International Journal of Biological Macromolecules 45 (2009) 8–11

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Short neuroprotective peptides, ADNF9 and NAP, are structurally disordered and monomeric in PBS

Tsutomu Arakawa [a,*], Takako Niikura [b], Fumio Arisaka [c], Yoshiko Kita [d]

[a] Alliance Protein Laboratories, 3957 Corte Cancion, Thousand Oaks, CA 91360, USA
[b] Department of Neurology, Georgetown University, Washington, DC 20057, USA
[c] Department of Molecular Bioprocessing, Tokyo Institute of Technology, Yokohama, Japan
[d] Department of Pharmacology, KEIO University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

ARTICLE INFO

Article history:
Received 12 February 2009
Received in revised form 23 March 2009
Accepted 24 March 2009
Available online 1 April 2009

Keywords:
ADNF9
NAP
Circular dichroism
Sedimentation equilibrium
Dodecylphosphocholine
Disordered

ABSTRACT

Activity-dependent neurotrophic factor 9 (ADNF9) and NAP are nine and eight amino acid peptides, which exhibit neuroprotective activity at femtomolar concentrations against cell toxic agents. We have here characterized their structures and interactions with dodecylphosphocholine (DPC) in phosphate-buffered saline (PBS). Circular dichroism analysis showed that ADNF9 and NAP are structurally disordered in PBS independent of peptide concentration and temperature, but appear to assume different secondary structure at increasing temperature. Sedimentation equilibrium analysis showed that both ADNF9 and NAP are monomeric at 37 °C, suggesting no self-association under physiological conditions. No secondary structure changes were observed in the presence of DPC, suggesting that ADNF9 and NAP do not interact with lipids.

1. Introduction

A short peptide, activity-dependent neurotrophic factor 9 (ADNF9), contains only nine amino acids (SALLRSIPA) and functions as a neuroprotective agent at femtomolar concentration ranges [1,2]. Another fM-active neuroprotective peptide, NAP, contains only eight amino acids (NAPVSIPQ) and, alone or along with ADNF9, exerts pharmacological effects on Down syndrome [3,4] and other disorders [5]. Because of potent pharmacological effects, the mechanistic understanding of their biological activities is urgently needed. Such an unusually high activity of these peptides, however, cannot be readily explained. First, fM means that there are only 6×10^5 molecules of the peptides per ml, which corresponds to ~6 molecules per cell, provided that a particular cell assay contains 10^5 cells per ml, more or less found in the standard cell-based assay [2]. Second, how does such a short sequence confer fM affinity for target molecules, a phenomenon difficult to understand when compared with many protein–protein interactions that have ~nM affinity [6–8]? Although little is known for the mechanism of ADNF9 function, there are some data for NAP that may shed light on their function. NAP has been shown to mediate neuroprotection through extracellular signal-regulated protein kinase and Akt pathways [9] and by binding to microtubules [10,11]. Involvement of self-association of ADNF9 has been proposed, which might enhance binding affinity for hitherto unidentified target molecules [12]. However, we have shown before that ADNF9 and its derivative are monomeric at 20 °C using sedimentation equilibrium [13,14]. Here we have further characterized the solution properties of this peptide and also NAP.

2. Materials and methods

ADNF9 and NAP were purchased from Peptide Institute (Osaka, Japan). Purity was confirmed by the manufacturer based on amino acid analysis and mass spectrometry. About 2 mg of the peptide was weighed into an eppendolf vial and dissolved with PBS at a final peptide concentration of 2 mg/ml. The stock solution was further diluted with PBS for CD and ultracentrifugation measurements. The peptide solution was also mixed with PBS containing 2% dodecylphosphocholine (DPC) for a final peptide concentration of 0.1 mg/ml. As in the previous cases, the pH of the solution was about 6.5–7.0 depending on the peptide concentration [13,14].

Far UV CD spectrum was determined at controlled temperature on a Jasco J-715 spectropolarimeter using a 0.1 cm cell. The temperature was controlled on a PTC-348WI temperature programmer and a Peltier cell holder. Solvent spectrum was obtained using PBS

* Corresponding author.
E-mail address: Tarakawa2@aol.com (T. Arakawa).

0141-8130/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijbiomac.2009.03.007

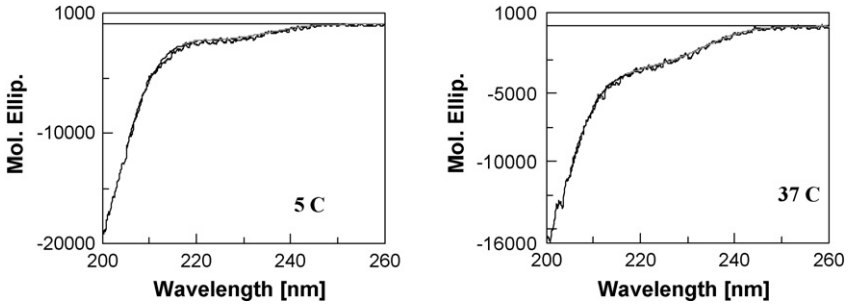

Fig. 1. Far UV CD spectra of ADNF9 at 5 °C (left panel) and 37 °C (right panel) in PBS as a function of peptide concentration. Peptide concentration: 0.1 (solid black line), 0.5 (dotted black line) and 2 mg/ml (gray line).

and subtracted from the sample spectrum. The solvent spectrum of 1% DPC in PBS was identical, within experimental error, to the PBS spectrum. The solvent-subtracted spectrum was converted to the mean residue ellipticity using the path-length of the cell (0.1 cm), the peptide concentration and the mean residue weight of 113 for both ADNF9 and NAP.

Sedimentation equilibrium experiments were carried out for ADNF9 and NAP in PBS on a Beckman analytical ultracentrifuge XL-I in a four-hole An60Ti rotor at 37 °C using double-sector centerpieces and quartz windows. In each cell, 120 μl of peptide solution in PBS was loaded on the sample side with reference side filled with PBS. Equilibrium concentration distribution of peptide was determined by absorbance at 233 nm: note that there are no aromatic residues in the peptide. Peptide concentrations of the load were 0.133, 0.200 and 0.333 mg/ml. The rotor speed was set at 40,000, 47,000 and 55,000 rpm. Scans were recorded every 2 h, from which sedimentation equilibrium was confirmed by unchanged profile of three consecutive scans. After equilibrium had been reached, data acquisition was initiated. All the scans were globally fit to a single species model by non-lin software implemented in the Beckman-Coulter software package. A theoretical partial specific volume of 0.7739 and 0.7420 for ADNF9 and NAP was used for molecular weight calculation [15].

3. Results and discussion

In the previous experiments, we first dissolved ADNF9 in water, as that was the standard protocol for bioassay [2,16]. We have here directly dissolved both ADNF9 and NAP peptides in PBS at 2 mg/ml, leading to an apparent complete dissolution. As shown later, sedimentation analysis of ADNF9 in PBS showed the peptide to be monomeric. This observation is in clear contrast to the property of another short neuroprotective peptide, Humanin (HN). HN and a Ser14Gly mutant, S14G-HN, could not be directly dissolved in PBS and can only be made soluble in water [17,18].

3.1. Concentration dependence of CD spectra

ADNF9 dissolved in PBS was diluted to 0.1 mg/ml for far UV CD analysis: note that ADNF9 does not contain aromatic amino acids, including disulfide bonds. Fig. 1(left panel) shows the spectrum measured at 5 °C, which is consistent with the disordered (irregular) structure [19] as previously reported [13,20,21]. Fig. 1 also shows the spectra at 0.5 and 2.0 mg/ml, which are identical to the spectrum at 0.1 mg/ml: note that at higher peptide concentrations, the CD measurements could not be done down to 200 nm due to high absorbance. The secondary structure at 5 °C does not change with peptide concentration, excluding a possibility of self-association and resultant conformational changes at this temperature. The results are essentially same at 37 °C. Fig. 1(right panel) shows the far UV CD spectra at 0.1, 0.5 and 2 mg/ml ADNF9 in PBS measured at 37 °C. The structure is still largely disordered independent of the peptide concentration; the spectra overlap with each other. Although the secondary structure of ADNF9 appears to be disordered at 37 °C, however, there is significant difference in the spectra between 5 and 37 °C as described later.

Fig. 2(left panel) shows the CD spectra of NAP at 0.1, 0.5 and 2 mg/ml in PBS at 37 °C. Similar to ADNF9, no concentration dependence was observed and the structure of NAP was largely disordered. At 5 °C or room temperature as well, the structure was essentially disordered, independent of the peptide concentration. It thus can be concluded that NAP also does not appear to undergo concentration-dependent self-association and concomitant conformational changes at any temperature. Fig. 2(right panel) compares the spectra of 0.1 mg/ml ADNF9 and NAP at 37 °C, showing a similar structure between them. Namely, they are highly disordered.

3.2. Temperature dependence of CD spectra

The far UV CD spectrum of 0.1 mg/ml ADNF9 was determined at 5, 37 and 60 °C: note that a temperature of 60 °C was arbitrarily chosen to examine the effects of temperature above the physiological

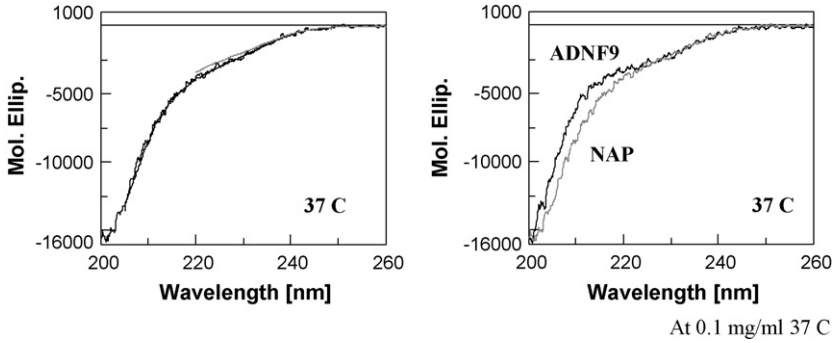

Fig. 2. Far UV CD spectra of NAP at 37 °C as a function of peptide concentration (left panel) and in comparison with ADNF9 (right panel). Left panel: 0.1 (solid black line), 0.5 (dotted black line) and 2 mg/ml NAP (gray line). Right panel: 0.1 mg/ml ADNF9 (solid black line) and 0.1 mg/ml NAP (gray line).