Chimeras between Single-stranded DNA-binding Proteins from Escherichia coli and Mycobacterium tuberculosis Reveal That Their C-terminal Domains Interact with Uracil DNA Glycosylases*

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Uracil, a promutagenic base in DNA can arise by spontaneous deamination of cytosine or incorporation of dUMP by DNA polymerase. Uracil is removed from DNA by uracil DNA glycosylase (UDG), the first enzyme in the uracil excision repair pathway. We recently reported that the Escherichia coli single-stranded DNA binding protein (SSB) facilitated uracil excision from certain structured substrates by E. coli UDG (EcoUDG) and suggested the existence of interaction between SSB and UDG. In this study, we have made use of the chimeric proteins obtained by fusion of N- and C-terminal domains of SSBs from E. coli and Mycobacterium tuberculosis to investigate interactions between SSBs and UDGs. The EcoSSB or a chimera containing its C-terminal domain interacts with EcoUDG in a binary (SSB-UDG) or a ternary (DNA-SSB-UDG) complex. However, the chimera containing the N-terminal domain from EcoSSB showed no interactions with EcoUDG. Thus, the C-terminal domain (48 amino acids) of EcoSSB is necessary and sufficient for interaction with EcoUDG. The data also suggest that the C-terminal domain (34 amino acids) of MtuSSB is a predominant determinant for mediating its interaction with MtuUDG. The mechanism of how the interactions between SSB and UDG could be important in uracil excision repair pathway has been discussed.

Uracil residues appear in DNA as a result either of spontaneous deamination of cytosine or of misincorporation of dUMP by DNA polymerase. Uracil DNA glycosylase (UDG) excises uracil residues from DNA and prevents mutations from arising to maintain genomic integrity. The UDG-directed base excision repair pathway has been shown to involve at least five proteins, UDG, AP endonuclease IV, RecJ, DNA polymerase I, and DNA ligase, and to utilize the single nucleotide gap-filling activity. The efficient excision of uracil from structured substrates led to a proposal that melting of such structures may facilitate efficient repair. We subsequently showed that the inclusion of Escherichia coli SSB (EcoSSB) in the reactions augmented uracil release from the structured substrates by E. coli UDG (EcoUDG; Ref. 3). The KMnO₄ footprint and Tₘ analyses showed that SSB melted the hairpin substrates (3, 4).

Recently, we cloned and purified SSB from Mycobacterium tuberculosis (MtuSSB; Ref. 5). Its biochemical characterization revealed that it is similar to EcoSSB in its oligomerization status and various DNA binding properties. Surprisingly, inclusion of MtuSSB in the reactions led to a decrease in uracil excision by EcoUDG (4). Thus, it appeared that the effect of SSB on uracil release from a structured substrate by EcoUDG was not merely a consequence of melting of the structured substrate. The binary (SSB-UDG) or the ternary interaction (DNA-SSB-UDG) may also influence the activity of UDG.

Single-stranded DNA-binding proteins (SSBs) are members of an important class of proteins that play an essential role in various DNA transactions (6). EcoSSB, the archetype of the prokaryotic SSBs, is a homotetramer consisting of monomeric subunits of 177 amino acids. The secondary structure prediction suggests that EcoSSB can be divided into two parts, an N-terminal domain (120 amino acids) rich in α-helices and β-sheets and a C-terminal domain (60 amino acids) having no defined structure (8, 9). Studies with EcoSSB N-terminal fragments obtained by cleavage at Arg₁¹⁵ by trypsin (SSBp) or at Trp₁³⁵ by chymotrypsin (SSBc) have established that the N-terminal domain is responsible for tetramerization and DNA binding. The C-terminal region has been suggested to be important in the interaction of SSBs with various proteins in E. coli (11).

The human homolog of single-stranded DNA-binding protein, replication protein A, was shown to interact with XPF-ERCC1 and participate in the nucleotide excision repair pathway (12). More recently, replication protein A has been shown to interact with human UDG and participate in the uracil excision repair pathway (13, 14). However, there are no reports wherein the prokaryotic SSBs have been shown to interact with UDGs or any other DNA glycosylases.

M. tuberculosis continues to be the pathogen that causes most casualties worldwide. To develop new means to control this pathogen, it is important to understand the biology of this organism. Because of the high G+C contents of its genome (15) and the habitat of the host macrophages where the bacterium resides, cytosine deamination may constitute a major form of DNA damage in these organisms, making UDG a crucial DNA repair enzyme. In this report, we have studied the interaction between UDGs and SSBs from E. coli and M. tuberculosis, and

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† The abbreviations used are: UDG, uracil DNA glycosylase; SSB, single-stranded DNA binding protein; EcoSSB, E. coli SSB; EcoUDG, E. coli UDG; MtuSSB, M. tuberculosis SSB; MtuUDG, M. tuberculosis UDG; EcoRRF, E. coli ribosome recycling factor; MtuEcoSSB, contains the N-terminal portion from MtuSSB and the C-terminal domain (48 amino acids) from EcoSSB; MtuEcoUDG, contains the N-terminal domain from MtuSSB and the C-terminal domain (34 amino acids) from EcoSSB.

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we show that the C-terminal domain of SSB mediates interaction with UDG, both in the absence and presence of DNA.

MATERIALS AND METHODS

Purification of SSBs and UDGs—The native and the chimeric SSBs (EcoSSB, MtuSSB, MtuEcoSSB, and EcoMtuSSB) were purified by the method already described (5, 16). Purification of EcoUDG and MtuUDG was carried out as described previously (4, 17). Purified proteins were estimated by modified Bradford’s dye binding assay using bovine serum albumin as a standard (18), analyzed by electrophoresis on 15% polyacrylamide gels containing 0.1% SDS under reducing conditions, and visualized by Coomassie Brilliant Blue R-250 staining (19).

Uracil DNA Glycosylase Reactions—10 pmol of a hairpin substrate, Loop-U2, containing uracil in the second position of the tetra loop, 5'-CTAGAGGCTTGGTATGC-3', was 5'-end labeled using [γ-32P]ATP and purified using Sephadex G-50 minicolumns (2, 3, 20). For assays, 1 pmol of Loop-U2 (20,000 cpm) was incubated in the presence or absence of 5 pmol of the various SSB tetramers in UDG buffer (50 mM Tris-HCl, pH 8.0, 1 mM Na3EDTA, 1 mM dithiothreitol, and 25 μg/ml bovine serum albumin) and treated with UDG (0.4, 4, or 40 pmol) at 37 °C for 10 min. The reaction was stopped with 5 μl of 0.2 M NaOH, heated at 90 °C for 10 min, dried in the Speed Vac (Savant), and dissolved in 10 μl of formamide dye (80% formamide, 0.05% xylene cyanol FF and bromphenol blue, and 1 μl Na3EDTA). Aliquots (5 μl) were analyzed on 18% polyacrylamide, 8 × urea gels (21), and the bands corresponding to substrate and product were quantitated by a Bioimage Analyzer (Fuji). Control reactions were treated exactly in a similar manner, except that no protein was added.

Association of the UDG Activity with SSBs—Loop-U2 (1 pmol, 20,000 cpm) was incubated with 5 pmol of SSBs or ribosome recycling factor (EcoRRF) in UDG buffer for 10 min at 37 °C. The reaction was terminated with 5 μl of 0.2 M NaOH and processed for the detection of the uracil excision activity as above.

Yeast Two-hybrid Assay System—The yeast two-hybrid analyses were performed using the HIS3 and β-galactosidase reporter systems (22). The open reading frames corresponding to EcoSSB, MtuSSB, EcoUDG, and MtuUDG were polymerase chain reaction-amplified from the respective pTrc99A based constructs, using forward (5'-GGATTC-CACAGGAAAACAGGCT-3') and reverse (5'-CTTTGTGGACAGCC-CAAACAGGCG-3') primers and Pfu DNA polymerase. The polymerase chain reaction products were digested with EcoRI and PstI enzymes and cloned into the corresponding sites of pGBT9 and pGAD424 vectors. The recombinants were verified by restriction analyses and nucleotide sequencing.

Electrophoretic Mobility Supershift Assays (EMSA)—The SSBs (10 pmol) were incubated with 1 pmol (20,000 cpm) of a 27-mer single-stranded oligomer, 5'-CACCTGATATCATATCTGCGCGAGCT-3' (16), with or without UDG (20 pmol) for 30 min in the binding buffer (20 mM Tris-HCl, pH 8.0, 50 mM NaCl, 5% glycerol (v/v), 50 mM Tris-HCl, pH 8.0, 1 mM Na3EDTA, 1 mM dithiothreitol, and 25 μg/ml bovine serum albumin) and separated on a native polyacrylamide (8%) gel (30:0.5, acrylamide:bisacrylamide) using 0.5x Tris borate-8%EDTA buffer for 1–2 h at 150 V (–8 V/cm) at 4 °C. The complex and the free DNA bands were visualized by autoradiography (21).

Binding of Chimeric SSBs to Loop-U2—Loop-U2 (1 pmol, 2 × 105 cpm) was incubated with MtuEcoSSB or EcoMtuSSB (0.1, 0.5, 1, 2.5, 5.0, 7.5, 10, or 15 pmol of the tetramer) in the binding buffer and analyzed by native polyacrylamide gels as in EMSA described above. The SSB-DNA complex and free DNA were quantified on a Bioimage Analyzer (Fuji), and the percentage of complex formed was plotted against the amounts of SSB in the reaction.

Far Western Blot Analysis—Proteins (MtuSSB, EcoSSB, MtuEcoSSB, EcoMtuSSB, EcoRRF, EcoUDG, and MtuUDG) were electrophoresed on 15% polyacrylamide gels containing 0.1% SDS under reducing conditions and transferred to a polyvinylidene difluoride membrane (0.45 μm; Amersham Pharmacia Biotech) by electroblotting (22). The transfer was carried out for 3 h at 200 mA. The membranes were blocked overnight with 1% bovine serum albumin in TBS (20 mM Tris-HCl, pH 7.4, 150 mM NaCl), washed with TBS, and overlaid with EcoUDG or MtuUDG (100 μg) in 7 ml of TBS for 2 h. After washing three times with TBS, the membranes were incubated with antibodies against EcoUDG or MtuUDG (1:2, 500 dilution) for 1 h at room temperature. After thorough washing, the blots were incubated with alkaline phosphatase-conjugated goat anti-rabbit IgG (Life Technologies, Inc.) at a 1:2000 dilution for 45 min at room temperature, washed three times with TBS, and developed with 12 μl 5-bromo-4-chloro-3-indolyl phosphate in 0.15 M Tris-HCl, pH 9.5, and 5 mM MgCl2 (23).

Surface Plasmon Resonance Studies—Experiments were performed exactly as described (4). The 5'-biotinylated single-stranded oligomer was immobilized on a SA5 sensor chip, and the various SSBs (EcoSSB, MtuSSB, MtuEcoSSB, and EcoMtuSSB) were passed over the surface to obtain a stable DNA-SSB platform. Thereafter, different concentrations of the EcoUDG and the MtuUDG (400–10,500 nM) were serially passed over the DNA-SSB surface using HBS buffer (10 mM Heps, pH 7.4, 50 mM NaCl, 3.4 mM Na3EDTA, 0.005% Tween 20). The increase in the response units indicated the interaction between the UDGs and the DNA-SSB complex. At the end of each injection of UDG, there was a fall in the response units, which indicated the dissociation of UDGs from the DNA-SSB surface. The sensograms thus obtained were utilized to evaluate the kinetic and equilibrium parameters using the BIAevaluation software.

RESULTS

Association of UDG Activity with SSBs—In our earlier studies, we purified SSBs from E. coli BW310, an ung+ strain (3, 4). In this study, we assayed the various SSB preparations from E. coli BW310 (ung+), and E. coli BL21(DE3) (ung+) for the presence of UDG activity. As shown in Fig. 1, EcoSSB and MtuSSB from the ung+ E. coli show complete excision of uracil from Loop-U2 (lanes 4 and 6), whereas those from the ung− strain do not exhibit any UDG activity (lanes 3 and 5). On the other hand, when a similar assay was carried out with EcoRRF purified from an ung− strain of E. coli BL21 (DE3), no uracil excision was seen (lane 7), indicating that the SSB-UDG interaction is specific. Thus, our first indication of a possible interaction between these two classes of proteins comes from the observation that the SSB preparations from ung− E. coli contained UDG activity despite the fact that the purification schemes for both of the proteins utilize significantly different steps (17, 24).

Yeast Two-hybrid Assays—The yeast two-hybrid approach was used to determine whether the SSBs and the UDGs are capable of interacting in vivo; this was carried out exactly as described earlier (16). As expected from the homotetrameric nature of the EcoSSB and MtuSSB, the yeast transformants harboring SSBs fused to the activation and the binding domains of GAL4 show growth on medium lacking histidine, tryptophan, and leucine and containing 10 mM 3-aminotriazole (Fig. 2, sectors 7 and 8, respectively). The Saccharomyces cerevisiae HF7c-alone strain harboring EcoUDG fused to the activation and the binding domains do not grow under the same conditions (Fig. 2, sectors 1 and 5, respectively). The transformants harboring EcoSSB or MtuSSB in combination with EcoUDG or MtuUDG showed growth (Fig. 2, sectors 2–4 and 6). These data show that in vivo, SSBs interact with UDGs in homologous (EcoSSB with EcoUDG or MtuSSB with Mtu-
and pGAD424 Mtu
UDG and pGAD424 Eco
tor 2, pGBT9 SSB; LacZ
reporter in that the
SFY256 (Table I) reflect the findings of the HIS3
S. cerevisiae
Miller units).

Because the C-terminal region of
SSB showed maximum variation in their C-terminal
domains from EcoSSB and MtuSSB. These chimeric SSBs
formed homotetramers and bound DNA akin to their native
counterparts (16). The native and the chimeric SSBs and
RRF were purified (Fig. 4
lanes 5) and assayed for
LacZ activity (see “Materials and Methods”). Average values are shown. Variation
between the two values for each combination was <10%. Full-length GAL4
activator expressed from pCL1 (LEU2) was used as a positive control.

| Protein fused to | Protein fused to | β-galactosidase |
|------------------|------------------|-----------------|
| GAL4 DB domain   | GAL4 AC domain   | Miller units    |
| EcoUDG           | EcoSSB           | 25.65           |
| EcoSSB           | EcoUDG           | 25.98           |
| EcoUDG           | MtuSSB           | 38.7            |
| MtuSSB           | EcoUDG           | 36.33           |
| MtuSSB           | MtuSSB           | 23.97           |
| MtuSSB           | MtuUDG           | 31.0            |
| MtuUDG           | EcoSSB           | 40.46           |
| EcoSSB           | MtuUDG           | 28.06           |
| GAL4             | EcoSSB           | 120             |
|                  | MtuSSB           | 0.855           |
|                  | MtuUDG           | 0.8             |
|                  | pGAD424          | 1.3             |

UDG) and heterologous (EcoSSB with MtuUDG or MtuSSB
with EcoUDG) combinations. The β-galactosidase assays using
S. cerevisiae SPY256 (Table I) reflect the findings of the HIS3
reporter in that the LacZ values obtained for the interaction
between SSB and UDG proteins varied from 24 to 40 Miller
units (Table I). Although these values are about 20–30% of that
of the native GAL4 activator, they are significantly higher than
the values obtained for various negative controls (0.8–1.3
Miller units).

Far Western Analyses—The sequence comparison of EcoSSB
and MtuSSB showed maximum variation in their C-terminal
domains downstream of amino acid position 129 (EcoSSB
numbering; Refs. 5 and 16). Because the C-terminal region of
EcoSSB has been implicated in its in vivo function, we generated
two chimeric constructs between the N- and C-terminal
domains of the two SSBs. The DNA sequence at amino acid
position 129 (EcoSSB numbering) contains a BamHI site in
MtuSSB. As a first step in generating the chimeric constructs,
the BamHI site was introduced into the EcoSSB gene by site-
directed mutagenesis (G129S mutation), and then the DNA
sequences corresponding to the N- and C-terminal domains
were swapped between the two SSBs (16) (Fig. 3). The construct
MtuEcoSSB contains the N-terminal portion from
MtuSSB and the C-terminal domain (48 amino acids) from
EcoSSB. Similarly, the chimeric construct EcoMtuSSB comprises
the N-terminal domain from EcoSSB and the C-terminal
domain (34 amino acids) from MtuSSB. These chimeric SSBs
formed homotetramers and bound DNA akin to their native
counterparts (16). The native and the chimeric SSBs
and RRF were purified (Fig. 4A) and used in a far Western
analysis. The interaction of EcoUDG or MtuUDG with the
SSBs was detected by respective polyclonal anti-UDG antibod-
ies (Fig. 4, B–E). Positive controls (EcoUDG and MtuUDG) in
lanes 6 (Fig. 4, B and C, respectively) showed that both of the
polyclonal antibodies detected UDGs with similar efficiencies.
Further, as expected, neither of the antibodies cross-reacted
with EcoRRF, which was used as a negative control (Fig. 4,
B–E, lanes 5). The membrane probed by anti-EcoUDG antibod-
ies revealed that EcoUDG interacted with both EcoSSB and
MtuEcoSSB (Fig. 4B, lanes 2 and 3) but not with MtuSSB and
EcoMtuSSB (Fig. 4B, lanes 1 and 4). Similarly, MtuUDG inter-
acted with MtuSSB and EcoMtuSSB (Fig. 4C, lanes 1 and 4)
but not with EcoSSB and MtuEcoSSB (Fig. 4C, lanes 2 and 3).
These observations suggest that the UDGs and SSBs from
homologous sources (belonging to the same organism) interact
with each other. Because the UDGs also interacted with the
chimeric SSBs containing C-terminal domains from SSB of the
homologous source, these observations suggest that the inter-
action between UDG and the SSB is mediated through the
C-terminal domain of the latter. When the amounts of SSBs
bound to the blots were increased (Fig. 4, D and E), the inter-
actions between the heterologous combinations such as those of
EcoUDG with MtuSSB (Fig. 4D, lane 1) and MtuUDG with
EcoSSB and MtuEcoSSB could also be discerned (Fig. 4E, lanes
2 and 3), suggesting that the nature of their interactions is
weak. Nevertheless, these interactions appear to be specific,
because no interactions of UDGs with EcoRRF were detected
(Fig. 4, D and E, lanes 5).

Electrophoretic Mobility Supershift Assays—The experi-
ments described thus far showed binary interactions between
SSBs and UDGs. To investigate the possibility of ternary in-
teractions involving DNA, SSB, and UDG, electrophoretic mo-
tility shift/supershift assays were carried out. As expected,
EcoSSB, MtuSSB, MtuEcoSSB, and EcoMtuSSB formed
complexes with DNA (Fig. 5A, compare lanes 2 and 5 with lanes 1

TABLE I
Interactions of various SSBs with UDGs as indicated by β-
galactosidase activity

Two transformants of each of the combinations were grown in liquid
medium till mid log phase (A660 ≈ 1.1) and assayed for LacZ activity
(see “Materials and Methods”). Average values are shown. Variation
between the two values for each combination was <10%. Full-length GAL4
activator expressed from pCL1 (LEU2) was used as a positive control.

-FIG. 2.
Interaction between MtuSSB, EcoSSB, MtuUDG, and EcoUDG using the yeast two-hybrid analysis. MtuSSB, EcoSSB, MtuUDG, and EcoUDG were cloned as fusions with the binding domain
(pGBT9) or the activation domain (pGAD424) of GAL4 protein and cotransformed into the host strain (HFTc). The host strain alone and the transfectants were streaked on medium lacking histidine, leucine, and
tryptophan and containing 10 mM 3-aminoantizole. Sector 1, HFTc sector
2, pGBT9EcoUDG and pGAD424EcoSSB; sector 3, pGBT9MtuUDG and
pGAD424 MtuSSB; sector 4, pGBT9MtuUDG and pGAD424MtuSSB; sector 5, pGBT9EcoUDG and pGAD424EcoSSB; sector 6, pGBT9MtuUDG and pGAD424EcoSSB; sector 7, pGBT9EcoSSB and pGAD424EcoSSB; sector 8, pGBT9MtuSSB and
pGAD424MtuSSB.

-FIG. 3.
Diagrammatic sketches of the various SSBs used.
EcoSSB and MtuSSB are similar in the region upstream of amino acid
position 129 (standard E. coli numbering, which corresponds to position
130 in the genes for both EcoSSB and MtuSSB). The MtuSSB gene
contains a conveniently located BamHI site at this position. A BamHI
site was introduced at position 129 of the EcoSSB gene and used to
generate chimeric constructs by domain swapping at the site (16). EcoMtuSSB contains the N- and C-terminal domains from EcoSSB and
MtuSSB, respectively. The MtuEcoSSB contains the N- and C-terminal
domains from MtuSSB and EcoSSB, respectively.
and D to a polyvinylidene difluoride membrane, incubated with EcoUDG antibodies (see "Materials and Methods").

B and UDGs (100 ng of each) (lanes 2 and E) were electrophoresed on SDS-PAGE, transferred to a polyvinylidene difluoride membrane, incubated with EcoUDG (B and D) or MtuUDG (C and E), and detected with the respective anti-UDG antibodies (see "Materials and Methods").

**FIG. 5. Supershift assays.** 5′-end-labeled single-stranded oligomer (1 pmol) was either not incubated with SSBs (lanes 1 and 4) or incubated with 10 pmol of EcoSSB or MtuEcoSSB without (lanes 2 and 5, respectively) or with 20 pmol of EcoUDG (lanes 3 and 6, respectively) for 30 min and analyzed as described under "Materials and Methods." B, all treatments are the same as described in A, except that MtuSSB and EcoMtuSSB were used.

and 4, and Fig. 5B, compare lanes 2 and 4 with lane 1). Further, consistent with the observations of the far Western analysis (Fig. 4B), when EcoUDG was added to DNA-EcoSSB or DNA-MtuEcoSSB complexes, a further shift was observed (Fig. 5A, lanes 3 and 6). However, under the same conditions, we failed to detect an interaction between MtuSSB or EcoMtuSSB and EcoUDG (Fig. 5B, lanes 3 and 5), corroborating our earlier finding of the far Western analysis (Fig. 4B, lanes 1 and 4). Thus, as was the case with binary interactions, transplantation of the C-terminal 48 amino acids of EcoSSB into the corresponding region of the MtuSSB was sufficient for a specific ternary interaction. However, when we performed a similar analysis utilizing MtuUDG with the SSBs, a supershift over and above the SSB-DNA complex could not be detected (data not shown). A possible reason for the lack of supershift by MtuUDG could be its high pI (9.5 as opposed to 6.6 of EcoUDG). In fact, when we performed a Western blot analysis of the native gels used for EMSA using polyclonal antibodies to MtuUDG, MtuUDG was foundlodged in the wells (data not shown).

**SPR Studies—**We have earlier used this methodology to detect interaction among the ternary complexes (DNA, SSBs, and UDGs) (4). We showed that the homologous combinations of SSBs and UDGs (EcoSSB with EcoUDG and MtuSSB with MtuUDG) show stronger affinity ( \( K_D \), 1.7 × 10⁻⁷ and 1.4 × 10⁻⁷ M, respectively). Of the heterologous combinations, MtuSSB with EcoUDG showed lower affinity ( \( K_D \), 8.4 × 10⁻⁶ M) relative to the homologous combinations of SSBs and UDGs, whereas the EcoSSB with MtuUDG did not show a detectable interaction (4). Because the far Western analysis (Fig. 4B) suggested that there was a weak interaction between EcoSSB and MtuUDG, we reinvestigated our SPR analysis with regard to this heterologous combination of proteins. Upon increasing the concentrations of MtuUDG (up to 10.5 μM), a weak interaction between these proteins could also be discerned ( \( K_D \), 7.67 × 10⁻⁶ M; Table II).

Use of chimeric SSBs in similar experiments shows that the MtuEcoSSB interacted with both the EcoUDG and MtuUDG but that the strength of these interactions ( \( K_D \), 2.44 × 10⁻⁶ and 4.46 × 10⁻⁶ M, respectively) is substantially lower than those of homologous combinations of SSBs and UDGs. However, a striking outcome of these observations and the far Western analyses (Fig. 4C) is that although the EcoMtuSSB interacts with MtuUDG ( \( K_D \), 2.4 × 10⁻⁶ M), it is incapable of interacting with EcoUDG. Therefore, the interaction of EcoUDG with EcoSSB and MtuEcoSSB but not with EcoMtuSSB suggests that the EcoSSB contacts EcoUDG through its C-terminal domain. Taken together, these findings show that the C-terminal do-

**TABLE II**

| SSB     | Kinetic parameters | EcoUDG | MtuUDG |
|---------|-------------------|--------|--------|
| EcoSSB  | \( k_0 \) (1/Ms)  | 6.2 × 10⁻⁶ | 4.6 × 10⁻⁷ |
|         | \( k_2 \) (1/s)   | 1.0 × 10⁻⁷ | 3.53 × 10⁻³ |
|         | \( K_D \) (M)     | 1.7 × 10⁻⁷ | 7.67 × 10⁻⁶ |
| MtuSSB  | \( k_0 \) (1/Ms)  | 1.7 × 10⁻⁶ | 1.16 × 10⁻⁴ |
|         | \( k_2 \) (1/s)   | 1.4 × 10⁻⁵ | 1.56 × 10⁻³ |
|         | \( K_D \) (M)     | 8.4 × 10⁻⁸ | 1.4 × 10⁻⁷ |
| MtuEcoSSB| \( k_0 \) (1/Ms) | 1.84 × 10⁻⁶ | 3.16 × 10⁻⁶ |
|         | \( k_2 \) (1/s)   | 4.5 × 10⁻⁴ | 1.4 × 10⁻³ |
|         | \( K_D \) (M)     | 2.44 × 10⁻⁶ | 4.46 × 10⁻⁶ |
| EcoMtuSSB| \( k_0 \) (1/Ms) | ND      | 1.6 × 10⁻⁶ |
|         | \( k_2 \) (1/s)   | ND      | 3.85 × 10⁻³ |
|         | \( K_D \) (M)     | 2.4 × 10⁻⁶ | ND      |

*These numbers were reported earlier (4) and are shown here for comparison.
domains of both EcoSSB and MtuSSB are primarily responsible for their interactions with the respective UDGs, and at least in the case of EcoSSB, the data allow us to conclude that its C-terminal domain is necessary and sufficient to engage EcoUDG as one of its interacting partners. On the other hand, the existence of weak interactions between MtuUDG and MtuEcoSSB or EcoSSB (Fig. 4E and Table I) does not permit us to completely rule out the contribution of the N-terminal domains of the SSBs (possibly through the conserved amino acids) for the interactions involving the mycobacterial proteins.

**Effect of Chimeric SSBs on Uracil Excision by UDGs**—We earlier showed that EcoSSB enhanced excision of uracil from Loop-U2 by EcoUDG and MtuUDG. On the contrary, although the inclusion of MtuSSB in the reactions containing the same substrate with MtuUDG stimulated uracil excision, it resulted in decreased activity of EcoUDG, most likely as a consequence of inappropriate interaction (4). Far Western analysis and the SPR studies showed a discernible interaction between the chimeric SSBs and UDGs (for most of the combinations). Therefore, it was of interest to investigate the effect of the chimeric SSBs on uracil excision activity of the UDGs using Loop-U2 as a substrate.

Neither MtuEcoSSB nor EcoMtuSSB alone showed any uracil excision activity (Fig. 6A, lanes 1 and 2). However, when included in the reactions along with EcoUDG, MtuEcoSSB stimulated the uracil excision activity (Fig. 6A, compare lanes 4 and 5 with lanes 7 and 8), and EcoMtuSSB showed a decrease in uracil excision activity of EcoUDG (compare lanes 4 and 5 with lanes 10 and 11). Furthermore, as observed before with the native SSBs, the chimeric SSBs led to a stimulation of uracil excision by MtuUDG (Fig. 6B, compare lane 3 with lanes 6 and 9).

Thus, these results suggest that the C-terminal domains within the chimeric SSBs are sufficient to mimic the typical effects of their native counterparts on the uracil excision activity of the two UDGs from Loop-U2. However, not surprisingly, the effects of chimeric SSBs, which contain the C-terminal domains in the context of heterologous N-terminal domains, on uracil excision are not as pronounced as seen before with the native SSBs (3). Analysis of the DNA binding activity of the chimeric SSBs revealed that they both bound Loop-U2 with similar efficiency (Fig. 7), suggesting that, as seen earlier with the mutants of EcoSSB deleted for the C-terminal domain (11), the DNA binding properties of SSBs can be mediated by the N-terminal domains, independent of the C-terminal domains. However, for the functional participation of the C-terminal domain with various cellular proteins, the context of the N-terminal domain is also important. Occurrence of such “cross-talk” between the N- and C-terminal domains of SSB could explain why in our earlier studies, the MtuEcoSSB chimera, despite containing the C-terminal domain from EcoSSB, failed to complement a ssb strain of E. coli (16).

**DISCUSSION**

SSBs play a vital role in a variety of DNA metabolic processes including replication, recombination, and repair (6). There is increasing evidence for the association of SSBs with several proteins such as the 𝜙 subunit of the DNA polymerase, primase, RecA, and the proteins involved in DNA repair such as UvrD, exonuclease I, MucB, and MucA (25–30). By using the chimeric constructs in studies employing the far Western analysis, SPR, and EMSA, we show that the C-terminal domain of SSB is responsible for interacting with UDG. The EcoSSB or a chimera containing its C-terminal domain interacts with EcoUDG in a binary (SSB-UDG) or a ternary (DNA-SSB-UDG) complex. However, neither MtuSSB nor the chimera containing the N-terminal domain from EcoSSB shows such interactions. Thus, the C-terminal domain (48 amino acids) of EcoSSB is necessary and sufficient for interaction with EcoUDG. Similarly, the C-terminal domain of MtuSSB mediated the interaction with MtuUDG. However, weak interactions of MtuUDG with EcoSSB and the chimeric SSB lacking the C-terminal domain of MtuSSB (MtuEcoSSB) suggested that the conserved amino acids in the N-terminal domains of SSBs might also contribute to their association. Further, from the activity assays shown in Fig. 6, it appears that the effect of SSBs on uracil
excision from the structured oligomer, Loop-U2, is mediated through the C-terminal domain. The crystal structure of EcoSSB shows the C termini as flexible arms extending out of the globular central structure (9). This extensible structure of the C terminus supports the view that it is able to mediate interactions with proteins in vivo.

Recent studies with the human UDG have shown that it interacts with the 34-kDa subunit and the trimeric form of the human homolog of the single-stranded DNA-binding protein, replication protein A (13). Subsequently it was demonstrated that UDG also interacts with proliferating cell nuclear antigen, suggesting that these proteins exist as a part of a multiprotein complex that UDG also interacts with proliferating cell nuclear antigen, polymerase δ, FEN1, and DNA ligase I at the replication foci (14). The increasing evidence for the involvement of replication protein A in excision repair sets a precedent for a similar scenario in the prokaryotic systems and hints at the universality of the phenomenon.

Thus, it appears that the SSB-UDG interaction would be important in uracil excision repair in the regions of the genome that become transiently single-stranded, such as the transcription bubble or replication fork. It is not surprising, therefore, that in humans, expression of UDG is cell cycle-regulated (31). From a physiological perspective, it would be preferable to have noninstructional lesions (AP sites) than the miscoding bases in the DNA during replication, because such lesions allow for postreplicative repair to occur. A DNA glycosylase could potentially alter a miscoding base into an AP site if it was associated with the protein complexes at the replication fork (32). Further, detection and removal of uracil from the newly synthesized DNA at the replication fork will also be ideal in avoiding the consequences of its misincorporation by the DNA polymerases.

The single-stranded regions in DNA are inherently prone to cytosine deamination. Because there is a greater potential of extrusion of DNA into complex structures in the single-stranded regions that may arise in the DNA as a consequence of various physiological processes, our studies on the uracil release from the hairpin DNA (3, 33, 34) and the effect of SSB on UDG activity on such substrates provide a good model system to examine the uracil excision repair pathway. We have earlier shown that one aspect of SSB-mediated stimulation of base excision repair is destabilization of the structured substrates (3, 4). The SSB-UDG interactions that we demonstrate here could also be crucial from the physiological perspective. The observation that UDG can interact with the SSB in a binary (SSB-UDG) or a ternary (DNA-SSB-UDG) complex suggests that SSB may facilitate recruitment of UDG to the site of the lesion by serving as a carrier. Alternatively, by virtue of the ternary interactions, the DNA-bound SSB may enhance the binding of the UDG to the site of the lesion. Further, the mechanism of how SSB switches its interacting partners including UDG, most of which localize to the C-terminal domain, remains to be clarified.

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REFERENCES

1. Krokan, H. E., Standal, R., and Slupphaug, G. (1997) Biochem. J. 325, 1–15
2. Kumar, N. V., and Varshney, U. (1994) Nucleic Acids Res. 22, 3737–3741
3. Kumar, N. V., and Varshney, U. (1997) Nucleic Acids Res. 25, 2336–2343
4. Purnapatre, K., Handa, P., Venkatesh, J., and Varshney, U. (1999) Nucleic Acids Res. 27, 3487–3492
5. Lohman, T. M., and Ferrari, M. E. (1994) Annu. Rev. Biochem. 63, 527–570
6. Weiner, J., Bertsch, L., and Kornberg, A. (1975) J. Biol. Chem. 250, 1972–1980
7. Weiner, J., Bertsch, L., and Kornberg, A. (1975) J. Biol. Chem. 250, 1972–1980
8. Senanayake, D., and Sambrook, J. (1989) EMBO J. 8, 4350–4354
9. Senanayake, D., and Sambrook, J. (1989) EMBO J. 8, 4350–4354
10. Williams, K. R., Spicer, E. K., LoPresti, M. B., Guggenheimer, R. A., and Varshney, U. (1997) J. Biol. Chem. 272, 6561–6566
11. Varshney, U., and van de Sande, J. H. (1991) Nucleic Acids Res. 19, 5131–5137
12. Varshney, U., and van de Sande, J. H. (1991) Nucleic Acids Res. 19, 5131–5137
13. Slupphaug, G., Olsen, L. C., Helland, D., Aasland, R., and Krokan, H. E. (1997) J. Biol. Chem. 272, 1906–1912
14. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Biochemistry. 28, 544–552
15. Varshney, U., and van de Sande, J. H. (1991) Nucleic Acids Res. 19, 5131–5137
16. Varshney, U., and van de Sande, J. H. (1991) Nucleic Acids Res. 19, 5131–5137
17. Varshney, U., and van de Sande, J. H. (1991) Nucleic Acids Res. 19, 5131–5137
18. Varshney, U., and van de Sande, J. H. (1991) Nucleic Acids Res. 19, 5131–5137
19. Varshney, U., and van de Sande, J. H. (1991) Nucleic Acids Res. 19, 5131–5137
20. Varshney, U., and van de Sande, J. H. (1991) Nucleic Acids Res. 19, 5131–5137
21. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Biochemistry.
