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Solution NMR structure and inhibitory effect against amyloid-β fibrillation of Humanin containing a D-isomerized serine residue

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ABSTRACT

Humanin comprising 24 amino acid residues is a bioactive peptide that has been isolated from the brain tissue of patients with Alzheimer's disease. Humanin reportedly suppressed aging-related death of various cells due to amyloid fibrils and oxidative stress. There are reports that the cytoprotective activity of Humanin was remarkably enhanced by optical isomerization of the Ser14 residue from L to D form, but details of the molecular mechanism remained unclear. Here we demonstrated that Humanin D-Ser14 exhibited potent inhibitory activity against fibrillation of amyloid-β and remarkably higher binding affinity for amyloid-β than that of the Humanin wild-type and S14G mutant. In addition, we determined the solution structure of Humanin D-Ser14 by nuclear magnetic resonance (NMR) and showed that D-isomerization of the Ser14 residue enables drastic conformational rearrangement of Humanin. Furthermore, we identified an amyloid-β-binding site on Humanin D-Ser14 at atomic resolution by NMR. These biophysical and high-resolution structural analyses clearly revealed structure–function relationships of Humanin and explained the driving force of the drastic conformational change and molecular basis of the potent anti-amyloid-β fibrillation activity of Humanin caused by D-isomerization of the Ser14 residue. This is the first study to show correlations between the functional activity, tertiary structure, and partner recognition mode of Humanin and may lead to elucidation of the molecular mechanisms of the cytoprotective activity of Humanin.

1. Introduction

Humanin (HN) was discovered in 2001 by Hashimoto et al. using a screening cDNA library constructed from the occipital lobe brain tissue of Alzheimer's disease (AD) patients [1]. HN is a peptide comprising 24 amino acids (MAPRGFSCLLLLTSEIDLPVKRRA) that has been encoded in the mammalian mitochondrial genome [1–3]. HN shows significant cytoprotective activity against neuronal cell death associated with familial AD and cell damage caused by inflammation or oxidative stress in hyperglycemia and ischemia [3–5].

Several HN mutants possessing greater cytoprotective activity, such as Ser14Gly mutant of HN (HN S14G) and HN variant containing D-form Ser14 residue (HN D-Ser14), have been reported [6]. Amyloid-β (Aβ) peptides are the most commonly investigated binding partners of HN at present, and HN S14G shows higher affinity [7]. Polypeptides have been found in which the incidence of isomerization of aspartate or serine residues within their sequence from the L to D form, caused by enzymatic post-translational modification, increases with aging [8]. Therefore, it is thought that HN D-Ser14 could be used as an intrinsic pro-drug against various neurodegenerative and aging-related diseases. Understanding the molecular mechanism that underlies the cytoprotective action of HN may lead to important insights and assist efforts to develop efficacious new drugs and therapeutic strategies to combat AD and other aging-related diseases.

Tertiary structures of HN wild-type and HN S14G in a 2,2,2-trifluoroethanol (TFE)/water mixtures have previously been revealed by Benaki and his co-workers using solution nuclear magnetic resonance (NMR) techniques [9,10]. Both HN wild-type and HN S14G form straight helical structures even though they

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Abbreviations

HN	Humanin
HN S14G	Ser14Gly mutant of Humanin
HN D-Ser14	Humanin variant containing D-form Ser14 residue
AD	Alzheimer's disease
Aβ	Amyloid-β
Aβ40	40 amino acid form of amyloid-β
TFE	2,2,2-trifluoroethanol
CD	Circular dichroism
TEM	Transmission electron microscopy
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
TOCSY	Totally correlated spectroscopy
HSQC	Heteronuclear single quantum coherence
DOSY	Diffusion-ordered spectroscopy

are predominantly disordered in alcohol-free water [9–11]. In the case of HN S14G it has been reported that the C-terminal site of the helical structure was slightly disordered relative to that of the HN wild-type [10]. However, it remains unknown if such site-specific conformational change is directly related to HN cytoprotective activity. In addition, the tertiary structure and functional insights of HN D-Ser14 are unknown, although D-isomerization of the Ser14 residue is expected to lead to structural and functional modulation of HN.

In an effort to elucidate molecular mechanisms of the cytoprotective activity of HN, the differences in function and tertiary structure between the HN wild-type, HN S14G, and HN D-Ser14 were carefully compared. We used Aβ as a binding partner of HN. Because the reported tertiary structures of HN wild-type and HN S14G were determined in an alcohol/water mixture [9,10], a HN binding partner should be analyzed in such a mixture for use in a structure–function correlation study. Physicochemical and structural studies of Aβ in alcohol/water mixtures have been widely conducted [12].

Here, we acquired circular dichroism (CD) spectra and performed electron microscopy of Aβ in the presence or absence of the HN variants to investigate how Aβ aggregation and/or fibrillation are influenced by interacting with HN wild-type, HN S14G, or HN D-Ser14. Then, the high-resolution tertiary structure of HN D-Ser14 was determined under the same solution condition as that used for the HN wild-type and HN S14G (alcohol/water mixture). Finally, binding modes between Aβ and the HNs were elucidated at atomic resolution by performing solution NMR experiments.

2. Materials and methods

2.1. Sample preparation

Sufficiently purified (purity >95%) peptides of HN wild-type, HN S14G, and HN D-Ser14 were purchased from Eurofins Genomics (Tokyo, Japan) and used without further purification. For NMR experiments of the HNs, peptides were dissolved in a 7:3 [v/v] H_2O/TFE-d_3 solution, and the peptide concentration was adjusted to 2.0 mM, as previously described [9,10]. Sufficiently purified (purity >95%) Aβ40 peptide was purchased from Zhejiang Ontores Biotechnologies (Zhejiang, China) and used without further purification.

2.2. Circular dichroism measurements

Far-UV CD spectra (195–250 nm) of 20-μM Aβ40 solutions after 4 days of incubation at 25 °C with ultrasonication were obtained

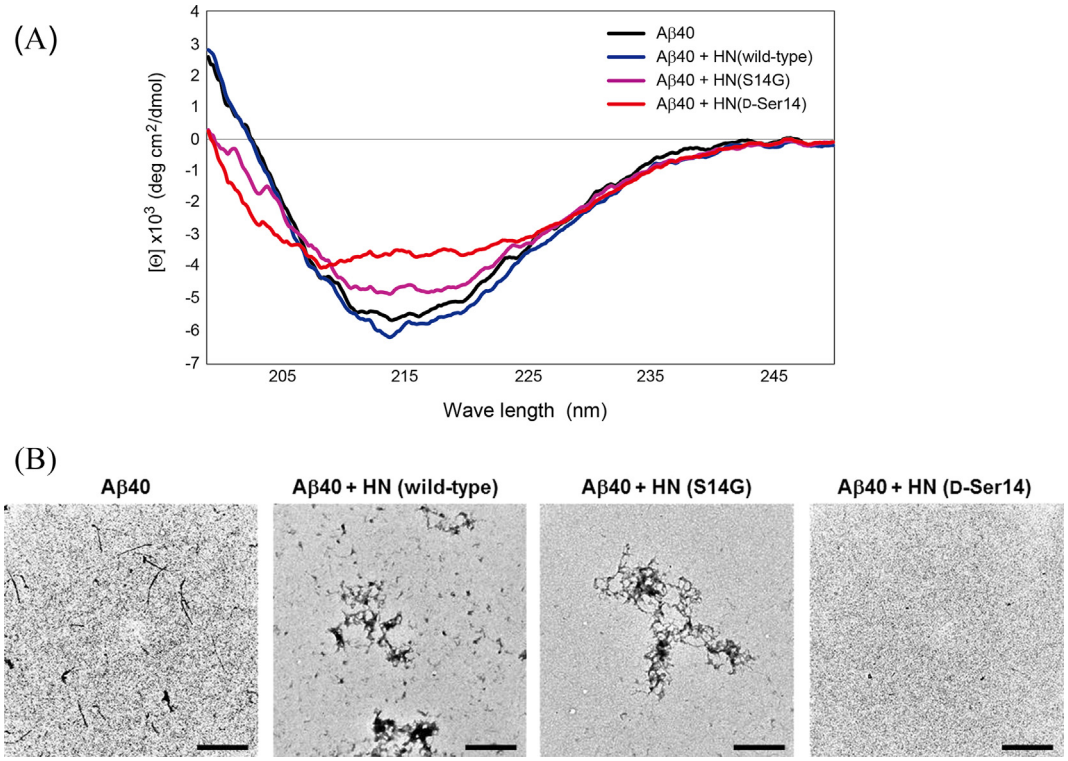

Fig. 1. Significant structural modulation of Aβ40 by HN D-Ser14. (a) Circular dichroism spectra of Aβ40 in the presence (Aβ40:HNs = 10:1) or absence of HNs. (b) Transmission electron microscopy of Aβ40 in the presence (Aβ40:HNs = 10:1) or absence of HNs. The black scale bars indicate 600 nm.