doi:10.1246/cl.2012.908
Published on the web August 25, 2012

Host–Guest-bridge Induces Irreversible Helix Folding in a Short Peptide

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Short peptides with or without host–guest-bridges on their side chains were prepared and the helix content was examined in the absence or in the presence of the host–guest-bridge breaker. While a short peptide without host–guest-bridge had 14% helix content, a short peptide with host–guest-bridge had 40% helix. This helix content hardly changed after the host–guest-bridge was removed. Host–guest-bridge acts as an initiator for helix folding in the short peptide.

Correct folding of protein is an essential event in living systems. Without correct folding, the protein may not work as it is expected, even worse, it may act as a poisonous molecule toward the organism.[1] All proteins have a unique structure and it should not be folded incorrectly in nature. Once the protein has folded, it rarely loses correct structure under physiological conditions, although it is not well known how the protein initiates correct folding and how it maintains this structure. When we consider the transition from random coil to helix of a short peptide, the first helical event is more difficult than the next adjacent helix. The first helical event must be a rate-determining step during whole folding process. A number of approaches have been shown for the stabilization of helix peptide with a bridge of a covalent bond between the side chains of the amino acid in the peptide.[2] Those bridges do stabilize helix structure in the short peptide. However, there are few examples of what happen if the bridge is deleted. Host–guest-bridging is one way to provide a bridge with a noncovalent bond in short peptides. Cyclodextrin (CD), a circular oligosaccharide, is capable of accommodating lipophilic molecules inside the cavity. This phenomenon could be used as a bridge with on and off switching in cyclodextrin–peptide hybrids (CD–peptides).[3] However, there are few examples of a host–guest-bridge affecting peptide folding. Even though CD–peptide has the same contents of host–guest-bridge, some of them show the same folding magnitude as native peptide sequences (without host–guest-bridge) or the bridge deletion promotes folding of the peptide. The relationship between host–guest-bridge and peptide folding has not been explained clearly.[4] CD–peptides bearing a fluorescence dye also can form association dimers.[5] In these cases, dimerization or higher level of association also stabilize folding of short peptides.[6] There are few examples of how single short peptides behave in the absence and in the presence of host–guest-bridge breakers.

The authors have hypothesized that the host–guest-bridge could be a driving force for the first helical event for the folding of short peptides. For the sake of proving this hypothesis, we have designed a novel CD–peptide which employed β-CD (seven-membered glucopyranoside) as a host and dansyl as a guest, then synthesized the CD–peptide and examined its structural behavior in an aqueous solution in response to the bridge breaker.

The CD–peptide was designed with 18 amino acids. A dansyl and a β-CD were placed on the side chains of the lysine and the glutamate at 3rd and 10th position from the N terminus,

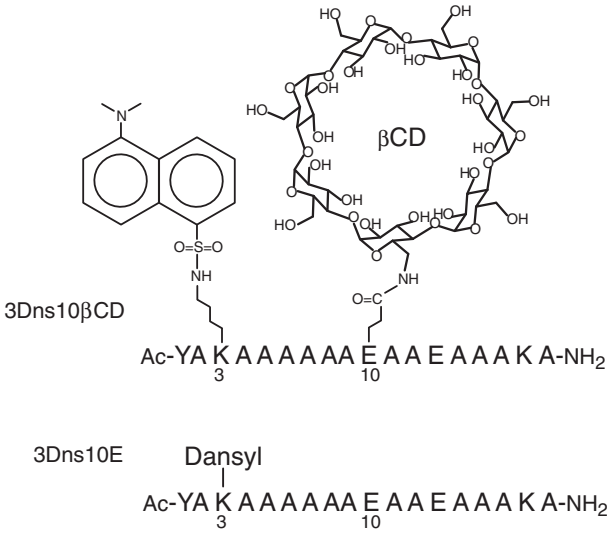

Figure 1. Schematic illustration of the CD–peptide, and the amino acid sequences of the peptides.

respectively. For solubility improvement, a pair of 13th glutamate and 17th lysine were installed (3Dns10β-CD, Figure 1). That could be an ion bridge in the helix peptide which stabilizes helix structure.[7] As a reference compound, dansyl-labeled peptide was synthesized (3Dns10E, Figure 1). The peptides were synthesized by solid-phase method and β-CD was introduced by condensation reaction between the side chain of the 10th glutamate and 6′-mono amino β-CD. The product was purified by reversed phase HPLC and evaluated by MALDI-TOF-MS.[8]

Binding magnitude between β-CD and dansylglysine was determined as the dissociation constant of 790 μM by fluorescent titration. Also the dissociation constant between 1-adamantanol and β-CD was determined to be 47 μM by competitive binding experiment. This shows that 1-adamantanol would be a good host–guest-bridge breaker as external guest molecule for the β-CD on the CD–peptide. These experiments, fluorescent titration, show that 1.5 mM of 1-adamantanol saturates complex formation with β-CD and this concentration is enough to break the host–guest-bridge in the CD–peptide.

An obvious difference was observed in the circular dichroism spectrum of 3Dns10β-CD and 3Dns10E (Figure 2). Both show typical α-helix pattern spectra with double negative maxima at 205 and 222 nm, and helix contents were determined to be 40% and 14%[9] for 13Dns10β-CD and 13Dns10E, respectively. This result clearly shows that the presence of the host–guest-bridge enhances helix content of the CD–peptide in 3Dns10β-CD. Upon the addition of 1-adamantanols, as a host–guest-bridge breaker in the CD–peptide, fluorescence intensity of 3Dns10β-CD was decreased (Figure 3) and the peak of the emission spectrum was red-shifted about 20 nm in the presence of 1.5 mM 1-adamantanol. This indicates that the dancyl moiety was excluded outside of the β-CD

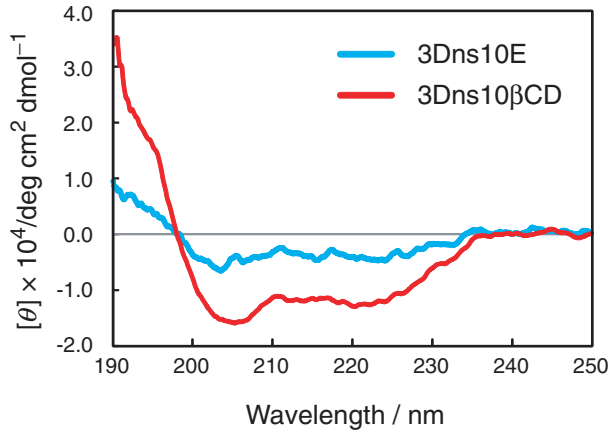

Figure 2. Circular dichroism spectrum of 3Dns10β-CD and 3Dns10E, 7.5 μM each, in 10 mM tris-HCl buffer pH 7.2.

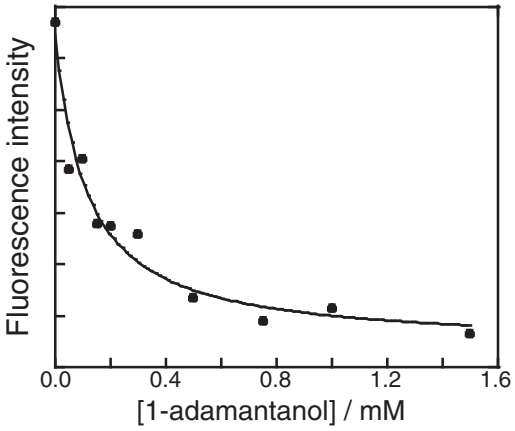

Figure 3. Fluorescence intensity of 3Dns10β-CD at 510 nm, as a function of the concentration of 1-adamantanol.

cavity by the inclusion of 1-adamantaol in the cavity. Then, the host–guest-bridge was canceled simultaneously. Over 80% of the host–guest-bridges were canceled in the presence of 1.5 mM 1-adamantanol.

Although fluorescence changed drastically, circular dichroism spectrum of 3Dns10β-CD barely changed in the presence of 1.5 mM 1-adamantanol, only when the concentration of 1-adamantanol reached 2.5 mM, the CD spectrum changed very slightly (Figure 4). Although helix content of the CD–peptide was reduced from 40% to 38%, these results indicate that even after the host–guest-bridge was broken, the CD–peptide retained its helical structure as that with the host–guest-bridge. Namely, host–guest-bridge induced irreversible folding of the CD–peptide as an α-helix. Secondary structure, such as α-helix, is constructed with hydrogen bonds of the main chain in the protein folding. On the coil to helix transition, the formation of the first helical element must have the largest energy barrier. Once the protein has fallen into the folded state, it is relatively stable enough to keep its own structure in the solution. However, some initiation reaction, hydrophobic interaction of the side chains of the amino acid sequence for example, must be needed for the correct folding of the protein. In this study, the host–guest-bridge of the CD–peptide might acts as an initiator for the formation of the first helical element in the CD–peptide. This study demonstrated that, similar

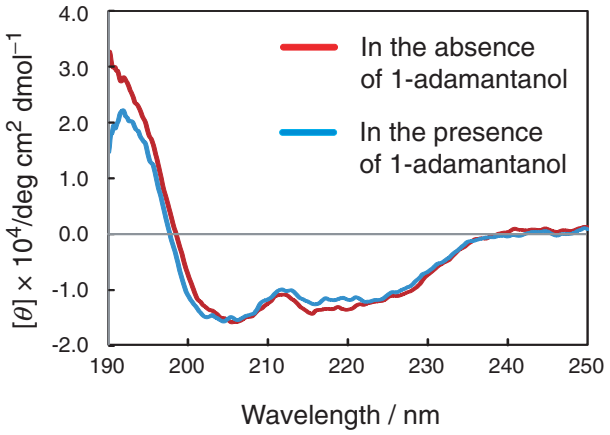

Figure 4. Circular dichroism spectrum of 3Dns10β-CD in the absence and in the presence of 1-adamantanol (2.5 mM).

to protein folding, once the CD–peptide reaches the folded state, it is considerably stable, maintaining its relative helical structure.

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