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HSP90-like artificial chaperone activity based on indole β-cyclodextrin

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Abstract—Indole β-cyclodextrin (β-1) was found to be able to prevent aggregation of citrate synthase (CS) on heating condition. As a result, β-1 showed anti-CS aggregation in this system by regulating in early stage. The depression mechanism of β-1 for aggregation of CS is as follows: the β-1 formed a complex with hydrophobic parts of the β-sheet structure of CS. From CD spectra, CS was changed own conformation was changed by β-1 addition. So, it was concluded that β-1 works as β-sheet inducer in thermal condition. On the other hand, native β-cyclodextrin (β-CyD) shows small suppression capability for CS aggregation.

1. Introduction

Protein aggregation coursed unfolding reaction on the thermal condition, both in vivo and in vitro, is well known to get low yields of native proteins.

Recently, there were a lot of developing strategies such as prevention of protein unfolding and constraint of aggregation and refolding native proteins artificially in the presence of any chemically synthesized compound such as detergents or urea.[1–5]

The strategy of artificial chaperone employs smaller molecules than protein, such as detergent, to promote folding from chemically denatured protein. Most of the studies on artificial chaperone were intended for chemically denatured state unfolded protein by detergent. It was reported that the detergent was stripping from protein-detergent complex by an addition of CyD.[2] In these reactions, it means that CyD works as remover of detergent, not as chaperone.

Our new strategy is targeted at prevention of thermal denaturation of protein and construction of a model like HSP90 ATP-independent chaperone based on modified CyD. CyDs are torus-shaped cyclic oligomers of D-glucopyranose unit named α-, β-, and γ- for 6, 7, and 8 units, respectively. The most interesting ability of CyDs is to exhibit inclusion with various organic molecules into the hydrophobic cavity in aqueous solution and has been widely used as functional units of supramolecular and molecular recognition systems.[6–8]

Previously, we have studied molecular recognition system of dansyl modified β-CyD for proteins such as HSP70 and 90.[9] It was indicated that dansyl modified β-CyD showed 50% chaperone activity for CS in comparison with HSP90 activity. For further an extension of the work, we synthesized 6-(2-indole-3-carboxylate-aminoethyl)-amino-6-deoxy-β-CyD (β-1) to investigate the effect of β-1 on the early stages of protein thermal denaturation. In this contribution, we would like to report the efficiency of a protection against CS thermal aggregation and transformation of CS to the secondary structure in the early stage by β-1.

2. Results and discussions

2.1. Anti-protein aggregation activity

The action in protein folding and unfolding reactions in vitro was studied in the presence or absence of β-1. Figure 1 shows the depression effect of native CyDs and β-1 on thermal aggregation of CS, in which

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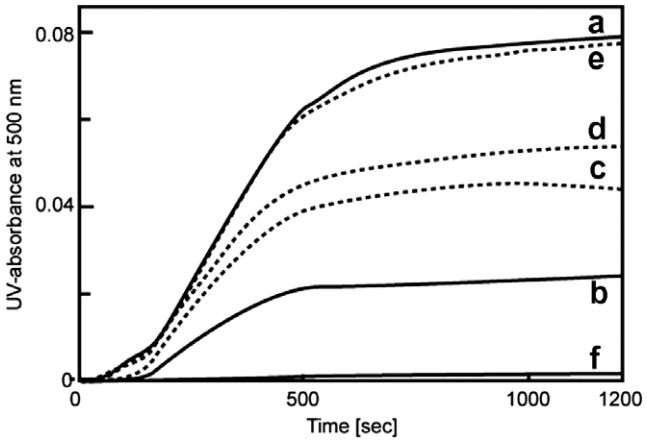


Figure 1. Chaperone activity of HSP90, β-1, and native CyDs for CS. CS (0.01 μM) aggregation on heat condition affected by the absence (a) and presence (b) of β-1 (8.0 μM), and in the presence of native CyDs (8.0 μM) (c: β-CyD, d: γ-CyD, and e:α-CyD) and HSP90 (0.04 μM) (f).

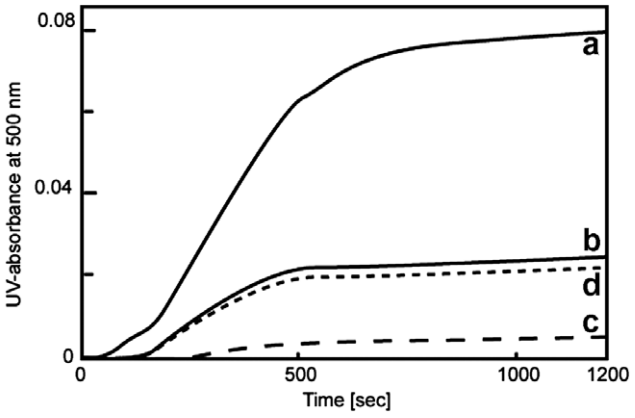


Figure 2. The effect of time-dependence of addition of β-1 on chaperone activity. CS (0.01 μM) aggregation in the absence (a) and presence (b) of β-1 (8.0 μM) applied at same time as heating, and in the presence of β-1 (c) applied in 10 s of heating, and applied in 30 s of heating (d).

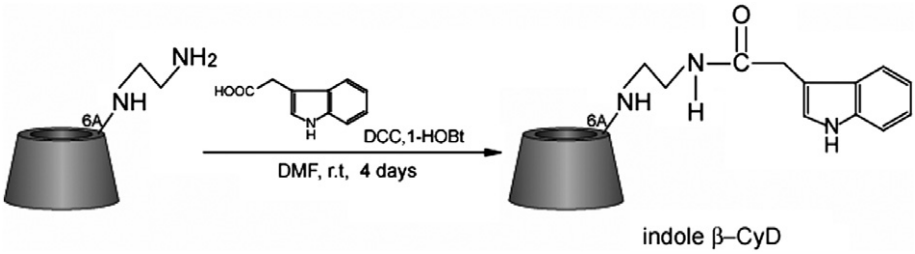
β-CyD showed still small capability for suppression of CS aggregation. On the other hand, α-CyD showed no ability for prevention of CS aggregation. However, γ-CyD showed a little ability for prevention of CS aggregation, but it is not so effective as that of β-CyD. β-CyD indicated the most effective ability among the native CyDs such as α-, β-, and γ-CyD. It was necessary to improve the ability of β-CyD, because the anti-aggregation effect of β-CyD is half the value of HSP90. So, we synthesized indole modified β-CyD named as β-1 as shown in Scheme 1, because it is shown in several reports[10,11] that indole unit is able to bind proteins with high affinity. However, it was indicated that indole-3-acetic acid did not show any activity for protein anti-aggregation. But, β-1 showed higher effective prevention of CS aggregation than β-CyD. It is suggested that the improvement of this capability was caused by modification of β-CyD with indole unit. It was really indicated that introduction of indole unit to β-CyD works to promote a suppression capability of thermal CS aggregation. When HSP90 was present in such a condition, CS was not aggregated (Fig. 1, line f). In the present study, it was shown that β-1 showed anti-aggregation activity for CS in thermal condition, but not enough in comparison with the activity of HSP90. The efficiency of β-1 for depression of CS aggregation increased by 50% compared to β-CyD.

Figure 2 shows aggregation profiles of CS, when β-1 was added since CS was heated at 50 °C. The addition of β-1 in 10 s of heating at 50 °C, CS aggregation was prevented more effectively than an addition of β-1 at just starting of heating, simultaneously. An addition of β-1 in 30 or 60 s since heating started, the prevention effort for aggregation of CS was not recognized. The time-dependent effort prevention CS aggregation was recognized when β-1 was added to CS under heating condition.

2.2. Circular dichroism spectra and conjugation of CS secondary structure

From circular dichroism (CD) spectra, we can estimate the content of secondary structure of protein. As shown in Figures 3–5, the CD spectra of CS depended on the time when β-1 was added to CS under heat processing. The α-helix has been characterized as follows; the negative band at 222 nm is due to n–π* transition of peptide, while the negative band at 208 nm and positive band at 192 nm resulted from the exciton splitting of the lowest π–π* transition. The β-sheet has been determined by the negative band at 216 nm, which is assigned as the n–π* transition and the positive band at 195 nm which belongs to exciton splitting of the lowest π–π* transition.[12]

It is well known that CS in native state consists of α-helix and no β-sheet. From PDB database, it is reported that CS only *consists of* α-helix in crystallography.[13] The CD spectra patterns of native CS showed the negative bands at 222 and 208 nm and the positive bands at 192 nm (Fig. 3, line a). The observed spectrum was in agreement with spectral assignment of α-helix and no assignment of β-sheet.



Scheme 1. Preparation of indole β-CyD.

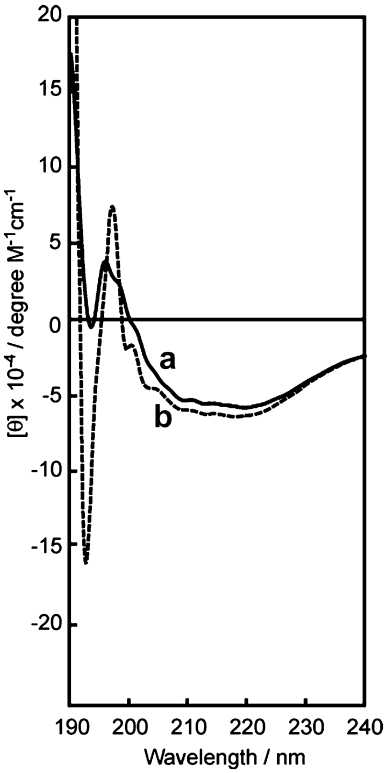


Figure 3. CD spectrum of CS (0.01 μM) native (a), heated for 1 min (b).

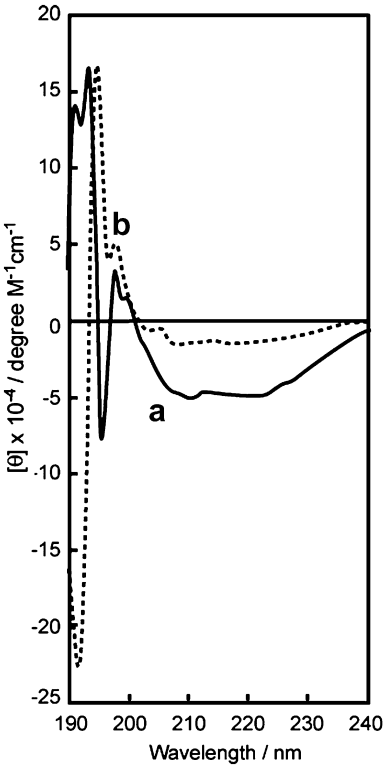


Figure 4. CD spectrum of CS (0.01 μM) heated for 1 min (a), heated for 2 min (b) in the presence of β-1 (8.0 μM).

The CD spectra of CS heated in 1 min showed the negative bands at 222 and 208 nm and positive band at 195 nm. It indicated that the secondary structure of CS was consisting of α-helix and β-sheet (Fig. 3, line b). Therefore, this result showed that the own structure of CS was easily changed by heat. But it did not completely progress toward aggregation on this condition.

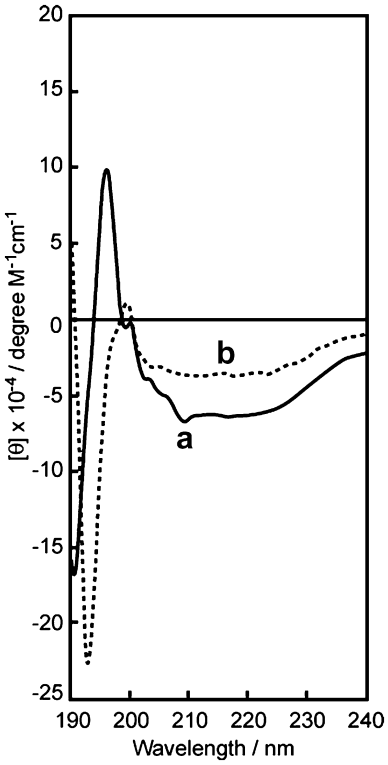


Figure 5. CD spectrum of CS (0.01 μM) heated for 1 min (a), heated for 2 min (b) in addition of β-1 (8.0 μM) applied in 10 s of heating.

Figure 4 shows the CD spectra of CS heated in the presence of β-1. In the case of 1 min heating, were recognized the negative bands at 222 and 207 nm and the positive 192 nm band. In this pattern, it is very similar to that of native CS and this pattern indicated that CS consisted of α-helix. However, in the case of 2 min heating, the spectra did not show the negative bands at 222 and 208 nm, which derived from α-helix. The positive band at 195 nm, which indicated β-sheet structure, is only observed. It was indicated that CS did not aggregate at this point. Figure 5 shows the CD spectra of CS in addition of β-1 in10 s of heating.

For 1 min heating, the negative 222 and 208 nm and the positive 195 nm bands were observed. It is indicated that the protein in this condition was consisting of α-helix and β-sheet. For 2 min heating, the CD spectra showed negative band at 192 nm and small positive band at 198 nm, which indicated that CS contains α-helix and β-sheet structures.

It is clear that time-dependence for addition of β-1 was recognized, because in 10 s addition, it was observed that CS contains α-helix and β-sheet, and an alternation of CS to secondary structure on thermal condition. In this study, it is suggested that β-1 has chaperone activity

for CS as well as act as an inducer of α-helix to β-sheet transformation of CS on thermal condition.

2.3. Estimation of the mechanism of prevention and conformational change of CS by β-1

The mechanism of stabilization of CS by β-1 seems to originate from the recognition capability of disordered proteins, which have many smaller hydrophobic moieties, which can be included in the CD cavity. In thermal condition of our experiment, disordered CS might have such moieties. It is well known that CyDs exhibit inclusion capability with a variety of organic compounds such as aromatic groups or steroidal compound into the hydrophobic cavity in aqueous media.[6] It might be possible that β-1 recognizes small local part of disordered CS, which came out in 10 s of heating. It means an addition of β-1 in 10 s later since heating was very effective for CS thermal aggregation.

CS in native state does not have any binding site, but in denatured state, CS own secondary conformation changed by heat, resulting in side chains of hydrophobic amino residues becoming exposed. This state was similar to molten globule (MG) state.[14,15] The MG state was viewed as protein disordered of local change. Disordered proteins were classified into two types, possessing a hydrophobic core and without hydrophobic core, as reported by Chang et al.[14]

The theory was formulated according to time-dependence for effective anti-aggregation of CS by β-1.

In native state, β-1 did not detect exposed aromatic or hydrophobic residues of CS because CS has only α-helix conformation. When it was heated for 10 s at 50 °C, denatured CS will have exposed aromatic or hydrophobic moieties, which were detected by β-1. If β-1 catches those moieties, indole unit of β-1 moves out from CyD cavity and binds a part of hydrophilic part of denatured CS as schemed in Figure 6, result in β-1 promoted transformation for β-sheet. It is well known that the indole unit is amphipathic. Therefore, addition of β-1 in 10 s of heating could make most effective prevention of thermal aggregation because hydrophobic residues of denatured protein make a complex with β-1.

Jakob and coworkers studied interaction of HSP90 with unfolding intermediates of CS on thermal condition.[16] They described the following protein aggregation model as shown in Scheme 2. They concluded that HSP90 binds transiently very early unfolding intermediates, prevents aggregation, and allows active species to stabilize. Our depression mechanism was very similar to Jakob's model as illustrated in Scheme 3. So, it is suggested β-1 showed a HSP90-like chaperone activity. The β-1 made a complex with disordered CS and suppressed irreversible thermal aggregation.

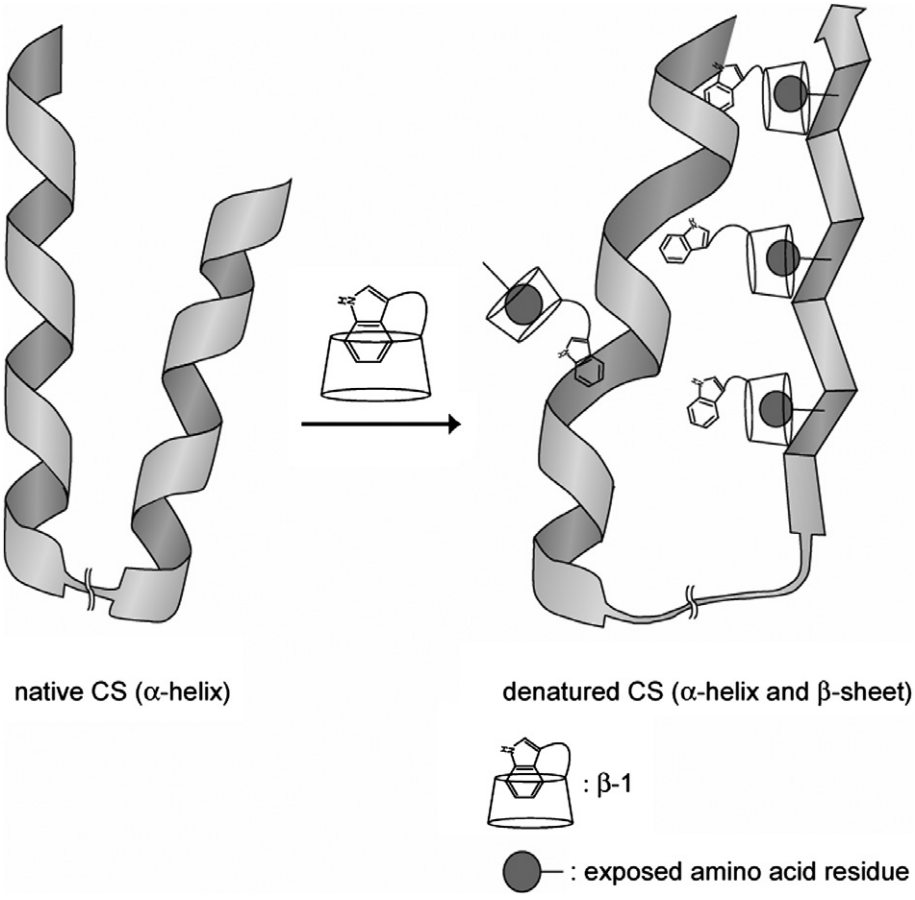
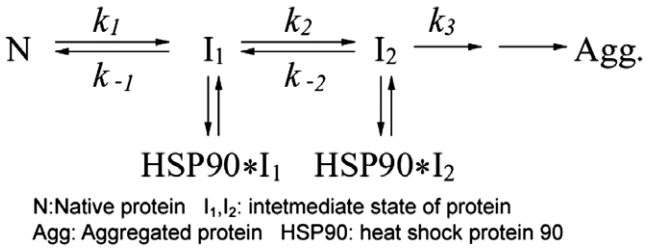


Figure 6. The complex model of denatured CS with β-1.



Scheme 2. Thermal unfolding pathway of CS in the presence of HSP90 proposed by Jakob et al.

3. Conclusion

We have synthesized β-1 to examine the ability of prevention of CS thermal aggregation. The β-1 showed more efficient chaperone activity than native β-CyD and time-dependence of β-1 addition for anti-thermal aggregation was recognized. From CD spectra, CS conformational change was effected by β-1 addition 10 s later of heating. The β-1 induced α-helix to β-sheet transformation of CS and resulted in resistance to irreversible thermal aggregation.

4. Materials and methods

4.1. Materials

Citrate synthase (CS) was purchased from Roche Diagnostics (Tokyo, Japan). CyDs were a gift from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). Indole-3-acetic acid and DMF were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). 1-Hydroxybenzotriazole (1-HOBt) and dicyclohexylcarbodiimide (DCC) were commercially available (Tokyo Chemical Industry Co., Ltd). 6-(2-Aminoethyl)-amino-6-deoxy-β-CyD was synthesized according to the procedure reported previously.[17]

4.2. Preparation of 6-(2-indole-3-carboxylate-aminoethyl)-amino-6-deoxy-β- CyD (β-1)

DCC (0.137 g, 0.66 mmol) was added to a cooled solution (−10 °C) of indole-3-acetic acid (0.107 g, 0.61 mmol) and 1-HOBt (0.089 g, 0.66 mmol) in 10 ml DMF. The reaction mixture was stirred at −10 °C for 2 h. To a stirred solution was added portionwise 6-(2-aminoethyl)-amino-6-deoxy-β-CyD (0.600 g, 0.51 mmol), the solution was stirred for another 1 h at −10 °C, and then the reaction mixture was stirred at room temperature for 4 days. After stirring, the reaction mixture was concentrated under reduced pressure. The residue was poured into ca. 300 ml of acetone. The resulting precipitates were filterted and dried. The water soluble fraction was applied to a CM-Sephadex C-50 column (350 × 50 mm). Stepwise elution from 1500 ml of water and 1200 ml of 0.1 vol% ammonia solution was applied to give the pure product of β-1. The fractions containing products were collected and evaporated in vacuo, and they were poured into 500 ml of acetone. The resulting precipitates were filtered and dried to afford 417 mg (61.4%, isolated yield) of pure β-1.

^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ = 3.2–3.7 (42H, m, C^2H–C^6H of CyD), 4.8–4.9 (6H, m, O^6H of CyD), 5.7–6.0 (14H, m, O^2H and O^3H of CyD), 6.9–7.1 (2H, m, aromatic-H of indole), 7.1–7.2 (1H, s, aromatic-H of indole), 7.3–7.4 (1H, d, aromatic-H of indole), 7.5–7.6 (1H, d, aromatic-H of indole).

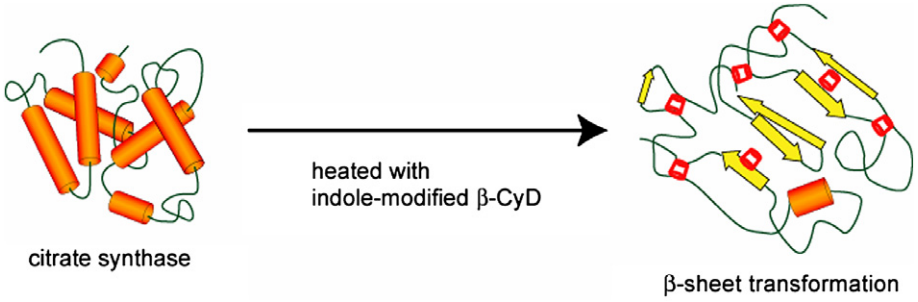
FAB-MS (*m*/*z*) 1334. Calcd for $C_{54}H_{83}N_3O_{35}\cdot 6H_2O$: C, 44.97;H, 6.63; N, 2.91. Found: C, 45.00; H, 6.33; N, 2.84.

4.3. Methods

4.3.1. Measurement of protein aggregation. The influence of HSP90 and β-1 on the thermal aggregation of CS at 50 °C was monitored as procedure described previously.[18] To monitor thermal unfolding/aggregation, the CS concentration was 0.01 μM in 50 mM HEPES buffer (pH 8.0), HSP90 (0.04 μM), and native CyDs (8.0 μM) and β-1 (8.0 μM). Light scanning of CS was monitored over 20 min by measuring the optical density at 500 nm in a spectrophotometer equipped with a temperature control unit using semi micro-cuvette (0.5 ml) with a path length of 10 mm. In this study, 1 arbitrary unit denotes an absorbance of 0.08 and at 500 nm.

4.4. Far UV–circular dichroism spectra

Circular dichroism (CD) spectra were measured using a J-720 spectropolarimeter (JASCO) as procedure described previously.[18,19] Fifty millimole HEPES buffer (pH 8.0) containing or absence of β-1 (8.0 μM) in a cuvette with a path length of 0.5 mm was used as a blank.



Scheme 3. The concept of prevention of thermal CS unfolding in the presence of β-1.

All scans were carried out at 25 °C in a region at 240–190 nm.

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