Single-stranded DNA bound to bacterial cold-shock proteins: preliminary crystallographic and Raman analysis

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The cold-shock response has been described for several bacterial species. It is characterized by distinct changes in intracellular protein patterns whereby a set of cold-shock-inducible proteins become abundant. The major cold-shock proteins of *Bacillus subtilis* (*Bs*-CspB) and *Bacillus caldolyticus* (*Bc*-Csp) are small oligonucleotide/oligosaccharide-binding (OB) fold proteins that have been described as binding single-stranded nucleic acids. *Bs*-CspB (M_r = 7365) and *Bc*-Csp (M_r = 7333) were crystallized in the presence of the deoxyhexanucleotide (dT)$_6$. Crystals of (dT)$_6$ with *Bs*-CspB grew in the orthorhombic space group $C222_1$, with unit-cell parameters a = 49.0, b = 53.2, c = 77.0 Å. Crystals with *Bc*-Csp grew in the primitive orthorhombic space group $P2_12_12$, with unit-cell parameters a = 74.3, b = 64.9, c = 31.2 Å. These crystals diffract to maximal resolutions of 1.78 and 1.29 Å, respectively. The presence of protein and DNA in the crystals was demonstrated by Raman spectroscopy.

1. Introduction

When bacteria are exposed to a sudden decrease of temperature, bulk transcription and translation will slow down or even cease. These cold-shock conditions are characterized by an increase in the synthesis rate of a specific set of proteins, including the family of major cold-shock proteins (Jones *et al.*, 1987, 1992; Graumann *et al.*, 1996, 1997; Graumann & Marahiel, 1999; Kaan *et al.*, 2002). Several cold-shock-inducible proteins interact with DNA or RNA and are possibly involved in the destabilization of unfavourable secondary structures of nucleic acids that may inhibit protein synthesis at low growth temperature. Examples of cold-shock-inducible proteins are the α-subunit of DNA gyrase from *Escherichia coli*, several initiation factors from *E. coli* or *Bacillus subtilis* and, prominently, the above-mentioned major cold-shock proteins.

The bacterial major cold-shock proteins (Csps) represent single cold-shock domains (CSDs). CSDs have been found in proteins from all forms of life (Wistow, 1990; Graumann & Marahiel, 1998; Manival *et al.*, 2001). CSDs are structured into a five-stranded antiparallel β-sheet that is folded into a β-barrel that is characteristic of the oligonucleotide/oligosaccharide binding (OB) fold (Schindelin *et al.*, 1993, 1994; Mueller *et al.*, 2000). It has been shown that the Csps are able to bind RNA as well as to single-stranded DNA (Schindelin *et al.*, 1993; Zeeb & Balbach, 2003) and also act as transcriptional activators by binding to the promoter regions of several cold-shock-induced genes (Jones *et al.*, 1992). Binding of Csp to mRNA has been proposed to prevent or destabilize secondary structures at reduced temperature. Csps would thus act as RNA chaperones (Jiang *et al.*, 1997). A common feature of CSDs is the presence of two conserved peptide motifs, RNP-1 and RNP-2 (Landsman, 1992; Schindelin *et al.*, 1993), which are also found in other RNA-binding proteins. Exposed aromatic and charged side chains of residues that belong to these motifs or are located nearby on the protein surface may be directly involved in nucleic acid binding.

Several NMR, fluorescence spectroscopic and mutational studies have been carried out in order to identify the residues that are involved in single-stranded DNA/RNA binding of CSDs in general (Schröder *et al.*, 1995; Schindler *et al.*, 1998; Lopez *et al.*, 1999, 2001; Kloks *et al.*, 2002; Zeeb & Balbach, 2003). We present here a preliminary X-ray diffraction and Raman analysis of crystals of the major cold-shock proteins from the mesophilic *B. subtilis* (*Bs*-CspB; 67 residues, M_r = 7365) and the thermophilic *Bacillus caldolyticus* (*Bc*-Csp; 66 residues, M_r = 7333) grown in the presence of (dT)$_6$.

2. Methods

### 2.1. Csp overexpression, purification, complex formation and crystallization

The genes encoding the cold-shock proteins *Bc*-Csp and *Bs*-CspB were overexpressed using the T7 RNA promoter system (Schindelin *et al.*, 1992). *Bc*-Csp and *Bs*-CspB were purified

as described previously (Schindler *et al.*, 1995; Mueller *et al.*, 2000) with minor modifications.

The HPLC-purified DNA fragments were purchased from BioTeZ (Berlin, Germany). For complex formation, protein and $(dT)_6$ were mixed in a 1:1.2 molar ratio. Excess DNA was separated using a self-packed G25 size-exclusion column (Amersham Biosciences, Freiburg, Germany) and the complexes were concentrated using Vivaspin 3 kDa concentrators (Vivascience, Hanover, Germany). Complex concentrations were in the range 6–11 mg ml^{-1} in 20 mM Tris pH 7.5, 50 mM KCl and 3 mM MgCl$_2$. Crystallization experiments were carried out at 293 K using the hanging-drop vapour-diffusion method.

2.2. Raman spectroscopic identification of thymine in the crystal

Raman spectra of single crystals of the *Bs*-CspB– and *Bc*-Csp–oligonucleotide complexes and their mother liquors were acquired using a T64000 Raman spectrometer (Jobin Yvon, Longjumeau, France) equipped with an Olympus BH-2 microscope. The crystals were suspended in hanging drops of the mother liquor. The microscope focus was adjusted either to the surface of the crystal or to a volume element of the solution. Raman scattering of the crystal and mother liquor was excited with
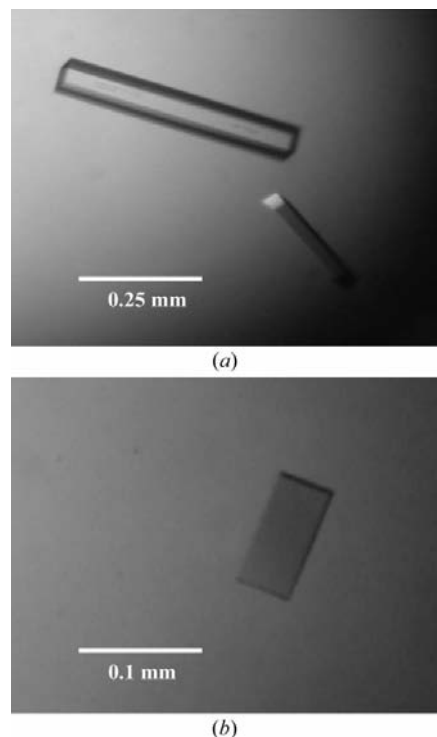
Table 1
Crystal parameters and X-ray diffraction data.

Data in parentheses are for the last shell.

	Bc-Csp–(dT)$_6$ (in-house)	*Bc*-Csp–(dT)$_6$ (BESSY ID14.2)	*Bs*-CspB–(dT)$_6$ (BESSY ID14.2)
Wavelength (Å)	1.54	0.9184	0.9184
Resolution (Å)	13.80–2.23 (2.35–2.23)	19.35–1.29 (1.40–1.29)	19.25–1.78 (2.00–1.78)
Space group	$P2_12_12$	$P2_12_12$	$C222_1$
Temperature (K)	110	100	100
Detector	MAR 345	MAR CCD	MAR CCD
Unit-cell parameters			
a (Å)	74.6	74.3	49.0
b (Å)	65.0	64.9	53.2
c (Å)	31.2	31.2	77.0
V_M (Å^3 Da^{-1})	2.1	2.1	2.8
No. of molecules per AU	2	2	1
Solvent content (%)	40	40	55
Unique reflections†	7737 (1059)	38042 (8013)	9874 (2832)
$I/\sigma(I)$	22.4 (15.0)	14.7 (5.0)	12.6 (3.4)
Data completeness (%)	98.7 (95.8)	97.9 (96.5)	99.2 (98.3)
R_{meas}‡ (%)	4.7 (8.3)	6.6 (32.4)	6.5 (42.8)

† Values are for $I/\sigma(I) \geq -3$. ‡ Multiplicity-corrected R_{sym} (Diederichs & Karplus, 1997).

488 nm light from an argon-ion laser with a power of ~40 mW at the sample. The Raman light was collected in 180° back-scattering geometry.

2.3. Data collection and processing

For diffraction experiments under cryo-conditions, the *Bs*-CspB–(dT)$_6$ crystals were transferred to a reservoir solution containing 10%(v/v) glycerol and then flash-frozen, whereas the *Bc*-Csp–(dT)$_6$ crystals were directly frozen in liquid nitrogen. 102 frames with an oscillation range of 1° were collected from a *Bc*-Csp–(dT)$_6$ crystal using a Rigaku RU H2B rotating-anode generator with a MAR 345 desktop beamline (MAR Research, Norderstedt, Germany) to 2.23 Å at 110 K with a crystal-to-detector distance of 200 mm. In addition, a data set was

collected at 100 K on the Protein Structure Factory beamline ID14.2 at BESSY, Berlin on a MAR CCD detector to 1.29 Å. The detector distance was 90 mm and 160 frames of 1° each were collected. Furthermore, a complete data set was collected from a *Bs*-CspB–(dT)$_6$ crystal at 100 K on BESSY ID14.2 to 1.78 Å with a detector distance of 140 mm. 293 frames with an oscillation range of 0.5° were recorded. All data were processed using *XDS* and *XSCALE* (Kabsch, 1993).

3. Results

Rod-like crystals of *Bc*-Csp suitable for data collection (Fig. 1*a*) grew in the presence of $(dT)_6$ within one week of mixing equal volumes of protein solution and reservoir

Figure 1
Crystals of (*a*) *Bc*-Csp and (*b*) *Bs*-CspB complexed with (dT)$_6$.
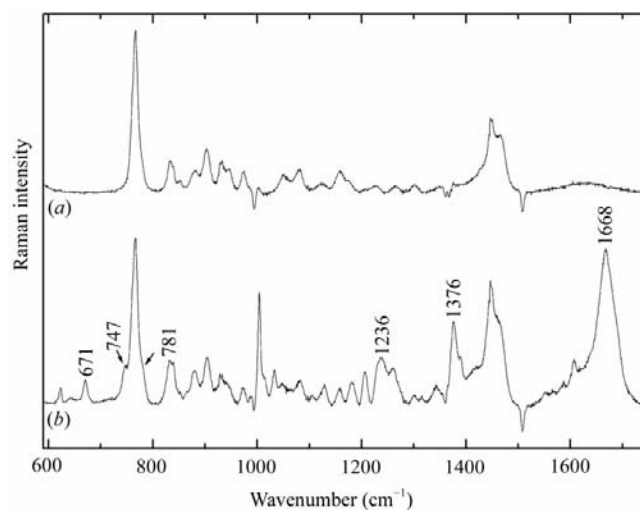
Figure 2
Raman spectra of (*a*) the mother liquor and (*b*) a single crystal of *Bc*-Csp in complex with the single-stranded hexanucleotide (dT)$_6$. Peaks in the spectrum of the crystalline complex are labelled with their wavenumbers at the positions of established thymine bands.

solution containing 35%(v/v) 2-methyl-2,4-pentanediol, 0.1 M sodium acetate and 0.02 M CaCl$_2$. Smaller plate-like single crystals of (dT)$_6$ bound to Bs-CspB (Fig. 1b) also grew. Here, two volumes of protein solution were mixed with one volume of reservoir solution containing 0.1 M sodium cacodylate pH 6.5, 0.2 M calcium acetate and 18%(w/v) PEG 8000.

To identify the macromolecular components within the crystal, a Raman spectroscopic analysis was carried out. Fig. 2 shows the spectra of the mother liquor (Fig. 2a) and the crystal (Fig. 2b) of Bc-Csp grown in the presence of (dT)$_6$. The spectra shown are averages of 30 exposures of 20 s each; they are not corrected for contributions from other buffer components. Peaks in the crystal spectrum are labelled by their wavenumbers at the positions of established thymine bands (Thomas & Tsuboi, 1993). The presence of these bands clearly indicates that thymine is a component of the crystal and permits us to conclude that a Bc-Csp–(dT)$_6$ complex has indeed been crystallized. A Raman analysis of the Bs-CspB crystals grown in the presence of (dT)$_6$ (not shown) similarly indicated the formation of a protein–DNA complex.

Data processing (Table 1) of the in-house data set of the Bc-Csp–(dT)$_6$ complex yielded a low distortion index for a primitive orthorhombic lattice, with an R_{meas} value of 4.6% after $XSCALE$. In space group $P2_12_12$, indicated by a systematic absence of odd-numbered axial reflections along a and b, two protein–DNA complexes in the asymmetric unit yields a Matthews coefficient of 2.1 Å^3 Da^{-1} (Matthews, 1968), which corresponds to a crystal solvent content of 40.5%. Processing of the synchrotron X-ray data of Bc-Csp complexed with (dT)$_6$ gave results nearly identical to the in-house data but with a higher resolution (1.29 Å). The crystals of Bs-CspB complexed with (dT)$_6$ grew in the orthorhombic space group $C222_1$ under the given conditions, with one complex in the asymmetric unit.

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