10C/1999G353

Unfolded structure of *Escherichia coli* co-chaperonin GroES

Takashi HIGURASHI[1], Kaoru ICHIMURA*[2], Tomohiro MIZOBATA[1], Yasushi KAWATA[1], Yuzuru HIRAGI[3]

[1]Dept. Biochem., Faculty of Engineering, Tottori Univ., Tottori, Tottori 680-0945, JAPAN
[2]Dept. Biochem., Dokkyo Univ. Sch. Med., Mibu, Shimotsuga, Tochigi 321-0293, JAPAN
[3]Inst. for Chem. Res., Kyoto Univ., Gokasho, Uji, Kyoto 611-0011, JAPAN

Introduction

Co-chaperonin GroES from *Escherichia coli*, a homoheptamer of identical subunit for molecular mass 10 kDa are reversibly unfolded under the conditions of heat and chemical denaturant such as guanidine hydrochloride. Thermal unfolded GroES has a tendency to form amyloid than unfolded GroES by guanidine hydrochloride, when protein is kept long time in the unfolded condition. We measured unfolded structure of GroES.

Materials & Methods

Chaperonin GroES

GroES proteins were purified from *E. coli* DH-1/pKY206, according to ref. [1]. Protein was dissolved in 50mM Tris-HCl with 50mM KCl at pH 7.8.

Thermal unfolding experiment

Protein was kept at designated temperature for 5 min before a measurement.

Results

Oligomeric GroES is dissociated to unfolded monomer at 1.5M guanidine hydrochloride [2] and at 70°C or more.

Radius of gyration (Rg) of native GroES was 31.9 Å, calculated from Giunier plot. Rg of unfolded GroES at 70 ℃ was 27.7 Å, on the other hand, Rg at 2.5M guanidine hydrochloride was 37.5 Å (data not shown).

Figure 1 shows Kratky plots of unfolded GroES (monomer) at 70°C and 2.5M guanidine hydrochloride. It was shown that the unfold structures between thermal and guanidine hydrochloride were clearly different.

The structure of thermal unfolded GroES polypeptide is more compact compared with that of guanidine-unfolded coil. These results would suggest that amyloid fiber was formed with a state of the thermal-unfold polypeptide, but not formed with that of 2.5M guanidine hydrochloride.

References

[1] T. Higurashi *et a*l, J. Mol. Biol., 291, 703 (1999)
[2] Y. Hiragi et al., PF News 17, 15(1999)
* ichimura@dokkyomed.ac.jp

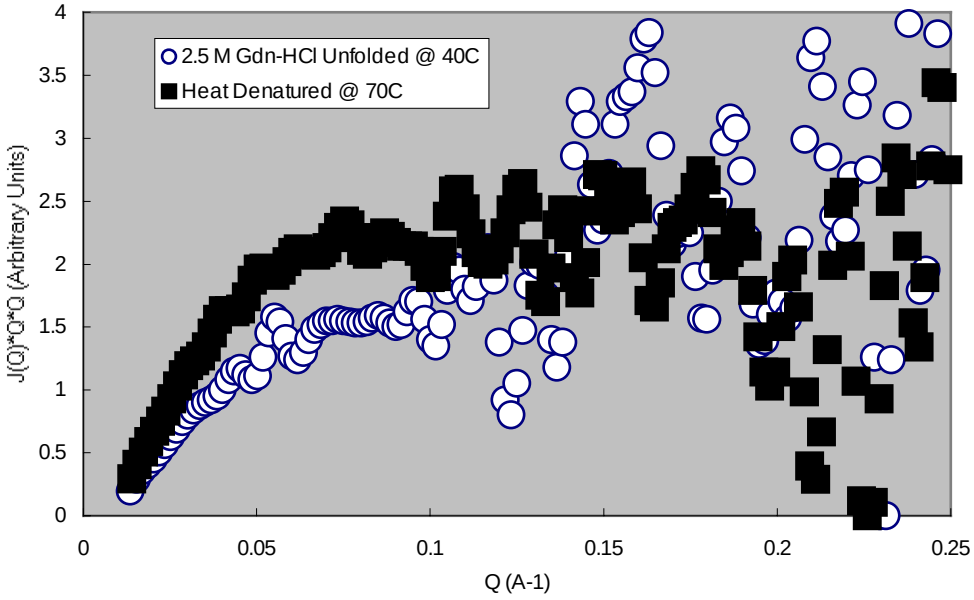

Fig.1 Kratky Plots of unfolded GroES under the condition at 70°C and of 2.5M guanidine hydrochloride.