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FOR THE RECORD

Crystallization and preliminary X-ray crystallographic properties of Hsc20, a J-motif co-chaperone protein from *Escherichia coli*

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Abstract: Hsc20 is a 20-kDa auxiliary protein that functions with the molecular chaperone Hsc66 in *Escherichia coli*. Crystals of Hsc20 suitable for X-ray diffraction analysis were grown using the hanging drop vapor diffusion technique in polyethylene glycol 400 containing dioxane as an additive to slow growth. The crystals are monoclinic and belong to the space group *C2* with unit cell dimensions a = 125.4 Å, b = 71.9 Å, c = 68.8 Å, and β = 97.0°. The crystals diffract to a minimum d-spacing of ~2.5 Å resolution, and a native data set was collected to 2.7 Å. The results of a self-rotation function analysis revealed threefold symmetry, suggesting three molecules of Hsc20 in the asymmetric unit and, hence, 12 molecules in the unit cell; this corresponds to a V_m value of 2.6 Å^3/Da and a solvent content of ~53% in the crystals. Structure determination by isomorphous replacement is in progress.

Keywords: crystallization; Hsc20; J-domain; molecular chaperone; X-ray diffraction

Hsc20 is a novel 20-kDa protein that is constitutively expressed in *E. coli* and that functions with the hsp70-type molecular chaperone Hsc66 (Kawula & Lelivelt, 1994; Vickery et al., 1997). The N-terminal 60 amino acids of Hsc20 exhibit sequence similarities to the J-domain found in co-chaperone proteins of the DnaJ/hsp40 class (reviewed by Caplan et al., 1993; Silver & Way, 1993; Cyr et al., 1994; Rassow et al., 1995), and Hsc20 stimulates the ATPase activity of Hsc66, suggesting that it functions as a regulatory protein in a manner similar to DnaJ/hsp40 proteins (Vickery et al., 1997). In contrast to the hsp40 class proteins, however, Hsc20 has a smaller and unique C-terminal domain with a sequence suggesting a coiled-coil structure rather than a zinc-finger motif. In addition, the amino acid sequence of the J-domain of Hsc20 is poorly conserved (~15% identity) compared to members of the hsp40 class of J-proteins. These differences raise the questions of the degree of structural similarity of Hsc20 to other J-proteins and also whether its mechanism of action is similar to other co-chaperones. While the complete structure of a hsp40 protein has not been elucidated, the structure of J-domain fragments of *E. coli* DnaJ (Szyperski et al., 1994; Hill et al., 1995; Pellecchia et al., 1996) and human hsp40 (Qian et al., 1996) were recently determined by NMR methods, and both peptides showed a novel fold with a general topology likely to be characteristic of members of the J-protein family. We have recently developed methods for high level expression and purification of Hsc20 and describe herein the crystallization and preliminary X-ray diffraction analysis of this new member of the J-protein family of chaperone proteins.

The Hsc20 protein was prepared as previously described (Vickery et al., 1997). *E. coli* DH5α cells harboring the expression plasmid pTrcHsc20 were induced with IPTG and grown for ~16 h. Hsc20 reached levels ~50% of the total cell protein, and Hsc20 could be readily isolated by anion exchange and size exclusion chromatography. The final preparation appeared homogeneous on SDS-PAGE analysis and revealed a single N-terminal sequence including Met-1; the overall yield of purified protein was ~0.5 g/L cell culture. Samples were routinely concentrated to >50 mg/mL and could be stored for several months at −70°. The general properties of Hsc20, including its stimulation of the ATPase activity of Hsc66, have been described (Vickery et al., 1997).

The hanging drop vapor diffusion method (McPherson, 1982) was used to screen crystallization conditions. Initial trials used a sparse matrix kit (Hampton Research) biased toward known crystallization conditions. Using protein concentrations in the range of 10–20 mg/mL, needle-like crystals formed spontaneously following overnight equilibration with well solutions of PEG400 (28–30%) in sodium Hepes buffer, pH 7.5, containing calcium chloride or magnesium chloride. Efforts to improve the size of the crystals by varying the concentration of PEG400, protein, or divalent cation, by altering the buffer or temperature, or by seeding pre-equilibrated drops did not significantly improve crystal morphology. When the effects of several additives were then tested, only dioxane (1–5% final concentration) was found to slow crystal growth

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Abbreviations: Hepes, N-[2-hydroxyethyl]piperazine-N′-[ethanesulfonic acid]; IPTG, isopropyl-β-D-thiogalactoside; PCR, polymerase chain reaction; PEG, polyethyleneglycol.

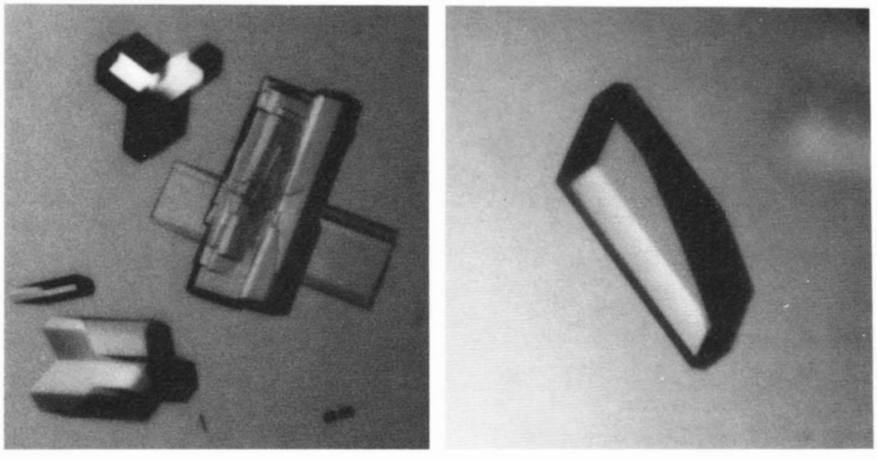

Fig. 1. Crystals of Hsc20 viewed under polarizing optics. Left panel: twinned crystalline forms. Right panel: single crystal used for diffraction analysis. (The single crystal on the right is approximately 0.8 × 0.3 × 0.1 mm.)

and to yield thicker crystals.[1] Examples of crystals obtained using an initial protein concentration of 12 mg/mL and a mother liquor containing 26% PEG400, 0.05M sodium Hepes, pH 7.5, 0.1 M magnesium chloride, and 2% dioxane are shown in Figure 1. The crystals most frequently appear as rods having a hexagonal cross-section but sometimes grow as thickened plates with a limiting dimension ~0.1 mm. Some twinning of crystals occurs and clusters of three rods/plates arranged symmetrically (left panel) are commonly observed. Single crystals (right panel) proved suitable for diffraction analysis.

Diffraction data on the Hsc20 crystals were collected using a Siemens rotating anode operating at 100 kv and 50 mA (CuKα radiation at 1.54 Å) and X-1000 area detector. Reflections could be measured to 2.5 Å, and the resolution did not appear to decay appreciably during data collection indicating little crystal decomposition as a result of exposure to the X-ray beam. The preliminary data were reduced and scaled using the XDS and XSCALE programs (Kabsch, 1993), and the crystals were found to belong to the space group $C2$ with unit cell dimensions a = 125.4 Å, b = 71.9 Å, c = 68.8 Å, and β = 97° ($\alpha = \gamma$ = 90°) and a unit cell volume ≅ 615 × 10^3 Å^3. Assuming a partial specific volume for the protein of 0.73 cm^3/g, the number of molecules per unit cell (Z) is predicted to be 8, 12, or 16 for water content values of 68, 53, or 37%, respectively. These values correspond to two, three, or four molecules per asymmetric unit and calculated crystal packing values, V_m, of 3.8, 2.6, and 1.9 Å^3/Da (Matthews, 1968). The V_m value of 2.6, corresponding to 3 molecules of Hsc20 per asymmetric unit, is close to the mean observed for protein crystals, while the other values are near the highest and lowest extremes. A native data set of 56,685 observations, including 17,911 unique reflections, was collected over the 33–2.6 Å range and scaled to give an overall R_{sym} = 9.0%. A summary of the completeness and redundancy of the data for several resolution ranges is given in Table 1.

To address the question of the number of molecules per asymmetric unit in the crystals a self-rotation analysis of the data was carried out with the program POLARRFN in the CCP4 program suite (1994). As shown in Figure 2 the contour section at κ = 120° shows a strong peak just off the C-axis. This indicates the presence of threefold non-crystallographic symmetry in support of the suggestion of three molecules of Hsc20 in the asymmetric unit as discussed above, and this may be useful for averaging diffraction data for structural analysis. Association of Hsc20 molecules (trimeric) was not anticipated, as size exclusion chromatography had indicated that the protein is predominantly monomeric in solution (Vickery et al., 1997). Gel filtration methods are relatively insensitive for weak interactions, however, and partial oligomerization may have gone undetected. Studies on other J-type co-chaperone proteins have suggested that oligomeric forms may be important in interactions with hsp70s (Wawrzynow & Zylicz, 1995), and the recent structure of the complex of the auxiliary protein GrpE with DnaK has revealed formation of a 2:1 complex (Harrison et al., 1997). While the conditions used for crystallization of Hsc20 are nonphysiological, the association observed suggests that the possibility of oligomerization should be further investigated.

We are presently searching for heavy atom derivatives to use for multiple isomorphous replacement and solution of the crystal structure. Because *E. coli* Hsc20 does not contain cysteine, it may prove useful to introduce cysteine residues by site-directed mutagenesis for site-specific heavy atom labeling. Recent studies have also shown that the PEG400-based mother liquor used for crystal growth allows the crystals to be frozen to −170° while retaining good diffraction quality; use of this cryoprotectant may allow for extended data collection times at low temperature and collection of higher resolution data.

Table 1. *Summary of crystallographic data*

Resolution range (Å)	Completeness (%)	Reflections	
		Total	Unique
∞–3.0	99.8	42,805	12,309
3.0–2.8	98.4	7,998	2,790
2.8–2.7	97.2	4,005	1,656
2.7–2.6	57.5	1,837	1,156

[1] A search of the Biological Macromolecule Crystallization Database (Gilliland et al., 1994) revealed 15 other examples of proteins crystallized in the presence of dioxane including one case (Grable et al., 1984) in which dioxane was used in combination with PEG400.

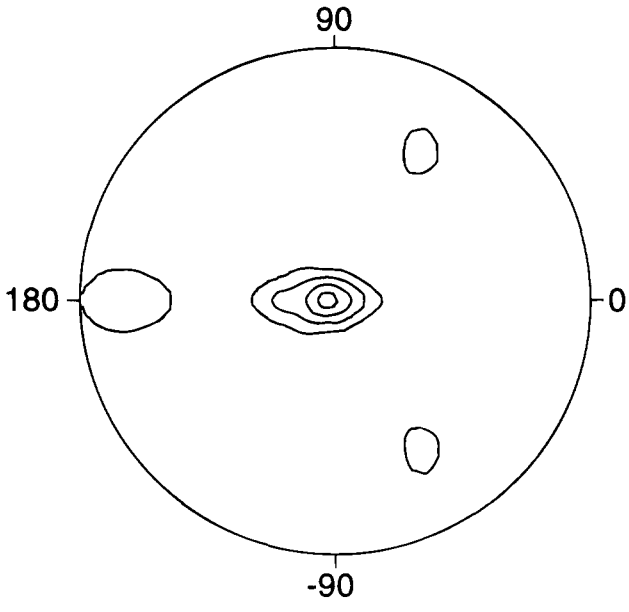

Fig. 2. Stereographic projection of the self-rotation function in spherical polar angles at $\kappa = 120°$. The analysis was carried out with the program POLARRFN from the CCP4 package (1994) using data in the 18–4 Å resolution shell and a Patterson function radius of 12.5 Å. Phi angles are marked on the circumference. The strong peak near the center (spherical polar coordinates $\phi = 180.0°$, $\psi = 2.1°$, $\kappa = 120.9°$) indicates the presence of a threefold symmetry axis.

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