Journal of Peptide Science
J. Pept. Sci. 2006; **12**: 639–642
Published online 11 July 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.773

JOURNAL OF PEPTIDE SCIENCE

The secondary structure analysis of a potent Ser14Gly analog of antiAlzheimer peptide, Humanin, by circular dichroism

TSUTOMU ARAKAWA,[a]* TAKAKO NIIKURA,[b] HIROHISA TAJIMA[b] and YOSHIKO KITA[b]

[a] Alliance Protein Laboratories, Thousand Oaks, CA 91360, USA
[b] Department of Pharmacology, KEIO University School of Medicine, Tokyo, Japan

Received 2 March 2006; Revised 4 May 2006; Accepted 4 May 2006

Abstract: The structure of a highly potent Ser14Gly analog of antiAlzheimer peptide, Humanin, was examined by circular dichroism (CD). The secondary structure is more disordered in water than in phosphate-buffered saline (PBS). The peptide structure in water is little dependent on both peptide concentration and temperature. On the contrary, the peptide structure was significantly different in PBS from the structure in water, which is more apparent at a higher peptide concentration and temperature. The observed different structure in PBS appears to be due to self-association of the peptide, which is enhanced by elevated temperature and, hence, via hydrophobic interactions. The wild-type Humanin also behaved similarly, i.e., it assumed a disordered structure in water but underwent conformational changes in PBS. Although high peptide concentrations for CD measurements are not encountered *in vivo*, the results suggest the tendency of the peptide to interact hydrophobically with other structures as well as with itself. Copyright © 2006 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: Humanin; secondary structure; circular dichroism; Alzheimer; Ser14Gly analog

INTRODUCTION

Humanin was identified as a neuroprotective peptide against Alzheimer's diseases [1–3]. The mutational analysis of the Humanin sequence showed that a Ser14Gly (S14G) mutation resulted in a large, approximately 1000-fold, increase in *in vitro* activity, although the mechanism of the observed activity enhancement has not been clarified [4]. While extensive studies have been carried out on the biological function of this peptide [1–7], there is little study done on the solution structure. Recently, the circular dichroism (CD) and NMR structure study on Humanin was carried out showing that the peptide was primarily disordered when dissolved in water [8]. Here, we compare the structure of the S14G analog of Humanin in water and in phosphate-buffered saline (PBS) by CD. While the structure of S14G analog is similar to that of wild-type peptides and is largely disordered in water, the structure of both its wild-type and S14G mutant is significantly different in PBS from their structure in water, as reported in this paper.

MATERIALS AND METHODS

Both wild-type Humanin and S14G analog peptides were prepared by Peptide Institute, Inc (Osaka, Japan). Both peptides were dissolved in water at 2 mg/ml by weight, as has been prepared in the biological studies (1–7), and used as a stock solution. The weight of the counter ion, trifluoroacetic acid, was ignored because of its unknown quantity in the lyophilized powder. Thus, there is ambiguity in the peptide concentration determined as above. Since the peptides have a maximum of five net positive charges, the highest possible concentration of trifluoroacetic acid is ~2 mM at 1 mg/ml peptide solution. There are no aromatic amino acids in the peptide for absorbance measurement, which gives an accurate peptide concentration for CD calculation. The stock solution was further diluted with water or PBS to make different peptide concentrations. The pH of peptide solution in water was around 3.5, while pH of peptide solution in PBS was around 7.

CD spectra were determined on a Jasco J-715 spectropolarimeter at room temperature using a 0.05-cm cell. For CD measurements at constant or variable temperatures, a Peltier cell holder controlled by PTC-348WI and a 0.1-cm cell were employed. The solvent spectrum was subtracted from the sample spectrum and the subtracted spectrum converted to the mean residue ellipticity (CD signal per amino acid) using the peptide concentration, the path-length of the cell and the mean residue weight. The mean residue weight was 110.7 for S14G analog and 112 for the wild-type peptide. The thermal melting was followed at 216 nm.

RESULTS

Humanin is highly soluble at 2 mg/ml in water and was subjected to far UV CD analysis. The spectra of S14G analog at 0.2 mg/ml and 1 mg/ml are shown in Figure 1 and are similar to each other, indicating no concentration dependence of the peptide secondary

*Correspondence to: T. Arakawa, Alliance Protein Laboratories, 3957 Corte Cancion, Thousand Oaks, CA 91360, USA;
e-mail: tarakawa2@aol.com

structure. The spectrum is close to that published for the wild-type Humanin, which was characterized by NMR as a random coil [8]. It appears that the S14G mutant as well is disordered in water. Figure 1 also shows the far UV CD spectrum of the mutant at 1 mg/ml in PBS (dashed line). It is significantly different from the spectra in water, indicating that the peptide assumes a different secondary structure in PBS.

The secondary structure of S14G mutant in PBS was examined at different peptide concentrations. Figure 2 shows the far UV CD spectra at 0.1, 0.5 and 1 mg/ml, which are significantly different from each other. Thus, the secondary structure in PBS of the peptide, unlike in water, depends on peptide concentration, suggesting self-association of the peptide in PBS, but not in water. The spectrum in PBS at 0.1 mg/ml is different from the spectrum in water at 1 mg/ml, indicating that the peptide assumes a different structure in PBS from the structure in water, regardless of the peptide concentration.

The far UV CD spectrum at 1 mg/ml in PBS shows a minimum at 216 nm. This signal increased as the peptide concentration was increased. Therefore, thermal melting was examined to see whether or not such signal change is also observed with increasing temperature. Figure 3 shows the change in CD signal at 216 nm as a function of temperature using 0.2 mg/ml peptide solution. In both water and PBS, the 216 nm signal decreased linearly with temperature, indicating that a higher temperature enhances the negative CD intensity. There is no cooperative transition in both solvents, indicating no apparent melting of the peptide structure by heat. In addition, there is no apparent change in turbidity during heating (data not shown).

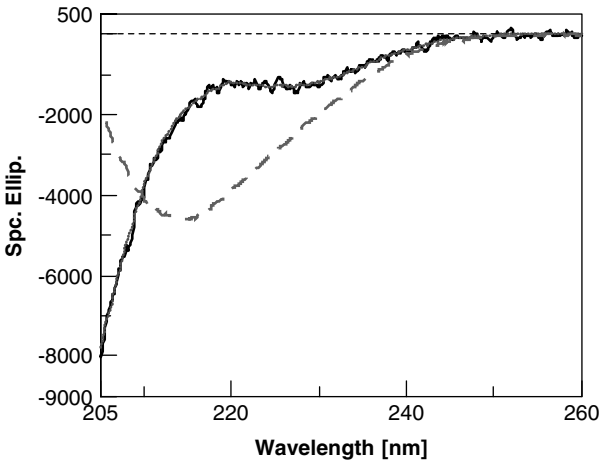

Figure 1 The far UV CD spectra of S14G mutant in water (solid line and dotted line) and in PBS (dashed line). Solid line, 0.2 mg/ml in water; dotted line, 1 mg/ml in water; dashed line, 1 mg/ml in PBS. As shown here and in the following figures, the data are obtained to 205 nm, because a high peptide concentration, and the use of PBS and a 0.1-cm cell, did not allow us to go below this wavelength.

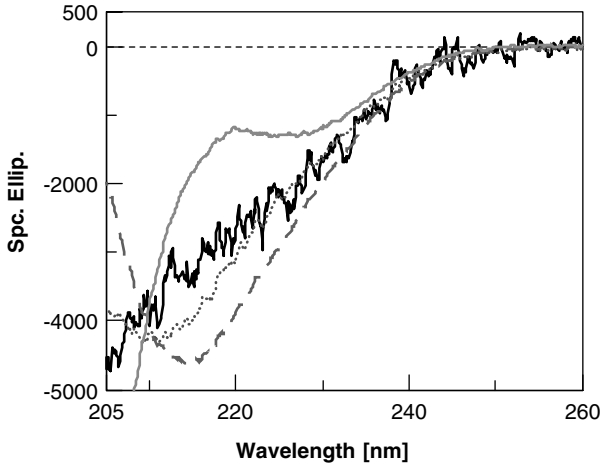

Figure 2 The far UV CD spectra of S14G mutant in PBS at room temperature as a function of peptide concentration. Solid line, 0.1 mg/ml; dotted line, 0.5 mg/ml; dashed line, 1 mg/ml. Spectrum in water (1 mg/ml) is shown in gray.

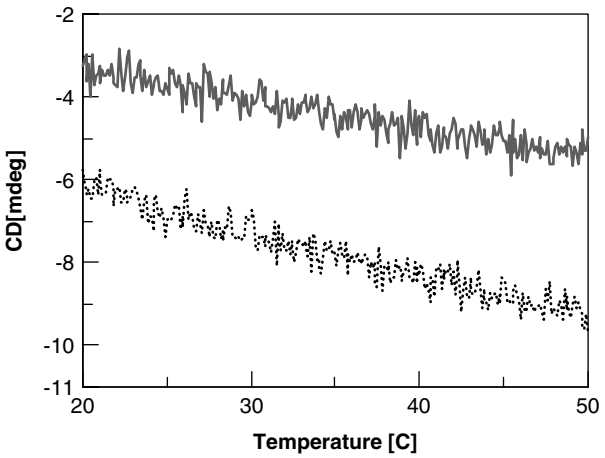

Figure 3 CD change monitored at 216 nm for 0.2 mg/ml S14G mutant in water (solid line) and PBS (dotted line). There is no apparent aggregation, as the absorbance (turbidity) at 216 nm changed little over the course of heating.

The secondary structure of S14G mutant in PBS at high temperature was then compared with its structure at high peptide concentration observed at room temperature. As shown in Figure 4, the far UV CD spectrum of 0.2 mg/ml sample was similar, if not identical, to that of 0.5 mg/ml sample at 60 °C, indicating a small peptide concentration dependence of secondary structure at 60 °C, while there was a much larger concentration dependence at room temperature as described above. These spectra are similar to the spectrum of 1 mg/ml peptide solution in PBS at room temperature. Thus, it may be concluded that both high peptide concentration and temperature convert the peptide into a similar structure.

Is the temperature-induced structure change reversible? It is not, at least in PBS within the time of

DOI: 10.1002/psc

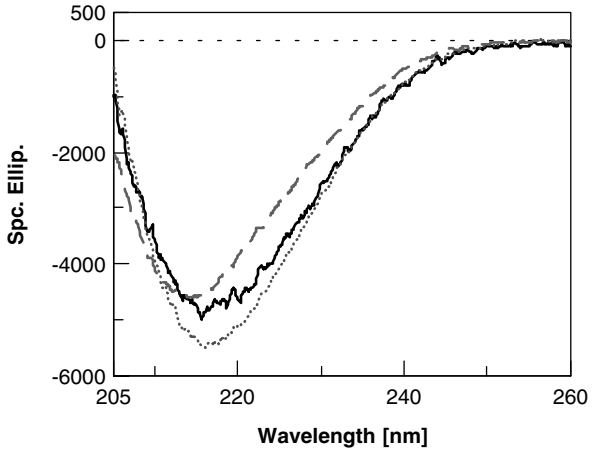

Figure 4 The far UV CD spectra of S14G mutant in PBS. Solid line, 0.2 mg/ml at 60 °C; dotted line, 0.5 mg/ml at 60 °C; dashed line, 1 mg/ml at room temperature.

incubation, as described below. As shown in Figure 5, there is a large change in the far UV CD spectrum at 60 °C (dotted line) compared with the spectrum at 20 °C (solid line). When the sample incubated at 60 °C over 40 min was brought to 20 °C and incubated for 30 min, the spectrum did not return to the original. Instead, the spectrum (dashed line) was closer to that at 60 °C, indicating, that at least, 30 min incubation at 20 °C does not restore the original structure in PBS.

On the contrary, high temperature structure change is reversible in water. As shown in Figure 6, there are some changes in the far UV CD spectrum at 60 °C, but not as drastically as in PBS, and the structure returns to the original when the 60 °C sample was cooled to 20 °C and incubated for 30 min. It may be concluded that the S14G mutant undergoes conformational transition in PBS as the peptide concentration was increased, indicating self-association of the peptide. Since the same conformational transition occurs at higher temperature, it appears that high temperature enhances such self-association, an indication that the self-association is due to hydrophobic interactions.

Finally, the structure of S14G mutant was compared with the wild-type Humanin (Figure 7). The structure in water is indistinguishable between the mutant and wild-type peptides, although an ambiguity exists in peptide concentration. The structure in PBS is similar between these two peptides, indicating that the wild-type Humanin peptide also undergoes structure change in PBS. There may be small, but significant, differences in the secondary structure in PBS between the mutant and wild-type peptides. Whether or not such structure differences between the wild-type and mutant peptides are related to the difference in their activity remains to be investigated.

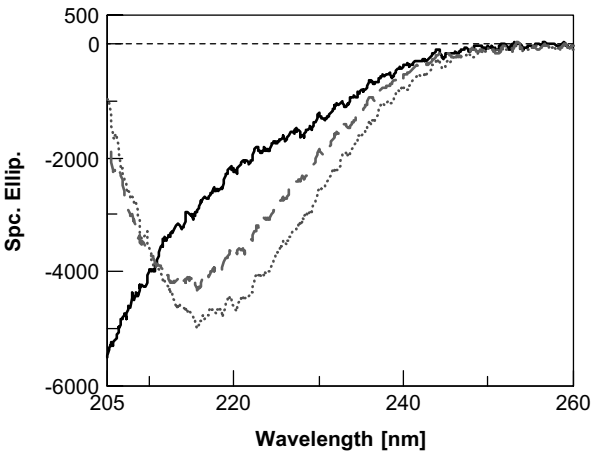

Figure 5 The far UV CD spectra of S14G mutant at 0.2 mg/ml in PBS as a function of temperature. Solid line, 20 °C; dotted line, 60 °C; dashed line, 20 °C after 60 °C heating.

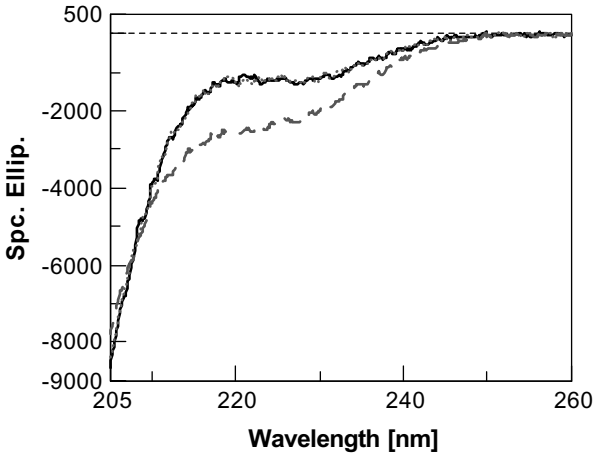

Figure 6 The far UV CD spectra of S14G mutant in water as a function of temperature. Solid line, 0.2 mg/ml at 20 °C; dashed line, 0.2 mg/ml at 60 °C; dotted line, 0.2 mg/ml at 20 °C after heating to 60 °C.

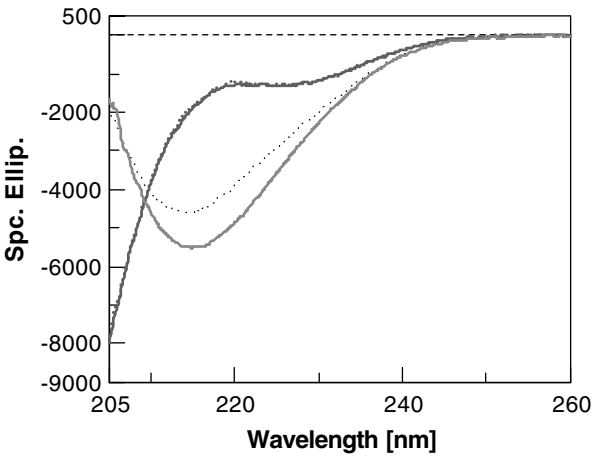

Figure 7 The far UV CD spectra of S14G mutant (dotted line) and wild-type (solid line) at 1 mg/ml in water (black) and PBS (gray). Note that the peptide concentrations of wild-type and S14G mutant were normalized on the basis of the absorbance at 210 nm.

DOI: 10.1002/psc

DISCUSSION

Short peptides are generally incapable of forming the stable secondary and tertiary structure because of the absence of long-range interactions, as observed here by the far UV CD for the wild-type Humanin and for the S14G analog in water. However, significant changes in the far UV CD spectra and, hence, the secondary structure were observed in PBS. Although CD is not a reliable technique in estimating the secondary structure rich in β-sheet and, additionally, as the spectra were obtained only to 205 nm, the structure in PBS of S14G mutant and wild-type peptides appears to be an immunoglobulin-domain type β-sheet structure. There are no other known secondary structure types that give rise to a minimum at 216 nm in the far UV CD spectrum. The structure-changes in PBS occur in the 0.1–1.0 mg/ml peptide concentration, suggesting that the affinity for self-association is in the 10–100 μM range. Although such a high peptide concentration is not encountered in biological systems, the observed results suggest a possibility that the Humanin peptide has a tendency to associate with other molecules as well as to itself, which may have resulted in the structure transition as observed in this study. In fact, self-association of Humanin into dimers and/or oligomers appears to occur under physiological conditions and seems to be required for biological activities [3,4].

Higher temperature was shown to enhance the structure-changes in PBS, suggesting the involvement of hydrophobic interaction. The S14G Humanin sequence, MAPRGFSCLLLLTGEIDLPVKRRA, contains a cluster of hydrophobic residues in the middle, which may be responsible for the temperature-dependent self-association of the peptides. In water (pH 3.5), the peptides have 4 net positive charges, which may be sufficient to prevent self-association due to charge repulsion, resulting in a disordered structure. In PBS, the decrease in net charge to 2 and a sufficient ionic strength reduce charge repulsion, leading to hydrophobic self-association of the peptides.

It should be noted that the mutation at position 14 from serine to glycine, which resulted in ~1000-fold higher biological activity [2,4], is near the above described hydrophobic cluster. The observed structure difference in PBS between the wild-type and S14G mutant may be due to the direct effects of this mutation on the structure and the hydrophobic nature of the cluster, leading to the large difference in the biological activities.

Acknowledgment

This paper is dedicated to late Prof. Ikuo Nishimoto, whose commitment to Alzheimer research resulted in the discovery of a potential therapeutic peptide, Humanin, against Alzheimer's disease. This study was supported by The Mochida Memorial Foundation for Medical and Pharmacological Research (T.N.) and Japan Society for the Promotion of Science (Y.K., T.N.).

REFERENCES

1. Hashimoto Y, Ito Y, Niikura T, Shao Z, Hata M, Oyama F, Nishimoto I. Mechanisms of neuroprotection by a novel rescue factor Humanin from Swedish mutant amyloid precursor protein. *Biochem. Biophys. Res. Commun.* 2001; **283**: 460–468.
2. Hashimoto Y, Niikura T, Tajima H, Yasukawa T, Sudo H, Ito Y, Kita Y, Kawasumi M, Kouyama K, Doyu M, Sobue G, Koide T, Tsuji S, Lang J, Kurokawa K, Nishimoto I. A rescue factor abolishing neuronal cell death by a wide spectrum of familial Alzheimer's disease genes and Aβ. *Proc. Natl. Acad. Sci. U.S.A.* 2001; **98**: 6336–6341.
3. Yamagishi Y, Hashimoto Y, Niikura T, Nishimoto I. Identification of essential amino acids in Humanin, a neuroprotective factor against Alzheimer's disease-relevant insults. *Peptides* 2003; **24**: 585–595.
4. Terashita K, Hashimoto Y, Niikura T, Tajima H, Yamagishi Y, Ishizaka M, Kawasumi M, Chiba T, Kanekura K, Yamada M, Nawa M, Kita Y, Nishimoto I. Two serine residues distinctly regulate the rescue function of Humanin, an inhibiting factor of Alzheimer's disease-related neurotoxicity: functional potentiation by isomerization and dimerization. *J. Neurochem.* 2003; **85**: 1521–1538.
5. Hashimoto Y, Niikura T, Ito Y, Sudo H, Hata M, Arakawa E, Abe Y, Kita Y, Nishimoto I. Detailed characterization of neuroprotection by a rescue factor Humanin against various Alzheimer's disease-relevant insults. *J. Neurosci.* 2001; **21**: 9235–9245.
6. Jung SS, van Nostrand WE. Humanin rescues human cerebrovascular smooth muscle cells from Aβ-induced toxicity. *J. Neurochem.* 2003; **84**: 266–272.
7. Niikuta T, Tajima H, Kita Y. Neuronal cell death in Alzheimer's disease and a Neuroprotective factor, Humanin. *Curr. Neuropharmacology* 2006; **4**: 139–147.
8. Benaki D, Zikos C, Evangelou A, Livaniou E, Vlassi M, Mikros E, Pelecanou M. Solution structure of Humanin, a peptide against Alzheimer's disease-related neurotoxicity. *Biochem. Biophys. Res. Commun.* 2005; **329**: 152–160.