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Characterization of Cold-Shock Proteins from Polar Microorganisms

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Polar organisms should have mechanisms to survive the extremely cold environment. We have isolated psychrophilic bacteria from Arctic and Antartic samples. Several genes encoding cold-shock proteins, which are small, cold-induced bacterial proteins, have been cloned from *Streptomyces* sp. AA8321, *Psychromonas artica*, and *Polaribacter irgensii*. Since the specific functions of any polar bacterial cold-shock proteins have not yet been determined, we examined the role of cold-shock protein A from *Streptomyces* sp. AA8321 ($CspA_{St}$). Gel filtration chromatography showed that purified $CspA_{St}$ exists as a homodimer under physiological conditions, and gel shift assays showed that it binds to single-stranded, but not double-stranded, DNA. Overexpression of $CspA_{St}$ in *Escherichia coli* severely impaired the ability of the host cells to form colonies, and the cells developed an elongated morphology. Incorporation of a deoxynucleoside analogue, 5-bromo-2-deoxyuridine, into newly synthesized DNA was also drastically diminished in $CspA_{St}$-overexpressing cells. These results suggest that $CspA_{St}$ play a role in inhibition of DNA replication during cold-adaptation. Overexpression of CspA from *P. artica* in *E. coli* conferred increased resistance to freezing and thawing. These results suggest that cold shock proteins from polar microorganisms play important roles in cold adaption.

Results and discussion

Samples from sea water, biofilms, coast sediments, and submarine deposits were taken near Dasan Station (Arctic area) and King Sejong Station (Antarctic area). Fifty eight pure cultures were obtained by repeated cultivation and purification of strains at 4°C. To determine the optimum and the maximum growth temperatures, each strains were grown at 4, 10, 15, 20, 25, 30, and 37°C on solid media for one week and diameters of each colonies were measured. Ten strains, which grow faster at low temperatures (4, 10°C) than at ambient or higher temperature, were selected for further study. Phylogenetic analysis using 16S rDNA sequences have identified *Streptomyces* sp. AA8321, *Psychromonas artica*, *Polaribacter irgensii*, and other species.

Exponentially growing cells express a number of cold-induced proteins upon temperature drops of more than 10°C (Jones *et al.,* 1987). Of the cold-induced proteins, the small proteins known as cold-shock proteins (Csps) are the most prominent. Although Csps are widely found in various bacteria, including psychrophiles, mesophiles, and even thermophiles (Ermolenko and Makhatadze, 2002; Phadtare *et al.,* 2003) and Csp sequences are highly conserved, their physiological roles seems to vary depending on the proteins and organisms. Most functional analyses were done on Csps from mesophiles and thermophiles, but not from psychrophiles, which have to survive the extremely cold environment. Therefore, we chose to characterize Csps from the polar microorganisms.

We have cloned *csp*-homologous genes from polar bacteria by polymerase chain reaction (PCR) using degenerative primers designed from the conserved sequences of Csps. Four *csp* genes from the Antarctic *Streptomyces* sp. AA8321, one from *Psychromonas artica*, and three from *Polaribacter irgensii*, were cloned and sequenced. The *cspA* gene from *Streptomyces* sp. AA8321 ($CspA_{St}$) was cloned into the plasmid pAED4, which allows inducible expression in *E. coli* BL21(DE3) pLysS cells under control the of *lpp-lac* promoter. Surprisingly, the viability of $CspA_{St}$-overexpressing cells, as assessed by colony-forming ability, was less than 1% of that of pAED4 control cells. When cell growth was assessed by optical density measurements of the culture at 600 nm, the cell mass continued to increase in $CspA_{St}$-overexpressing cells although at the lower levels than that of control cells carrying the pAED4. Since protein production, as reflected by optical density, continued to increase, both transcription and translation continued to occur at moderate rates in $CspA_{St}$-expressing cells. When cell morphology was observed under a microscope, *E. coli* cells carrying the pAED4 vector were short and rod-shaped, whereas $CspA_{St}$-producing cells were elongated without a noticeable increase in cell width.

$CspA_{St}$ protein was purified by anion-exchange column chromatography and size-fractionation, as revealed by a single protein band with an apparent molecular mass of 7 kDa on SDS-PAGE. The purified protein exhibited a circular dichroism spectrum typical of β-strand-dominated proteins, suggesting that $CspA_{St}$ adopts a cold-shock domain-fold structure. Gel filtration of $CspA_{St}$ on a Superdex-75 column resulted in an elution profile consistent with that of a 15-kDa protein, indicating that $CspA_{St}$ exists exclusively as a homodimer under native conditions. In gel-shift assays with heat-denatured ssDNA as a probe, addition of purified $CspA_{St}$ retarded migration of the probe band in a concentration-dependent manner. In contrast, dsDNA did not gel-shift upon addition of $CspA_{St}$. DNA synthesis was severely impaired in the $CspA_{St}$-overproducing cells, as revealed by low BrdU incorporation into genomic DNA of $CspA_{St}$-overproducing cells (about 4% that of control cells).

The study suggest a role for $CspA_{St}$ in halting DNA replication until the cell adjusts itself upon sudden temperature drops (Kim *et al*., 2007). In our $CspA_{St}$-overproduction experiments, the amounts of $CspA_{St}$ protein declines at 5 h after induction, and the cell viability resumes simultaneously. The *cspA* gene

from *P. artica* was also cloned into the plasmid pAED4, the expression vector in *E. coli*. Overexpression of *P. artica* CspA has drastically increased cell survival upon repeated freezing and thawing, compared to the control cells. The mechanism of cold-resistance conferred by CspA from *P. artica* will be further studied.

References

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