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Serum albumin impedes the amyloid aggregation and hemolysis of human islet amyloid polypeptide and alpha synuclein

Aleksandr Kakinen[a], Ibrahim Javed[a], Ava Faridi[a], Thomas P. Davis[a,b,*], Pu Chun Ke[a,**]

[a] ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, VIC 3052, Australia

[b] Department of Chemistry, University of Warwick, Gibbet Hill, Coventry CV4 7AL, United Kingdom

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ABSTRACT

Protein aggregation is a ubiquitous phenomenon underpinning the origins of a range of human diseases. The amyloid aggregation of human islet amyloid polypeptide (IAPP) and alpha synuclein (αS), specifically, is a hallmark of type 2 diabetes (T2D) and Parkinson's disease impacting millions of people worldwide. Although IAPP and αS are strongly associated with pancreatic β-cell islets and presynaptic terminals, they have also been found in blood circulation and the gut. While extensive biophysical and biochemical studies have been focused on IAPP and αS interacting with cell membranes or model lipid vesicles, the roles of plasma proteins on the amyloidosis and membrane association of these two major types of amyloid proteins have rarely been examined. Using a thioflavin T kinetic assay, transmission electron microscopy and a hemolysis assay here we show that human serum albumin, the most abundant protein in the plasma, impeded the fibrillization and mitigated membrane damage of both IAPP and αS. This study offers a new insight on the native inhibition of amyloidosis.

1. Introduction

Human islet amyloid polypeptide (IAPP) and alpha synuclein (αS) are two major classes of amyloid proteins serving both functional and pathogenic roles in biology. Specifically, monomeric IAPP assists insulin for glycemic control, while monomeric αS is thought to be associated with the modulation of neurotransmitter dopamine release, endoplasmic reticulum (ER)/Golgi trafficking, and synaptic vesicles [1]. The endogenous stabilization of IAPP is provided by insulin, physiological metal ions, low pH and zinc-mediated complexation of IAPP and C-peptide [2–8]. Likewise, transitions of αS from disordered monomers to partially folded intermediates and amyloid fibrils may be promoted by changes in local pH, ionic strength and temperature, as well as mutation [9,10].

A large collection of literature has revealed that amphiphilic environments, such as cell membranes and lipid vesicles, can initiate electrostatic interactions between the N-termini of amyloid proteins and the anionic membranes [11–16], facilitated by hydrophobic interaction and hydrogen bonding between the interactants. Such interactions have been shown to convert the proteins from disordered monomers to α-helix and then β-sheet rich oligomers and protofibrils and, eventually, cross-beta amyloid fibrils. The oligomers and amyloid fibrils show a high propensity for partitioning into cell membranes, causing pore formation in a porin-like manner or extracting lipid content to compromise membrane fluidity [17–20]. The endpoints of such interactions range from generation of reactive oxygen species (ROS) to damage to ER, mitochondria, and DNA, followed by cell degeneration. Furthermore, it has been found that the aromatic side chains of LANFLVH, a fragment of IAPP, are nonessential in amyloid formation or membrane leakage [21], while cholesterol showed contrasting effects on IAPP toxicity and disruption to model membranes [22]. In addition, inhibition of IAPP amyloidosis and increased IAPP resistance to protease degradation by copper (II) has also been reported [23].

IAPP amyloid aggregation has been implicated as causative to the onset of type 2 diabetes (T2D). Likewise, the amyloid aggregation of αS into Lewy bodies or Lewy neurites is closely associated with motor symptoms and Parkinson's disease. While IAPP fibrils and plaques are generally found in the extracellular space of pancreatic β-cell islets, Lewy bodies and Lewy neurites are located intracellularly. Regardless, oligomeric IAPP and αS have been widely acknowledged as the toxic species based on in vitro and animal studies [24,25].

IAPP is stored at millimolar concentrations in pancreas beta islets and readily fibrillates at micromolar concentrations [3]. Upon release into the bloodstream, IAPP is immediately exposed to a milieu of

* Correspondence to: T.P. Davis, Monash University, 381 Royal Parade, Parkville, VIC 3052, Australia.
** Correspondence to: P.C. Ke, Monash University, 381 Royal Parade, Parkville, VIC 3052, Australia.
E-mail addresses: thomas.p.davis@monash.edu (T.P. Davis), pu-chun.ke@monash.edu (P.C. Ke).

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plasma proteins and lipids [26,27]. Consequently, we have recently applied the concept of protein 'corona' [28,29] originated from the field of nanomedicine for describing the structural transformation as well as the toxicity of IAPP in circulation [27]. Here the protein corona was rendered by model plasma proteins adsorbed on fibrillating IAPP or mature, hydrophobic IAPP fibrils through electrostatic and hydrophobic interactions as well as H-bonding, similarly to the formation of a protein corona adsorbed on nanoparticles [27,29]. On the other hand, αS has recently been found in the heart and the gut, with the latter being hypothesized as a possible origin of the protein through the propagation of intestinal microbiota [30,31]. Interestingly, human serum albumin (HSA), which accounts for 55% of all plasma proteins, has recently been found to possess a chaperone-like capacity against the amyloid aggregation of amyloid beta and transthyretin [32–34], and this capacity of HSA was postulated to be realized through a "monomer-competitor" mechanism for amyloid beta [35]. Accordingly, in the present study we examined the binding of HSA with IAPP and αS, two major types of amyloid proteins with significant health implications. We evaluated the effects of their binding on the conformation and toxicities of the amyloid proteins using a thioflavin T (ThT, for fibrillization kinetics) assay and high-resolution transmission electron microscopy (TEM, for mesoscopic morphology), as well as a red blood cell (RBC) hemolysis assay (cell association and membrane leakage). Our study revealed a prolonged lag phase of IAPP and suppressed fibrillization of αS with the increase of HSA concentration. Additionally, the hemolysis assay demonstrated a protective effect of HSA on the toxicities of IAPP and αS towards RBCs. Together, these results offered a new perspective on the natural inhibition of IAPP and αS amyloidosis through the mediation of plasma proteins.

2. Results and discussion

2.1. Effects of HSA on IAPP and αS fibrillization

The fibrillization kinetics of IAPP and αS in the presence of different HSA concentrations was examined using the amyloid specific dye ThT (Fig. 1). The lag phase of IAPP (50 μM) fibrillization at 37 °C was approximately 20 min, followed by an elongation phase and a saturation phase after 2 h of incubation (Fig. 1A). In the presence of 10 μM HSA (5:1 molar ratio of IAPP to HSA) the lag phase was increased considerably to 4.2 h and the elongation phase to 8 h. With the HSA concentration increased to 50 μM (i.e., 1:1 IAPP to HSA molar ratio), the lag time was extended to 5.4 h while the elongation phase up to 11 h. At the highest tested HSA concentration of 100 μM (1:2 IAPP to HSA molar ratio), the lag time was further extended to 9 h while the saturation phase was reached after 13 h. This result demonstrates the deceleration effect of HSA on the fibrillization kinetics of IAPP, which could be due to the attraction between cationic IAPP and negatively charged HSA at physiological pH, or through hydrophobic interaction between the amyloidogenic region of IAPP (i.e. residues 20–29) and the hydrophobic domains of HSA to reduce IAPP availability for nucleation and elongation. This observation is consistent with the aggregation inhibition of amyloid beta in the presence of HSA at comparable HSA/amyloid protein ratios [35].

Our electron microscopy imaging confirmed the formation of IAPP amyloid fibrils after 17 h for the control (Fig. 2A, B) and for IAPP incubated with HSA (Fig. 2C–H). However, the presence of HSA altered the IAPP fibril morphology. Namely, at IAPP to HSA molar ratio of 5:1 the IAPP amyloid fibrils were stacked together into multi-fibrillar bundles (Fig. 2C, D). At a higher HSA concentration of 50 μM, IAPP fibrillated into large aggregates displaying significant intertwining (Fig. 2E, F). At excess HSA concentration of 100 μM we observed clear co-aggregation of fibrillar IAPP with HSA (Fig. 2G, H).

In addition, our statistical analysis of the TEM images revealed that HSA exerted a concentration-dependent influence on IAPP fibril length and rigidity (Fig. 4A–D). The persistence length (λ), a major indicator of rigidity [36], is 2885 nm for (control) IAPP fibrils (Fig. 4A), which is comparable to that of beta lactoglobulin and amyloid-beta fibrils [37,38]. The lowest HSA concentration did not significantly affect the persistence length or contour length of IAPP (Fig. 4B). However, at 1:1 IAPP to HSA molar ratio we noted a three-fold softening of the amyloid fibrils (λ = 846 nm) and a narrower distribution of the fibril contour length (Fig. 4C). It should be acknowledged that the analysis of amyloid aggregates is not straightforward and may result in underestimated fibril contour length. Interestingly, the highest HSA concentration yielded more rigid fibrils (λ = 3684 nm, or 27% increase from that of the control) (Fig. 4D).

Understandably, the kinetics of αS fibril formation was markedly slower compared to IAPP, partly due to the much larger molecular weight of the neuronal protein (140 residues vs. 37 residues, or 14,460 Da vs. 3904 Da for αS vs. IAPP) as well as other different physicochemical attributes of the amyloid proteins (sequence, charge, and hydrophobicity etc.). The lag phase of αS at 50 μM, 37 °C and with 500 rpm of shaking was up to 60 h, followed by a steep elongation phase up to 125 h (Fig. 1B; Fig. 3A, B). In the presence of the lowest HSA concentration (10 μM, 5:1 IAPP to HSA molar ratio), αS

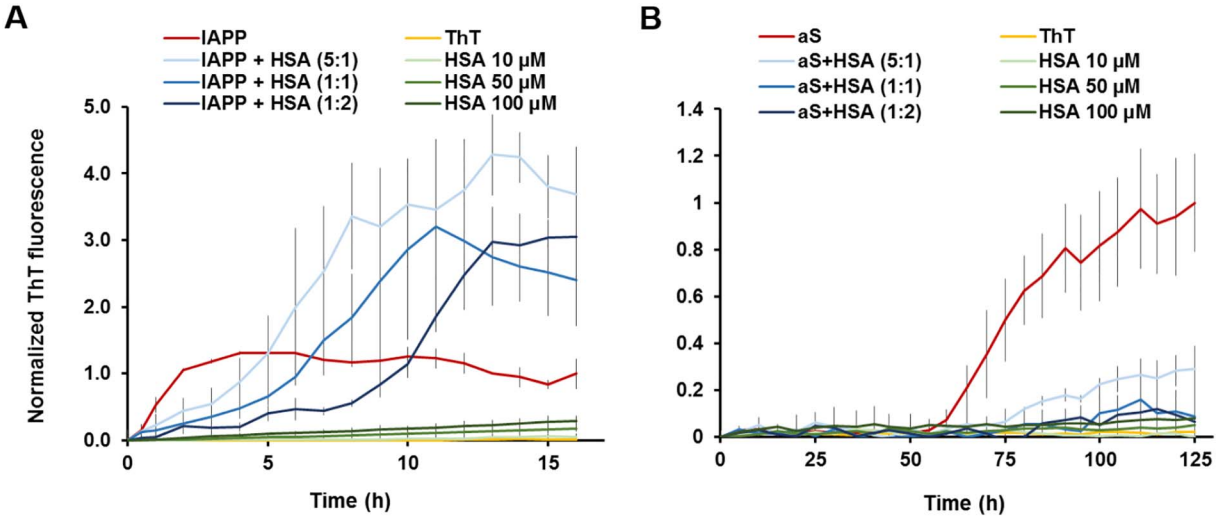

Fig. 1. Effects of human serum albumin (HSA) on the fibrillization kinetics of (A) IAPP (50 μM) and (B) αS (50 μM). IAPP fibrillization in presence of 10 μM (5:1 IAPP to HSA molar ratio), 50 μM (1:1) and 100 μM (1:2) of HSA revealed a prolonged lag phase with the increase of HSA concentration. Fibrillization of αS was observed w/o the presence of HSA and with 10 μM (5:1 molar ratio) of HSA. However, αS amyloid formation was inhibited in the presence of higher HSA concentrations (50 μM, 100 μM).