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Novel ssDNA-binding properties of SSB2 and SSB3 from *Thermoanaerobacter tengcongensis*

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Abstract: [Objective] SSB2 and SSB3 are ssDNA-binding proteins of *Thermoanaerobacter tengcongensis*. This work aimed to disclose novel properties of both proteins. [Methods] We performed electrophoretic mobility shift assays (EMSAs) using oligonucleotides spanning the replication origin of *T. tengcongensis* and non-denaturing polyacrylamide gels. Western blotting assays were used to study the expression patterns of both proteins. [Results] SSB2 bound to 35-nt, 59-nt and 70-nt ssDNA spanning the replication origin and formed one, two or three DNA-protein complexes. The number of the SSB2-DNA complexes was determined by both the length of the ssDNA and the concentration of SSB2. SSB3 formed one more DNA-protein complex with 59-nt or 70-nt ssDNA in comparison with SSB2. Storage of the proteins at −70℃ led to the disappearance of one SSB2-(70-nt) complex, or two SSB3-(59-nt) complexes or three SSB3-(70-nt) complexes in the EMSA, indicating the distinct loss of the SSBs's conformations. Moreover, SSB2 and SSB3 displayed different expression patterns at variable incubation temperatures *in vivo*. [Conclusion] SSB2 and SSB3 could bind ssDNA with various conformations that were determined by the length of ssDNA, the concentration of the proteins, as well as the temperature of treatment. To our knowledge, this is the first disclosure of the characteristics of SSB2 and SSB3 on 35-70 nt oligonucleotides.

Keywords: Single-stranded binding protein (SSB); ssDNA-binding profile; protein conformation; protein expression pattern

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Thermoanaerobacter tengcongensis is an extreme thermophilic eubacterium, which was isolated from Tengcong hot spring in Yunnan, China[1]. To adapt to the high-temperature condition, the replication-related proteins including single-stranded DNA-binding protein (SSB) should be thermostable. There are three SSBs in *T. tengcongensis*, designated as SSB1, SSB2 and SSB3. SSB1 was being studied in our group and displayed quite different biochemical features (unpublished data) from other known SSB members. SSB2 and SSB3 were studied recently by Olszewski et al[2]. They found that both SSB2 and SSB3 contain an OB fold, which displays the single-stranded DNA-binding activity[3]. When the DNA substrates were 35-76 nt oligo(dT), these proteins can form a single DNA-protein complex. If the substrate was 120 nt oligo(dT), two DNA-protein complexes were detected[2]. However, these results were obtained using electrophoretic mobility shift assay (EMSA) in agarose gels[2].

We have found that EMSA using native polyacrylamide gel can provide better resolution of the DNA-protein interaction than using agarose gel. Therefore, to get more detailed information of the DNA binding functions of SSB2 and SSB3, we performed EMSAs with polyacrylamide gel. Although SSB proteins are known to be able to interact with ssDNA without

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sequence specificity, we considered that the native DNA from *T. tengcongensis* is more biologically relevant substrates for SSB2 and SSB3. Moreover, SSB proteins perform ssDNA protecting activity during DNA replication, repair, and recombination[4-7]. Therefore, we used the oligonucleotides corresponding to the DNA replication origin of *T. tengcongensis* in the EMSAs instead of oligo (dT), since the replication origin was most likely involved in all the above DNA processes. To study the possible effect of DNA length on the binding property of SSB2 and SSB3, three oligonucleotides with different lengths, i.e., 35-nt, 59-nt and 70-nt, were used to study the DNA-binding properties of SSB2 and SSB3.

All the above strategies led us to obtain novel, subtle and different DNA-binding properties of both SSB2 and SSB3.

1 MATERIAL AND METHODS

1.1 Strains and growth conditions

T. tengcongensis MB4^T was isolated from Tengcong hot spring in Yunnan, China[8]. It was routinely incubated in modified MB medium at 75℃ according to Xue et al.[1,9]. *Escherichia coli* C41 (DE3) and C43 (DE3) were used for the overexpression of SSB2 and SSB3, respectively[10-11].

1.2 Overexpression and purification of SSB2 and SSB3

Chromosomal DNA was isolated from *T. tengcongensis* as previously described[12]. Based on the published sequence of *T. tengcongensis*, the *ssb2* coding region was amplified by PCR using chromosomal DNA as template and oligonucleotides P1 (5′-GGGCATATGAGCCTTAACAAAGTCATGC-3′, NdeI site underlined) and P2 (5′-GGGCTCGAGAACAGTAAAAAGATTTTCC-3′, *Xho*I site underlined) as forward and reverse primers. Meanwhile, the *ssb3* coding region was amplified by PCR using P3 (5′-GGGCATATGTTAAATAAAGTAATTCTGAT-3′, *Nde*I site underlined) and P4 (5′-GGGCTCGAGAAAAGGCAAGTCATCTTC-3′, *Xho*I site underlined) as primers. The PCR products digested with *Nde*I and *Xho*I were inserted into the same sites of pET23b to generate the expression plasmids pET23b::*ssb2* and pET23b::*ssb3*, respectively. The authenticity of *ssb2* and *ssb3* sequences was confirmed by DNA sequencing (Sunbiotech Company, China).

For protein overexpression, pET23b::*ssb2* or pET23b::*ssb3* was introduced into *E. coli* C41 (DE3) or C43 (DE3), respectively. The resulting strain was grown at 37℃ in 200 mL LB medium with 100 ($\mu g \cdot mL^{-1}$) ampicillin to an OD_{600} of 0.6. IPTG was then added to a final concentration of 0.1 ($mmol \cdot L^{-1}$) and the cultures were further incubated for 14 h at 28℃. The cells were harvested by centrifugation (8000 × g, 4℃, 10 min), and re-suspended in 30 ml of binding buffer [500 ($mmol \cdot L^{-1}$) NaCl, 20 ($mmol \cdot L^{-1}$) Tris-HCl and 5 ($mmol \cdot L^{-1}$) imidazole (pH 7.9)]. The cell suspension was treated by sonication on ice and centrifuged (14000 × g, 4℃, 20 min) to remove the cellular debris. The supernatant was then applied to Ni-NTA (Ni^{2+}-nitrilotriacetate)-agarose columns (Novagen, UK), pre-equilibrated with binding buffer[13]. The column was washed with 20 mL of washing buffer [500 ($mmol \cdot L^{-}$) NaCl, 20 ($mmol \cdot L^{-1}$) Tris-HCl and 150 ($mmol \cdot L^{-1}$) imidazole (pH 7.9)]. After then, the His-tagged protein was specifically eluted from the resin with 10 mL of elution buffer [500 ($mmol \cdot L^{-1}$) NaCl, 20 ($mmol \cdot L^{-1}$) Tris-HCl and 250 ($mmol \cdot L^{-1}$) imidazole, pH 7.9] and concentrated to about 5 ($\mu g \cdot \mu L^{-1}$) by ultrafiltration (Mili-pore membrane, 3 kDa cut-off size). Protein purity was determined by Coomassie blue staining after SDS-PAGE on a 12% polyacrylamide gel. Protein concentrations were determined by spectrophotometric absorbance at a wavelength of 562 nm, using BCA Protein Assay Kit (Novagen, UK). The purified protein was used immediately in the EMSA or stored in elution buffer at −70℃.

1.3 Preparation of DNA probes

Three oligonucleotides spanning the replication origin of *T. tengcongensis* were synthesized by Sangon (Shanghai Sangon Biological Engineering and Technology and Service Company, China). They were designated as P5, (nt 1-35, 5′-TTTTTCTTATTGATAATCTGTTGATAATTTGCTAT-3′), P6 (nt 1-59, 5′-TTTTTCTTATTGATAATCTGTTGATAATTTGCTATTATAGAAGTAAACCTGTTGATAAC-3′), and P7 (nt 1-70, 5′-TTTTTCTTATTGATAATCTGTTGATAATTTGCTATTATAGAAGTAAACCTGTTGATAACTTAAATAAATT-3′). All three oligos contain the same 1-35 nt of the replication origin. The oligonucleotides were 5′-end labeled with [γ-^{32}P]-ATP using T4 polynucleotide kinase

(Promega,USA) for 30 min at 37℃, then heated for 2 min at 90℃ to inactivate the T4 kinase. The unincorporated label was removed with Qiaquick Nucleotide Removal Kit (Qiagen,Germany).

1.4 Electrophoretic mobility shift assay (EMSA)

Electrophoretic mobility shift assays (EMSAs) were performed as previously described with modifications[7]. Briefly, 0.05 pmol 32P-labeled oligonucleotide was incubated with varying amounts of SSB2 or SSB3 at 25℃ or other indicated temperatures for 5 min in 20 μL reaction buffer [20 (mmol·L^{-1}) Tris,25 (mmol·L^{-1}) NaCl,1 (mmol·L^{-1}) EDTA,1 (mmol·L^{-1}) DTT and 5% glycerol]. After incubation, protein-bound and free DNA were separated by electrophoresis on non-denaturing 6% polyacrylamide gels (mono/bis, 80∶1) with running buffer [40 (mmol·L^{-1}) Tris-HCl (pH 7.8),20 (mmol·L^{-1}) boric acid, and 1 (mmol·L^{-1}) EDTA] at 10 (V·cm^{-1}) for 1.5 h and at 25℃. Gels were dried and exposed to Biomax radiographic film (Kodak). All the experiments were done three times and the same results were obtained.

1.5 Protein extraction and Western blotting analysis

T. tengcongensis MB4^T was respectively incubated in anaerobic bottles with modified MB medium at 45℃, 55℃,65℃, and 75℃ without shaking. Cells harvested by centrifugation (8000×g,4℃,3 min) were suspended in binding buffer (see above) and lysed by sonication. The resulting lysate was centrifuged (14000×g,4℃,20 min) to remove the cellular debris. Protein concentrations of the supernatants of different cell lysates were determined as described above. 10 μL of the samples containing 100 μg soluble proteins were heated to 100℃ for 5 min. After brief cooling, the sample was further centrifuged at 14000×g. The suspension was subjected to 12% SDS-PAGE and transferred to a PVDF membrane. Western blotting analysis was performed using mouse polyclonal antibodies against SSB2 at 1∶5000 dilutions or that against SSB3 at 1∶2000 dilutions. Signals were detected with the ECL Western Blotting Detection Kit (Promega,USA)[14].

2 RESULTS AND DISCUSSION

2.1 The novel ssDNA-binding profile of SSB2

It has been reported that SSB2 of *T. tengcongensis* was able to bind oligo(dT) 35-76 and form one DNA-protein complex[2]. When we used the oligonucleotides spanning the replication origin of *T. tengcongensis* in the EMSAs, SSB2 exhibited novel and complex binding profiles in comparison with those obtained by Olszewski et al[2]. Highly purified His-tagged SSB2 was obtained (Fig. 1-A). SSB2 protein could bind to the ssDNA of the replication origin at the protein concentrations ranging from 0.005 to 0.5 (μmol·L^{-1}). It formed different numbers of SSB2-DNA complexes with the 35-nt P5,59-nt P6 or 70-nt P7 at the saturated protein concentration of 0.5 (μmol·L^{-1}) (Fig. 1B). A single protein-DNA complex was formed at low or saturated protein concentration when SSB2 was incubated with P5 (Fig. 1B lanes 2-4). Meanwhile, one or two DNA-protein complexes could be formed at low or saturated protein concentration, while SSB2 was incubated with P6 (Fig. 1-B lanes 6-8). When P7 was used as the substrate, SSB2 could interact with it and form one to three DNA-protein complexes with the increase of protein concentration (Fig. 1-B lanes 10-12). These results indicate that: (i) SSB2 could bind 35 nt,59 nt and 70 nt ssDNA substrates and displayed various binding profiles; (ii) the formation of two or three DNA-protein complexes with P6 or P7 revealed the existence of various conformations of SSB2; (iii) for the longer oligonucleotide, such as 70 nt P7 substrate, SSB2 displayed multiple DNA-binding profiles that is distinctively correlated with the SSB2's concentration. These findings are different from that reported by Olszewski[2]. In their study, only one SSB2-DNA complex was detected when $(dT)_{35}$, $(dT)_{60}$, $(dT)_{70}$ or $(dT)_{76}$ was used as the substrate. Two SSB2-DNA complexes were detected only when $(dT)_{120}$ was used as the substrate[2]. The following reasons may be responsible for the discrepancies: (i) the fluorescein-labeled probe that Olszewski et al used was not sensitive enough to show the positions of all DNA-protein complexes. Rather, only the dominant ones could be detected because of their stronger fluorescence; (ii) 2% agarose gel had much larger aperture incapable of separating the different SSB2-DNA complexes formed with the same ssDNA substrate; (iii) the EMSA reaction system and the electrophoresis conditions used by Olszewski et al might not be efficient enough to dissolve

multiple DNA-protein complexes formed with the same oilgonucleotide shorter than 76 nt.

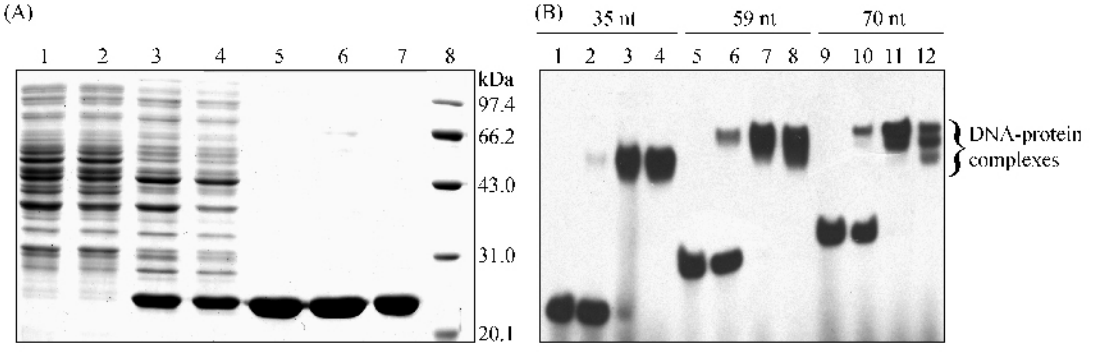

Fig. 1 The purification of SSB2 and its binding profile with oligonucleotides spanning the replication origin. (A) The overexpression and purification of SSB2 from *E. coli* C41 (DE3)/pET23b::*ssb2*. *Lanes 1 and 2* Total and soluble protein extracts from *E. coli* C41 (DE3)/ pET23b after IPTG induction; *lanes 3 and 4* Total and soluble protein extracts from *E. coli* C41 (DE3)/pET23b::*ssb2*; *lanes 5, 6 and 7* The purified SSB2 after chromatography on Ni-NTA (Ni^{2+}-nitrilotriacetate)-agarose columns; *lane 8* The molecular mass weight marker of proteins. (B) Binding of SSB2 to the oligonucleotides spanning replication origin of *T. tengcongensis*. The reaction solution contained 0.05 pmol of the 5′-end labeled oligonucleotide and 0.1, 1.0 or 10 pmol of SSB2. *Lanes 1, 5 and 9* Free probes of 35 nt, 59 nt and 70 nt; *lanes 2, 6 and 10* Probes were incubated with 0.1 pmol SSB2; *lanes 3, 7 and 11* Probes were incubated with 1 pmol SSB2; *lanes 4, 8 and 12* Probes were incubated with 10 pmol SSB2.

2.2 The effect of temperature on the ssDNA-binding activity of SSB2

To determine the effect of temperature on the ssDNA-binding activity of SSB2, EMSAs were performed at different temperatures using freshly prepared SSB2 at saturated concentration. The results showed that SSB2 could bind to P6 or P7 and form two or three DNA-protein complexes at 50℃, 75℃, and 95℃, similar to that at 25℃ (Fig. 2A). Therefore, the freshly prepared SSB2 displayed the same ssDNA-binding activity at higher reaction temperatures as that of 25℃, indicating that SSB2 was of thermostability.

During the study of the ssDNA-binding properties of SSB2, we found that SSB2 showed altered ssDNA-binding profiles after it was stored at −70℃ for several days. The low-temperature-stored SSB2 could still bind to P5, P6, and P7 at 25℃ or 75℃, in which the binding profiles of SSB2 with P5 and P6 were not significantly affected. In contrast, only two, instead of three, SSB2-P7 complexes were detected at the saturated protein concentration (Fig. 2B). This phenomenon could be explained as that: the storage of SSB2 at −70℃ might reduce the conformations of SSB2 in the interaction with 70 nt ssDNA. Thus, freshly prepared SSB2 should be used in the EMSA in order to obtain its detailed biochemical functions.

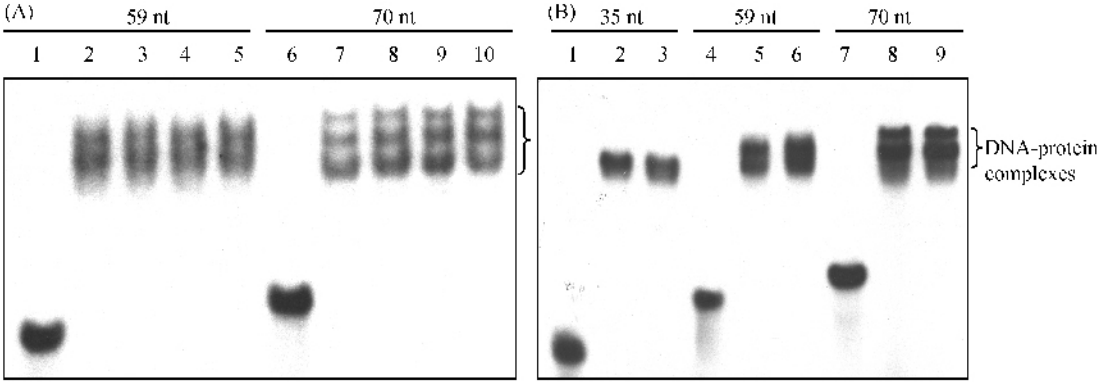

Fig. 2 The thermostability and low-temperature-sensitivity of SSB2. (A) The thermostability of freshly prepared SSB2. 59-nt probe (lane 1) was incubated with SSB2 at 25℃, 50℃, 75℃, and 90℃ respectively for 5 min (lanes 2-5); 70-nt probe (lane 6) was incubated with SSB2 at the similar conditions (lanes 7-10). (B) The effect of low-temperature storage on the ssDNA-binding property of SSB2. The SSB2 was stored at −70℃ for one week and then used in the following EMSAs when required. *Lanes 1, 4 and 7* Free labeled probes of 35 nt, 59 nt and 70 nt; *lanes 2, 5 and 8* The probes were incubated with SSB2 at 25℃; *lanes 3, 6 and 9* The probes were incubated with SSB2 at 75℃.

2.3 The novel ssDNA-binding profile of SSB3

To gain more insights into the properties of SSB3, EMSAs were performed between SSB3 and the oligonucleotides P5, P6 and P7 at 25℃. SSB3 was overexpressed in *E. coli* and purified to homogeneity (Fig. 3A). SSB3 protein formed one DNA-protein complex with P5, one to three DNA-protein complexes with P6, or one to four DNA-protein complexes with P7 at the protein concentrations ranging from 0.005 to 0.5 μM (Fig. 3B). These results clearly indicated that the substrate of SSB3 could be 35-nt, 59-nt, or 70-nt ssDNA of the replication origin of *T. tengcongensis*, and that the number of DNA-protein complexes or the DNA-binding profile of SSB3 was determined by both the length of the oligonucleotide substrate and the protein concentration.

Interestingly, SSB3 formed one more protein-DNA complex with 59-nt or 70-nt ssDNA than SSB2 at their saturated protein concentration (compare Fig. 1B to Fig. 3B). It is speculated that SSB3 may have more conformations or oligomerizations than SSB2 in the binding with comparatively longer ssDNA substrates. The other different property between SSB2 and SSB3 was that 0.05 pmol of P6 or P7 could be completely bound by at least 1 pmol of SSB2 or 10 pmol of SSB3 (compare lanes 7 and 11 in Fig. 1B with lanes 8 and 12 in Fig. 3B), indicating that SSB2 might have higher affinity to 59-nt or 70-nt ssDNA than SSB3. These different features were not disclosed by Olszewski et al.

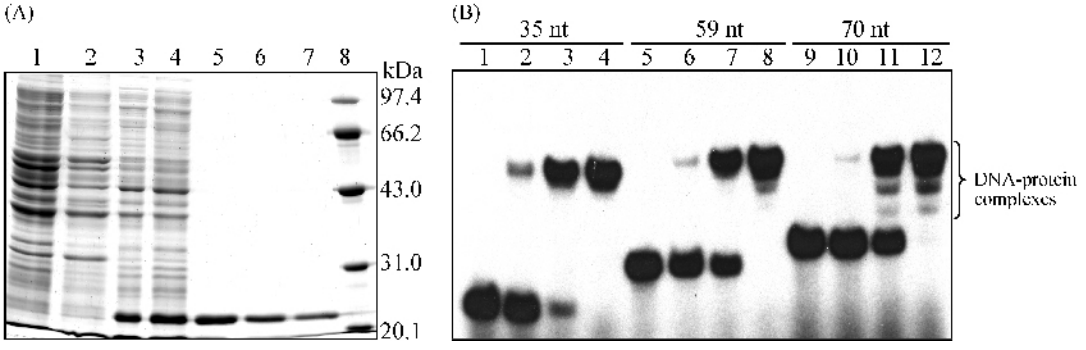

Fig. 3 The purification of SSB3 and its binding profile with oligonucleotides spanning the replication origin. (A) The expression and purification of SSB3 from *E. coli* C43 (DE3)/ pET23b::*ssb3*. *Lanes 1 and 2* Total and soluble protein extracts from *E. coli* C43 (DE3)/pET23b after IPTG induction; *lanes 3 and 4* Total and soluble protein extracts from *E. coli* C43 (DE3)/pET23b::*ssb3* after IPTG induction; *lanes 5, 6 and 7* The purified SSB3 after chromatography on Ni-NTA (Ni^{2+}-nitrilotriacetate)-agarose columns; *lane 8* The molecular mass weight marker of proteins. (B) Binding of SSB3 to the oligonucleotides spanning replication origin of *T. tengcongensis*. The reaction solution contained 0.05 pmol of the 5′-end labeled oligonucleotide and 0.1, 1.0 or 10 pmol of SSB2. *Lanes 1, 5 and 9* Free probes of 35 nt, 59 nt and 70 nt; *lanes 2, 6 and 10* Probes were incubated with 0.1 pmol of SSB3; *lanes 3, 7 and 11* Probes were incubated with 1 pmol of SSB3; *lanes 4, 8 and 12* Probes were incubated with 10 pmol SSB3.

2.4 The effect of temperature on the ssDNA-binding activity of SSB3

To investigate the potential effect of low-temperature storage on the ssDNA-binding activity of SSB3, EMSAs were performed. The binding profile of the cold-treated SSB3 with P6 or P7 was markedly different (Fig. 4A), in comparison with that of the freshly prepared SSB3 (Fig. 3B). Only one DNA-protein complex could be formed between the low-temperature-stored SSB3 and P5, P6, or P7 at the saturated protein concentration (Fig. 4A). Compared with the DNA-binding profile of freshly-prepared SSB3 in Fig. 3B, two of SSB3-P6 complexes and three of SSB3-P7 complexes were absent in the EMSA gel of Fig. 4B. These results indicated that the storage of SSB3 at −70℃ can lead to the decrease of its conformational forms and their interaction properties with ssDNA. However, the storage at −70℃ did not seem to reduce SSB3's binding capacity with ssDNAs, as all of the oligonucleotides were incorporated into the SSB3-DNA complexes, with no free DNA probes left (Fig. 4A).

To study the thermostability of SSB3, freshly purified SSB3 was heated at 50℃, 75℃, 90℃, and 100℃ respectively for 10 min or 20 min. After the treatment, SSB3, at saturated concentration, was incubated with P5 at 25℃ for 5 min (Fig. 4B). The EMSA results showed that SSB3 preheated at 50℃ for 10 or 20 min had similar ssDNA-binding profile as the untreated protein, indicating

that 50℃-heating does not affect the ssDNA-binding activity of SSB3. Similar result was obtained with the SSB3 preheated at 75℃ for 10 min. In contrast, after pretreated at 75℃ for 20 min, or at 90℃ and 100℃ for 10 or 20 min, SSB3 bound to P5 but formed less amount of protein-DNA complex indicated by leftover free ssDNA. This suggests that the ssDNA-binding activity of SSB3 can be affected by heat treatment for longer time or at higher temperature (Fig. 4B).

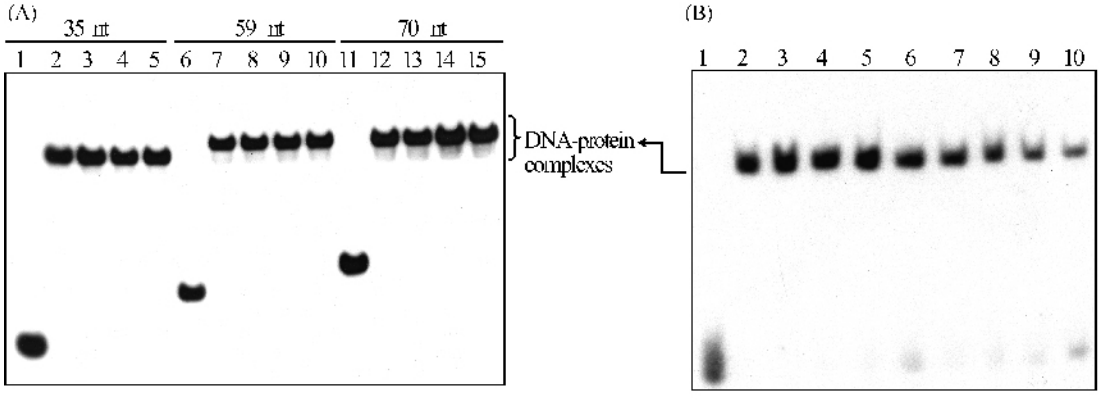

Fig. 4 The effect of low-temperature storage and preheating on the ssDNA-binding activity of SSB3. (A) The effect of low-temperature storage on the ssDNA-binding activity of SSB3. SSB3 was stored at −70℃ for one week and then used in the following EMSAs at saturated concentration when required. 35 nt probe (lane 1) was incubated with SSB2 for 5 min respectively at 25℃, 50℃, 75℃ and 90℃ (lanes 2-5). 59 nt probe (lane 6) was incubated with SSB2 for 5 min respectively at 25℃, 50℃, 75℃ and 90℃ (lanes 7-10). 70 nt probe (lane 11) was incubated with SSB2 for 5 min respectively at 25℃, 50℃, 75℃ and 90℃ (lanes 12-15). (B) The effect of pretreatment of the protein at high temperature on the ssDNA-binding activity of SSB3. *Lane 1* The free 35-nt P5 was labeled and used as a probe; *lane 2* The untreated SSB3 was incubated with P5 at 25℃; *lanes 3 and 4* SSB3, pretreated at 50℃ for 10 and 20 min respectively, was incubated with P5 at 25℃; *lanes 5 and 6* SSB3, pretreated at 75℃ for 10 and 20 min respectively, was incubated with the P5 at 25℃; *lanes 7 and 8* SSB3, pretreated at 95℃ for 10 and 20 min respectively, was incubated with P5 at 25℃; *lanes 9 and 10* SSB3, pretreated at 100℃ for 10 and 20 min respectively, was incubated with the P5 at 25℃.

2.5 The expression patterns of SSB2 and SSB3 *in vivo*

The EMSA results showed that SSB2 and SSB3 displayed different ssDNA-binding properties which were determined by the protein concentrations or influenced by the heat-/cold-treatment. This prompted us to analyze the expression level of SSB2 and SSB3 in *T. tengcondensis* at different incubation temperatures. Because *T. tengcongensis* can grow properly between 45℃ and 75℃, but poorly at temperatures exceeding 90℃ or lower than 40℃, western blotting analysis was performed with the clear lysates of *T. tengcongensis* grown at 45℃, 55℃, 65℃, and 75℃ respectively using antibodies against SSB2 or SSB3. We collected equal amount of cells at each incubation temperature and they could give nearly the same amount of soluble proteins after sonication. The western blotting assays showed that both SSB2 and SSB3 could be expressed in *T. tengcongensis* at 45℃, 55℃, 65℃, and 75℃ (Fig. 5). However, the expression patterns were different: SSB2 was expressed at high level at 45℃ to 65℃, but at a slightly decreased level at 75℃. In contrast, SSB3 was expressed at a lower level at 45℃, but at constant and higher level at 55℃, 65℃, and 75℃. These results indicate that both proteins can be expressed constitutively at temperatures ranging from 45℃ to 75℃ and may play important roles in the ssDNA binding and protection processes. The different expression patterns of SSB2 and SSB3 implies that their expression may be regulated by unknown mechanisms, such as a temperature-responsive mechanism. SSB2 or SSB3 may confer its own dominant function under specific conditions, for instance, SSB2 may be more important than SSB3 at 45℃, whereas SSB3 may play a more significant role at 75℃.

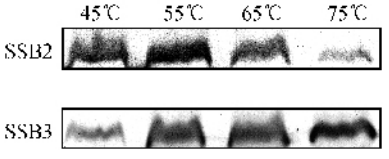

Fig. 5 The effect of incubation temperature on the expression levels of SSB2 and SSB3 in the *T. tengcongensis*. Samples containing 100 μg soluble proteins were used for the Western blotting analysis.

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腾冲嗜热菌单链 DNA 结合蛋白 SSB2 和 SSB3 新的单链 DNA 结合特性

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摘要：【目的】揭示腾冲嗜热菌中两个单链 DNA 结合蛋白 SSB2 和 SSB3 的全新的底物结合功能及其不同的体内表达模式。【方法】利用腾冲嗜热菌复制起始位点附近的长度较短的单链 DNA 为底物，采用非变性聚丙烯酰胺凝胶电泳及 Western blot 方法，研究 SSB2 和 SSB3 体外单链 DNA 结合特征和体内表达模式。【结果】SSB2 与 35nt 的复制起始区单链 DNA(ssDNA)结合，形成单个 SSB2-DNA 复合物；当与 59 nt ssDNA 结合时，可以随着蛋白浓度的递增形成一个或两个 SSB2-DNA 复合物；而与 70nt 的单链 DNA 结合时，则形成 1 ~ 3 个 SSB2-DNA 复合物。这些结果说明 SSB2 在与长度小于 70 nt 的单链 DNA 结合时，存在着多种构型。而这些构型的形成取决于单链 DNA 的长度、蛋白的浓度，并与 SSB 蛋白所受的预处理温度和反应温度有关。SSB3 与 59nt 和 70 nt 结合时，最多形成 3 个或 4 个复合物。低温保存和高温下反应对 SSB3 蛋白的功能影响比 SSB2 更为显著，表现为构型明显减少或结合 ssDNA 能力下降。此外，在腾冲嗜热菌中，SSB2 和 SSB3 的表达水平随温度变化而变化，SSB2 在 45℃ ~ 65℃间表达水平很高，在 75℃时表达水平下降，而 SSB3 在 45℃时表达水平较低，在 55℃到 75℃间表达水平较高，说明二者在腾冲嗜热菌中的表达可能受到培养温度的调控。【结论】腾冲嗜热菌中 SSB2 和 SSB3 具有全新的底物结合特征及其不同的体内表达模式。

关键词：单链 DNA 结合蛋白 蛋白构型 蛋白表达模式

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