Journal of Pharmaceutical and Biomedical Analysis 97 (2014) 88–96

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

The protease activity of transthyretin reverses the effect of pH on the amyloid-β protein/heparan sulfate proteoglycan interaction: A biochromatographic study

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ARTICLE INFO

Article history:
Received 6 February 2014
Received in revised form 18 April 2014
Accepted 21 April 2014
Available online 2 May 2014

Keywords:
Transthyretin
pH effect
Heparan sulfate proteoglycan.

ABSTRACT

Patients suffering of Alzheimer's disease (AD) are characterized by a low transthyretin (TTR) level in the brain. The effect of pH and TTR concentration in the medium on the β-amyloid protein (Aβ)/heparan sulfate proteoglycan (HSPG) association mechanism were studied using a biochromatographic approach. For this purpose, HSPG was immobilized via amino groups onto the amino propyl silica pre-packed column, activated with glutaraldehyde, by using the Schiff base method. Using an equilibrium perturbation method, it was clearly shown that Aβ can be bound with HSPG. This approach allowed the determination of the thermodynamic data of this binding mechanism. The role of the pH was also analyzed. Results from enthalpy–entropy compensation and the plot of the number of protons exchanged versus pH showed that the binding mechanism was dependent on pH with a critical value at pH = 6.5. This value agreed with a histidine protonation as an imidazolium cation. Moreover, the corresponding thermodynamical data showed that at pH > 6.5, van der Waals and hydrogen bonds due to aromatic amino acids as tyrosine or phenylalanine present in the N-terminal (N_T) part governed the Aβ/HSPG association. Aβ remained in its physiological structure in a random coil form (i.e. the non-amyloidogenic structure) because van der Waals interactions and hydrogen bonds were preponderant. At acidic pH (pH < 6.5), ionic and hydrophobic interactions, created by histidine protonation and hydrophobic amino acids, appeared in the Aβ/HSPG binding. These hydrophobic and ionic interactions led to the conversion of the random coil form of Aβ into a β-sheet structure which was the amyloidogenic folding. When TTR was incubated with Aβ, the Aβ/HSPG association mechanism was enthalpy driven at all pH values. The affinity of Aβ for HSPG decreased when TTR concentration increased due to the complexation of Aβ with TTR. Also, the decrease of the peak area with the increase of TTR concentration demonstrated that this Aβ/TTR association led to the cleavage of Aβ full length to a smaller fragment. For acidic pH (pH < 6.5), it was shown that the importance of the hydrophobic and ionic interactions decreased when TTR concentration increased. This result confirmed that Aβ was cleaved by TTR in a part containing only the N_T part. Our results demonstrated clearly that TTR reversed the effect of acidic pH and thus played a protective role in AD.

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1. Introduction

Alzheimer's disease (AD) is one of the major causes of dementia and death among the elderly (Hardy et al., 1992). In this pathology, brains are characterized by neurofibrillary tangles and the presence of senile plaques (SPs). SPs primarily consist of amyloid β protein (Aβ). Aβ is a 4 kDa protein with two common isoforms, Aβ(1–40) (which is the major Aβ sequence ≈90% found circulating

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http://dx.doi.org/10.1016/j.jpba.2014.04.021
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in cerebrospinal fluid CSF) [1] and Aβ(1–42) [2] generated from the proteolytic cleavage of a large transmembrane glycoprotein, amyloid β precursor protein (APP). Hardy and Higgins [3] and Hardy and Selkoe [4] hypothesized that the deposition of Aβ is the causative agent of AD and neurofibrillary tangles, cell loss, vascular damage, and dementia as a direct result of the deposition, which is known as the "amyloid cascade hypothesis". As Aβ has a high propensity to form aggregated β-sheet [5] protein deposits in the SPs, AD is considered as the most common form of amyloidoses in the brain. Structurally, two domains in Aβ can be distinguished: a hydrophilic N-terminal (N_T) region with residues 1–28 and a hydrophobic C-terminal (C_T) region with residues 29–40, which always exists as a β-sheet, independently of the pH and the temperature. The structure of the N_T region depends on the type of interactions involved. Zhang et al. [6] showed that the stabilization of the random coil conformation was due to van der Walls bonds [7], and the β-sheet structure is formed by hydrophobic and ionic interactions [8,9]. It is well-known that extrinsic or environmental factors, such as the pH, influence the relative proportions of the random coil, α-helix and β-sheet solution structures and modulate the aggregation of the Aβ peptide into amyloid. The relative proportions of these structures and the fibrillogenesis are highly pH-dependent [5]; pH greatly influences the conformation of the first 28 amino acids. This segment unfolds into a random-coil conformation between pH 1–4 and above pH 7. Between pH 4 and 7, Soto et al. [5] demonstrated that it rapidly precipitates into an oligomeric β-sheet structure. The structure of the N_T region determines the beginning of the fibrillogenesis. Physiologically, Aβ is probably degraded as a physiological event in the endosomal/lysosomal pathway, where the pH is below 4 in the lysosomes and below 6.5 in the endosomes [10]. Moreover, acidosis in the brain, where the pH value is under 6.6, is correlated with AD [11]. As in many amyloidoses, several other proteins with the ability to modulate amyloid fibril formation accumulate in SPs, particularly heparan sulfate proteoglycan (HSPG) [12], which has been found to be associated with all amyloid deposits. HSPG is a biologic macromolecule characterized by a core protein to which glycosaminoglycan side-chains (GAG) are covalently attached. The main role of HSPG is still unknown but it seems to be a scaffold for amyloid fibril formation. Although, due to the sulfur group of HSPG, electrostatic interactions contribute a great amount of the binding energy, hydrogen-bonding, van der Waal interactions, and hydrophobic effects are also involved in the interactions with proteins [13]. It has been reported that Aβ is sequestrated by extracellular proteins present in CSF as apolipoprotein E, apolipoprotein J, and APP [14]. Furthermore, Schwarzman et al. [15] demonstrated that TTR could bind to Aβ and they hypothesized that TTR sequestrated Aβ in CSF and plasma, therefore preventing amyloid fibril formation and deposition. TTR is a thyroxin and retinol protein transporter, which is located in both serum and CSF. In serum, TTR can lead to transthyretin amyloidoses. However, in the brain, its role is undefined. Studies have shown that the TTR level in the brain is correlated with the presence of SPs. The TTR level decreases with the SP growth [16].

In order to understand the role of the TTR in the Aβ-aggregation, the effect of pH and different concentrations of TTR in the medium on the Aβ(1–40)/HSPG association was analyzed using a biochromatographic approach.

2. Materials and methods

2.1. Reagents

Heparan sulfate proteoglycan (HSPG), isolated from basement membrane of Engelbreth-Holm-Swarm mouse sarcoma, and transthyretin from human plasma were obtained from Sigma-Aldrich (Paris, France). Amyloid β protein: the Aβ(1–40) isoform was purchased from Abcam (London, England). Potassium dihydrogen phosphate and dipotassium hydrogen phosphate, used for the preparation of the mobile phases, were of analytical grade and purchased from Merck (Paris, France).

2.2. Apparatus

The HPLC system consisted of a Shimadzu LC-10ATvp pump (Champs-sur-Marne, France), a Rheodyne 7725 injection valve (Cotati, CA, USA) fitted with a 20 μL sample loop, and a Shimadzu SPD-10A UV–vis detector (Noisiel, France). The Silice Uptisphere® 120 Å 3 μm NH_2 column (50 mm × 4.6 mm column size) was furnished by Interchim (Montluçon, France). The preparation of the heparan sulfate proteoglycan column via the in situ technique is described below. Throughout the study, a constant flow-rate of 0.3 mL/min was maintained and the temperature varied between 10 and 40 °C.

2.3. Covalent immobilization technique of HSPG on silica-NH_2 particles

The "*in situ*" immobilization technique to prepare this new HSPG column was previously used by our group [17] for the immobilization of acetylcholinesterase on an ethylenediamine (EDA) monolithic convective interaction media (CIM) disk. The immobilization of HSPG, via the amino groups of the protein, onto the amino propyl silica pre-packed column, activated with glutaraldehyde, by using the Schiff base method, was thus carried out as follows. Firstly, the column was washed with phosphate buffer saline (PBS) (pH 6.5, 0.01 M) for 30 min at a flow-rate of 0.5 mL/min. Then, the silica-NH_2 particles were activated by recycling 10% of glutaraldehyde in PBS (pH 6.5, 0.01 M) for 10 h at the same flow-rate, followed by washing with PBS (pH 6.5, 0.01 M) during 1 h. 0.2 mg of HSPG was dissolved in 50 mL of PBS (pH 7.4, 0.01 M) and the protein solution continuously circulated through the column at a flow-rate of 0.3 mL/min for at least 24 h. The column was then injected with PBS (pH 7.4, 0.01 M) for 1 h and 0.1 M of sodium cyanoborohydride in 100 mL PBS (pH 7.4, 0.01 M) circulated during 5 h for reductive amination. The column was then washed with PBS (pH 7.4, 0.01 M) for 1 h and a solution of 0.2 M ethanolamine was injected for 3 h to deactivate the aldehyde groups. To finish, 0.1% of sodium azide circulated during 1 h for column conservation purposes.

The total mass of HSPG (192 μg) in the column was determined by elemental analysis. For this analysis, four fractions of the stationary phase were removed from the head to the end of the column. The maximum relative difference of the amount of immobilized HSPG between these different measurements was always 0.5%, therefore providing a homogeneous HSPG distribution in the column from the ends to the center.

2.4. *Aβ incubation with TTR and chromatographic operating conditions*

The effect of different concentrations of TTR on Aβ/HSPG binding was determined by using the experimental method described below. Aβ, at a concentration of 20 μM, was incubated at room temperature during 3 h with different concentrations of TTR (x) (x = 0, 2, 5, 10, 15 μM). 20 μL of Aβ + x in PBS 0.01 M at pH = 7.4 were injected and the resulting retention factor was determined at seven temperatures, i.e. 283, 288, 293, 298, 303, 308 and 313 K. 20 μL of TTR, at a concentration of 20 μM, were also injected in the chromatographic system in similar conditions. The mobile phase consisted of a phosphate buffer saline (0.01 M), which was prepared by mixing equimolar solutions of mono- and dibasic sodium phosphate to

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