluorescently labelled secondary antibody. The A β _{1–42} fibrils were immediately detected by the primary antibodies and gave off bright fluorescence upon secondary antibody binding (Fig. 6b). In contrast, A β _{1–42} fibrillar corona in excess of plasma rendered fewer fluorescent spots (Fig. 6c,d), indicating that plasma proteins concealed the fibrillar morphology to effectively prevent binding of their primary antibody irrespective of the time of incubation. This result is consistent with an earlier study by Pilkington et al., concerning the formation of a plasma protein corona on IAPP fibrils.²⁰ Collectively, these studies revealed the prevalence of the protein coronae associated with amyloid fibrils, which may hinder antibody-based therapeutic strategies against amyloid diseases.

Effects of A β protein corona on IAPP fibrillization, toxicity and cross seeding

The fibrillization kinetics for IAPP cross-seeded with 5% A β _{1–42} seeds with or without protein corona was measured (Fig. 7a). The control IAPP (50 μ M) showed a lag phase of 2.9 $\pm$ 2 h, followed by elongation with a fibrillization rate constant k of 0.011 $\pm$ 0.0053 h^{–1}. Mature fibrils formed within 13 h of incubation displayed a reaction half-time $t_{1/2}$ of 6 h.

IAPP fibrillization was accelerated by A β ₁₋₄₂ seeds, with the lag phase reduced to 1.6 h and the fibrillization rate constant k rose to 0.022+0.01 h⁻¹. The reaction half-time $t_{1/2}$ was shortened to 1.2 h, while the saturation phase was reached in 3.5 h. The A β ₁₋₄₂ protein corona accelerated the fibrillization process without an apparent lag phase, due to increased aggregation of IAPP monomeric units in the presence of plasma proteins. Mature fibrils were formed within 5 h of incubation, with a fibrillization rate constant k rose further to 0.048+0.08 h⁻¹. These results suggest that A β ₁₋₄₂ seeds with their protein corona served as nucleation centers for the fibril extension of IAPP, when compared to IAPP fibrillization cross-seeded with regular A β ₁₋₄₂ seeds. TEM images further revealed that the cross-seeded IAPP fibrils possessed a similar morphology to mature IAPP fibrils (Fig. 7c, e), whereas the cross-seeded IAPP fibrils with protein corona showed fibrils coated with plasma proteins along their contours (Fig. 7f). The cytotoxicities of IAPP and its cross-seed species were studied (Fig. 7b), where monomeric IAPP elicited the highest cell death of 40% as indicated by PI positive nuclei. The cell mortality was reduced to 13% for IAPP cross-seeded with A β ₁₋₄₂ seeds and to 22% for IAPP with A β ₁₋₄₂ seeds pre-coated with a human plasma corona. In contrast, the A β ₁₋₄₂ seeds were non-toxic. This indicates that the IAPP toxicity in pancreatic beta cells was mitigated by cross seeding the peptide with A β ₁₋₄₂ seeds and their protein corona. This result is in contrast to the case of cross-seeding IAPP in the brain of AD mice, which resulted in enhanced aggregation and elevated AD pathology and memory impairment.²⁷

4. CONCLUSION

A β fibrils acquired distinct coronae through spontaneous protein adsorption in human blood plasma. The compositions of the coronae were identified by LC-MS/MS and examined by protein network analysis. Due to their comparable structural properties, A β ₁₋₄₂ and A β ₁₋₄₀ fibrils rendered coronal proteins similar in composition and physicochemical properties. In total, 58 proteins were unique to the more hydrophobic A β ₁₋₄₂ samples and 31 proteins unique to the A β ₁₋₄₀ samples, and the proteins unique to either A β ₁₋₄₂ or A β ₁₋₄₀ were mostly immune-related indicating their toxic potential. Furthermore, both coronae consisted of proteins significant in complement activation, inflammation and protein metabolic pathways, known to be involved in the biological processes implicated in AD. Molecular docking revealed that the coronal proteins were often of multi-domain, possessing conformational flexibility to interact with the fibrils through hydrophobic moieties. IHC assay further confirmed a significantly impaired capacity of A β fibril recognition by its antibodies. The protein corona of A β seeds further accelerated IAPP fibrillization, but without elevating IAPP toxicity likely due to faster conversion of toxic oligomer species to amyloid fibrils and off-pathway amorphous species. This study highlights the need of taking into consideration of the structural and functional transformations of A β amyloids for the development of new AD therapeutics (Scheme).⁵² As the binding of amyloid proteins and their environmental proteins is driven by nonspecific forces and is therefore a general phenomenon, this study has implications for delineating the *in vivo* transformation of amyloid proteins and their aggregates in the extracellular space or in the blood, where a myriad of proteins co-exist to entail biological and pathological manifestations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

TEM images of a) mature Aβ₁₋₄₂ fibrils, b) mature Aβ₁₋₄₀ fibrils, c) Aβ₁₋₄₂ fibril-human plasma protein corona, d) Aβ₁₋₄₀ fibril-human plasma protein corona, e,f) diameter analysis of mature fibrils and Aβ₁₋₄₂ and Aβ₁₋₄₀ fibrillar coronae. Scale bars: 100 nm.

Figure 2.

a) Venn diagram representing the number of proteins identified in each sample group in comparison with plasma control. The most abundant proteins are identical in both Aβ fibril coronal samples and top-70 proteins with higher intensity than plasma control are mentioned. b) Heatmap of the most abundant proteins in the coronae of Aβ₁₋₄₂ and Aβ₁₋₄₀ fibrils having intensities compared with control human serum. c) Classification of the identified proteins into five groups based on their biological and cellular functions from Uniport.³⁴

Figure 3. Physicochemical properties of the identified proteins. The frequency of GRAVY score and pI values of the most abundant coronal proteins plotted with point size as $A\beta_{1-40}/A\beta_{1-42}$ ratio. The $A\beta$ associating proteins tend to have a wide range of hydropathy scores (<0 are more likely globular hydrophilic proteins, >0 are more likely hydrophobic proteins), with pI values mostly in the neutral or slightly acidic range.

Figure 4.

Protein network analysis of the top coronal proteins using string resources. The network was generated for 64 out of the 70 proteins (immunoglobulin sidechains not included) with a 0.9 confidence interaction score and categorized into 4 primary nodes based on K-means clustering.

Figure 5.

$A\beta$ fibril structures and their binding with top coronal proteins. a) $A\beta_{1-42}$ and e) $A\beta_{1-40}$ fibrils are composed of β -sheet peptides (grey) shown in cartoon format with electrostatic potential surfaces estimated with PyMOL. Binding of top identical coronae proteins b) serum albumin (orange), c) complement C3 (purple), d) serotransferrin (green), f) alpha-2-macroglobulin (cyan), g) complement C4-A (pink), and h) plasminogen (blue) with the $A\beta_{1-42}$ fibril was estimated by aligning them with maximum contact surface areas. Plasma proteins are shown in cartoon format.

Figure 6. Immunohistochemistry for A β fibrillar protein corona recognition by antibodies. (a) The A β_{1-42} fibril control showed bright fluorescence (b) while the A β_{1-42} fibrillar corona formed in excess of plasma rendered few fluorescent spots (c,d) irrespective of time of incubation. Scale bars: 100 μ m.

Figure 7.

a) ThT fibrillization kinetics for IAPP cross-seeded with 5% A β ₁₋₄₂ seeds with or without protein corona. Cross seeding with 5% A β ₁₋₄₂ promoted IAPP nucleation and fibrillization. The A β ₁₋₄₂ protein corona accelerated the fibrillization process with no visible lag phase due to increased aggregation of monomeric units in the presence of plasma proteins. Error bars indicate standard deviation of averaged data set for n=3. TEM images revealed structures of mature fibrils formed by cross seeding. c) mature IAPP fibrils (50 μ M) d) A β ₁₋₄₂ seeds (25 μ M), e) IAPP (50 μ M) + 5% A β ₁₋₄₂ seeds, f) IAPP (50 μ M) cross seeded with 5% A β ₁₋₄₂ seeds pre-coated with protein corona. Scale bars: 100 nm. b) Viability of β TC6 cells exposed to IAPP, IAPP monomers with 5% A β ₁₋₄₂ seeds and IAPP monomers with 5% A β ₁₋₄₂ seeds pre-incubated with plasma proteins. Error bars indicate standard deviation of averaged data set for n=3 (ns, $p > 0.05$ and $p < 0.001$).

Scheme.

Characterization methodologies and implications of the protein coronae of A β _{1–42} and A β _{1–40} fibrils.

Table 1.

Top-10 proteins identified in both A β _{1–42} and A β _{1–40} fibril coroneae.

#	Protein	Uniprot	# residues	Charge	PDB	Classification
1	Serum albumin	P02768	609	–11	1AO6 (25–609)	α
2	Complement C3	P01024	1663	–18	2A73(23–665,673–1663)	multi-domains
3	Serotransferrin	P02787	698	–2	6D05 (1–698)	multi-domains
4	Haptoglobin	P00738	406	–6	5HU6 (148–406)	α - β
5	Alpha-2-macroglobulin	P01023	1474	–22	4ACQ (24–1474)	multi-domains
6	Vitamin D-binding protein	P02774	474	–13	1LOT (17–474)	α
7	Hemopexin	P02790	462	–4	1QHU (48–462)	multi-domains
8	Complement C4-B	P0C0L5	1744	–4	4XAM (20–675)	multi-domains
9	Complement C4-A	P0C0L4	1744	–7	4FXK(20–675,680–1446,1454–1744)	multi-domains
10	Plasminogen	P00747	810	–1	4DUU(20–810)	multi-domains

Table 2a.Top-10 unique proteins identified in A β ₁₋₄₂ fibril corona.

Protein	# residues	Charge	Structure
Immunoglobulin kappa variable 1-27	117	+2	6CR1(1-117) 95.79%
Immunoglobulin heavy variable 1-45	117	+4	5O5O(1-117) 75.21%
Immunoglobulin kappa variable 1D-13	117	+1	4M6O(1-117) 100%
Immunoglobulin heavy variable 4-61	118	+5	5UTZ(1-118) 92.93%
Immunoglobulin lambda constant 7	106	+2	6AXK(1-106) 99.06%
Transmembrane protein 212	194	+2	N/A
Histone-lysine N-methyltransferase 2D	5537	-148	6O7G(1503-1562) chain B 3UVK(5337-5347) chain B 4Z4P(5382-5536) chain A
PH domain leucine-rich repeat-containing protein phosphatase 1	1717	-29	N/A
ATP-dependent DNA helicase Q5	989	+20	5LB8(11-526) chain A
Coagulation factor XIII A chain	732	-12	1EVU(2-732) chain A

Table 2b.

Top-10 unique proteins identified in A β _{1–40} fibril corona.

Protein	# residues	Charge	Structure
Extracellular matrix protein 1	540	–9	N/A
carboxypeptidase n subunit 2	545	–11	N/A
Properdin	469	+9	1WOR(28–469) chain A
sex hormone binding globulin	402	–5	1LHV(30–218) chain A
Ficolin-2	313	–2	2J3U(97–313) chain A
Carboxypeptidase B2	423	+1	3D66(24–423) chain A
Hepatocyte growth factor activator	655	–1	2R0K (373–655) chain A
Immunoglobulin heavy variable 3–72	119	+1	5U68 (1–119) 79.49%
Immunoglobulin heavy variable 3–23	117	+2	1OHQ(20–116) chain A
Immunoglobulin heavy variable 3–7	117	0	2FL5(20–117) chain B