Unusual dimerization of a *Bc*Csp mutant leads to reduced conformational dynamics

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Introduction.

Cold shock proteins (Csp) constitute a family of ubiquitous small proteins that act as RNA-chaperones to avoid cold-induced termination of translation. All members contain two subdomains composed of 2 and 3 β-strands respectively, which are connected by a hinge loop and fold into a β-barrel. *Bacillus caldolyticus* Csp (*Bc*Csp) is one of the most studied members of the family in terms of its folding, function and structure. This protein has been described as a monomer in solution, although a recent crystal structure showed dimerization via domain swapping (DS). In contrast, other cold shock proteins of the same fold are known to dimerize in a non-swapped arrangement. Hypothesizing that reducing the size of the hinge loop may promote swapping as in several other DS proteins with different folds we deleted two residues from these region (*Bc*CspΔ36-37) in order to study this particular kind of oligomerization.

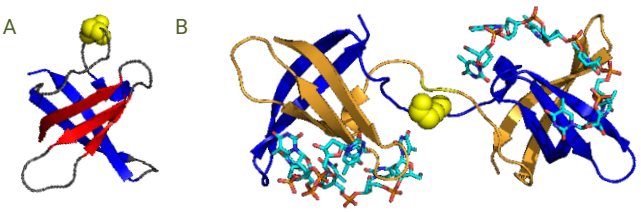

Fig. 1. Crystallographic structures of *Bc*Csp. *Bc*Csp has been reported as a monomer (A; 1C9O) and a swapped dimer in presence of its ligand dT_6 (B; 2HAX). Both share the same architecture but differ in their topology and orientation of residues from the hinge loop (yellow spheres).

The double deletion Δ36-37 forms a soluble dimer without significantly altering the stability and function of *Bc*Csp.

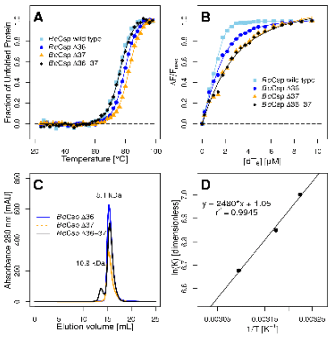

Table 1. Thermodynamic parameters of wild-type *Bc*Csp and its single and double deletion mutants

Protein	T_m	$\Delta G_{70°C}$ [kcal·mol^{-1}]	K_D [μM]	$K_D/K_{D,wild\text{-}type}$
wild-type *Bc*Csp	75.9 (± 0.1)	1.0 (± 0.14)	0.04	1
*Bc*CspΔ36	79.2 (± 0.1)	1.5 (± 0.14)	0.37	9
*Bc*CspΔ37	82.0 (± 0.1)	2.0 (± 0.14)	1.56	39
*Bc*CspΔ36-37	76.6 (± 0.1)	1.1 (± 0.14)	1.46	36

Table 2. Thermodynamic analysis of dimer dissociation of *Bc*CspΔ36-37 at several temperatures

T [°C]	$K_D \times 10^{-9}$ [M]	ΔH_D [kcal·mol^{-1}]	ΔG_D [kcal·mol^{-1}]
50 ± 0.1	1.26 (± 0.12)	4.91 (± 0.29)	4.27 (± 0.41)
42 ± 0.1	1.06 (± 0.30)	4.91 (± 0.29)	4.28 (± 0.41)
37 ± 0.1	0.91 (± 0.10)	4.91 (± 0.29)	4.29 (± 0.40)

Fig. 2. Biophysical characterization of hinge loop deletion mutants of *Bc*Csp (A) Thermal stability acquired by thermal unfolding at 1 °C/min measured by circular dichroism. All mutants are similar to wild-type *Bc*Csp, showing slight differences in their T_m and $\Delta G_{70°C}$. Parameters for thermal two-state unfolding model are shown in **Table 1**. (B) Ligand binding measured by ligand titration, whose fit parameters are shown in **Table 1** (C) Oligomeric state for each mutant was determined by SEC using a Superdex 75 column. (D) van't Hoff's analysis of the monomer-dimer equilibrium of *Bc*CspΔ36-37. The K_D was determined at three temperatures and are shown in **Table 2**.

Protective effect of dimer formation on the conformational dynamics of *Bc*Csp

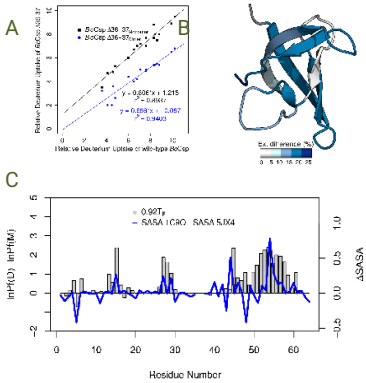

Fig. 5. Protective effect of dimerization of *Bc*CspΔ36-37 in conformational dynamics. (A) Correlation of relative deuterium uptake of monomeric (black) and dimeric *Bc*CspΔ36-37 (blue) against wild-type *Bc*Csp. (B) Cartoon representation of the difference in relative deuterium uptake upon dimerization of *Bc*CspΔ36-37. (C) Structure-based models qualitatively recapitulate the effects of dimerization on the structure of *Bc*CspΔ36-37. The difference in lnPf between dimer and monomer at $0.92T_F$ is compared to the difference in SASA (blue line) obtained by comparing wild-type monomeric *Bc*Csp (PDB 1C9O) and dimeric *Bc*CspΔ36-37 (PDB 5JX4).

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Crystal structure of *Bc*CSPΔ36-37 reveals a dimer formed *via* its ligand-binding surface

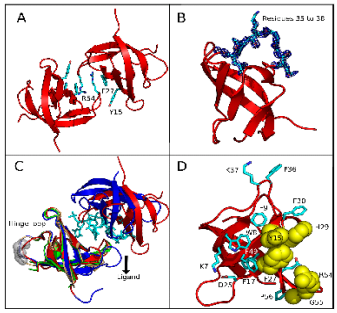

Fig. 3. Crystallographic structure of the non-swapped dimer of *Bc*CspΔ36-37 (A) Cartoon representation of the structure of *Bc*Csp Δ36-37, whose parameters are in **Table 3**. (B) Electron density of the hinge loop of *Bc*CspΔ36-37. (C) Structural alignment of wild-type *Bc*Csp (orange), *Bc*Csp Δ36-37 (red), *Bs*CspB in complex with dT_6 (PDB 2ES2, green).

Table 3. Data collection and structure refinement parameters

Data Collection	*Bc*Csp
Space group	$P2_1$
Cell dimensions (Å) a, b, c (°) α, β, γ	34.0, 44.1, 36.5; 90.00, 99.28, 90.00
Detector	R-AXIS IV++
X-ray source	MicroMax 007 HF
Wavelength (Å)	1.5418
Resolution range (Å)	18 – 1.799 (1.863-1.799)
Multiplicity	3.5 (2.5)
Rmeas (%)*	5.7 (14.8)
CC (1/2)	99.8 (96.5)
Completeness (%)	95.10 (75.18)
Total reflections	18342 (1243)
Unique reflections	9538 (736)
I / σ(I)	23.6 (8.8)
Refinement parameters	
Reflections used for refinement	9514
R (%)**	15.00
R_{free}(%)**	18.21
No. of protein atoms	1049
No. of water molecules	221
B (Å^2)	
Protein	7.48
Ligands	22.81
Water	16.21
Error estimates	
Coordinate Error (Å)	0.13
Phase error (°)	16.60
Ramachandran Plot	
Favored (%)	98.44
Allowed (%)	1.56
Outliers (%)	0.00
All-atom clashcore	7.66
RMSD from ideal geometry	
r.m.s. bond lengths (Å)	0.013
r.m.s. bond angles (°)	1.335
PDB accession number	5JX4

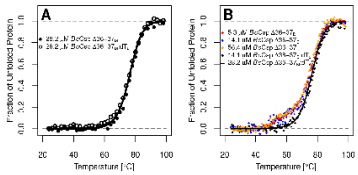

Fig. 4. Dimerization of *Bc*CspΔ36-37 occurs throughout the ligand-binding surface. (A) Thermal unfolding of monomeric *Bc*CspΔ36-37 with (empty circle) and without (filled circle) its ligand dT_6. (B) Thermal unfolding of isolated dimeric *Bc*Csp Δ36-37 at several protein concentrations in comparison with unfolding of dimeric *Bc*CspΔ36-37 in the presence of dT_6.

Concluding Remarks

- Single deletion of hinge loop residues in BcCsp does not lead to a dimeric form of this protein. However, the double deletion Δ36-37 generates a larger species that corresponds to a dimer.
- The crystallographic dimer obtained corresponds to a dimer of BcCspΔ36-37 in solution.
- The dimerization interface is located along the ligand-binding surface, whose resifues change their occlusion state upon dimerization.
- Dimer formation leads to a dramatic decrease of the conformational dynamics of BcCspΔ36-37, hinting at protective effect upon dimerization.