10C/2001G375

Clustering and protein-aggregate inducing effect of fluorinated alcohol

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Introduction

Fluorinated alcohols as 2,2,2-trifluoroethanol (TFE) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) are known to act as a strong denaturant of proteins breaking their secondary and tertiary structures and inducing α-helices in them. We reported in a previous work that HFIP induces α-helical segments in human calcitonin (hCT), a peptide hormone with 32 amino acid residues, to promote formation of fibrous aggregates as β-amyloid. The aim of this paper is to provide structural evidence for the interaction between HFIP molecules and hCT aggregates from analysis of solution x-ray scattering (SXS) profiles.

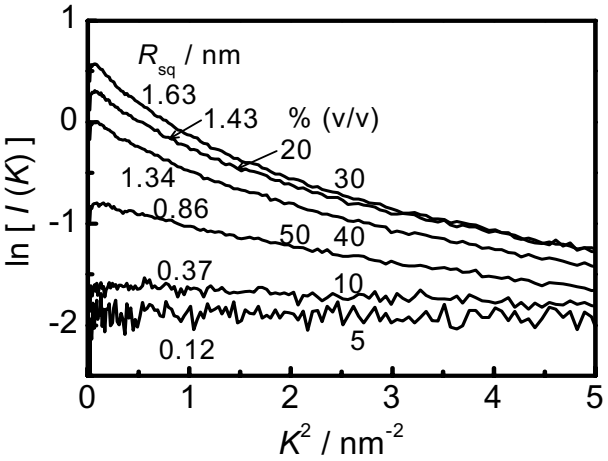
Method

Solution x-ray scattering experiments have been made using the PF beam line BL10C. First, SXS profiles of HFIP-water mixtures were measured to determine dependence on HFIP concentration of the mean-square radius of the HFIP cluster formed in solution. Then, SXS profiles of hCT in HFIP-water mixture were measured at various HFIP concentrations. To minimize damages of hCT molecules by x-ray irradiation, SXS measurement was made flowing continuously the sample solution through a sample cell. The actual x-ray irradiation time of each hCT molecule is 0.5 s. SXS data were accumulated for 5 minutes. The SXS profile of hCT in each sample solution was obtained by subtracting the SXS profile of solvent from that of solution.

Results and Discussion

The figure shows SXS profiles in the Guinier to the medium K region for HFIP-water mixtures with different HFIP concentrations. The forward scattering intensity I_f increases with increase in HFIP concentration C_{al} to reach a maximum at 30 %v/v, and then sharply decreases with its further increase. Consistently with the dependence, the mean square radius R_{sq} obtained from Guinier analysis of SXS profiles exhibits dependence on C_{al} similar to I_f: R_{sq} reaches a maximum of 1.63 nm at the same HFIP concentration of C_{al} =30 %v/v. This result indicates that HFIP molecules exist mostly in the monomeric state at C_{al} lower than 5 %v/v and, with increasing C_{al}, they begin to form clusters whose average size reaches a maximum at C_{al} =30 %v/v. It was also found from the shape of Kratky profile that the HFIP cluster is not globular but of the random-coil chain form.

We have confirmed from circular-dichroism (CD) studies that (1), in aqueous solution with pH2.0, hCT is in



the random-coil state, but (2), in HFIP-water mixture, α-helices are induced in hCT with increasing HFIP concentration. The SXS profile for hCT in the α-helical state clearly showed that hCT molecules form globular aggregates. Combining the two pieces of information, we can assume that hCT molecules, each of which has α-helical conformation, form aggregates.

Interestingly, the SXS profile of hCT, which is given by the difference between those for an hCT solution and an HFIP-water mixture as its solvent, was found to show negative values at K larger than 0.2 nm^{-1}. If the peptide solution is a simple mixture of hCT aggregates and the solvent or the HFIP-water mixture, such a negative SXS profile will never be observed. Conversely, the above result means that there exist some specific interactions of hCT aggregate with solvent or HFIP molecules. A possible mechanism for interpreting the observation will be as follows: As described above, HFIP molecules will form molecular clusters in aqueous solution. When hCT molecules are added in the solution, they will form α-helical aggregates with an aid of surrounding HFIP molecules. Then, the density of HFIP molecules in the bulk region of solvent becomes lower than that of the initial solvent, which decreases the size of HFIP clusters to decrease actual scattering intensity of solvent. Hence, the negative profile observed will be an apparent effect due to not considering this situation.

References

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