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Structure of a new crystal form of human Hsp70 ATPase domain

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Hsp70 proteins are highly conserved proteins induced by heat shock and other stress conditions. An ATP-binding domain of human Hsp70 protein has been crystallized in two major morphological forms at pH 7.0 in the presence of PEG 8000 and $CaCl_2$. Both crystal forms belong to the orthorhombic space group $P2_12_12_1$, but show no resemblance in unit-cell parameters. Analysis of the crystal structures for both forms shows a 1–2 Å shift of one of the subdomains of the protein. This conformational change could reflect a 'natural' flexibility of the protein which might be relevant to ATP binding and may facilitate the interaction of other proteins with Hsp70 protein.

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1. Introduction

Hsp70 proteins were originally described as a highly conserved 70 kDa protein group induced by heat stress. They are found in eukaryotic and prokaryotic organisms (for reviews, see Bukau & Horwich, 1998; McKay *et al.*, 1994) and, even under normal conditions, they are among the most abundant proteins in cells.

Eukaryotic genomes usually encode multiple Hsp70s which are localized in cytoplasm and cellular organelles such as the mitochondria and chloroplasts. Prokaryotic Hsp70s show high homology to their eukaryotic counterparts (Bukau & Horwich, 1998; Bardwell & Craig, 1984). Hsp70s function as molecular chaperones that bind unfolded polypeptides, stabilize them and assist in their correct folding, and therefore they are crucial to many cellular processes such as translation or protein transport across membranes. Chaperones are also involved in assembly and disassembly of multimeric complexes. Finally, they can protect and reactivate other proteins under stress conditions, such as heat shock or chemical agents, which may denature proteins (Bukau & Horwich, 1998).

The Hsp70 proteins consist of two major domains. The amino-terminal 44 kDa domain binds and hydrolyses ATP. The carboxyl-terminal domain exhibits protein-binding and regulatory activities. Both domains have been shown to interact with several protein cofactors such as Hip, Hop, HDJ-1 and BAG-1 proteins (Bukau & Horwich, 1998). A number of structural studies of both domains have been reported. The ATPase domain of bovine Hsc70 was first described by Flaherty *et al.* (1990) and since then this group has published several structures of ATPase mutants. More recently, the *Escherichia coli* DnaK peptide-binding domain in complex with peptide substrate and the structure of the complex of *E. coli* DnaK ATPase domain and GrpE protein have been described (Harrison *et al.*, 1997; Zhu *et al.*, 1996). Our laboratory has determined the high-resolution structure of the human Hsp70 ATPase domain complexed with ADP and P_i (Sriram *et al.*, 1997).

Here, we describe a new crystal form of the human Hsp70 ATPase domain. The refined structure of this new crystal form is compared with that previously reported (Sriram *et al.*, 1997) and provides evidence for the flexibility of the IIB subdomain of the structure, which corresponds to residues 229–306 (Flaherty *et al.*, 1990).

2. Materials and methods

Expression and purification of the human Hsp70 ATPase domain has been described previously (Freeman *et al.*, 1995). The protein was crystallized in 25 m*M* imidazole buffer pH 7.0, 8% polyethylene glycol 8000, 10 m*M* KCl, 10 m*M* $CaCl_2$ and 1 m*M* γ-S-ATP using the hanging-drop vapor-diffusion method. These conditions are similar to those described earlier (Sriram *et al.*, 1997). Two morphologically distinct crystal forms can be obtained: the first type grow as bipyramids diffracting to 1.84 Å resolution and the second type grow as rods diffracting to 2.3 Å resolution using synchrotron radiation. The 1.84 Å crystal structure of the former, which we refer to as the type I form, has been described recently (Sriram *et al.*, 1997).

Diffraction data for both crystal forms were collected at 100 K using synchrotron radiation

from the NSLS X12B beamline at Brookhaven National Laboratory. Data were processed and reduced with the *HKL* suite of programs (Otwinowski & Minor, 1995). A summary of the results is given in Table 1. The starting model used for refinement was the 1.84 Å refined coordinates of the type I crystal form, which were placed in the correct position of the new unit cell using the *CCP*4 (Collaborative Computational Project, Number 4, 1994) version of the program *AMoRe* (Navaza, 1994). The structure was refined with the *CNS* suite (Brünger *et al.*, 1998) using data from 9 to 2.3 Å resolution (Table 2). The final round of refinement converged to an *R* factor of 20.3%. The $R_{\rm free}$ value is 25.3%. A total of 199 water molecules were identified in the structure. The overall quality of the electron-density maps was similar to the trend observed in the type I form, with poor density for the loop regions 186–191, 212–214 and 360–362.

Table 1
Summary of data processing for the type II crystal form.

Values in parentheses are for the highest resolution shell 2.4–2.3 Å.

Resolution (Å)	9–2.3
Number of observations	56187
Number of unique reflections	20763
Space group	$P2_12_12_1$
Unit-cell parameters (Å, °)	a = 70.22, b = 72.56, c = 98.93, $\alpha = \beta = \gamma$ = 90
Completeness (%)	90.7 (80.7)
$R_{\rm merge}$ † (%)	5.0 (20.5)
$I/\sigma(I)$	22.1 (5.5)

† $R_{\rm merge} = \sum_{hkl}\sum_{i=1}^{N}|\langle I_i^{hkl}\rangle - I_i^{hkl}|/\sum_{hkl}\sum_{i=1}^{N}|I_i^{hkl}|$.

3. Results and discussion

The two crystal forms characterized in our laboratory were obtained under identical crystallization conditions. The crystals were analysed directly by SDS–polyacrylamide gel electrophoresis, which revealed a single protein band at 42 kDa for both crystal forms, corresponding to the molecular weight of the ATPase domain of human Hsp70 protein (data not shown). Both crystal forms belong to the orthorhombic space group $P2_12_12_1$ but differ significantly in their unit-cell constants. The type I crystals have unit-cell parameters of a = 144.69, b = 64.03, c = 46.28 Å, $\alpha = \beta = \gamma = 90°$, which are very close to those described previously (Flaherty *et al.*, 1990; Sriram *et al.*, 1997). The new type of ATPase crystals, referred here as type II crystals, have unit-cell parameters $a = 70.22, b = 72.56, c = 98.93$ Å, $\alpha = \beta = \gamma = 90°$. The difference in unit-cell size gives rise to two distinct arrangements of the protein in the respective unit cells (Fig. 1). In the type I crystal form, the protein is densely packed with small irregular solvent channels between adjacent molecules in the unit cell, whereas the arrangement of the protein molecules in the type II crystals form a network of large square solvent channels. These features of the two crystal forms are reflected in the mechanical properties of crystals, with the type I bipyramidal crystals being much less fragile than the long rectangular-shaped type II crystals, which tend to fracture easily.

In our comparison of the two crystal forms, we use the domain nomenclature of Flaherty *et al.* (1990). The ATPase molecule consists of two major domains. Domain I

Table 2
Summary of refinement statistics for the type II crystal form.

Numbers of atoms	
Total	3172
Protein	2944
ADP	27
Water	199
Other†	2
Mean atomic displacement parameters (Å^2)	
Total	30.4
Protein	30.2
ADP	19.7
Water	35.8
Other†	31.9
R factor‡ (%)	20.3
$R_{\rm free}$§ (%)	25.3
R.m.s. deviations	
Bond lengths (Å)	0.0058
Bond angles (°)	1.13
Isotropic atomic displacement parameter restraints (r.m.s., σ)	
Main-chain bond	1.5, 1.5
Main-chain bond angle	2.5, 2.0
Side-chain bond	2.3, 2.0
Side-chain angle	3.2, 2.5
Ramachandran plot	
Residues in most-favored regions (%)	90.5
Residues in additional allowed regions (%)	9.5
Residues in generously allowed and disallowed regions	0.0

† Calcium and chloride ions. ‡ R factor = $100 \times \sum_{hkl}||F_o| - |F_c||/\sum_{hkl}|F_o|$. § $R_{\rm free}$ was calculated for 10% of the data, which were omitted from refinement.

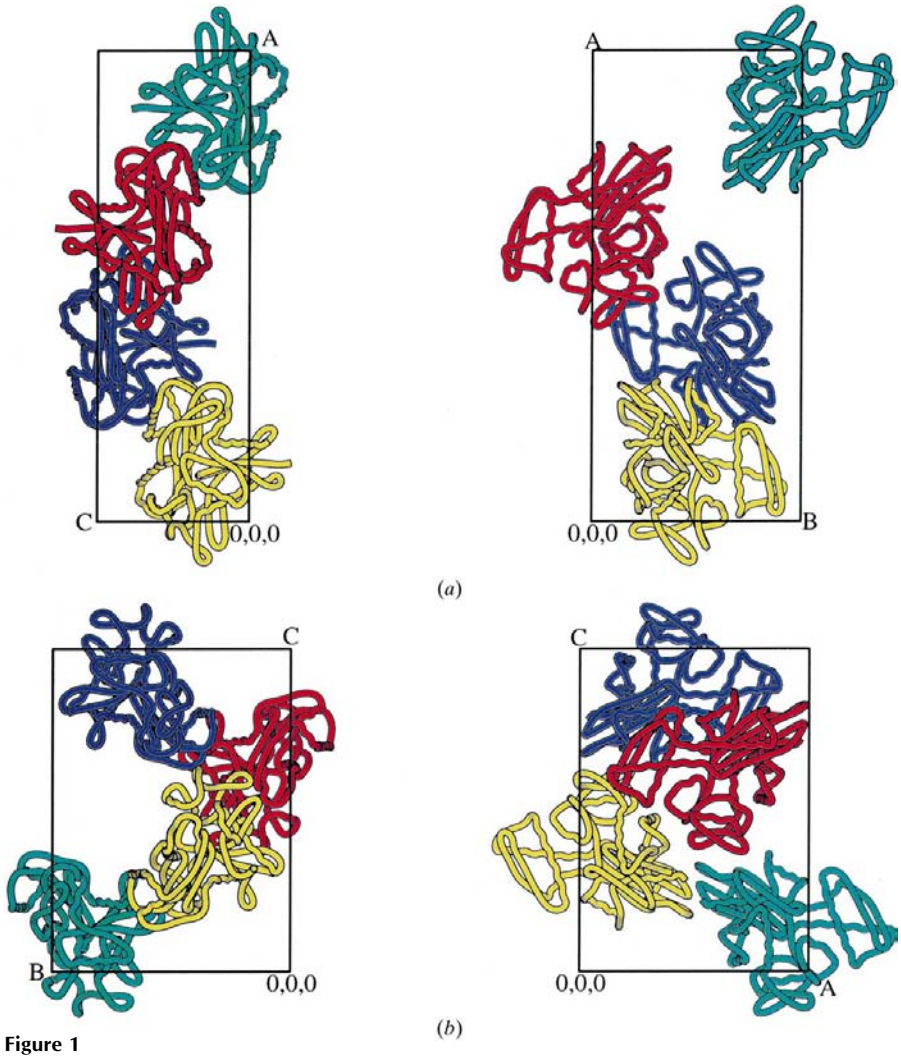

Figure 1
Arrangements of the molecules in the unit cell of Hsp70 ATPase. Two orthogonal views of type I (*a*) and type II (*b*) crystals are shown.

consists of residues 3–188 and domain II consists of residues 189–382. The latter domain has been divided further into two sub-domains, namely IIA (129–228 and 307–382) and IIB (229–306). The comparison of the two ATPase structures shows domain I of the ATPase (residues 3–188) to be the most well conserved. A least-squares superposition (Kabsch, 1978) of residues 4–186 of the two structures resulted in an r.m.s. deviation in the C^{α} positions of 0.27 Å, which is comparable to the experimental error of the atomic coordinates. In the same superposition, the r.m.s. deviation of C^{α} atoms for the other domain of ATPase was 0.86 Å (domain II, residues 189–382, excluding disordered regions), with the largest displacement of 1.9 Å occurring at residue 291. The most visible difference in the two structures was a concerted shift of subdomain IIB (residues 229–306) shown in Fig. 2. There were no significant changes in the internal structure of subdomain IIB, which is reflected by an r.m.s. deviation in the C^{α} atoms of 0.28 Å on superposition of the subdomains themselves of the two independent structures. These data suggest the existence of a hinge mechanism in the ATPase structure at the edge of subdomain IIB allowing the protein main chain to bend. Analysis of the crystal packing in both crystal forms shows that the movement of subdomain IIB is not influenced by the arrangement of the molecules in the respective unit cells. The subdomain movement draws us to the comparison made by Harrison *et al.* (1997), who have suggested that binding of the protein GrpE to DnaK from *E. coli* induces a 14° rotation of subdomain IIB in DnaK relative to its position in the bovine Hsc70 structure (Harrison *et al.*, 1997). In our case, the change in protein conformation of the analogous part of the protein is not so remarkable. However, here we are comparing two structures of the same protein as opposed to comparing proteins from phylogenetically distant species. In addition, the movement of the Hsp70 subdomain differs, being nearly parallel to the ATPase cleft rather than a subdomain rotation.

The observed subdomain movement in the two crystal forms of the human Hsp70 ATPase protein may have biological relevance. The change in structure probably reflects the local flexibility of Hsp70 protein which may be important for ATP binding and ADP release in the absence of a release factor. In addition, by analogy with the DnaK–GrpE protein complex structure, we can speculate that movement of the Hsp70 domain may also help in interactions with other proteins and cofactors.

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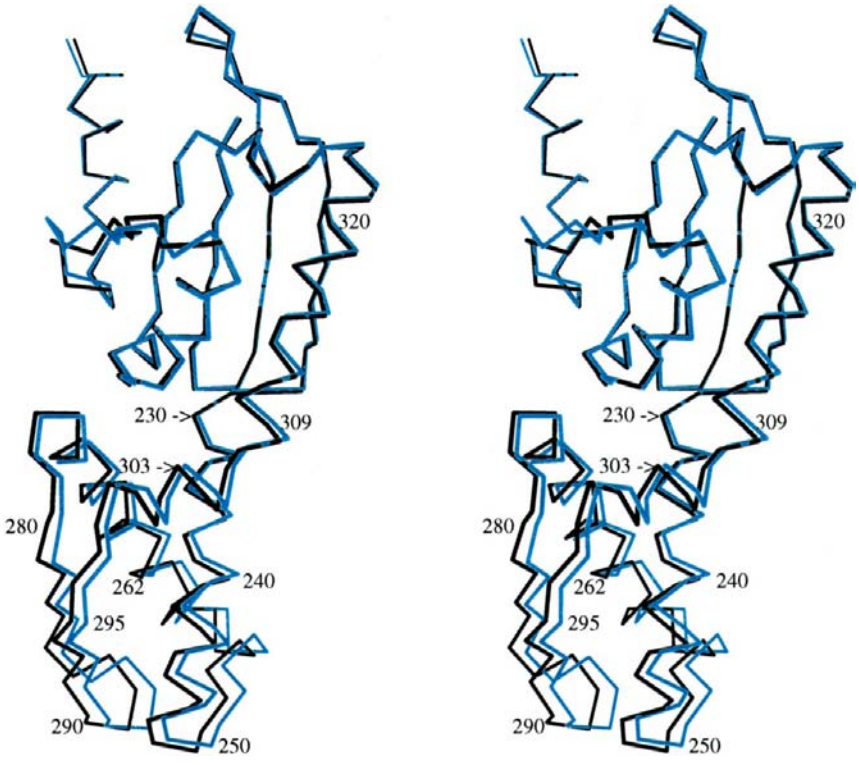

Figure 2
Least-squares superposition of type I (black) onto type II (cyan) Hsp70 molecules using C^{α} atoms of the N-terminal domain. Only domain II (residues 189–382) of the protein is shown.

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