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Chiral discrimination and spontaneous crystallization of thiaheterohelicenes through uptake by zein protein: A new function of protein structure modified with sodium dodecyl sulfate

Hiroko Nakagawa *, Masashi Moriyama, Tomoko Umahashi, Yuichi Masuoka, Koh-ichi Yamada

Faculty of Pharmaceutical Sciences, Josai University, Sakado, Saitama 350-0295, Japan

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ABSTRACT

[5]Thiaheterohelicene in rapid equilibrium between the P and M enantiomers was incorporated into zein protein slightly modified by sodium dodecyl sulfate, and totally converted into the M enantiomer, which was released from the protein as pure fine crystallines.

1. Introduction

Proteins have specific functions in a specific folded conformation regulated by interactions such as hydrogen bonding, van der Waals, electrostatic, and hydrophobic interactions. Thus, if the folded conformation is changed to some extent to an unfolded one, the specific functions of these proteins are abrogated. However, relatively small changes in the protein secondary or tertiary structures can alter protein reactivity upon treatment with chemical reagents.[1] If the modified structures could provide increased accessibility of substrates to the reactive sites of protein, other functions could be readily developed.

The zein protein, a major storage protein of corn, is a type of prolamin and is practically insoluble in water; it also requires a high concentration (60–95%) of ethanol in aqueous systems to dissolve it while maintaining a folded conformation.[2,3] In the presence of an anionic surfactant, for example, sodium dodecyl sulfate (SDS), the zein protein becomes soluble in water ([SDS] > 2 mM). In such a solution, SDS molecules are incorporated into the zein, which is spread out by electrostatic interactions, forming small hydrophobic microdomains at an SDS concentration below approximately 20 mM.[4] The hydrophobic properties of the microdomains formed by the above treatment have been determined by the inclusion of pyrene molecules applied as a fluorescence probe.[5] Herein, we report the uptake and some properties of thiaheterohelicenes in such microdomains to identify a new function of the protein by circular dichroism (CD) spectroscopy.

The compound thieno[3,2-e:4,5-e′]di[1]benzothiophene ([5]thiaheterohelicene, 5TH), which was investigated as a guest molecule in the protein, and concurrently as a chiral probe, is hydrophobic and insoluble in water. In organic solvents, 5TH readily racemizes in equilibrium between the right-handed P and left-handed M enantiomers (Scheme 1), because of a rapid inversion of the molecular helix. However, the racemate 5TH is converted into either the P or M enantiomeric excess in various chiral circumstances because of the shifting of the equilibrium, to exhibit intense induced CD absorptions depending on the properties of the chiral circumstances.[6] On the other hand, the congener 7TH,[†] which has a complete skeletal helix, is stable in solution as each enantiomer and must be heated at over 180 °C to invert its helical moiety.[7]

2. Results and discussion

Proteins have been widely known to exhibit CD absorptions at 178–250 nm[8] owing to their secondary structures, and at 250–300 nm[9] owing to their tertiary structures affected by aromatic amino acid residues in chiral peptide chains.[10] Thus, we examined the effect of SDS concentration on the conformation change of the zein protein. The dependences of the CD relative intensities monitored at 223 nm (A) and 277 nm (B) on the SDS concentration in aqueous solutions are shown in Figure 1. The ordinates shown in Figure 1 were obtained from the ratio of the intensity at each concentration to the intensity at 5 mM for A and B, respectively. The intensities based on the tertiary structures (B) showed a sensitive large decrease, which was almost half the value in the narrow range of low SDS concentrations, whereas those of the secondary structures (A) seemed to undergo a small change. This suggests that the formation of microdomains in the zein protein is mainly due to the change in the tertiary structures of the protein. From the results shown in Figure 1, we fixed the SDS concentration to 15 mM in further experiments.

When 25 μl of a dioxane solution of 5TH (1×10^{-2} mol/l) was added to 5 ml of aqueous solution of the zein–SDS complex ([zein] = 0.1% = 4×10^{-5} mol/l and [SDS] = 1.5×10^{-2} mol/l), a transparent solution was immediately obtained without CD peaks

* Corresponding author. Tel.: +81 49 271 7686; fax: +81 49 271 7714.
E-mail address: hnaka@josai.ac.jp (H. Nakagawa).

† IUPAC name: bisthieno[3′,2′:4,5]benzo[1,2-b:4,3-b′]di[1]benzothiophene.

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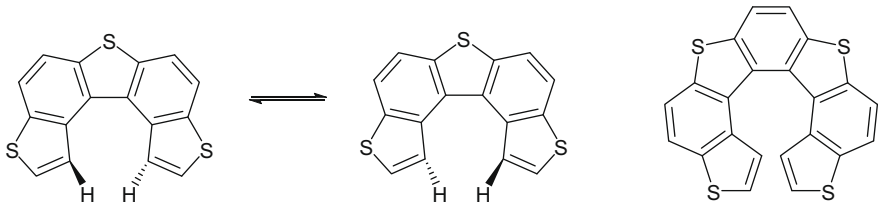


Scheme 1. Equilibrium of 5TH and 7TH.

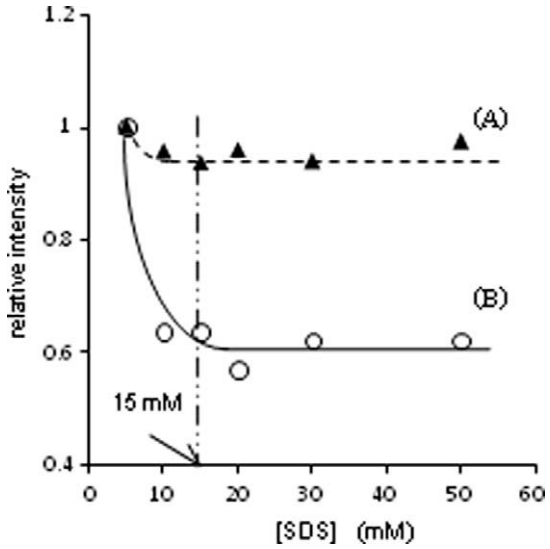


Figure 1. Effect of SDS concentration on CD intensities based on the secondary (A, monitored at 223 nm) and tertiary (B, monitored at 277 nm) structures.

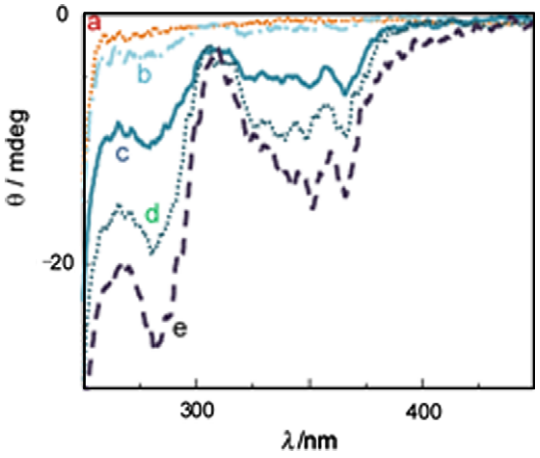


Figure 2. Change in CD spectra of 5TH in zein–SDS complex solution at 18 °C. [zein] = 4×10^{-5} M, [SDS] = 1.5×10^{-2} M and [5TH] = 5×10^{-5} M. (a) 0 min, (b) 30 min, (c) 60 min, (d) 90 min, (e) 120 min.

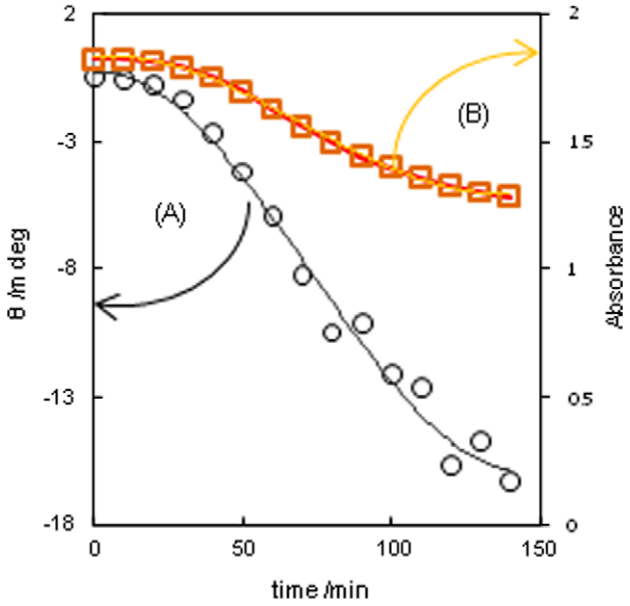


Figure 3. Time course of θ (A, monitored at 350 nm) and absorbance (B, monitored at 331 nm) of 5TH in zein–SDS complex solution.

in the absorption region of 5TH. On standing the solution for about 30 min, CD absorption peaks appeared with a negative Cotton effect at approximately 350 nm, which was attributable to the absorption of an M enantiomer of 5TH.[11] The absorption intensity at 350 nm was gradually enhanced with time (Figs. 2 and 3A). Simultaneously, fine brilliant crystalline plates with a size of less than 50 μm were generated in the solution. Increasing the degree of deposition of the crystallines, the UV absorbance at 350 nm decreased (Fig. 3B). The crystallines were separated by filtration after being left to stand overnight. The UV measurement of the filtrate showed that little 5TH remained, and no CD absorptions were observed near 350 nm. The crystallines thus obtained were confirmed to be almost pure 5TH by NMR[‡] and UV analyses. In particular, nitrogen could almost not be detected by elemental analysis,[‡] indicating that the crystallines were not zein–5TH inclusion complexes, because the observed nitrogen content of intact zein was 14.4%. The CD spectrum of the crystallines was measured by dispersing the sample in liquid paraffin (Fig. 4), since dissolving the crystallines in organic solvents negated the CD absorption because of rapid racemization. Although the spectrum shown in Figure 4 was fairly noisy owing to the difficulty in dispersing the solid homogeneously, the Cotton effect at approximately 350 nm was reproducible as a negative sign and, as a whole, was almost the same as the Cotton effect shown in Figure 2. These results indicate that the racemate 5TH was converted into M enantiomer crystallines via uptake by the zein protein. The enantiomer crystals of 5TH are thermodynamically metastable compared with stable racemic crystals,[12] just as other congener thiaheterohelicenes. Furthermore, when 5TH was added to the above-mentioned filtrate, the crystallines were gradually generated again along with induction of CD absorption. After the separation of these M-form crystallines, further addition of 5TH to the

‡ ^{1}H NMR: 270 mHz, in DMSO-d_6: δ = 8.331 (d, 1H, J = 5.61 Hz), 8.257 (d, 1H, J = 8.58 Hz), 8.122 (d, 1H, J = 6.10 Hz), 8.118 (d, 1H, J = 8.24 Hz), others are very small peaks except for water. Elemental analysis, Found: C, 61.68; H, 3.11; N, 0.18. Found for zein alone: C, 52.91; H. 7.57; N, 14.44. Calcd. for 5TH ($C_{16}H_8S_3$): C, 64.83; H, 2.72.

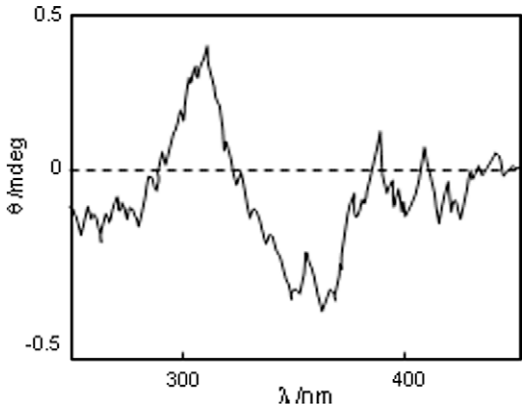


Figure 4. CD spectrum of the crystallines dispersed in liquid paraffin.

new filtrate led to almost the same situation as above, and this cycle was repeated several times. These facts imply that the zein protein modified with surfactant SDS has acquired the ability to recognize the chirality of 5TH and to induce the repeated crystallization of only the M enantiomer.

To clarify these chirality recognitions and discriminations between enantiomers for the crystallization behavior of 5TH by the zein protein, the enantiomerically stable P and M enantiomers of 7TH were used in similar experiments. Each enantiomer of 7TH was readily incorporated into the zein–SDS complex, giving a transparent solution. The solutions of both enantiomers provided intense CD absorptions that are nearly mirror images of each other, corresponding to the chirality of 7TH (Fig. 5). For both solutions, the intensities of CD absorption gradually decreased with time, together with the appearance of fine crystallines. Accompanied by the decrease in CD intensity, the UV absorptions of both solutions also decreased with an increase in the amount of crystallines generated. Therefore, the speed of crystalline formation was tentatively estimated from the decrease in UV absorbance. Figure 6 shows the time course of the UV absorbance changes at 388 nm. The decrease in absorbance for (M)7TH was completed within 30 min, whereas that for (P)7TH took over 6 h. Therefore, it was considered that the crystalline production of (M)7TH is much faster than that of (P)7TH. In other words, the zein protein preferred the crystallization of (M)7TH over that of (P)7TH as a result of the chiral discrimination between both enantiomers, in good agreement with the crystallization tendency of 5TH.

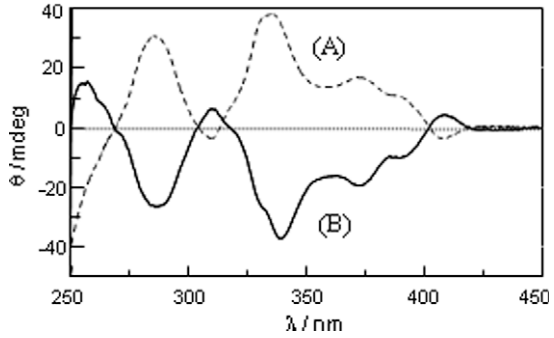


Figure 5. CD spectra of (P)7TH (A) and (M)7TH (B) in zein–SDS complex solution at 18 °C. [zein] = 4×10^{-5} M, [SDS] = 1.5×10^{-2} M and [7TH] = 1.2×10^{-5} M.

A mechanistic explanation of the aforementioned phenomenon in the case of the 5TH guest is proposed, as illustrated in Scheme 2. (1) The electrostatic interaction between the ionic head groups of the zein protein and the negatively charged SDS molecules[13]

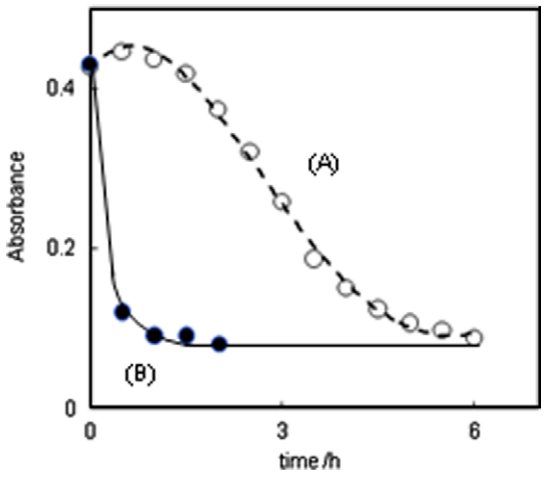
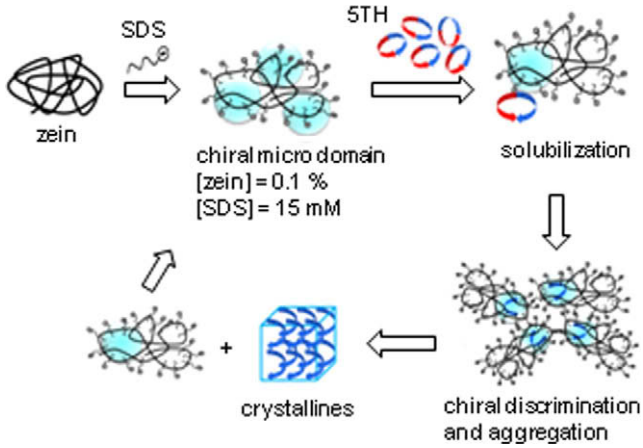


Figure 6. Time course of absorbances of (P)7TH (A) and (M)7TH (B) at 388 nm.

causes a change in the tertiary structure of the zein protein, along with a small change in the secondary structure, and stimulates the formation of hydrophobic microdomains. (2) Guest 5TH molecules are dissolved by adsorption on the surface of zein–SDS complexes. In this step, no chiral discrimination occurs yet, yielding no CD absorption. (3) Gradually, the subsequent uptake of 5TH into the chiral microdomains allows the chiral discrimination between the 5TH enantiomers by the protein, totally converting the racemate 5TH into the M enantiomer. (4) Many zein–SDS complexes carrying (M)5TH in their microdomains, associate with each other, presumably because of the mutual hydrophobic attraction, forming a chiral field for the crystallization of chiral 5TH molecules. (5) The growing crystallines of (M)5TH are slowly released from such a field, thereby gradually increasing CD intensity.



Scheme 2. Proposed mechanism for chiral discrimination and crystallization of 5TH by zein protein.

3. Conclusion

The similar chiral discrimination and chirality conversion that we have described here for thiaheterohelicenes using the zein protein, have been reported for various enzymes and proteins, for example, lipase[14] and serum albumins of some species.[15,16] However, the successive processes of chiral discrimination and chiral crystallization of small molecules as substrates have been noted for the first time in this study. Therefore, it is presumed that these phenomena may confer a new function on various proteins whose

structures are modified by various surfactants. In fact, similar chiral crystallizations such as the aforementioned process are observed for other proteins such as lysozyme and lactoglobulin; they are now under extensive investigation.

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