

Chapter 2

Structural Diversity Based on Variability in Quaternary Association. A Case Study Involving Eubacterial and Related SSBs

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Abstract

Eubacterial and related single-stranded DNA-binding proteins (SSBs) exhibit considerable variability in their quaternary association in spite of their having the same tertiary fold. The variability involves differences in the orientation of dimers in the tetrameric molecule (or of two-domain subunits in the dimeric molecule) and that of monomers in each dimer. The presence of an additional strand in mycobacterial and related SSBs, which clamps the dimers together, is a major determinant of the mode of quaternary association in them. The variability in quaternary structure has implications to the stability of the protein and possibly to its mode of DNA binding.

Key words: Eubacterial SSBs, Mycobacterial SSBs, OB fold, Quaternary structure, Protein stability, DNA binding

1. Introduction

Proteins employ a variety of strategies to produce structural diversity based on the same folding motif. One of them is variability in quaternary association. The first major systematic attempt to explore this variability, mainly using X-ray crystallography, was made in relation to legume lectins. It was established that legume lectins are a family of proteins in which small alterations in essentially the same tertiary structure lead to large variations in quaternary association (1–7). Since then such variability has been observed and explored in several other proteins, including other classes of plant lectins (6, 8–11). Eubacterial and related single-stranded DNA-binding proteins (SSBs) constitute an important family of proteins which exhibit substantial variation in quaternary association among its members while retaining the basic fold of the subunit.

DNA transactions such as replication, recombination, and repair involve unwinding of duplex DNA into single-stranded DNA (ssDNA). SSBs are involved in protecting vulnerable ssDNA from chemical and nuclease attacks and formation of aberrant secondary structures (12). They bind ssDNA with high affinity and in a sequence-independent manner. They have been found in all classes of organisms performing similar function, but displaying little sequence similarity. They exhibit some common features in three-dimensional structure. The commonality is most pronounced among the subunit structures of eubacterial and related SSBs. Among them, *E.coli* SSB (*EcSSB*) (13) and human mitochondrial SSB (*HMtSSB*) (14) were the first to be structurally characterized using X-ray crystallography. Like other similar SSBs, both consist of a globular N-terminal domain and a disordered C-terminal domain. While the N-terminal domain binds DNA, the C-terminal domain is believed to be involved in interacting with other proteins (12). The DNA-binding domain has the OB fold (15) in *EcSSB* as well as *HMtSSB*. Both of them form similar tetramers with 222 (D2) symmetry. The structure of the SSB from *Mycobacterium tuberculosis* (*MtSSB*) subsequently became available (16). *MtSSB* has the same tertiary structure as in *EcSSB* and *HMtSSB*, but with a somewhat different quaternary arrangement. It is then that the variability in the quaternary association of SSBs came into focus. The subsequent structure determination of SSBs from other sources showed this variability to be fairly extensive, as indeed has been noted in earlier reports. The present review attempts a comprehensive treatment of this variability and related issues.

2. Methods

The crystal structures reported in the literature and/or the coordinates of which have been deposited in the Protein Data Bank (PDB) (17) form the data used in the present analysis. They have been determined by the well-known methods of macromolecular crystallography. Several computer programs such as ALIGN (18), NACCESS (19), COOT (20), CALCOM (21), CHIMERA (22), and routines in Collaborative Computation Project, Number 4 (CCP4) (23) were used for analysis of the available structures. PYMOL (24) and CHIMERA were used for preparing figures. Sequence alignment was carried out using CLUSTALW (25).

3. Database

The crystal structures of eubacterial and related SSBs from the following sources have been reported:

1. *E. coli* (*EcSSB*) ([13](#)).
2. Human mitochondria (*HMtSSB*) ([14](#)).
3. *Mycobacterium tuberculosis* (*MtSSB*) ([16](#)).
4. *Deinococcus radiodurans* (*DrSSB*) ([26](#)).
5. *Mycobacterium smegmatis* (*MsSSB*) ([27](#)).
6. *Thermus aquaticus* (*TaSSB*) ([28](#), [29](#)).
7. *Thermotoga maritima* (*TmSSB*) ([30](#)).
8. *Mycoplasma pneumoniae* (*MpSSB*) ([31](#)).
9. *Streptomyces coelicolor* (*ScSSB*) ([32](#)).
10. *Mycobacterium leprae* (*MlSSB*) ([33](#)).

DNA complexes of SSBs from the following sources have been published:

1. *E. coli* (*EcSSB* + DNA) ([34](#)).
2. *Helicobacter pylori* (*HpSSB* + DNA) ([35](#)).

Coordinates of the structures of SSBs from the following sources are available in the PDB, but the results are yet to be published:

1. *Thermus thermophilus* (*TtSSB*) (PDB code 2cwa).
2. *Bartonella henselae* (*BhSSB*) (3pgz).
3. *Synechococcus sp* (*SsSSB*) (3koj).

The coordinates of the structure of a complex of *MsSSB* with DNA are available in the PDB (3a5u), but the results have not been published.

4. Results

4.1. Structural Features

Among the SSBs listed above, all except four are tetramers with 222 symmetry (Fig. [1](#)). Each subunit is less than 200 amino acid residues long (177 in *E. coli*). The C-terminal stretch of varying length (about 60 residues in *E. coli*) involved in interacting with other proteins is disordered in all the crystal structures analyzed so far. Parts of it are believed to be intrinsically disordered. The larger N-terminal domain, which binds DNA, is characterized by the OB

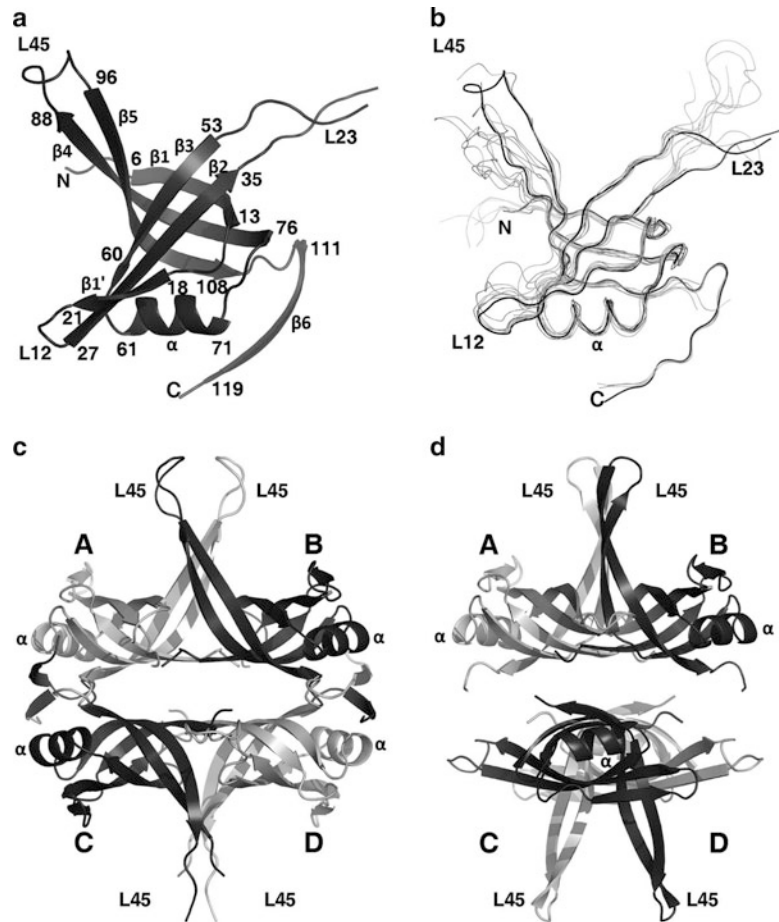


Fig. 1. (a) The structure of the N-terminal domain of *MtSSB*, (b) superposition of the N-domain of tetrameric SSBs (the line corresponding to *MtSSB* is thick), and the quaternary structure of (c) *MtSSB* and (d) *HpSSB*. In (c) and (d), the AB dimer has the same orientation.

fold in all cases (Fig. 1a). The DNA-binding domain has nearly the same structure in all the concerned SSBs, except in the flexible loops (Fig. 1b). The SSB tetramer can be described as a dimer of dimers (Fig. 1c, d). Partly for convenience and partly on the basis of intersubunit interactions in a majority of the SSBs considered here, subunits A and B can be treated as the dimer. The interactions between A and B include inter-subunit hydrogen bonds of the type, which occur in antiparallel β -sheets. They are between the two N-terminal stretches (as in Fig. 2b).

Mycobacterial SSBs and *ScSSB* have some distinctive additional structural features. The ordered DNA-binding domain in them contains an additional stretch ($\beta 6$ in Fig. 1a), which is absent in other SSBs. This additional feature results in the clamping of the two dimers together at two extremities (Fig. 1c), which lends

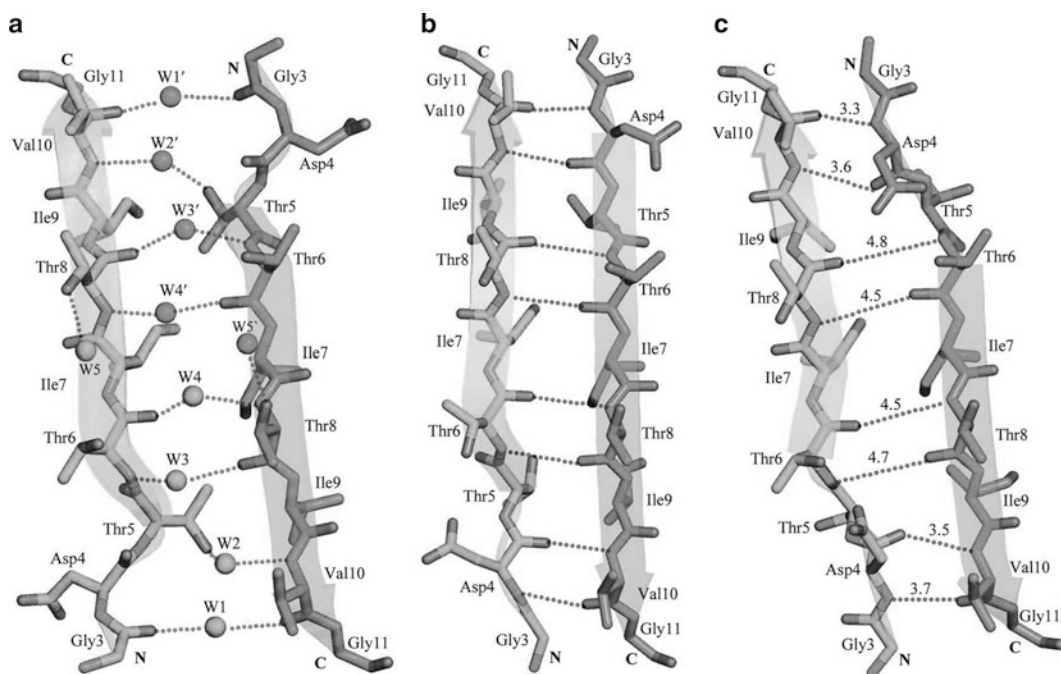


Fig. 2. Interactions between monomers (a) in form I *M/SSB*, (b) in form II *MtSSB*, and (c) at the AB interface in form II *M/SSB*. The distances in (c) indicate that the four inner interactions are water bridges. Reproduced with permission from Acta Cryst (2010) D66, 1048–1058.

additional stability to the tetramer. Furthermore, in these SSBs, the interactions between the N-terminal stretches in the AB dimer could be direct hydrogen bonds or water bridges (Fig. 2). Crystal structures of two forms of *MtSSB*, one form of *M_sSSB*, and two forms of *M/SSB* are available. The two subunits are interconnected by water bridges in form I of *MtSSB*, *M_sSSB*, and form I of *M/SSB* (Fig. 2a). Direct hydrogen bonds exist between them in form II *MtSSB* (Fig. 2b), while an intermediate situation is seen in form II of *M/SSB* (Fig. 2c). The interconnection is through water bridges in *ScSSB* as well. Direct hydrogen bonds exist in all other tetrameric SSBs. The similarity between mycobacterial SSBs and *ScSSB* is reflected in amino acid sequences as well (Table 1). The N-domains of the three mycobacterial SSBs have a sequence identity of 94–95 % among themselves. The sequence identity between these SSBs and *ScSSB* ranges between 73 and 76 %.

DrSSB, *TaSSB*, and *TtSSB* form another group. They are dimers, but each subunit is made up of two OB domains in such a way that the structure of the subunit is similar to the AB dimer in tetrameric SSBs. Therefore, the overall structure of these dimeric SSBs is similar to that of the more common tetrameric SSBs. The sequence identity between the two domains in each subunit of the dimeric SSBs is typically a little over 30 %, which is roughly comparable to the sequence identity of each such domain with the

Table 1

Alignment scores among N-terminal domains of SSBs with known structure. N and C refer to the N-terminal and C-terminal domains of dimeric SSBs

	<i>MI</i>	<i>Mt</i>	<i>Ms</i>	<i>Sc</i>	<i>Ec</i>	<i>Tm</i>	<i>Bh</i>	<i>Hp</i>	<i>HMT</i>	<i>Ss</i>	<i>Mp</i>	<i>DrN</i>	<i>TaN</i>	<i>TtN</i>	<i>DrC</i>	<i>TaC</i>	<i>TtC</i>
<i>MI</i>		95	95	73	30	30	23	22	16	17	7	30	28	28	31	35	35
<i>Mt</i>			94	75	33	30	25	24	15	16	2	29	28	29	32	35	37
<i>Ms</i>				76	33	31	25	25	17	16	2	30	29	29	33	35	37
<i>Sc</i>					30	26	26	24	18	19	2	28	22	25	33	29	32
<i>Ec</i>						39	54	37	31	20	10	35	30	31	36	41	40
<i>Tm</i>							33	36	25	24	9	34	25	29	28	33	33
<i>Bh</i>								27	33	14	8	26	25	23	31	26	27
<i>Hp</i>									23	10	8	27	28	31	32	33	33
<i>HMT</i>										5	5	23	16	16	21	24	22
<i>Ss</i>											8	22	16	13	23	22	19
<i>Mp</i>												5	4	5	3	1	3
<i>DrN</i>													46	48	31	37	40
<i>TaN</i>														75	28	33	32
<i>TtN</i>															23	30	31
<i>DrC</i>																52	53
<i>TaC</i>																	91
<i>TtC</i>																	

domains of tetrameric SSBs (Table 1). Among the SSBs considered here, *Mp*SSB is unique in that it is a dimer with each subunit made up of a single OB domain. Each single domain subunit shares a alignment score of only 2–10 with the subunits of tetrameric SSBs. Despite this very low sequence identity, the structure of dimeric *Mp*SSB is very similar to that of the AB dimer in tetrameric SSBs.

4.2. Variability in Quaternary Association

The variability in the quaternary association of tetrameric SSBs arises from the change in the mutual orientation of the two dimers and of the two monomers within a dimer. The changes are of course consistent with the 222 symmetry of the whole tetramer. In *Mp*SSB, only the orientation between the two monomers is relevant. On the other hand, in dimeric SSBs with each subunit containing two OB domains, only the orientation similar to that between the two dimers in tetrameric SSBs is relevant.

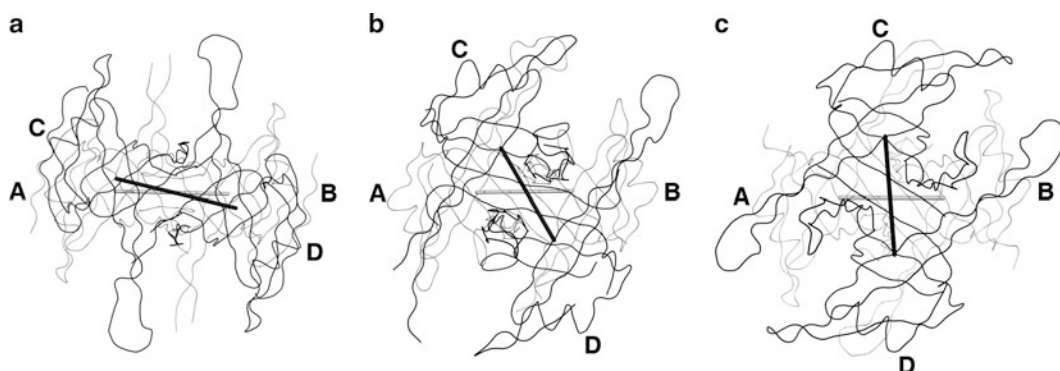


Fig. 3. Mutual orientation of the CD dimer (*thick line*) with respect to the AB dimer (*thin line*) in the tetramer in (a) *MfSSB*, (b) *EcSSB*, and (c) *HpSSB*. Broad straight lines join the centroids of A and B (*gray*) and C and D (*black*).

The variability in the mutual orientation of the dimers (or the two monomers in the case of SSBs with two domains in each subunit) is extensive (Fig. 3, Table 2). In mycobacterial SSBs and *ScSSB*, the two dimers almost eclipse each other with their longest dimensions pointing in the same direction. On the other extreme, the longest dimensions are nearly perpendicular to each other in *HpSSB*. In terms of orientation between the two dimers, *EcSSB*, *HMtSSB*, and *BhSSB* cluster together closer to *HpSSB*. *TmSSB* exhibits an orientation still closer to *HpSSB*, while the orientation in *SsSSB* involves a movement in the opposite direction. The three dimeric SSBs cluster together with an orientation closer to that in mycobacterial SSBs and *ScSSB*. The variability in the mutual orientation of the two monomers within a dimer is much less extensive and is best described in terms of the distance between the centers of masses of subunits A and B and the angle the two centers subtend at the center of mass of the tetramer (Fig. 4, Table 2). Mycobacterial SSBs and to some extent *ScSSB* again cluster together in terms of these parameters. *EcSSB*, *HMtSSB*, and *BhSSB* also cluster together as in the case of the orientation between the tetramers.

The variability in quaternary association outlined above leads to interesting differences in the surface area buried on oligomerization (Table 2). The monomer–monomer interface in the dimer involves a long loop which is not only highly flexible, but also disordered and hence undefined to different extents in different structures. Therefore, the estimate of surface area buried on dimerization could have some error. Yet it is clear from the table that the surface area buried on dimerization is somewhat lower in mycobacterial SSBs and *ScSSB* than in other SSBs. This could partly be on account of the comparatively high separation between the two monomers and partly because of the occurrence of water molecules in water bridges in the interface in some of the structures. The two dimers almost eclipse each other in the first group and the surface area buried at the AC (and equivalent BD) interface on oligomerization

Table 2

(1) Mutual orientation ($^{\circ}$) of dimers with respect to that in mycobacterial SSBs, (2) distance (\AA) between the centers of mass of subunits A and B, and surface areas (\AA^2) buried on oligomerization

Source	PDB code	(1)	(2)	Tetramer	AB	AC	AD
<i>MtSSB</i>	1ue1	0	23.7	8,966(5,554)	1,708(1,021)	2,771(1,740)	83(22)
<i>MiSSB</i>	3afp	0	23.9	9,104(5,300)	1,799(985)	2,800(1,724)	17(1)
<i>MsSSB</i>	1x3e	0	24.3	8,932(5,446)	1,573(895)	2,770(1,735)	35(20)
<i>ScSSB</i>	3civ	4	27.0	9,312(5,661)	1,654(917)	2,838(1,777)	17(13)
<i>SrSSB</i>	3koj	40	23.5	6,895(4,525)	2,138(1,457)	580(373)	906(595)
<i>EcSSB</i>	3kaw	44	20.3	6,542(3,553)	2,319(1,135)	458(389)	637(424)
<i>HMtSSB</i>	3ull	45	18.3	7,132(4,239)	2,777(1,482)	588(391)	772(417)
<i>BhSSB</i>	3pgz	49	18.4	6,905(3,757)	2,440(1,209)	453(407)	815(487)
<i>TmSSB</i>	1z9f	59	21.4	7,935(5,143)	2,594(1,599)	387(368)	1,257(832)
<i>HpSSB</i> + DNA	2vw9	71	20.0	5,690(3,774)	2,367(1,601)	94(93)	500(360)
<i>TaSSB</i>	2fxq	21		2,413(1,430) ^a			
<i>DrSSB</i>	1se8	23		2,189(1,429) ^a			
<i>TtSSB</i>	2cwa	24		3,290(1,709) ^a			
<i>MpSSB</i>	2hql		18.3		3,178(2,084)		

The nonpolar component is given in parentheses. In case of more than one crystal form of the same SSB, only one is used

^aThe values represent the area buried on dimerization of SSBs with two-domain subunits

is substantial. The buried area at this interface is, as expected, much lower in the group constituted by the rest of the tetrameric SSBs. The situation is the opposite at the AD (and BC) interface, although the difference between the two sets of values is less pronounced. The observed variability in the quaternary association of SSBs does not exhibit any obvious linear correlation with the differences in amino acid sequence. However, some broad correlation is discernible. In terms of sequence similarity, mycobacterial SSBs and *ScSSB* form one group. They form one group also in terms of the different components of surface area buried on oligomerization. The rest of the tetrameric SSBs form another group with similar surface areas buried at different interfaces in the oligomer. The sequence identity among them is not in general higher than that between them and mycobacterial SSBs; yet they form a group distinct from mycobacterial proteins in terms of oligomerization. Perhaps, the additional β strand (β_6) which clamps the two

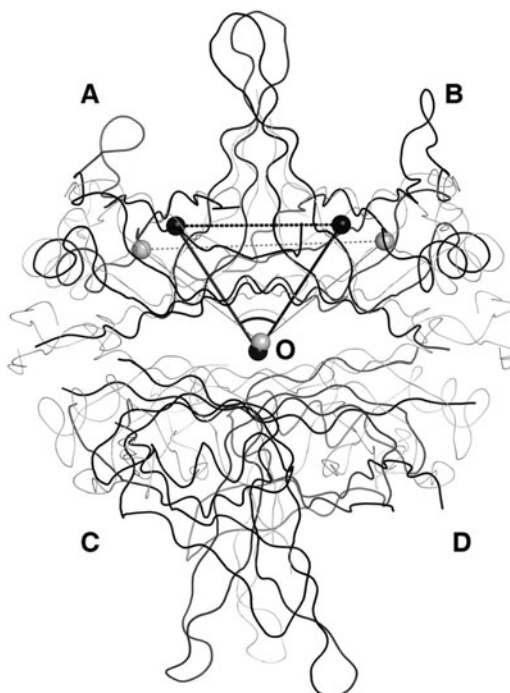


Fig. 4. Mutual orientation of A and B in HmtSSB (*black*) and ScSSB (*gray*). The balls represent centers of mass of A and B and the tetramer. The distance between the center of mass of A and B in HmtSSB is 18.3 Å and they subtend an angle of 66.5° at the center of mass of the tetramer. The corresponding values in ScSSB are 27.0 Å and 102.7°, respectively. Intermediate values are found in other SSBs of known structure.

dimers together in mycobacterial SSBs and ScSSB is a substantial contributor to this difference. It is also interesting that the total area buried on tetramerization is higher in mycobacterial SSBs and ScSSB than in other tetrameric SSBs. This observation is in consonance with the higher stability of MtSSB compared to EcSSB in the presence of guanidine hydrochloride (36).

5. Discussion

Despite their similar function and almost identical tertiary structures based on the OB fold, eubacterial and related SSBs exhibit considerable variability in quaternary association as evidenced by the crystal structures of their DNA-binding domains from different sources. The major source of this variability is changes in the mutual orientation of the two dimers in the tetrameric molecules with 222 symmetry. The mutual orientation of the two subunits in dimeric SSBs, in which each subunit is made of two OB domains, is equivalent to that between the two dimers in tetrameric SSBs. A secondary source of

variability in oligomerization is changes in the mutual orientation of the two monomers in the dimer.

In terms of quaternary structure, tetrameric SSBs of known three-dimensional structure can be broadly classified into two categories; mycobacterial SSBs and *Sc*SSB belong to one category and the rest to the second category. The difference between the two appears to result from the presence of $\beta 6$ in the first category of SSBs. They clamp the two dimers together in the tetramer resulting in the nearly eclipsed mutual orientation of the dimers. In the absence of this clamp, the molecules in the second category move away from this eclipsed disposition; the mutual orientations in them vary, but the variation is within about 30° . The clamps also loosen the interactions between the two monomers in the dimer in the first category. This allows in some instances water molecules to enter into the monomer–monomer interface. These observations are also consistent with the plasticity of the molecules in terms of relatively rigid and flexible regions, estimated earlier taking advantage of the availability of the structures of several crystallographically independent subunits of the same SSB. For example, in *Mt*SSB, which belongs to the first category, much of the dimer–dimer interface is relatively rigid, but the monomer–monomer interface in the dimer is relatively flexible (33). The opposite is true in the case of *Ec*SSB, which belongs to the second category.

The difference in the mode of oligomerization in the two categories appears to have implications to the stability of the tetramer, as evidenced by the area buried on oligomerization in the two cases and the experimental observation of the higher stability of *Mt*SSB than that of *Ec*SSB in the presence of guanidine hydrochloride (36). *M. tuberculosis* has to withstand considerable environmental stress during dormancy and growth in macrophages and the higher stability of *Mt*SSB could be of advantage in coping with and protecting genomic integrity from the stress (16). ssDNA with higher G + C content tends to form larger and more stable secondary structures and this might also dictate the requirement of more stable SSB in mycobacteria which have G + C-rich genomes. The structural results along with a comparative analysis of sequences of SSBs from eubacterial and mitochondrial SSBs had earlier indicated that the insertion of the critical $\beta 6$ tends to occur in all high G + C Gram-positive bacteria (27). Thus the sturdy quaternary association of SSBs found in the first category could well occur in such bacteria.

It has been demonstrated through complementation studies that *Mt*SSB cannot perform the function of *Ec*SSB and vice versa (36). The barrier could not be overcome even when chimeras constructed by swapping the C-terminal domains of the two SSBs were used. Therefore species specificity cannot be attributed to the C-terminal region of the protein. The difference in the quaternary structures of the two SSBs could also contribute to the species barrier (16).

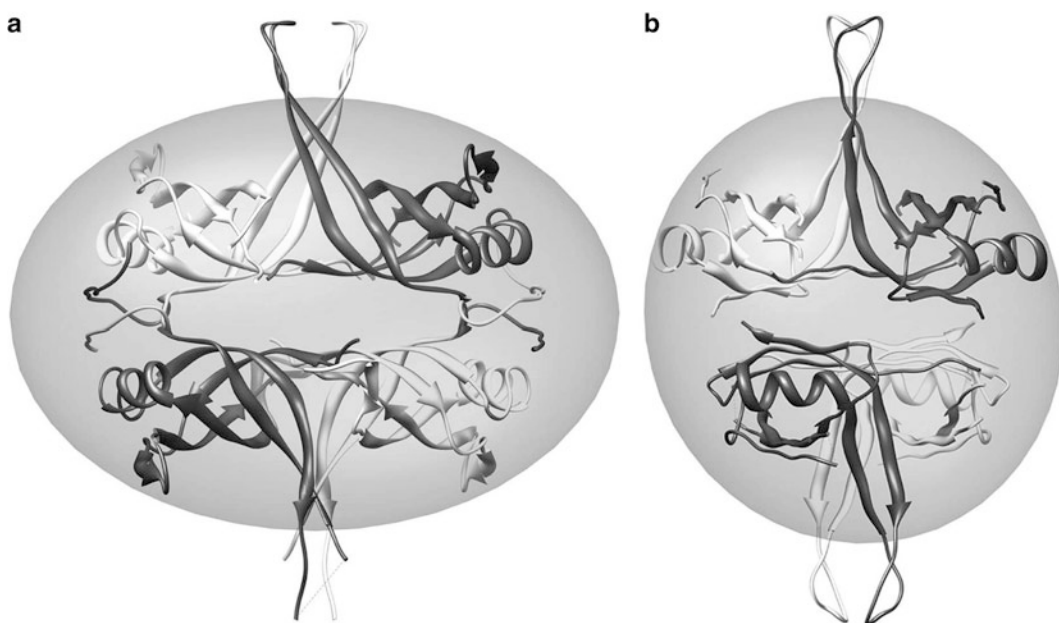


Fig. 5. Ellipsoidal representation of the core of the tetramer in (a) *MtSSB* and (b) *EcSSB*.

The most obvious overall difference between the SSBs of the two categories is in their shapes. For example, *MtSSB* is approximately ellipsoidal whereas *EcSSB* is approximately spherical (Fig. 5). Modeling based on this difference suggested that the length of DNA required to wrap around an *MtSSB* tetramer is lower than that required to wrap around an *EcSSB* tetramer (16). The length of DNA which binds to tetrameric *EcSSB* has been established as 65 ± 3 nucleotides (37), but the corresponding length with respect to *MtSSB* is yet to be determined. Crystal structures of the complexes of shorter DNA fragments with *EcSSB* (34) and *HpSSB* (35) have been reported. Coordinates of a similar complex with *MtSSB* (3a5u) are available in the PDB. The three structures together provide an interesting picture(s) (38). In all the cases, the electron density for DNA was discontinuous, perhaps reflecting disorder; partial occupancy was also indicated. Therefore, the structure of DNA in the complexes was partly modeled. Again, the DNA molecules do not obey the symmetry of the tetrameric protein. The paths followed by DNA in the three cases exhibit substantial differences, probably reflecting the differences in the quaternary structure of the proteins. There are notable commonalities as well in some regions of the path of DNA and the amino acid residues involved in interactions with DNA. The structural features of eubacterial SSBs, the commonalities among them, and the variations in their quaternary structure are very well characterized. The same cannot be said about SSB–DNA

interactions. Further work is needed to fully structurally characterize them, to discern commonalities among them, and to elucidate the effect of variability in the quaternary association of SSBs on the features of their DNA binding.

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