*Journal of Inclusion Phenomena and Macrocyclic Chemistry* **40:** 83–88, 2001.



# The Effect of Dansyl-modified β-Cyclodextrin on the Chaperone Activity of Heat Shock Proteins

FUMIO HAMADA[1,*], MIYUKI NARITA[1], AKIRA MAKABE[1] and HIDEAKI ITOH[2,*]
[1]*Department of Materials-process Engineering and Applied Chemistry for Environments, Faculty of Engineering and Resource Science, Akita University, Tegata, Akita 010-8502, Japan;* [2]*Department of Biochemistry, Akita University School of Medicine, Hondo, Akita 010-8543, Japan*

(Received: 28 July 2000; in final form: 1 November 2000)

*Key words:* molecular recognition, β-cyclodextrin, dansyl, HSP70, HSP90, chaperone activity, trypsin degradation.

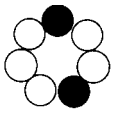
**Abstract**

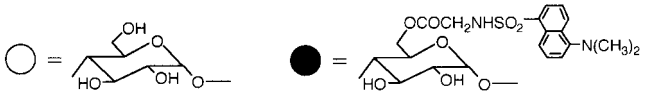
The effect of dansyl modified β-cyclodextrin (**1**) on the chaperone activity of heat shock proteins such as HSP70 and HSP90 has been studied. The fluorescence intensity of **1** was decreased when HSP70 and HSP90 were added to the host solution. This phenomenon suggested that host–guest complexation was occuring. The binding constants of **1** were obtained using a 1 : 1 complex formation type equation by employing the guest-induced fluorescence variations. Host **1** exhibited a higher binding ability for HSP70 than for HSP90. The effects of **1** on the chaperone activity and degradation of HSP70 and HSP90 were studied by measuring the absorption of aggregation of citrate synthase (C.S.) and sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis of trypsin degradation, respectively. Host **1** can contribute to regulate C.S. aggregation and promote trypsin degradation of HSP70 and HSP90.

## Introduction

Cyclodextrins are torus-shaped cyclic oligomers of D-glucopyranose and named α-, β- and γ- for the hexamer, heptamer and octamer, respectively. They can include a variety of organic compounds in their hydrophobic cavity in an aqueous solution [1, 2]. For at least a decade, we have discussed fluorescent molecular sensing systems based on cyclodextrins modified with fluorescent active units such as naphthalene [3], anthracence [4], fluorescein [5], terphenyl [6], pyrrolinone [7], anthranilate [8–12], and dansyl [13–16]. In these papers, we described how these fluorescent cyclodextrin derivatives showed high sensing abilities for bile acids such as lithocholic acid, chenodeoxycholic acid and ursodeoxycholic acid. It is especially interesting that bis dansyl-modified β-cyclodextrins showed higher sensitivity and selectivity for these guests than bis dansyl-modified γ-cyclodextrins [16]. As mentioned above, these fluorescent cyclodextrin derivatives show high sensitivity and selectivity for small guests.

In contrast, there are few reports which describe an interaction between cyclodextrins and large molecules such as biopolymers. Ikeda *et al.* reported some interaction between thryl(alkylamino)cyclodextrin and DNA [17]. For that reason, we have tried to study the molecular recognition property of **1** with proteins such as heat shock proteins. Heat shock proteins (HSP) are synthesized in response to external stresses [18, 19] such as a sudden increase in temperature [20]. The predominant classes of stress proteins including GroE, HSP70, HSP90, and small HSPs have been implicated in protein folding as molecular chaperones [21]. Although the crystal structure of the N-terminal fragment of HSP90 (about 25 kDa) has been reported recently [22, 23], the complete crystal structure of HSP90 has not been reported.






In the present study, we describe the binding ability of **1**, as illustrated in Scheme 1, for HSP70 [24] and HSP90 [25, 26] with molecular weights of 70,000 and 90,000 (dimer 180,000 *in vivo*), respectively, and the influence of this fluorescent cyclodextrin on the chaperone activity of citrate synthase (C.S., MW = 160,000, dimer) and trypsin degradation activity for these heat shock proteins. Fluorescence spectra showed that host **1** recognizes these heat shock proteins, changing the fluorescence spectra, and measurement of the absorption of aggregation of C.S. shows as much as 25% chaperone activity for C.S. in the presence of HSP90. Furthermore, hydrolysis of HSP70 and HSP90 by trypsin by measuring sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis exhibits different behaviors in the pres-

* Authors for correspondence.

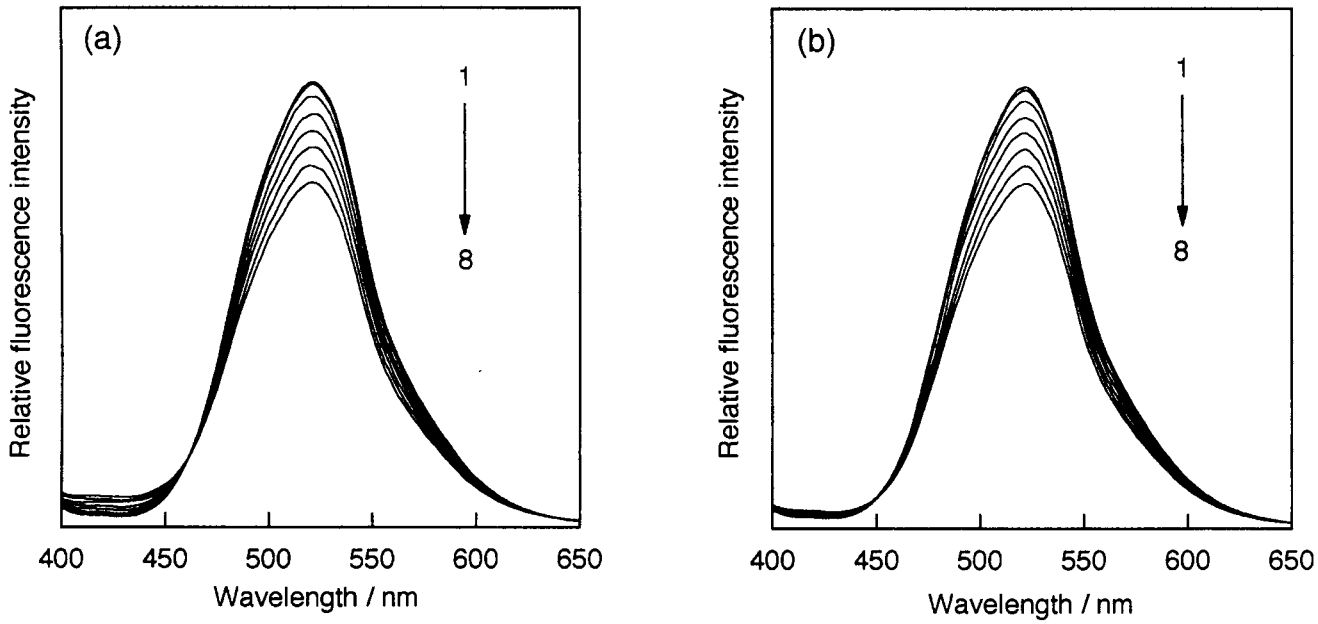



*Figure 1.* Fluorescence spectra of host **1** ($1.0 \times 10^{-6}$ M) in a1 vol.% ethylene glycol HEPES ($1.0 \times 10^{-4}$ M, pH7.5) solution at various concentrations of HSP70 (a) and HSP90 (b) (1: 0, 2: $1.6 \times 10^{-5}$, 3: $3.8 \times 10^{-5}$, 4: $7.4 \times 10^{-5}$, 5: $1.1 \times 10^{-4}$, 6: $1.4 \times 10^{-4}$, 7: $1.7 \times 10^{-4}$, 8: $1.9 \times 10^{-4}$ M).

ence and absence of **1** or ATP, in which the host indicates promotion of trypsin degradation for these proteins in the absence of ATP.

## Experimental

### *Materials*

All chemicals were of general purpose reagent grade unless otherwise stated. $6^A$,$6^D$-Bis dansyl-modified $\beta$-cyclodextrin was prepared according to the previously reported procedure [16]. Bovine HSP90 was purified from brain [27, 28] and bovine HSP70 was purified from brain using ATP-Sepharose as described previously [29].

### *Analytical techniques*

Spectrophotometric readings were taken using a Perkin-Elmer LS 40B fluorescence spectrophotometer, a JASCO J-700 spectropolarimeter and a Pharmacia Uitrospec 3000 UV-Vis spectrophotometer.

For the fluorescence measurements, the excitation wavelength of the fluorescence spectra was 340 nm and excitation and emission slits were 10 nm. Ethylene glycol (1 vol.%) HEPSE (*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid, $1.0 \times 10^{-2}$ M) solution was used as a solvent. The guests species, HSP70 and HSP90, in HEPES solution were injected into 2.5 mL of a 1 vol.% ethylene glycol HEPES solution of the host to make a sample solution with a host concentration of $1.0 \times 10^{-6}$ M and guest concentration of $1.6 \times 10^{-5}$, $3.8 \times 10^{-5}$, $7.4 \times 10^{-5}$, $1.1 \times 10^{-4}$, $1.4 \times 10^{-4}$, $1.7 \times 10^{-4}$, and $1.9 \times 10^{-4}$ M.

The influence of HSP90 and the fluorescent cyclodextrin on the thermal aggregation of mitochondrial C.S. (Boehringer Mannheim) at 43 °C was monitored as described [30]. To monitor thermal unfolding/aggregation, the C.S. concentration was 0.075 $\mu$M in 40 mM HEPES buffer (pH 7.4) in the presence or absence of bovine serum albumin (15 $\mu$M), HSP90 (0.15 $\mu$M) and cyclodextrin (1 $\mu$M). Light scattering of C.S. was monitored over 90 min by the optical density at 500 nm in a spectophotometer equipped with a temperature control unit using semi micro-cuvettes (1 mL) with a path length of 10 mm. In this study, 1 arbitrary unit denotes an absorbance of about 0.15 and at 500 nm.

The effect of fluorescent cyclodextrin on the trypsin digestion of HSP70 and HSP90 was analyzed using SDS-polyacrylamide gel electrophoresis by the method of Laemmli [31]. Purified HSP70 or HSP90 (1 mg/mL) was digested mildly by 0.1 or 0.01 mg/mL trypsin treated with TPCK. (*N*-tosyl-L-phenylalanine chloromethyl ketone which is a nonreversible inhibitor for chymotrypsin) in the presence or absence of fluorescent cyclodextrin ($1.0 \times 10^{-6}$ M) and ATP (1 mM) at 37 °C and 60 min.

## Results and discussion

### *Fluorescence spectra*

Figure 1 shows the fluorescence spectra of the host (**1**) in a 1 vol.% ethylene glycol HEPES solution in the presence and absence of HSP70 and HSP90. The fluorescence spectra of the host are composed of monomer emission with a peak at around 526 nm, and the fluorescence intensity decreases with increasing guest, HSP70 and HSP90 concentrations. We have reported that the decrease of a guest-induced fluorescence means that the appended moiety is moving from the cyclodextrin cavity [15, 16]; in contrast, an enhancement means the appended moiety is moving more deeply into the

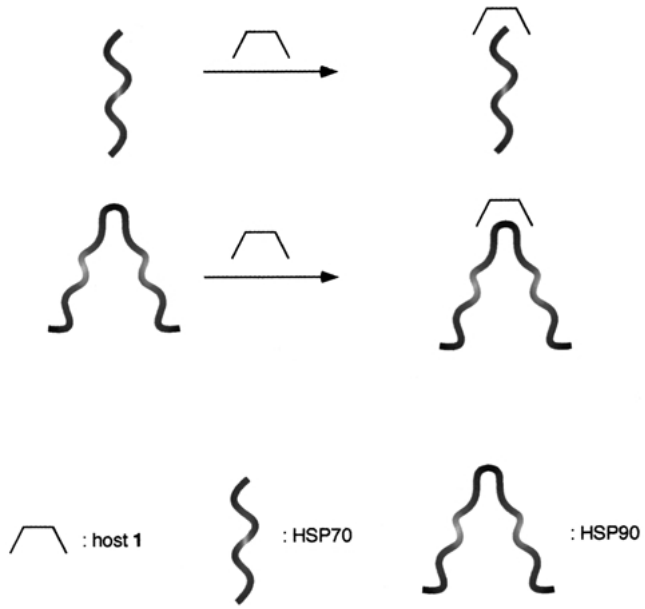



*Figure 2.* Binding behaviors of host **1** for HSP70 and HSP90.

cyclodextrin cavity [12]. The result obtained from the fluorescence spectral changes of the host suggests that the dansyl moieties are excluded from the cyclodextrin cavity while simultaneously a part of HSP70 or HSP90 is included into the cyclodextrin cavity. The binding mechanism between **1** and such a heat shock protein might be as envisaged in Figure 2.

The guest-induced fluorescence variation at 526 nm was employed to calculate the binding constants of the host using a Benesi–Hildebrand type equation, Equation (1), for 1 : 1 complex formation as shown in Figure 3, as reported previously [15, 16].

$$\frac{1}{I_f - I_f^0} = \frac{1}{a[\mathrm{CD}]} + \frac{1}{a[\mathrm{CD}]K} \times \frac{1}{[\mathrm{G}]} \qquad (1)$$

Here, $I$ is the fluorescence intensity at 526 nm ($I_f$ for complex, $I_f^0$ for the host alone), [CD] is the total host concentration, [G] is the total guest concentration, $a$ is a constant. The binding constants of the host were obtained in order to examine the correlation between them and the fluorescence variations. The binding constants of the host for HSP70 and HSP90 are 830 ± 110 ($dm^3\ mol^{-1}$) and 280 ± 10 ($dm^3\ mol^{-1}$), respectively. It is obvious that the binding ability of the host for HSP70 is higher than that for HSP90. It indicates that HSP70 is more easily associated with the host than HSP90. It is reasonable that a part of HSP70 is included more easily into the cyclodextrin cavity than HSP90 because HSP90 exists as a dimer, which might cause some hindrance to forming a host–guest complex between **1** and HSP90.

*Effect of host **1** on the chaperone activity for HSP90*

It is reported that HSP70 and HSP90 exhibit chaperone activity [21], which regulates the folding of proteins to prevent aggregation and insolubilization. To analyze the effect of **1** on the chaperone activity of HSP90, HSP90 action with and without the host in protein folding and unfolding reactions in vitro was studied. As an assay system, the thermal unfolding and aggregation of mitochondrial C.S. was used, because C.S. is inactivated and aggregates rapidly upon incubation at 43 °C [32]. Figures 4a and b show the chaperone activity of HSP90 using C.S. in the presence and absence of the host by measuring the absorption of aggregation of C.S. In the absence of the host as shown in Figure 4a, C.S. aggregation with and without HSP90 gives values of zero and 0.04, respectively, indicating that C.S. aggregation was regulated completely by HSP90. It is obvious that the chaperone activity of HSP90 was performed perfectly without the host. In Figure 4b, the values of C.S. aggregations in the presence of the host with and without HSP90 are 0.015 and 0.020, respectively, indicating that C.S. aggregation without HSP90 was reduced by 50% as compared with C.S. aggregation in the absence of HSP90 and the host as shown in Figure 4a. It suggests that the host promotes chaperone activity for C.S. in the absence of HSP90 with double the value of that for C.S. in the absence of HSP90 and the host, including a part of C.S. into its cavity to protect C.S. from its spontaneous aggregation. Furthermore, C.S. aggregation with HSP90 in the presence of the host was reduced by more than 25% compared with that without HSP90 in the presence of the host. In other words, the host shows a 25% increase in chaperone activity for C.S. in the presence of HSP90 compared with its absence. It indicates that HSP90 and C.S. in the presence of the host show an aggregation, whereas HSP90 and C.S. without the host can hardly exhibit aggregation. It is estimated that core formation, which is like an assembly such as a cluster or micelle colloid, occurs between these proteins, HSP90 and C.S., and the host.

*Influence of Host **1** on trypsin degradation for HSP70 and HSP90*

The influences of the host on the trypsin degradation of HSP70 and HSP90 was investigated by SDS-polyacrylamide gel electrophoresis analysis as shown in Figures 5 and 6, respectively. HSP70 or HSP90 were mildly or strongly digested with trypsin by the methods described previously. In the absence of ATP, HSP70 was partially degraded: the new protein band showed molecular masses of 68-, 60-, 33-, and 25 kDa on SDS-polyacrylamide gel (Figure 5, lanes1, 2 and 5, 6). There was no effect of the host under these conditions. In contrast, we could observe the effect of the host in the presence of ATP. In the presence of ATP, HSP70 was digested to a greater extent by trypsin and the new protein bands (68-, 45-, 43-, 38-, 33- and 25 kDa) were detected (Figure 5, lane 3). In the presence of the host under the same conditions, the digestion of HSP70 was well inhibited. The digestion pattern (Figure 5, lane 4) was different from lane 3. In the presence of the host, there were no digested peptide bands of 45-, 43- and 38 kDa (Figure 5, lane 4). This result indicated that cyclodextrin affected the resistance of HSP70 to the protease.

With a high concentration of trypsin (0.1 mg/mL) digestion of HSP70, the protein was almost completely digested in the presence of ATP (Figure 5, lanes 5 and 6). On the other

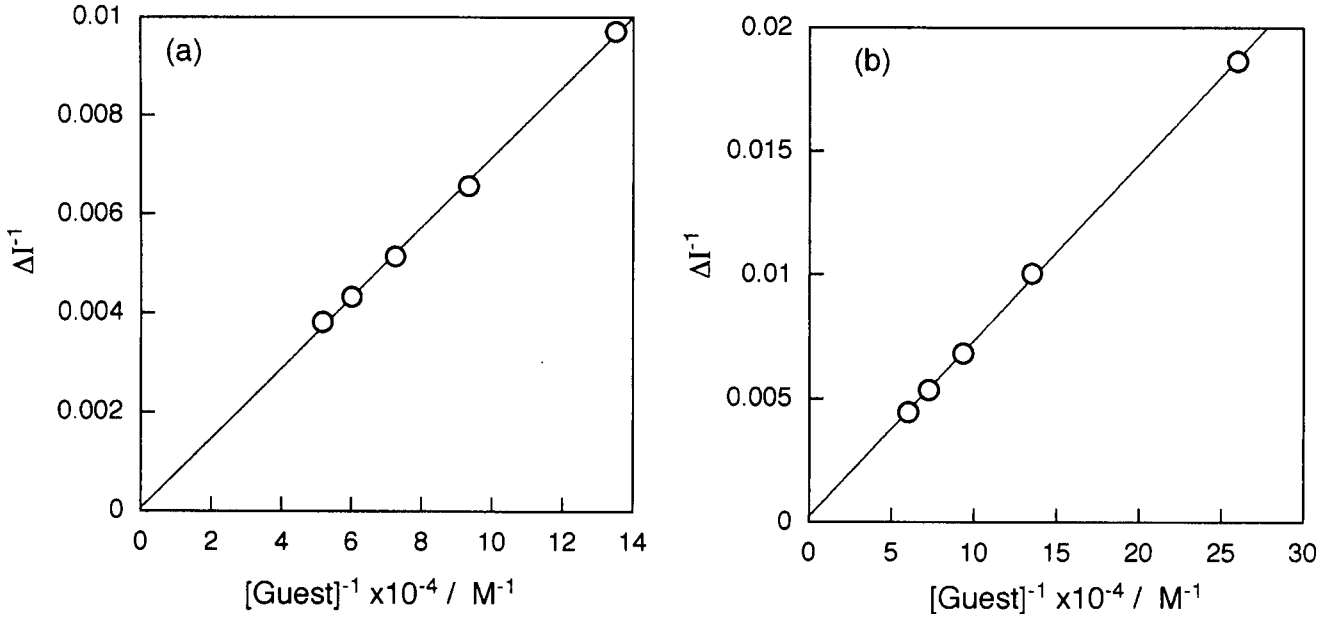



*Figure 3.* Binding curves of host **1** ($1.0 \times 10^{-6}$ M) in a 1 vol.% ethyleneglycol HEPES ($1.0 \times 10^{-4}$ M, pH7.5) solution for HSP70 (a) and HSP90 (b).

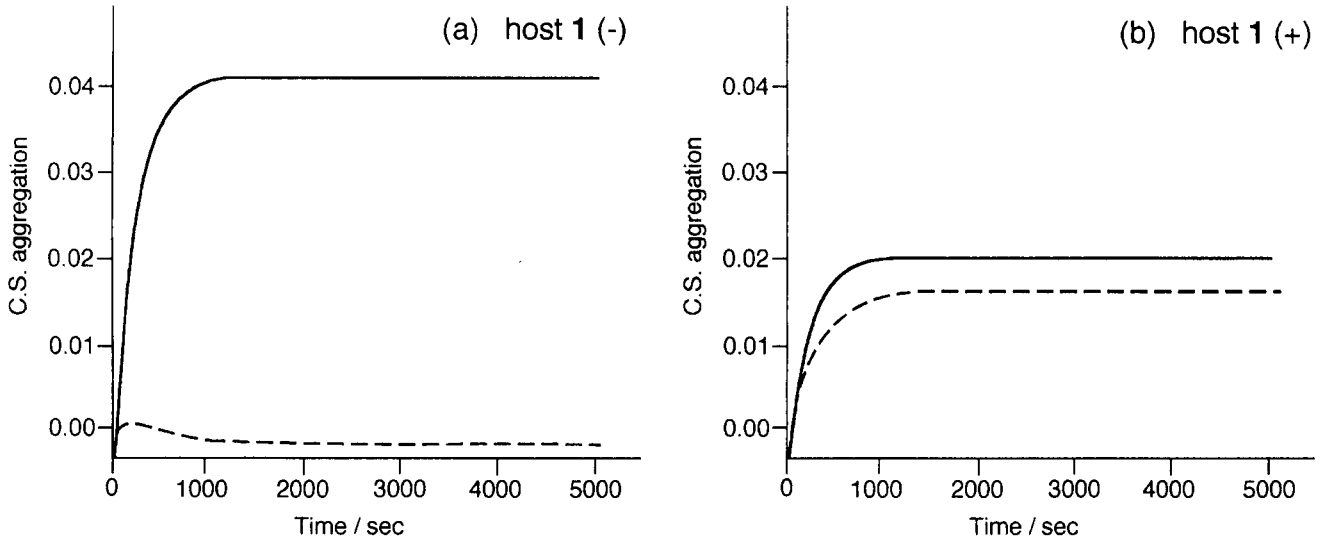



*Figure 4.* C.S. ($0.075 \times 10^{-6}$ M) aggregation affected by the absence (a) and presence (b) of host **1** in ($1.0 \times 10^{-6}$ M) without (——) and with (– –) HSP90 ($0.15 \times 10^{-6}$ M).

hand, we could observe the partly digested 33 kDa peptide band in the presence of both ATP and the host (Figure 5, lane 8). These results suggested that cyclodextrin protected the proteolysis of HSP70 by trypsin in the presence of ATP and the host promoted trypsin digestion of HSP70 in the absence of ATP.

In Figure 6, HSP90 in the absence of ATP was digested by trypsin and new protein bands showed molecular masses of 20–68 kDa (lanes 5 and 6), and in particular the density of the elution bands of HSP90 was much lower in the presence of the host (line 6) than in its absence (line 5). HSP90 in the presence of ATP was digested by trypsin and new protein bands showed molecular masses of 20–94 kDa (lanes 7 and 8) and the density of the HSP90 protein bands in the digest containing the host was higher (lane 8) than those in the absence of the host (lane 7). It indicates that HSP90 without ATP is completely degraded by trypsin (line 6) whereas HSP90 with ATP is less degraded by trypsin (line 8), when the host was present. These results suggest that the host regulates or promotes degradation of HSP70 and HSP90 with trypsin in the presence and absence of ATP, respectively, by including a part of HSP70 or HSP90 into the cyclodextrin cavity. It is assumed to be doubtful that the cyclodextrin can complex with such a big molecule because the cyclodextrin cavity size is too small to include molecules like HSP70 and HSP90. Recently, it was reported that some oligosaccharide-branched cyclodextrins interacted with concanavalin A, which was an indication that the cyclodextrin was capable combining with big molecules [33]. We assume that the behavior of the interaction is as follows: there should be a stereospecific molecular recognition between the correctly fitting **1** and HSP70 or HSP90.

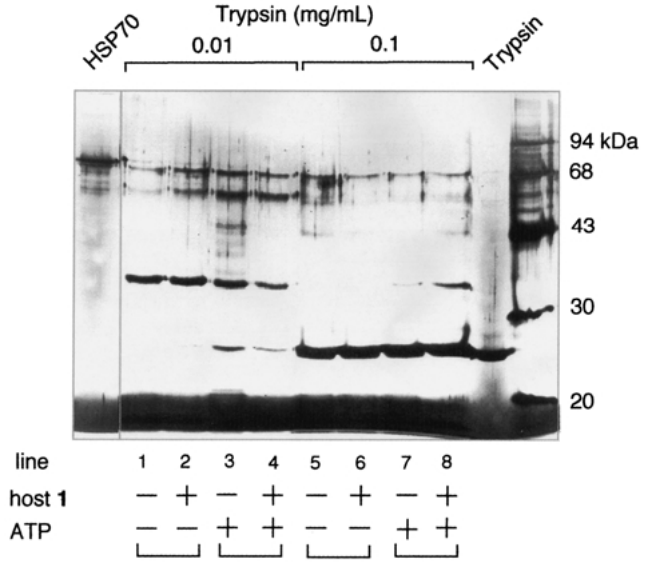



*Figure 5.* Trypsin degradation for HSP70 (1 mg/mL) influence by **1** ($1.0 \times 10^{-6}$ M) analyzing on SDS-polyacrylamide gel electrophoresis.

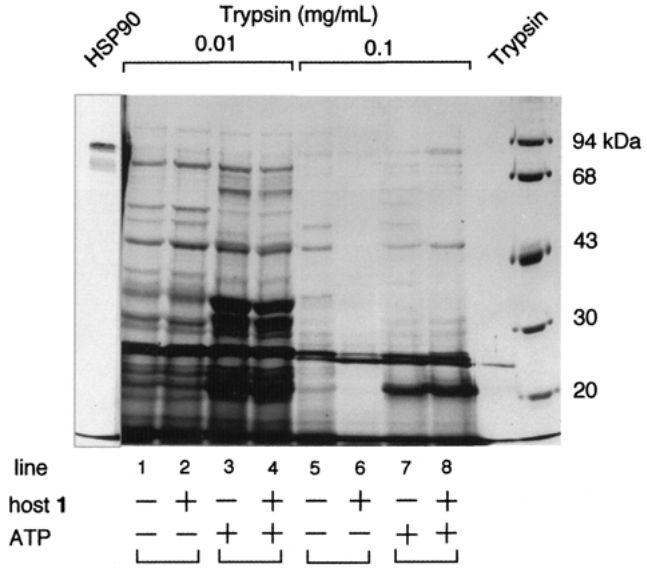



*Figure 6.* Trypsin degradation for HSP90 (1 mg/mL) influenced by **1** ($1.0 \times 10^{-6}$ M) analyzing on SDS-polyacrylamide gel electrophoresis.

## Conclusions

It was found that a host–guest complexation can occur between $\beta$-cyclodextrin analogs and HSP70 and HSP90, and also the host influences both the chaperone activity and trypsin degradation of these proteins. The host recognized these proteins with high binding abilities, as indicated by fluorescence spectral changes, similar to the detection of bile acids and terpenoids. It is clarified that this fluorescent host can exhibit 50% chaperone activity for C.S. compared with HSP90, and also the activity is increased more than 25% in the presence of HSP90, in which a part of the proteins can be included in the cyclodextrin cavity. It is clear that the host promoted trypsin degradation of HSP70 and HSP90 in the absence of ATP with 0.1 mg/mL of trypsin, in which HSP70 was incompletely degraded whereas HSP90 was completely degraded. In this study, it is obvious that the cyclodextrin hydrophobic cavity can provide a hydrophobic domain in which proteins can be recognized and their bio-activity, such as molecular chaperone and trypsin degradation, altered. As a further extension of our work, the interaction with other fluorescent cyclodextrins such as $\gamma$-cyclodextrin analogs and heat shock proteins such as HSP70 and HSP90 will be investigated.

## Acknowledgement

This study was supported by a Grant-in-Aid for Specially Promoted Research (No. 404: Molecular Synchronization for Design of New Materials System) from the Ministry of Education Science, Sports and Culture of Japan.

## References

1. J.-M. Lehn: *Supramolecular Chemistry*, VCH, Verlagsgesellshaft (1995).
2. J. Szejtli: *Cyclodextrin Technology*, Kluwer, Dordrecht (1998).
3. F. Hamada, S. Minato, T. Osa, and A. Ueno: *Bull. Chem. Soc. Jpn.* **70**, 1339 (1997).
4. A. Ueno, F. Moriwaki, T. Osa, F. Hamada, and K. Murai: *J. Am. Chem. Soc.* **110**, 323 (1998).
5. F. Hamada, K. Ishikawa, Y. Higuchi, Y. Akagami, and A. Ueno: *J. Incl. Phenom. Mol. Recognit. Chem.* **25**, 283 (1996).
6. S. Ito, M. Narita, and F. Hamada: *Int. J. Soc. Mat. Eng. Resource* **7**, 156 (1999).
7. M. Narita, S. Koshizaka, and F. Hamada: *J. Incl. Phenom. Macrocyclic Chem.* **35**, 605 (1999).
8. F. Hamada, Y. Kondo, K. Ishikawa, H. Ito, I. Suzuki, T. Osa, and A. Ueno: *J. Incl. Phenom. Mol. Recognit. Chem.* **17**, 267 (1994).
9. F. Hamada, K. Ishikawa, R. Ito, S. Hamai, I. Suzuki, T. Osa, and A. Ueno: *J. Incl. Phenom. Mol. Recognit. Chem.* **20**, 43 (1995).
10. F. Hamada, K. Ishikawa, I. Tamura, and A. Ueno: *Anal. Sci.* **11**, 935 (1995).
11. F. Hamada, K. Ishikawa, I. Tamura, K. Murai, Y. Akagami, and A. Ueno: *Int. J. Soc. Mat. Eng. Resource* **5**, 69 (1997).
12. M. Narita, F. Hamada, I. Suzuki, and T. Osa: *J. Chem. Soc., Perkin Trans. 2*, 2751 (1998).
13. A. Ueno, S. Minato, I. Suzuki, M. Fukushima, M. Ohkubo, T. Osa, F. Hamada, and K. Murai: *Chem. Lett.* 605 (1990).
14. F. Hamada, Y. Kondo, R. Ito, I. Suzuki, T. Osa, and A. Ueno: *J. Incl. Phenom. Mol. Recognit. Chem.* **15**, 273 (1993).
15. M. Narita, F. Hamada, M. Sato, I. Suzuki, and T. Osa: *J. Incl. Phenom. Macrocyclic Chem.* **34**, 421 (1999).
16. M. Sato, M. Narita, N. Ogawa, and F. Hamada: *Anal. Sci.* **15**, 1199 (1999).
17. T. Ikeda, K. Yoshida, and H.-J. Schneider: *J. Am. Chem. Soc.* **117**, 1453 (1995).
18. H. Itoh and Y. Tashima: *Int. J. Biochem.* **23**, 1185 (1991).
19. W.J. Welch: *Physiol. Rev.* **72**, 1063 (1992).
20. L. Nover: *The Heat Shock Response*, CRC Press, Boca Raton, FL (1991).
21. R.I. Morimoto, A. Tissiäres, and C. Georgopoulos: *The Biology of Heat Shock Proteins and Molecular Chaperones*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1994).
22. C.E. Stebbins, A.A. Russo, C. Scheide, N. Rosen, F.U. Hartl, and N.P. Pavlentch: *Cell* **89**, 239 (1997).
23. C. Prodromou, S.M. Roe, R. O'Bien, J.E. Ladbury, P.W. Piper, and L.H. Pearl: *Cell* **90**, 65 (1997).
24. T. Bhattacharyya, A.N. Karnezis, S.P. Murphy, T. Hoang, B.C. Freeman, B. Phillops, and R.I. Morimoto: *J. Biol. Chem.* **270**, 1705 (1995).
25. S.K. Moore, C. Kozac, E.A. Robinson, S.J. Ullrich, and E. Appella: *J. Biol. Chem.* **264**, 5343 (1989).

26. Y. Minami, Y. Kimura, H. Kawasaki, K. Suzuki, and I. Yahara: *Mol. Cell. Biol.* **14**, 1459 (1994).
27. H. Itoh and Y. Tashima: *Eur. J. Biochem.* **193**, 429 (1990).
28. H. Itoh, I. Toyoshima, H. Mizunuma, R. Kobayashi, and Y. Tashima: *Archiv. Biochem. Biophys.* **282**, 290 (1990).
29. H. Itoh and Y. Tashima: *Int. J. Biochem.* **25**, 69 (1993).
30. H. Itoh, M. Ogura, A. Komatsuda, H. Wakui, A.B. Miura, and Y. Tashima: *Biochem. J.* **343**, 697 (1999).
31. U.K. Laemmli: *Nature* **227**, 680 (1970).
32. U. Jacob, H. Lilie, L. Meyer, and J. Buchner: *J. Biol. Chem.* **270**, 7288 (1995).
33. H. Imata, K. Kubota, K. Hattori, M. Aoyagi, and C. Jindoh: *Polymer Journal* **29**, 563 (1997).