Tetrameration and Single-stranded DNA Binding Properties of Native and Mutated Forms of Murine Mitochondrial Single-stranded DNA-binding Proteins*

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We examined previously unexplored aspects of the tetramerization and single-stranded DNA (ssDNA) binding properties of native, precursor, and mutated forms of mitochondrial ssDNA-binding protein (mtSSB) from a mammalian organism (mouse). Tetrameric forms of mtSSB reassemble spontaneously after thermal denaturation and undergo subunit exchange. Binding of mtSSB to ssDNA as a function of protein concentration is nonlinear, suggesting a concentration-dependent transition in intrinsic binding affinity and in the topology of the DNA-protein complex. The cleavable presequence at the amino terminus of the precursor form of mtSSB does not disrupt tetramer formation but has a specific inhibitory effect on DNA binding that is not seen in a fusion protein that substitutes a bulkier peptide moiety in this position. Mutated forms of mtSSB bearing amino acid substitutions at highly conserved amino acid positions exhibit subtle or severe defects in ssDNA binding activity and/or tetramerization, even when assembled into heterotetramers in combination with wild-type mtSSB monomers. These experiments provide new insights into structural and functional properties of mammalian mtSSB and have implications for the pathogenesis of human diseases resulting from defects in mtDNA replication.

Single-stranded DNA-binding proteins (SSBs)1 are involved in DNA replication, repair, and recombination in prokaryotic organisms and in both nuclei and mitochondria of eukaryotes. Fundamental properties of SSBs have been explored most thoroughly with respect to the Escherichia coli protein, the function of which is dependent on three types of intermolecular interactions: self-association to form homotetramers, binding to ssDNA, and interaction with other proteins of the replication complex (1–3). The N-terminal portion of SSB is responsible for tetramerization and ssDNA binding (4, 5), whereas the carboxy-terminal region contains domains that interact with heterologous proteins (6, 7). Naturally occurring point mutations in E. coli SSBs cause pleiotrophic defects in replication, repair of UV-damaged DNA, and recombination (8–11).

The amino acid sequence of mitochondrial SSB (mtSSB) has been determined previously in several species, including human, rat, Xenopus, and Saccharomyces cerevisiae (12–16). These eukaryotic proteins include a number of conserved residues shared with E. coli SSBs in their amino-terminal regions but are divergent otherwise. Although no spontaneous mutations in mtSSB genes of humans or other metazoan organisms have been described, defects in mitochondrial DNA replication or repair produce a variety of rare but devastating human diseases (17–20), and mtSSB may participate directly or indirectly in the pathogenesis of these disorders.

In this report, we define previously unexplored aspects of the tetramerization and ssDNA binding properties of mtSSB from a mammalian organism (mouse). A murine mtSSB cDNA was cloned by PCR amplification, and several forms of recombinant mtSSB were purified following expression in bacteria: the mature native protein; an epitope-tagged mtSSB; an unprocessed mtSSB bearing the presequence required for mitochondrial import; a fusion protein in which glutathione S-transferase (GST) is linked to the amino terminus of mtSSB; and mtSSB-bearing amino acid substitutions at each of four highly conserved amino acid positions. We used gel mobility shift assays, equilibrium sucrose gradient centrifugation, and immunoprecipitation to characterize the ssDNA binding properties and the dissociation and re-assembly of mtSSB homo- and heterotetramers following thermal denaturation.

MATERIALS AND METHODS

Cloning of mtSSB and Plasmid Constructions—A mouse cDNA encoding the precursor form of mtSSB was amplified by reverse transcription-PCR from mouse heart mRNA, using primers based on an alignment of mtSSB sequences from other mammals (16). The amplified product was cloned into a pBluescript vector (Stratagene, La Jolla, CA), and both strands were sequenced using an ABI automatic sequencer (Foster City, CA). Murine mtSSB cDNA sequences were cloned into bacterial expression vectors pGEX-VP or pGEX-VH for production of GST-fusion proteins bearing a cleavage site for a plant protease (TEV) at the junction between GST and mtSSB. Separate expression plasmids were constructed to encode either the wild-type mature form of mtSSB (WT), mature mtSSBs bearing a 14-amino acid tag (-Leu-Asn-Gly-Leu-Phe-Val-Val-Asp-His6) located at the carboxyl terminus (WH), or the unprocessed precursor form of mtSSB bearing the amino-terminal presequence required for mitochondrial import (WP). The orientation and sequence of inserted DNA was examined by endonuclease restriction and sequencing. Recombinant mtSSB proteins cleaved with TEV protease include two extra alanines at the amino terminus by comparison to the native endogenous proteins.

Site-directed Mutagenesis—Point mutations were introduced by PCR into expression plasmids encoding murine mtSSB. Briefly, each of two complementary oligodeoxynucleotide primers harboring the desired

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1 The abbreviations used are: SSB, single-stranded DNA-binding protein; mtSSB, mitochondrial SSB; PCR, polymerase chain reaction; GST, glutathione S-transferase; WT, wild-type mature; WH, His-tagged mtSSB; WP, unprocessed precursor form of mtSSB.

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2 pGEX-VP and pGEX-VH were constructed from pGEX-cs (23) by Drs. Yih-Sheng Yang and Donald Capra.
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FIG. 1. A, comparison of protein sequences from mouse, human, Xenopus, and yeast mtSSB and the mature wild-type mtSSB (WT), open box) and variant forms bearing the native presequence for mitochondri al import (WP, black box) or cleavage by TEV-protease (1 h at 30°C). After dialysis against storage buffer (50 mM Tris-HCl, pH 7.5, 200 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, and 10% glycerol), the concentration and purity of recombinant proteins were assessed by a quantitative Coomassie Blue reaction.

Expression and Purification of Recombinant Proteins—Recombinant proteins were expressed in an E. coli host (DH5α) and purified from clarified cell lysates by binding to glutathione-agarose beads (Pharmacia Biotech Inc.) (24), followed by elution with 5 mM glutathione (GSTMtSSB) or cleavage by TEV-protease (1 h at 30°C). The resulting cDNA fragments, each bearing a specific point mutation, were subcloned into a GST-fusion protein vector, pGEX-VP. Inserts were sequenced in their entirety to confirm the substitution of the substitution and to eliminate clones bearing other mutations introduced by Taq polymerase.

Equilibrium Sucrose Gradient Centrifugation—Step gradients were set up by layering 0.8 ml of 20, 15, 10, and 5% sucrose each in a 3.8-ml tube. The full-length mouse mtSSB cDNA encodes a precursor protein that extends 148 amino acid residues. Using a bacterial expression system, we purified recombinant mtSSB proteins (W and M) are mixed, five different varieties of tetramer are possible: W4, W3M, W2M2, WMM3, and M4. The reassembly of W68A and subunit exchange between W68A and wild-type tetramers after heat denaturation supports the assumption that incorporation of individual monomers is a random process.

RESULTS

Expression of Wild-type and Mutant mtSSB Proteins—Mouse mtSSB shares >90% sequence identity with mtSSBs from other vertebrates (14, 16), and certain residues are shared with both yeast mtSSB from S. cerevisiae (15) and bacterial SSB from E. coli (26) (Fig. 1A). The single-stranded competitor DNA (ssDNA) is phage M13 DNA in concentrations of 25, 50, 100, or 200 ng in each reaction.
residues in the amino-terminal region of mtSSBs, we also introduced point mutations into mouse mtSSB and purified the mutant proteins to 90% purity. Each of the point mutations resulted in the substitution of a neutral alanine residue for the amino acid found in the native sequence (Fig. 1 A). The four mutant proteins were named by reference to the position of the substitution within the wild-type murine mtSSB sequence: Trp49 to Ala (W49A), Trp68 to Ala (W68A), His69 to Ala (H69A), and Phe74 to Ala (F74A).

ssDNA Binding Activity of Wild-type and Variant mtSSBs—

We chose a 34-mer single-stranded oligonucleotide as a probe of sufficient length to wrap completely the mtSSB tetramer, based on prior calculations that each monomer of rat mtSSB occupies eight nucleotides (13). Gel mobility shift assays of equilibrium binding reactions demonstrated that both WT and WH forms of mtSSB assume a tetrameric structure (data not shown). Even upon lowering the WT protein concentration from 1 to 0.04 μM, we were never able to detect monomeric or dimeric forms of WT mtSSB bound to DNA using the gel mobility shift assay.

When ssDNA binding was assessed as a function of increasing protein concentrations at a constant and excess concentration of probe, two different types of ssDNA binding activities were observed as judged by a change of slope in the activity/concentration function. At 0.4 μM or lower protein concentrations, the ssDNA-protein interaction was weaker, and at 0.6 μM or higher concentrations, the reaction changed to a stronger binding affinity. This transition occurred within a narrow range of protein concentrations from 0.4 to 0.6 μM (Fig. 3, A and C). In a parallel control experiment (Fig. 3 B), ssDNA binding activity was stimulated only marginally by increasing concentrations of a nonspecific protein (lysozyme). The inflection point in the binding isotherm must, therefore, reflect an increase in the intrinsic binding affinity of mtSSB for ssDNA.

ssDNA Binding Activity of Mutant mtSSBs—Gel mobility shift assays performed with varying concentrations of protein demonstrated subtle defects in ssDNA binding activity of W49A, H69A, and F74A (Fig. 4 A) and a profound defect in W68A. This latter mutation completely abolished ssDNA binding at protein concentrations of 0.4 μM or lower, even with the probe labeled to higher specific activity (Fig. 4 B). However, some binding activity of W68A was evident at higher protein concentrations. This concentration-dependent difference in the binding defect manifested by W68A is not simply an artifact of the detection limit of the assay, because extended exposure of the gel image confirmed the complete absence of binding activity at protein concentrations at or below 0.4 μM (data not shown).

As a reference point for quantitative measurements of ssDNA binding activity, we included E. coli SSB at a concentration of 0.1 μM in each reaction. The larger E. coli protein forms a complex with DNA that can be distinguished on the
basis of different mobility from complexes formed with mtSSB. In this comparison, the mutations found in H69A and F74A displayed less than 50% of the ssDNA binding activity of WT. The mutation in W49A had the least effect, but ssDNA binding displayed less than 50% of the ssDNA binding activity of WT. In this comparison, the mutations found in H69A and F74A permitted renaturation (Fig. 5C). As shown by sucrose gradient centrifugation, the reduction in ssDNA binding reflects dissociation of tetramers (Fig. 5D). Note that monomers were not detected in this particular gradient because their concentrations fell below the minimum level for detection. Further lowering of mtSSB concentration to 0.2 μM resulted in almost complete loss of ssDNA binding activity after heating to 100 °C (data not shown).

The dissociation and reassembly of mtSSB tetramers were characterized further by mixing the larger, His-tagged mtSSB (WH) with WT and immunoprecipitating with anti-His antibody. When the two forms were mixed and denatured at temperatures of 60 °C or lower, we observed no evidence for subunit swapping and formation of heterotetramers upon renaturation. Heterotetramers were formed, however, when the mixture of WT and WH was heated to 70 °C or higher prior to renaturation (Fig. 6). These heterotetramers retained normal ssDNA binding activity in the gel mobility shift assay (Fig. 10B).

The thermal stability and reassembly of mtSSBs bearing point mutations were tested in a similar manner using gel mobility shift assays. Mutant proteins W49A and F74A exhibited thermal stability similar to WT (Fig. 7). The mutation introduced into H69A, however, had different and distinctive effects. Although this mutant form of mtSSB exhibited only a modest impairment in ssDNA binding when studied at room temperature (Fig. 4A), profound defects were noted after preincubation at higher temperatures (Fig. 7). A more detailed study (Fig. 8) demonstrated that the activity of this mutant protein was not stable even at 25 °C, and ssDNA binding activity was almost completely lost after 1 h of preincubation at temperatures within the physiological range (37 °C to 42 °C). The effective half-life of H69A under the assay conditions we used was estimated to be 3.5 ± 1 min, 7 ± 2 min, and 35 ± 10 min at 42 °C, 37 °C, and 25 °C, respectively.

**Tetramerization of Mutated mtSSBs**—As a direct test of tetramer formation by mutant mtSSBs, we used equilibrium sucrose gradient centrifugation under the same conditions used in ssDNA binding assays. W49A, W68A, and F74A appeared to be fully tetramerized at the 1–5 μM monomer concentration (Fig. 9A). This experiment established the basis for deficient ssDNA binding of W68A as a defect in contacting DNA, rather than a defect in tetramer formation. In contrast, H69A exhibited an obvious, temperature-sensitive defect in tetramerization. Incubation of the protein at 50 °C for 5 min (Fig. 9B) or 37 °C for 1 h (Fig. 9C) resulted in a complete transition from tetramers to monomers. The time course of the tetramer-monomer transition at 37 °C was parallel to that of the loss of ssDNA binding activity. In contrast to WT, thermal dissociation of H69A tetramers was insensitive to increasing protein concentration and could not be reversed by reducing the temperature gradually.
Heterotetramer Formation by Mutated SSBs—Fig. 10A demonstrates that W68A was coimmunoprecipitated with WH, indicating formation of heterotetramers. In contrast, H69A could not be coimmunoprecipitated with WH, consistent with the temperature-sensitive tetramerization defect exhibited by H69A in the sucrose gradient assay. The intensity of the W68A band relative to the WH band following immunoprecipitation underestimates the extent of heterotetramer formation, because of a higher efficiency of precipitation of (WH)_1(W68A)_1 complexes in comparison to (WH)_2(W68A)_2 and (WH)_1(W68A)_3 heterotetramers. The same phenomenon is evident following coimmunoprecipitation of WT and WT:W68A subunits by comparison to that present in WT homotetramers, or the presence of WT subunits in the heterotetramer increases the affinity of W68A monomers by comparison to mutant homotetramers. We regard the latter possibility as more likely.

Using gel mobility shift assays, DNA-protein complexes formed by WH homotetramers can be distinguished from those formed by heterotetramers or mutant homotetramers on the basis of their electrophoretic mobility in the gel (Fig. 10B). This experiment confirmed the conclusions drawn from immunoprecipitation reactions and sucrose gradient analysis; heterotetramers containing W49A, W68A, and F74A were readily detected, but H69A monomers could not renature to a conformation capable of tetramer formation. The inability of W68A homotetramers to bind DNA also is evident in this figure.

DNA Binding Properties of W68A/WT Heterotetramers—Because W68A is capable of forming tetramers but defective in ssDNA binding, we deemed it likely that heterotetramers formed with W68A and wild-type mtSSB monomers would exhibit ssDNA binding properties distinct from those of the WT homotetramer. However, the precise manner in which ssDNA binding would be altered by the presence of one, two, or three W68A monomers within the tetramer was not obvious a priori.

We analyzed ssDNA binding by W68A/WT heterotetramers under conditions where total monomer concentration was held constant at 0.2 μM while the percentage of WT versus W68A was varied, and under conditions where the concentration of WT monomer was held constant at 0.2 μM while increasing concentrations of W68A were added. The starting concentration of free probe was constant in all experiments. In the first experiment (constant total protein), ssDNA binding activity diminished linearly as the percentage of W68A was increased (Fig. 11, A and C). However, the observed binding isotherm for WT:W68A heterotetramers runs consistently higher than the binding curve predicted simply from the percentage of WT monomers in the population using the formula described in “Materials and Methods” or the binding curve observed in a control experiment in which the same proportion of WT and W68A were mixed without heat denaturation so that heterotetramers were not formed.

We conclude from this experiment that all heterotetramers that contain at least one WT monomer can bind ssDNA, but the relative affinity of the tetramer is proportional to the ratio of WT versus W68A subunits. In addition, the higher binding activity of heterotetramers by comparison to the simple percentage of WT monomers suggests one of two possibilities; either the binding affinity of a WT monomer is increased by incorporation into a heterotetramer with W68A subunits by comparison to that present in WT homotetramers, or the presence of WT subunits in the heterotetramer increases the affinity of W68A monomers by comparison to mutant homotetramers. We regard the latter possibility as more likely.

In the second experiment (constant concentration of WT), the relationship between ssDNA binding activity and the amount of W68A is defined best by division into three phases: 1) a linear increase from 0.2 to 0.6 μM of total proteins; 2) an exponential increase from 0.6 to 1 μM of total protein; and 3) a plateau phase from 1 to 1.8 μM of total protein (Fig. 11, B and D). The first two phases resemble the transition in binding mode at higher protein concentrations observed for WT homotetramers. At a low (0.2 μM) concentration of WT homotetramers, increasing the concentration of mutant heterotetramers (the control experiment without heat denaturation) increased binding activity. This effect was different, however, when heterotetramer formation was induced; a greater concentration-dependent transition in binding mode transition was evident, although this was still impaired by comparison to a uniform population of WT proteins.

Effect of Presequence on ssDNA Binding and Tetramerization—Vertebrate mtSSB is synthesized as a precursor protein in the cytosol. A 16-amino acid presequence is removed by import into mitochondria (13, 16). We assessed the ability of the unprocessed precursor form of mtSSB (WP) to form tetramers and bind ssDNA. The sucrose gradient profile performed at a monomer concentration of 2 μM showed that WP can form tetramers (Fig. 12A). However, ssDNA binding activity of the WP tetramer is markedly reduced by comparison with WT (Fig. 12B). Thus, the presence of the presequence is permissive for protein-protein contacts among mtSSB monomers but blocks the DNA binding site. This effect is not simply a consequence of steric hindrance from additional residues at the amino terminus, because replacement of the presequence with a bulky GST moiety restored ssDNA binding activity (Fig. 12B). Both the precursor form and the GST-substituted forms of mtSSB may have subtle defects...
in tetramer formation, because WP and GST-WT dimers could be observed at protein concentrations where WT mtSSB was found completely in tetrameric complexes. These results suggest that the presequence of mtSSB not only provides the mitochondrial import signal but serves also to attenuate tetramerization and ssDNA binding activity within the cytosolic or nuclear compartments.

**DISCUSSION**

The ssDNA binding activity assessed by gel mobility shift assays under conditions of constant and excess concentrations of probe revealed two different types of binding activities, depending on the protein concentration. We propose that the transition in ssDNA binding affinity reflects a different topology of the DNA-protein complex that most likely results from a conformational change in the protein (Fig. 13). At monomer concentrations below 0.4 μM, mtSSB binds to the oligonucleotide probe with lower affinity, and presumably, each tetramer is wrapped completely by one molecule of probe. At higher protein concentrations (above 0.6 μM), a conformational change must occur to generate higher binding activity. The molecular mechanism of this transition remains unknown. Because the migration of protein-DNA complexes within the gel is not markedly altered, we favor the hypothesis that the higher affinity state is associated with conformational changes in a single bound tetramer, rather than the binding of two tetramers to a single molecular of probe.

This interpretation is supported by several lines of evidence. Previous studies of *E. coli* SSB indicate that the protein interacts with ssDNA in one of several topological binding modes. At low binding density (protein:DNA ratio) of the bacterial protein, DNA appears to wrap around the tetramer (27, 28), whereas alternative binding modes are observed when a higher protein binding density is used. These include a linear array of tetramers aligned on ssDNA where only two of the four subunits contact DNA (27, 29, 30). Rat mtSSB also appears to wrap ssDNA around the tetramer at low protein concentrations (0.17–0.25 μM) (13).

We have analyzed the functional consequences of alanine...
substitution mutations in each of four highly conserved amino acids of mouse mtSSB. Three of the mutant proteins, W49A, W68A, and F74A form stable tetramers but exhibit defects in DNA binding ranging from mild to severe. We conclude from these results that the conserved residues at these three positions are not involved in maintaining a tertiary structure required for subunit associations but either make direct contacts with DNA or support the tertiary structure of the DNA binding surface. Using different methods (fluorescent quenching assay), previous studies of mutations in analogous positions of E. coli SSB (see Fig. 1) also revealed defects in ssDNA binding (31–36). Likewise, mutations W68T and W68Y in human mtSSB appeared to have a similar effect as a mutation at W54 in the bacterial protein (37).

Our data extend these previous results in showing that W68A is absolutely required for mtSSB to bind ssDNA at low protein concentrations (0.4 μM or below), but this residue is less critical for binding activity at higher protein concentrations (0.6 μM or higher). These observations reinforce the working model that the DNA binding surface of mtSSB tetramers can assume a different conformation at high protein concentrations.

The mutation of H69A produced distinctive results, with important implications for structure-function relationships of mtSSB. H69A is capable of DNA binding but temperature-sensitive for tetramerization and incapable of spontaneous refolding after thermal denaturation. This mutation, therefore, compromises the tertiary structure required to maintain subunit interactions. An analogous mutation H55Y occurred naturally in E. coli SSB (31). This mutation, as well as other site-directed mutations at this position in the bacterial protein, H55K, H55E, and H55F, results in biochemical defects examined in vitro and in vivo phenotypes that include temperature-sensitive lethality, defective DNA repair, and impaired recombination. Our data provide the first experimental evidence that H69 in mammalian mtSSB has structural functions similar to H55 in E. coli SSB (31, 35, 36).

All known mtSSBs are encoded by nuclear, rather than mitochondrial, genes and are synthesized as precursor proteins on cytoplasmic ribosomes (13, 16). A presequence for mitochondrial targeting is cleaved after import into mitochondria to generate the mature form of the protein. We observed that the short presequence of only 16 residues exerts intramolecular effects that markedly alter the functional properties of mtSSB. Specifically, subunit interactions are weaker within tetramers formed with precursor rather than mature mtSSB monomers (most evident at low protein concentrations), and DNA binding activity is markedly impaired. These features of the precursor protein may have important consequences within the cell by limiting ectopic, extra-mitochondrial assembly of mtSSB tetramers, the disassembly of which prior to mitochondrial import would have a high energy cost. The inability of the precursor form of mtSSB to bind DNA also would limit potentially deleterious consequences of DNA binding within the nucleus if ectopic tetramer formation were to occur.

Defective binding of ssDNA by tetramers formed with precursor forms of mtSSB is not simply a consequence of steric hindrance from additional residues at the amino terminus of the protein but a specific effect related to the structure of the reactions contained the same amount of probe. The radioactive oligodeoxynucleotide probe was diluted by 2 ng of cold probe. A, gel mobility shift assay for WT and W68A heterotetramer formation. Each reaction contained the same concentration of total proteins with the ratios of WT and W68A indicated on each lane. All reactions contained the same amount of probe, which was diluted by 2 ng of cold probe. B, gel mobility shift assay for WT and W68A heterotetramer formation. Each reaction contained the same concentration (0.2 μM) of WT protein with increasing W68A concentrations as indicated on each lane. Heterotetramer formation was induced by preheating the protein mixture at 80 °C for 5 min and cooling gradually. All of the reactions contained the same amount of probe. The radioactive oligodeoxynucleotide probe was diluted by 12 ng of cold probe. C and D, quantitative plots of A and B and data from control experiments in which the same concentration and ratio of WT and W68A proteins were mixed without heat denaturation. The tetramer-ssDNA binding signal in the lane only containing 0.2 μM of WT protein was used to define 100% (A) or 1 unit (B) of binding activity.
prosequence peptide, which is thought to form an amphipathic α-helix (38, 39). Introduction of a much larger protein moiety, 31 kDa, as an amino-terminal fusion to the mature form of mtSSB could function in a dominant manner to generate abnormal mitochondrial genomes.

The assembly of protein monomers into stable homo- or hetero-oligomeric complexes is essential for many cellular functions (40). The formation of oligomeric complexes may expand the potential of protein monomers for interactions with other macromolecules and may facilitate additional regulatory controls over protein function. Both E. coli SSB and mtSSBs form tetramers in solution that allow these proteins to bind ssDNA with higher affinity and to form a variety of different topological structures in association with DNA (27–29, 41, 42). The forces involved in mtSSB tetramerization are exclusively non-covalent in nature, because no cysteine residues are present in mtSSB, and the complex is completely dissociable by thermal denaturation.

In this report, we provide new information about the assembly, dissociation, and functional properties of mtSSB tetramers from a mammalian organism. The stability of the mtSSB tetramer is remarkable in that subunit dissociation can be detected only at temperatures above 60 °C. Even under more extreme conditions, 100 °C, mtSSB monomers refold rapidly into an active conformation and form tetramers spontaneously without a requirement for molecular chaperones. Our studies indicate that subunit association is a slower process than renaturation of monomers and, therefore, is rate-limiting to the restoration of ssDNA binding activity. The rate of tetramer assembly following thermal denaturation increases directly as a function of the concentration of monomers. At low protein concentrations (below 1 μM of monomer), reassociation of monomers into tetramers following thermal denaturation and rapid cooling was not measurable. Finally, the minimal temperature at which tetramers dissociate also appears to provide a threshold for mtSSB to assume an “open” structure consistent with measurable rates of subunit exchange.

Subunit exchange following thermal denaturation also provided a new approach to explore relationships between structure and function of heterotetramers formed with wild-type and mutant mtSSB monomers. Our data indicate clearly that mutant forms of mammalian mtSSB form heterotetramers with the wild-type protein, and that the incorporation of mutant monomers into heterotetramers may influence the affinity and topological mode of ssDNA binding. Heterotetramers with mutant subunits, like WT homotetramers, exhibit a transition in binding mode as protein concentrations are increased. This supports the hypothesis that a conformational change accounts for the transition between a low affinity and a high affinity state. In addition, our findings have potential implications for efforts to understand the pathogenesis of human diseases that arise from defects in mitochondrial DNA replication. The formation of heterotetramers with altered DNA binding properties illustrates a possible mechanism by which mutant alleles of mtSSB could function in a dominant manner to generate abnormal mitochondrial genomes.

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