Ss-LrpB from *Sulfolobus solfataricus* Condenses about 100 Base Pairs of Its Own Operator DNA into Globular Nucleoprotein Complexes*

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Ss-LrpB from the hyperthermophilic crenarchaeote *Sulfolobus solfataricus* P2 is a member of the Lrp-like family of Bacterial/Archaeal transcription regulators that binds its own control region at three regularly spaced and partially conserved 15-bp-long imperfect palindromes. We have used atomic force microscopy to analyze the architecture of Ss-LrpB-DNA complexes with a different stoichiometry formed with the wild type operator and with an operator mutant. Binding of dimeric Ss-LrpB to all three target sites is accompanied by the formation of globular complexes, in which the protein induces strong DNA deformations. Furthermore, DNA contour length foreshortening of these complexes indicates DNA wrapping, with about 100 bp being condensed. The average bending angle is 260°. The establishment of protein-protein contacts between Ss-LrpB dimers in these globular complexes will contribute to the cooperativity of the binding. The profound remodeling of the control region is expected to have a strong impact on gene expression and might constitute the key element in the autoregulatory process.

Archaenal transcription is a chimera of eukaryotic and bacterial features (for a recent review, see Ref. 1). The basal transcription apparatus resembles that of eukaryotes. The archaenal polymerase is a homologue of RNA polymerase II and initiation requires the basal transcription factors TATA Box-binding protein and transcription factor B. In contrast, archaenal transcription regulators are mainly of the bacterial type. This is a surprising finding and the question arises as to how these bacterial type regulators (mainly helix-turn-helix proteins) interact with this multicomponent archaenal type basal transcription machinery. To date, little information is available on archaenal regulators. Most of the best characterized proteins belong to the leucine-responsive regulatory protein (Lrp) family (2). At present, the crystallographic structure of only two members of this family is known: LrpA from *Pyrococcus furiosus* (3) and FL11 from *Pyrococcus OT3* (4). *Sulfolobus solfataricus* contains at least five Lrp-like regulators (5–8), including Ss-LrpB (9). Their physiological role is still unclear, except for LysM that is involved in the regulation of lysine metabolism (7).

DNA-binding proteins, especially architectural nucleoid-associated proteins, polymerases, general transcription factors such as TATA Box-binding protein, and transcription regulators, often change the DNA conformation upon binding as part of their mode of action. This can result in DNA bending or even wrapping, DNA looping, DNA stiffening, or other forms of DNA remodeling. Intrinsinc bending, protein-induced remodeling of the DNA, and the conformability of operator segments, even if they constitute linker regions that are not directly contacted by the regulatory protein(s), may play an important role in the proceeding of a regulatory response. The determination of the architecture of the regulatory nucleoprotein complex(es) is therefore a crucial step in the analysis of gene regulation.

The structural analysis of nucleoprotein complexes can be performed by biochemical analyses, crystallography, or microscopy techniques such as atomic force microscopy (AFM) or electron microscopy. AFM is becoming a widespread and very valuable technique in the study of protein-DNA complexes at nanometer resolution (10). This technique is straightforward, and sample preparation and analyses occur under near native conditions, thereby limiting the risk of artifacts. AFM allows the simultaneous analysis of many aspects of the complexes (e.g. morphology, contour length, and bending angle), resulting in both qualitative and quantitative information. As a consequence, the architecture of several protein-DNA complexes formed by bacterial and, to a lesser extent, eukaryal regulators and RNA polymerases have been studied (for a review on bacterial studies, see Ref. 11). To our knowledge, this has not yet been done for archaenal transcription regulators. Here, we use the high resolution imaging capacity of AFM to study the architecture of various Ss-LrpB-DNA complexes.

Previously, we have shown that Ss-LrpB binds to its own control region at three specific and regularly spaced binding sites called Box1, Box2, and Box3 (9). This Ss-LrpB-DNA interaction occurs with an apparent dissociation constant $K_D$ of $\sim 10$ nM, and is cooperative. Each binding site is 15 bp long, semilandromic, and exhibits a variable degree of sequence identity with the consensus sequence $5'-TTGYAW-WWWWTGCAA-3'$ (Y = pyrimidine, R = purine, W = weak bp). Box1, the most promoter proximal target, shows an overlap of 1 bp with the BRE promoter element. In-gel footprinting demonstrated that the two outermost binding sites that show a high degree of sequence identity with the consensus sequence are occupied before the middle site, Box2, is bound. Box2 shows less sequence identity with the consensus and is a low affinity site. Protein-protein interactions and DNA deformations are supposed to play an important role in this cooperative interaction (9).

Here, we determine the oligomeric state of Ss-LrpB in solution and
present a detailed analysis of the three-dimensional structure of protein-DNA complexes exhibiting different stoichiometries, formed by binding of Ss-LrpB on the own control region (wild type and mutant forms). Measurements of contour length and bending angle of the complexes indicate that Ss-LrpB binding to the three targets accompanies pronounced DNA bending and condensation. This leads us to the hypothesis that the regulator wraps the control region DNA. These observations provide further insights into the autoregulatory mechanism that is employed by Ss-LrpB.

**EXPERIMENTAL PROCEDURES**

**Protein Purification**—Recombinant Ss-LrpB protein was obtained by a combination of heat treatment and ion exchange chromatography. Ss-LrpB was purified from a 300-ml culture of *Escherichia coli* BL21(DE3) containing pET24Ss-lrpBNde-null (9). The culture was grown in rich medium containing kanamycin (30 μg ml⁻¹) at 30 °C. Expression was induced at a cell density of 13.5 × 10⁶ cells ml⁻¹ by adding 1 mM isopropyl β-D-thiogalactopyranoside followed by overnight incubation. Cells were collected by centrifugation at 7000 rev min⁻¹ for 10 min (Sorvall RC5B Plus, rotor SLA-1500) and resuspended in 6 ml of extraction buffer (20 mM piperidine buffer (pH 10.7)). Cells were broken by sonication for 6 min at 20% of the maximal amplitude (Vibracell, Bioblock Scientific) in a cell cooled at 4 °C. After centrifugation of the disrupted cells at 10,000 rev min⁻¹ for 10 min (microcentrifuge), the supernatant was incubated at 80 °C for 10 min. Denatured proteins were removed by centrifugation at 13,000 rev min⁻¹ for 3 min (microcentrifuge). The extract was then loaded on a 6 ml ResourceQ anion exchange column (Amersham Biosciences), equilibrated with extraction buffer. Ss-LrpB was eluted by applying a linear gradient of 0 to 1.0 M NaCl. Fractions containing Ss-LrpB were identified by SDS-PAGE and electrophoretic mobility shift assay (EMSA) and pooled.

This resulted in ~4 mg of electrophoretically pure Ss-LrpB. All protein concentrations were determined by a MicroBCA assay (Pierce) and are expressed in Ss-LrpB monomer equivalents.

An aliquot of purified Ss-LrpB was analyzed by gel filtration chromatography on a Superdex 75 16/60 column (Amersham Biosciences). The column was equilibrated with 20 mM phosphate buffer (pH 7.4), 1 mM MgCl₂, 0.1 mM dithiothreitol, 12.5% glycerol, 50 mM NaCl, 0.4 mM EDTA in a total volume of 15 μl. The mixture was then diluted 2-fold in adsorption buffer (40 mM Hepes (pH 6.8), 10 mM NiCl₂, 6H₂O) and 15 μl was deposited on freshly cleaved mica. All AFM images were flattened prior to analysis using NanoScope III atomic force microscope (Digital Instruments/ Veeco) operating in tapping mode at room temperature. We used Nanoprobe SPM tips, type TESP (Veeco), with 125-μm cantilevers with a nominal spring constant of 50 N m⁻¹ and resonant frequencies in the range from 279 to 362 kHz. The scan rate was 2 Hz and the scan size was 1.5 × 1.5 μm. All images in one analysis were obtained with the same tip and deposition.

**DNA Manipulations**—The DNA fragments used in the AFM experiments were prepared by PCR approach. To obtain the wild type operator fragment, we started from the template DNA pBendBox1 + Box2 + Box3 (9) and amplified a 579-bp region containing the three boxes. This was done by using ReadyMix TaqPCR reaction mix (Sigma) and the oligonucleotides 5’-GGTTCCGGCCACATTTCCCAGG-3’ and 5’-CGGCAATACCAAGCCTATG-3’ as primers. Following the PCR, the DNA fragments were separated from parasite DNA on a 1.5% agarose gel, excised, and eluted using a GenElute gel extraction kit (Sigma). All oligonucleotides used in this work were purchased from Sigma Genosys.

**RESULTS**

*Ss-LrpB Behaves Mainly as a Dimer in Solution*—DMSI cross-linking is a classical method used for the determination of the oligomeric state(s) of a protein (12). DMSI forms covalent cross-links between lysyl
residues by amination of the primary amino groups. Fig. 1A shows a DMSI cross-linking experiment carried out with purified Ss-LrpB (theoretical monomer molecular mass of 17.5 kDa). One major band of ~32.2 kDa and four minor bands of ~52.2, 67.3, 78.2, and 94.1 kDa appeared after cross-linking. These correspond to cross-linked dimers and cross-linked trimers, tetramers, pentamers, and hexamers, respectively (Fig. 1A). Even at the highest DMSI concentrations used, the Ss-LrpB dimer constitutes by far the predominant cross-linked species (>85%). Tetramers and trimers made up between 5 and 10% of the cross-linked protein at DMSI concentrations of at least 1.25 mg/ml. Cross-linked hexamers and pentamers only appeared at the highest DMSI concentrations used. Therefore, in solution Ss-LrpB occurs mainly as a dimer. In parallel, we performed similar experiments with two other Lrp-like proteins, Ss-Lrp and Sa-Lrp from S. solfataricus and Sulfolobus acidocaldarius, respectively, which have a characterized oligomeric state (8). DMSI cross-linking confirmed that Ss-Lrp exists primarily as a tetramer in solution and that Sa-Lrp forms different oligomeric species up to a dodecamer (data not shown).

The oligomeric state of Ss-LrpB as determined by cross-linking was supported by size exclusion chromatography, in the sense that no Ss-LrpB oligomeric forms higher than a dimer were detected. Purified Ss-LrpB was applied to the column at a concentration of 5.2 μM. Only one peak, eluting with a maximum corresponding to 22.4 kDa (Fig. 1B), contained Ss-LrpB perceptible by SDS-PAGE. This molecular mass lies containing Ss-LrpB as analyzed by SDS-PAGE. This molecular mass lies between 17.5 and 32.2 kDa, which is in agreement with the elution profile of an aliquot of purified Ss-LrpB (5.2 μM) applied to a Superdex 75 16/60 column. The arrow indicates the sole peak containing Ss-LrpB as analyzed by SDS-PAGE.

**Visualization of DNA Molecules by AFM—** Purified 579-bp-long PCR amplicons were used containing the three regularly spaced binding sites of the Ss-LrpB control region near the center of the fragment (Fig. 2A). Each Box is 15 bp long. The center-to-center distance between Box1 and Box2, and Box2 and Box3, is 32 and 31 bp, respectively (Fig. 2B). Tapping mode AFM in air allowed visualization of these DNA molecules (Fig. 2C). The contour length (L) of 355 DNA molecules was measured by tracing the molecules from one end to the other using ImageJ. Overlapping DNA molecules or molecules that contained visible anomalies were omitted from the analysis. This resulted in an average length L of 173 ± 16 nm (Fig. 2A). The calculated axial base pair rise is 0.30 nm/bp (L/579), which is a value lower than the rise of regular B-form DNA (0.34 nm/bp) but in agreement with the bp rise determined in previous AFM studies. The difference with the theoretical bp rise can be explained by the smoothing procedure that rounds sharp bends and the limited resolution of the microscope, incapable of resolving bends within a small range (15).

A primordial concern in AFM studies is whether the DNA molecules and the protein-DNA complexes are able to freely equilibrate on the mica surface before capture. This is in contrast to kinetic trapping, a process that results in the projection of the three-dimensional conformation of the molecules (16). The latter would result in irrelevant data on the conformation of the complexes. This can be assessed by analyzing DNA persistence length (P). P describes the bendability of a DNA molecule and is a measure of the average length at which thermal energy causes the DNA molecule to bend in another direction. The mean square end-to-end distance $\langle R^2 \rangle$ can be expressed as a function of L and P for both cases $\langle R^2 \rangle_{2D}$ or $\langle R^2 \rangle_{proj}$ (16). Assuming a P of 53 nm, which is the value for DNA molecules in solution (independent of the length of the molecules), and a L of 173 nm for the operator DNA, $\langle R^2 \rangle_{2D}$ would correspond to 18,597 nm² and $\langle R^2 \rangle_{proj}$ to 8623 nm². The experimentally obtained $\langle R^2 \rangle$ of this set of DNA molecules is 18,494 nm² which is a value consistent with the two-dimensional (2D) model. P was also determined when applying simulation-based bending analysis software (see below; Ref. 14). The normalized R distribution of these molecules was fitted using distributions obtained by simulations of DNA molecules exhibiting no protein-induced bending. This resulted in a L/P value of 3.2, corresponding to a P of 54 nm. Therefore, free equilibration can be assumed.

**Visualization of Distinct Ss-LrpB-Operator Complexes—** AFM allowed visualization of the conformational change of the operator DNA molecules caused by binding of Ss-LrpB (Fig. 2). To obtain a stoichiometrically uniform population representing full occupation of the operator, Ss-LrpB-DNA complexes were allowed to be formed at a Ss-LrpB concentration (180 nM) that favors binding to all three Boxes. Many complexes were observed, most of them being single complexes, containing only one DNA molecule (Fig. 2D). Single complexes with different morphologies were observed (Fig. 2, E–H). They have in common that their complexed region is typically globular shaped with two DNA arms of nearly identical length. This globular shape and the sharp angle formed between the in- and out-going DNA arms are indicative of DNA wrapping.

Besides the single complexes, multiple complexes were observed, containing several DNA molecules linked by higher oligomeric Ss-LrpB forms (Fig. 2, D and F). Their number might be underestimated because larger complexes might not attach as firmly to the mica surface as smaller ones. In a single case, a complex was formed with two adjacent
globular regions (Fig. 2, D and I). This morphology is an indication of the existence of rare complexes (at this protein concentration) with a different stoichiometry. Likely, this molecule represents binding of two Ss-LrpB oligomers to two binding sites. The existence of these types of complexes was confirmed when analyzing complexes with a mutant operator fragment (see below). For the remainder of the analysis with WT operator complexes, only single complexes are considered.

Ss-LrpB Shortens the DNA Contour Length upon Full Occupation of the Operator—Measuring the contour length of protein-DNA complexes can provide more information on protein-induced conformational changes. In case of DNA wrapping a foreshortening can be expected. The contour length of AFM-visualized complexes can be measured in different ways (Fig. 3, B and C). The visible contour length corresponds to the total length of the two naked DNA arms. This might result in an underestimation of the length because the “shadow” of the protein causes a partial occlusion of the naked DNA (17). The read-through contour length is measured when the entry and exit points of the DNA are distant; the DNA measured through the complexed region could then be part of the DNA that is condensed by the protein.

We measured both visible and read-through contour length for 353 Ss-LrpB-DNA complexes with the WT operator fragment (Fig. 3, B and C). It can be assumed that in most complexes all three Boxes are bound (due to the high Ss-LrpB concentration). The visible contour length was on average $130 \pm 19$ nm, which is a difference of 43 nm or 143 bp with the contour length of unbound DNA molecules. The read-through contour length was $158 \pm 18$ nm, which corresponds to a difference of 15 nm or 50 bp. Therefore, it can be concluded that Ss-LrpB considerably condenses the DNA upon full occupation of the operator, a very strong indication of DNA wrapping. The exact amount of bps condensed by the protein will probably be situated between 50 and 143 bp. The three Boxes and linkers correspond to 78 bp.

Ss-LrpB Bends the DNA with an Average Bending Angle of $26^\circ$—Circu-
complexes, which were also used for contour length measurements. The bending angle was calculated by subtracting the measured angle from 180° (Fig. 4A). This resulted in an apparent bending angle of 88°/H11006 45° (Fig. 4B). This is a very broad distribution that likely reflects the effect of thermal fluctuations and the flexibility of the complexes, rather than the existence of several "microstates" exhibiting different bending angles. The latter would result in separate populations with different mobilities in EMSAs, which is not the case (9). This broad distribution is consistent with other AFM studies of protein-induced DNA bending (15, 18).

Recently, a method was developed to allow bending angle analysis of AFM images based on the end-to-end distance (R) distribution of the nucleoprotein complexes (14). The R distribution, normalized by L, is fitted to histograms based on simulations using least squares minimization (14). The bin size was chosen to be optimal. The application of the simulation-based bending analysis software (Bending Analysis) indicated a best fit apparent bending angle of 100° (Fig. 4C and D). This value is higher than the apparent angle obtained by direct measurement (tangent method) and will probably be a better approximation of the real bending angle. If the DNA is being wrapped, an apparent bending angle of 100° would correspond to a 260° bend in the opposite direction (Fig. 4A).

Ss-LrpB Binding to the Box2 Mutant Operator—To further analyze the structural identity of the globular nucleoprotein complexes observed with the WT operator, we have analyzed complexes formed with an operator mutant carrying a three bp substitution (CAA to TTT) in the downstream half-site of Box2 (Fig. 5A). As shown previously by EMSA, this mutation inhibits but does not completely abolish the formation of complexes with all three sites bound (9). In the DNA fragment used for AFM the operator has an asymmetric position on the fragment (Fig. 5A). We observed DNA molecules with one (Fig. 5B and E), two (Fig. 5C), or in rare instances even three (Fig. 5D) distinct complexed regions. Molecules showing two complexed regions most likely have one Ss-LrpB oligomer bound to each one of the outermost high affinity binding sites (Box1 and Box3). Molecules showing a single globular region may consist of a mixture of DNA molecules with one Ss-LrpB oligomer bound to all three Boxes (equivalent to the globular complexes formed with the WT operator). The difference was
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FIGURE 5. AFM analysis of Ss-LrpB-operator complexes formed with the Box2TTT mutant operator. A, schematic representation of the fragment used in AFM experiments, with indication of the relative positions of the PstI and BamHI cloning sites, the three Boxes (indicated 1, 2, and 3), and the CAA to TTT substitution in Box2 (mut). The sequence of the fragment between the two cloning sites corresponds to the cloned S. solfataricus DNA comprising the Ss-lrpB promoter/operator region. B, example of an AFM image of an Ss-LrpB-DNA complex with one globular region, assumed to have one Box bound. C, example of an AFM image of an Ss-LrpB-DNA complex with two complexed regions. D, example of an AFM image of an Ss-LrpB-DNA complex with three regions bound. E, example of an AFM image of an Ss-LrpB-DNA complex with one globular region, assumed to have three Boxes bound with DNA wrapping. F, frequency distribution of apparent volumes of complexed regions for complexes with two or three Ss-LrpB oligomers bound (white) and for complexes with a single globular region (black).

not always evident by means of visual inspection; the distinction was made after volume analysis of the complexes (see below). Previously we have shown by circular permutation assay that the binding of Ss-LrpB to a single Box or to the two outer Boxes results in a DNA bending angle of about 59 or 80°, respectively (9). Although this is not evident based upon the examples shown in Fig. 5, A and B, it was obvious for other complexes that Ss-LrpB also induced DNA bending upon binding one or two Boxes and complexes bound at three Boxes with DNA wrapping. Hence, the apparent volume of the complexed region of complexes with all three sites bound is on average about three times larger than the apparent volume of the distinct globular regions of one-, two-, and three-site bound complexes, when no DNA wrapping occurs. This result was confirmed with other data sets from other depositions and measured with different tips. Therefore, these data sets were not merged. The result is consistent with the binding of three interacting Ss-LrpB oligomers (likely dimers).

DISCUSSION

In this study, we present a detailed analysis of the architecture of the nucleoprotein complexes formed by binding of Ss-LrpB to the operator region of its own gene. To the best of our knowledge this is the first case of an archaeal transcription regulator system studied by AFM. With the WT operator fragment and at a high protein concentration, both single and multiple complexes were observed. The latter are complexes containing more than one DNA molecule. The existence of this type of complexes provides a possible explanation for the supershifting observed previously in EMSAs at high protein concentrations (9). This is most likely caused by protein aggregation, which is a characteristic feature of Ss-LrpB, rather than by cumulative binding at nonspecific sites on the DNA.

Previously, we have shown by EMSA and in-gel footprinting that the two outer Boxes are both bound before the weaker middle Box2 (9). AFM experiments with a Box2 mutant operator fragment allowed distinct visualization of complexes bound at one (Box1 or Box3), two (Box1 and Box3), or three Boxes. The ability to observe the individual protein oligomers bound to adjacent sites reflects the high resolution of AFM which is in this case at least 4.8 nm (corresponding to the 16-bp linker). Based on these observations suggestions can be made regarding the stoichiometry of the complexes. Ss-LrpB behaves mainly as a dimer in solution and it might be hypothesized that each Box is bound by one dimer. The semipalindromic nature of the binding sites also reinforces the idea of recognition by protein dimers. When all three Boxes are

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bound, close associations occur between the DNA-bound dimers. This is supported by volume analysis of the globular regions of these complexes. Combined, the results presented here indicate that the globular nucleoprotein complexes formed with the WT operator consist of DNA wrapped around three interacting Ss-LrpB dimers bound to three regularly spaced binding sites. DNA wrapping and the establishment of protein–protein contacts appear to be tightly linked in this system and might be responsible for the apparent cooperativity in the binding (9).

Ss-LrpB induces extensive DNA curvature upon binding. Previously, circular permutation assays indicated that the operator is increasingly deformed by Ss-LrpB binding to one, two, and three boxes, culminating in an apparent bending angle of 148° (9). However, this technique has its drawbacks and is not valid for bending angles above 120° (23). It is clear that Ss-LrpB-induced DNA bending exceeds this angle, given the observation that the DNA is condensed by wrapping around the protein. The DNA bending was quantified using two independent methods: the tangent method and bending analysis based on the EED/L distribution. It has been shown that the tangent method leads to an underestimation of the bending angle, especially when the DNA is strongly bent (14), and this was indeed the case. Bending analysis based on the end-to-end distance distribution revealed an average apparent bending angle of 100°, which corresponds to 260° when assuming DNA wrapping (see Fig. 4A).

Ss-LrpB binding to all three binding sites resulted in a DNA fore-shortening. The exact amount of bps that are being condensed is bordered by a lower limit of 50 bp (possibly underestimated when measuring the read-through contour length) and an upper limit of 143 bp (possibly overestimated when measuring visible contour length). Previously, it was shown that DNase I footprinting resulted in a global region of protection of 86 bp (9). Therefore, we propose that the real amount of bps being condensed by Ss-LrpB is near the average between the read-through and visible contour length which is about 100 bp. This clearly indicates DNA wrapping at full occupation of the Boxes resulting in the formation of globular nucleoprotein structures. DNA wrapping around Ss-LrpB maximizes the contact area between the DNA and the protein while allowing the Ss-LrpB dimers to establish intermolecular interactions in order to stabilize the complex (see below). This observation contributes to the aforementioned hypothesis that structurally very different Ss-LrpB–operator complexes exhibiting different stoichiometries would exert different autoregulatory effects (9). At low Ss-LrpB concentrations, when only one Box is bound, a positive regulation might occur. At higher concentrations, when all three Boxes are bound, severe alterations in DNA conformation, caused by the DNA wrapping, would result in a negative autoregulation. Binding to the third Box is cooperative (9). Therefore, small changes in Ss-LrpB concentration result in large changes in binding site occupancy and, accordingly, DNA conformation. This can lead to a fine-tuned “switch” between positive and negative autoregulation, and wrapping might be the key component of this switch. Direct confirmation of this hypothesis will be sought by in vitro transcription.

Assuming 100 bp to be wrapped around a protein core, this would correspond to a globular structure with a diameter of 95 Å. It seems likely that these molecular dimensions agree with three Ss-LrpB dimers having the DNA wrapped around them. Similarly, a model was built of an FL11 octamer with the DNA wrapped around exhibiting a diameter of 90 Å (4). These dimensions are also comparable with the molecular size of an LrpA octamer (96 × 96 × 110 Å) (3). Besides, these globular structures are reminiscent of the structure of eukaryotic (H1–H4)2 nucleosomes that have a diameter of ~100 Å and of archaean Hm/f or HTz tetrasomes (24, 25).

If the DNA molecule behaves as a worm-like chain, the energy required to bend the DNA is dependent on the persistence length, temperature, bending angle, and the length over which the bend is extended (15). To bend the Ss-LrpB operator with 260° over a length of 100 bp, 47 kJ/mol are required (taking a complex equilibration temperature of 37 °C into account). Assuming a similar nucleoprotein complex conformation at higher temperatures, 54 kJ/mol would be required at a temperature of 80 °C, which is the optimal growth temperature of S. solfataricus P2 (26). This energy would mainly be compensated by the formation of additional favorable Ss-LrpB–DNA interactions and especially by protein–protein interactions between the three distinct Ss-LrpB dimers. Only a few weak interactions would suffice to compensate for 40–60 kJ/mol. Also, intrinsic bending of the operator region might reduce this energetic cost, which is the case for the Ss-LrpB operator. Indeed, fragments containing the three Boxes show an intrinsic bending with an average bending angle of 35°, as demonstrated by a circular permutation assay (9).

The formation of higher order nucleoprotein structures with the DNA wrapped around multiple protein molecules appears to be a general propensity of regulators belonging to the Lrp family. All cases reported so far can be divided in two classes. The first class consists of Lrp-like proteins that specifically recognize DNA sequences and bind these sites cooperatively (on the same face of the DNA helix). Each binding site undergoes deformation upon binding. In some cases, this will eventually lead to a structure in which the DNA is wrapped around multiple Lrp units. It might be speculated that each Lrp dimer establishes interactions with one binding site. This is the case for the binding of the global regulator Lrp from E. coli to the operator regions of certain regulon members (e.g. ilvIH; Ref. 27), for the abovementioned FL11 (4), and is also suggested for LrpA from P. furiosus based on its three-dimensional octameric structure (3). In contrast to FL11 and LrpA, Ss-LrpB is proposed to form an array of three instead of four dimers and interacts with three regularly spaced sites. Furthermore, it is striking how well conserved these binding sites are. In other cases, the Lrp-like regulators often recognize clusters of strongly degenerated binding sites (E. coli Lrp, Ref. 28, Ptl2 from Methanocaldococcus jannaschii, Ref. 29). The second class consists of Lrp-like proteins that seem to bind specific structures instead of an array of base-specific groups or that seem to bind nonspecifically. Examples are Smj12 from S. solfataricus (nonspecific binding; Ref. 6) and LrpC from Bacillus subtilis (structure-specific binding; Ref. 30), which both introduce positive supercoils by right-handed wrapping. Besides being a global regulator, E. coli Lrp is also suggested to be a less-specific DNA-wrapping protein (31). Often, these proteins are suggested to play a role in the global organization of the nucleosome.

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