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Subunit architecture of silkworm small heat shock protein and its temperature-dependent change

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Introduction

Abrupt increase in environmental temperature induces the expression of a variety of small heat shock protein (sHSP) genes. sHSP is a family of proteins having an α-crystalline domain and suppressing the thermal aggregation of other proteins. sHSP19.9 and sHSP20.8 are two of six sHSPs from the silkworm, *Bombyx mori*. Each is composed of identical polypeptides, and each of polypeptides has a single cysteine residue: Cys-123 and Cys-43 in sHSP19.9 and sHSP20.8, respectively. In both sHSPs, some sulfhydryl groups remain free, others form inter-subunit disulfide bonds [1,2]. However, little is known about their assembly structures. This paper reports structural characteristics of sHSP19.9 and sHSP20.8 at 30°C measured by small-angle x-ray scattering (SAXS).

Materials and methods

sHSP19.9 and sHSP20.8 were overproduced by *E. coli* cells as N-terminal His-tagged proteins. The detailed preparation procedures were described elsewhere [2]. The purified samples were examined by SDS-PAGE and dialyzed against HEPES buffer (pH 7.7) containing 10 mM NaCl, where no radiation damage was observed. SAXS measurements were done at BL10C. The camera length was 2,000 mm, calibrated by meridional reflection of collagen. Data collection time and temperature were 600 sec, 30°C, respectively. Radius of gyration (R_g) and forward scattering *I(0)* were determined by *lnI(S)* vs. S^2 plot, Guinier plot of zero concentration extrapolated profiles, for which five protein concentrations (0.4-2.0 mg/ml) were employed [3]. The molecular weight of sHSPs were estimated by comparing *I(0)/C* relative to that of dihydrolipoyl transacetylase, E2 (Mw=2.96MDa). Pair distribution function, *P(r)* was calculated by using GNOM software [4].

Results and Discussions

Judging from the concentration dependency of SAXS profiles as well as linearity in Guinier plots, both sHSPs form monodisperse oligomers independent of protein concentrations that were apart from association-dissociation equilibrium. The SAXS parameters of sHSP 19.9 and sHSP 20.8 were then determined as listed in Table 1. According to *I(0)*, the oligomerization number of sHSP19.9 and sHSP20.8 were *ca.* 120-mer and 60-mer, respectively. Since the disulfide bond content of sHSP19.9 is reported to be less than sHSP20.8 [1], the difference in content might be responsible for that in number of subunits.

Table 1: SAXS parameters of sHSPs at 30°C

samples	Rg (A)	I(0)/C	Estimated Mw
SHSP19.9	77.2 A ± 0.2 A	50800 ± 240	2.78 MDa
SHSP20.8	59.6 A ± 0.2 A	27240 ± 100	1.49 MDa

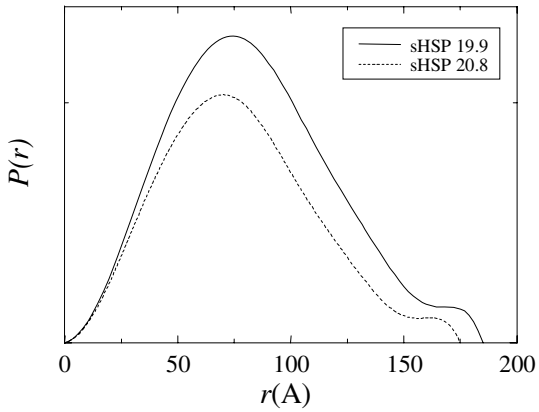

Figure 1: *P(r)* functions of sHSPs

P(r) function of sHSP19.9 and sHSP20.8 did not show any drastic shape changes depending on the positional difference of cysteine residue, which does not contradict with the identical *S* values in analytical centrifugation [1]. *Ab initio* models suggested that both sHSPs form an oblate shape with a similar axial ratio. These sHSPs solubilize aggregations, however, no indication of columnar shape like other chaperone protein complexes, was observed. Temperature-induced changes of these sHSPs are now in progress.

References

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