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The binding constant for amyloid Aβ40 peptide interaction with human serum albumin

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Abstract

Human serum albumin (HSA) is the major carrier of Aβ peptides in blood plasma. 1:1 interaction stoichiometries were established in previous indirect antibody-based studies for both Aβ40 and Aβ42, but corresponding binding constants were not provided. In this study we applied direct titrations of HSA with Aβ40 monitored using circular dichroism spectroscopy and obtained a dissociation constant (K_d) of 5 ± 1 μM for a HSA complex with Aβ40. The interaction resulted in an increase of the α-helical contents in the complex, compared to its components, which is quantitatively consistent with the known ability of Aβ40 to adopt a partially α-helical conformation in a hydrophobic environment. The relevance of these findings for the role of HSA in Aβ physiology is discussed.

Keywords: Alzheimer's disease; Amyloid β40 peptide; Human serum albumin; Circular dichroism; Dissociation constant; α-Helix

Alzheimer's disease (AD) is the major cause of disability and death in the elderly. While the etiology and pathological mechanisms of AD are under debate, the accumulation of amyloid plaques in the brain is its major pathological hallmark [1]. Amyloid-β-peptides of 40 and 42 amino acid residues, Aβ40 and Aβ42 in short, are predominant components of these plaques [2]. These peptides, which result from the alternative cleavage of the transmembrane amyloid precursor protein (APP), are also present as soluble monomers and oligomers. The complicated process of conversion of Aβ peptides from their soluble to insoluble forms via oligomeric aggregates is considered a key event in the pathogenesis of AD [2–4].

The Aβ40 and Aβ42 peptides circulate in cerebrospinal fluid (CSF) and in blood. An equilibrium between these compartments is regulated dynamically mainly by two transmembrane receptors present in brain endothelium, specialized in transporting them across the blood–brain barrier (BBB). The LRP receptor transports Aβ peptides from CSF into the bloodstream and the RAGE receptor is responsible for shuttling the peripheral Aβ into the brain [5–7]. Apolipoproteins also participate in this transport [8]. The equilibrium across the BBB is affected by plaque formation [9].

Agents that do not penetrate across the BBB but bind Aβ peptides in blood serum promote the efflux of soluble brain Aβ towards the periphery. The peripheral administration of an anti-Aβ antibody resulted in the increased transport of CNS-derived Aβ peptide into blood [10,11]. Shifting the peripheral/brain Aβ equilibrium in favor of the bloodstream may therefore be a therapeutic target in AD [5–7].

Human serum albumin (HSA) is the most abundant protein in both human blood serum and CSF. In plasma it is present at a concentration of ca. 640 μM (42 mg/ml)

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[12]. Its CSF concentration is ca. 0.5% of that of the blood serum, 3 μM [13]. It may increase significantly, up to 10% of the serum value and more, when the blood–CSF barrier is impaired [14]. Such impairment often accompanies AD and other neurodegenerative conditions [15].

HSA serves as one of the most important physiological transport proteins for numerous endogenous and exogenous compounds. Not surprisingly, HSA has been reported to be the major Aβ binding protein in the circulation. More than 90% of both Aβ40 and Aβ42 detected in blood serum were reported to be bound to HSA under physiological conditions. Several relatively less abundant proteins, such as lipoproteins, as well as erythrocyte membranes also participate in HSA binding in the bloodstream [16–18]. CSF and blood serum components inhibit the Aβ polymerization. This ability was specifically decreased in both serum and CSF obtained from AD patients [19,20]. HSA is also one of the most potent inhibitors of Aβ polymerization [21,22]. A detailed, quantitative characterization of interactions of Aβ peptides with HSA is therefore crucial for a better understanding of its role in the transport and metabolism of Aβ peptides, leading to the design and development of antiamyloid drugs. Such an attempt was undertaken by an indirect immunological method. Binary (1:1) stoichiometries were indicated for both Aβ40 and Aβ42, but the binding constants were not reported explicitly [18]. Here we report a binding constant for a binary complex of HSA with Aβ40, determined directly by circular dichroism-monitored titrations.

Materials and methods

Materials. HSA, fatty acid and globulin free, and sodium phosphates were purchased from Sigma; NaF, NaOH, HCl were obtained from Merck and ammonium acetate from Fluka. ProTEV protease was purchased from Promega.

Expression, purification, and identification of Aβ1-40 peptide. The gene encoding Aβ40 was cloned into pET30a(+) (Novagen) downstream of a leader sequence encoding a highly expressing variant of bovine pancreatic trypsin inhibitor [23]. For selective cleavage, the ProTEV cleavage site (GluAsnLeuTyrPheGlu) was localized as a linker between the leader and Aβ40 [24]. The transformed *E. coli* strain BL21(DE3) cells were grown from overnight cultures in LB medium supplemented with 33 μg/ml kanamycin at 37 °C to $A_{600} = 0.8$. Protein overexpression was induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside, followed by incubation for 3 h. The cells were harvested by centrifugation and stored at −80 °C. Frozen cells were thawed on ice and resuspended, lyzed in 100 mM NaH_2PO_4, 10 mM Tris–Cl, 6 M GuHCl (pH 8), and sonicated on ice. The fusion protein was purified by Ni-NTA affinity (Qiagen) according to the manufacturer instructions, followed by purification to homogeneity on a Vydac C_{18} semi-preparative HPLC column and lyophilized. The purified fusion protein was resuspended in a small amount of hydrochloric acid (pH 2) and then diluted to a final concentration of 0.4 mg/ml with 50 mM Tris–HCl containing 0.5 mM EDTA and 1 mM DTT (pH 8). Next, 100 μl of ProTEV per 4 mg of fusion protein was added in order to initiate the cleavage and the release of Aβ peptide. After 72 h the mixture was purified by HPLC using a Vydac polymer semi-preparative column and a standard acetonitrile gradient in 5 mM ammonium acetate, pH 8.0. The Aβ40 peptide was eluted at 25% acetonitrile and concentrated using SpeedVac system until acetonitrile was evaporated completely. The identities of both the fusion protein and Aβ40 were positively verified by electrospray mass spectrometry on a Q-ToF Premier ESI-MS instrument (Waters). Aβ40 stock solutions were controlled by SDS–PAGE, ESI-MS, and UV–vis. Electrophoresis demonstrated an absence of covalent oligomers, mass spectrometry indicated that the content of monomeric species exceeded 90%, the remainder being low Aβ40 oligomers, largely dimers and trimers, and the lack of turbidity in spectroscopic measurements demonstrated the essential absence of fibrils. The stocks were stored at 4 °C and used within 48 h. Their monomeric status was not changed significantly during storage.

CD spectroscopy. HSA was dissolved in a 10 mM sodium phosphate/10 mM NaF buffer, adjusted to pH 7.4. Stock solutions of Aβ40 were prepared in the ammonium acetate buffer at pH 7.4. Prior to use HSA and Aβ40 solutions were filtered through 0.22 μm sterile syringe filters. Concentrations of HSA and Aβ40 solutions were determined spectrophotometrically (Cary 50 Bio) at 280 nm, using molar extinction coefficients of 39,800 [25] and 1490 M^{-1} cm^{-1}, the latter calculated from the amino acid composition [25]. CD spectra were recorded between 300 and 190 nm at 0.2 nm/s and 25 °C on an Aviv Model 202 instrument, using 1 mm cuvettes. Initial HSA concentrations were varied between 0.7 and 9.8 μM. Three Aβ40 stock solutions, at 42, 60, and 77 μM, were obtained from three separate expression and purification procedures. Titrations were performed up to Aβ40/HSA molar ratios of 5 or 10. In a control experiment a 2 μM HSA sample was titrated with pure ammonium acetate buffer in order to account for spectral effects of HSA dilution and buffer mixing.

Results

Circular dichroism was used to establish the binding constant for the Aβ40 interaction with HSA. Preliminary experiments demonstrated that experiments could only be successfully performed at low micromolar HSA concentrations. Further HSA dilutions resulted in spectral effects too weak to be quantified, while saturation of the binding could not be approached at HSA concentrations close to 10 μM, due to limitations of Aβ40 stock solutions, which exhibited a tendency for oligomerization at concentrations exceeding ca. 100 μM, as demonstrated by ESI-MS. However, previous studies suggested that this concentration range should be appropriate for the binding constant determination [18,21]. Fig. 1 presents an example of the CD titration of a 2 μM HSA sample with a 42 μM Aβ40 solu-

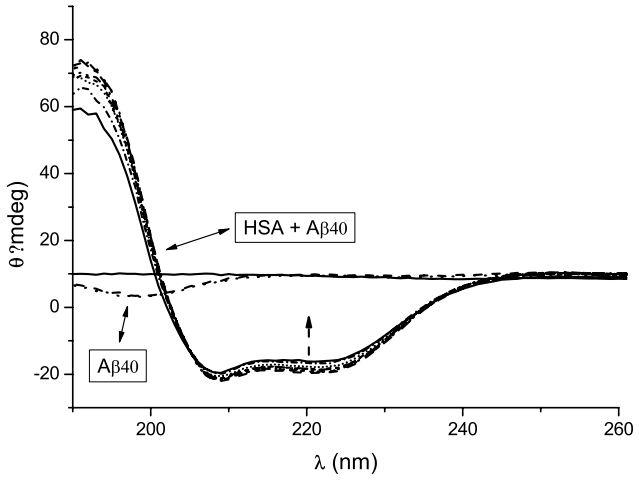

Fig. 1. A titration of 2 μM HSA with 42 μM Aβ40 from 0 to 5 mol equivalents. The dashed arrows marks the direction of changes. Two spectra of 14 μM Aβ40 are shown for a comparison.