

Abstract

The Characteristics of New SSB Proteins from Metagenomic Libraries and Their Use in Biotech Applications [†]

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Abstract: Single-stranded DNA binding proteins (SSBs) bind to single-stranded DNA in a sequence-independent manner to prevent formation of secondary structures and protect DNA from nuclease degradation. These ubiquitous proteins are present in prokaryotes, eukaryotes, and viruses, and play a pivotal role in the following major cellular processes: replication, recombination, and repair of genetic material. In DNA replication, SSB proteins specifically stimulate DNA polymerase, increase fidelity of DNA synthesis, assist the advance of DNA polymerase, and organize and stabilize replication forks. Here, we present our characterization of four SSB proteins of different origins. One of them was isolated from *Clostridium* sp. phage phiCP130 (SSB C1: 124 aa, Mr = 13,905). Three others (SSB M2: 136 aa, Mr = 15,009; SSB M3: 144 aa, Mr = 16,106; and SSB M5: 138 aa, Mr = 15,851) were isolated from metagenomics libraries. They show high similarity to SSB proteins from *Caldanaerovirga acetigigens*, *Caldanaerobius fijiensis*, and *Fervidobacterium gondwanense*. The recombinant proteins were overproduced in *E. coli* Rosetta (pRARE), except for SSB M5, which was overproduced in *E. coli* BL21. Proteins were purified using a metal-affinity chromatography as His-tagged fusion proteins. Electrophoretic mobility shift assay was used to examine their DNA binding activity with fluorescein-labeled oligonucleotide (dT40) used as a substrate. Thermal stability analysis revealed that they are stable at elevated temperatures, with the exception of SSB protein C1, which loses its activity above 65 °C. The other proteins are active at high temperatures, SSB M3 up to 85 °C, while SSB M2 and SSB M5 are active up to 98.7 °C. The subunit structure of proteins was analyzed by gel filtration on Superdex 75 column (AKTA). This allowed us to conclude that in solution, the analyzed proteins exist in oligomeric form, a feature which is characteristic of other SSB proteins. Purified SSB proteins were tested to improve specificity of PCR-based DNA amplification.

Keywords: DNA-binding protein; single-stranded DNA; oligomers



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