DNA Reshaping by MukB

RIGHT-HANDED KNOTTING, LEFT-HANDED SUPERCOILING

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MukB is a bacterial SMC (structural maintenance of chromosome) protein required for faithful chromosome segregation in Escherichia coli. We report here that purified MukB introduces right-handed knots into DNA in the presence of type-2 topoisomerase, indicating that the protein promotes intramolecular DNA condensation. The pattern of generated knots suggests that MukB, similar to eukaryotic condensins, stabilizes large right-handed DNA loops. In contrast to eukaryotic condensins, however, the net supercoiling stabilized by MukB was negative. Furthermore, DNA reshaping by MukB did not require ATP. These data establish that bacterial condensins alter the shape of double-stranded DNA in vitro and lend support to the notions that the right-handed knotting is the most conserved biochemical property of condensins. Finally, we found that MukB can be eluted from a heparin column in two distinct forms, one of which is inert in DNA binding or reshaping. Furthermore, we find that the activity of MukB is reversibly attenuated during chromatographic separation. Thus, MukB has a unique set of topological properties, compared with other SMC proteins, and is likely to exist in two different conformations.

SMC (structural maintenance of chromosome) proteins are involved in various aspects of higher order chromatin dynamics in organisms ranging from bacteria to humans. They act as a part of multisubunit complexes that are required for such diverse cellular functions as chromosome cohesion and condensation, recombination, repair, and dosage compensation (1–6).

In solution, SMC proteins form a distinctive V-shaped structure composed of two molecules joined via their hinge domains (7–9). Two long coiled-coils protruding from the hinge terminate in globular head domains of SMCs. The head domains themselves can dimerize, creating a composite ATP binding pocket at the interface of the two head domains. ATP binding and hydrolysis has been proposed to drive conformational changes in the interacting head domains, which eventually result in the formation and disruption of protein rings (10–12) or macromolecular assemblies (13–15). Mutations within the P-loop or signature motif of SMCs inactivate the proteins in vivo (12, 16). However, the role of ATP in DNA reshaping by SMCs remains unclear.

In eukaryotes, the structurally similar condensins and cohesins have very distinct roles in chromosome dynamics. In vitro, the purified SMCs act according to their intracellular functions. Condensins promote intramolecular DNA condensation, which can be detected as an increase in DNA knotting in the presence of type-2 topoisomerase (17–19). Similar reaction carried out with cohesins produces catenanes, indicating that DNA fragments from different DNA molecules are brought together (20). Rad50 protein, an SMC protein involved in double strand break repair in yeast, oligomerizes upon binding to the ends of linear DNA (15). The mechanism of DNA reshaping by SMC complexes remains unknown. It is not even clear how much of the biochemical specificity resides within the SMC core of the complex and how much is conferred by its non-SMC subunits.

Perhaps the most comprehensive picture of DNA reshaping has been established for 13 S condensin from frogs (17). In a reaction coupled to DNA strand transport by type-2 topoisomerase, the five-subunit 13 S condensin promoted the ATP-dependent formation of chiral knots in nicked circular DNA. The majority of generated knots were right-handed trefoils, the three-noded knots. Five-noded torus knots and six-noded granny knots, which can be formed after two strand passages by topoisomerase, were also detected. Based on this knotting pattern, the authors suggested that 13 S condensin introduces right-handed loops into DNA (17). Besides knotting, 13 S condensin promoted positive supercoiling of relaxed plasmids in the presence of type-1 topoisomerase (21). Both knotting and supercoiling are indicative of DNA compaction (1, 22), and thus it is not entirely clear which of the two reactions is primary in DNA condensation. Condensins from other organisms were reported to support either positive supercoiling (23) or knotting (18) or knotting without concomitant supercoiling (19, 24). The SMC protein from Bacillus subtilis has not been tested for knotting or supercoiling. Instead, the protein was reported to promote ATP-dependent aggregation of single-stranded DNA (25). This result is surprising, since condensins are believed to act on double-stranded DNA. It was interpreted therefore as an indication that B. subtilis SMC preferentially binds to negatively supercoiled DNA (25, 26).

Here, we investigated DNA reshaping by the Escherichia coli SMC protein MukB. MukB is the first discovered member of the SMC protein family (27). Purified MukB forms a stable complex with double-stranded DNA and binds ATP and GTP in the presence of Zn^{2+} (28). Inside the cell, MukB acts in a complex with two other, non-SMC proteins, MukE and MukF (29, 30). Cells deficient in MukB function show chromosome decondensation and cutting and produce normal size chromosomes (15). MukB is unique among SMCs in that it is not conserved between the species. In contrast to eukaryotic condensins, MukB has very distinct roles in chromosome dynamics. In eukaryotes, the structurally similar condensins and cohesins have very distinct roles in chromosome dynamics. In vitro, the purified SMCs act according to their intracellular functions. Condensins promote intramolecular DNA condensation, which can be detected as an increase in DNA knotting in the presence of type-2 topoisomerase (17–19). Similar reaction carried out with cohesins produces catenanes, indicating that DNA fragments from different DNA molecules are brought together (20). Rad50 protein, an SMC protein involved in double strand break repair in yeast, oligomerizes upon binding to the ends of linear DNA (15). The mechanism of DNA reshaping by SMC complexes remains unknown. It is not even clear how much of the biochemical specificity resides within the SMC core of the complex and how much is conferred by its non-SMC subunits.

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Here, we investigated DNA reshaping by the Escherichia coli SMC protein MukB. MukB is the first discovered member of the SMC protein family (27). Purified MukB forms a stable complex with double-stranded DNA and binds ATP and GTP in the presence of Zn^{2+} (28). Inside the cell, MukB acts in a complex with two other, non-SMC proteins, MukE and MukF (29, 30). Cells deficient in MukB function show chromosome decondensation and cutting and produce normal size anucleate cells (27). The phenotype of mukB can be suppressed by mutations in DNA topoisomerases that increase DNA supercoiling (31) and thereby result in greater compactness of the chromosome. It was proposed therefore that MukB promotes DNA knotting in the presence of type-2 topoisomerase. The pattern of generated knots, mostly right-handed trefoils, was similar to that found earlier for other condensins (17–19, 24). Thus, this aspect of DNA reshaping by condensins is highly conserved between the species. In contrast to eukaryotic condensins, however, the net supercoiling generated by MukB was negative. Furthermore, the topological activities of MukB did not require ATP. It appears, therefore, that MukB stabilizes DNA in a compact static structure that is prone to produce knots and supercoils upon treatment with topoisomerases. As an unexpected bonus, we find that MukB can be
eluted from a heparin column as two peaks. The two eluted forms of MukB differ in DNA binding and reshaping and thus appear to represent two conformations of MukB. These two forms of MukB might correspond to different conformations of the protein during the chromosome condensation-decondensation cycle.

**MATERIALS AND METHODS**

**Plasmids and Strains**—The MukB-deficient strain GC7528 (27) was a kind gift of Dr. Sota Hiraga. pBB10 is a pBAD/Myc-His6B (Invitrogen)-based plasmid that contains the mukB-His10 gene under the control of arabinose-inducible promoter pBAD.

**Protein Purification**—Phage T2 topoisomerase was a generous gift of Dr. Nicholas R. Cozzarelli. Wheat germ topoisomerase I was purified as described (34). E. coli topoisomerase I was purified by nickel-chelate chromatography using a polyhistidine tag cloned onto its C terminus.

MukB-His10 was purified from DH5α cells harboring plasmid pBB10. The cells, grown in LB, were induced at 1% L-arabinose, collected by centrifugation 3 h later, resuspended in Buffer B plus 50 mM NaCl, 400 mM imidazole and 2) Buffer B plus 50 mM NaCl. The protein mixture was layered on top of 20% polyethylene glycol 20000 in Buffer B plus 200 mM NaCl, 0.5 mM MgATP, the protein was then eluted with 5 column volumes of 20 mM HEPES, pH 7.7, 50 mM NaCl, 400 mM imidazole, 1 mM phenylmethylsulfonyl fluoride. Eluted protein was supplemented with 2 mM EDTA and 1 mM DTT, applied to a heparin-agarose (Sigma) column, and washed with 5 volumes each of 1) Buffer B (20 mM HEPES, pH 7.7, 5% glycerol, 2 mM EDTA, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride) plus 50 mM NaCl, 400 mM imidazole and 2) Buffer B plus 50 mM NaCl. The protein was then eluted with a 10-volume gradient of 50–500 mM NaCl in Buffer B. The peak fractions were pooled; concentrated by dialysis against 20% polyethylene glycol 20000 in Buffer B plus 200 mM NaCl; dialyzed against 20 mM HEPES, 200 mM NaCl, 2 mM EDTA, 1 mM DTT, 50% glycerol; and stored at −20 °C. Analytical fractionation of purified MukB was done using a 1-ml HiTrap heparin column (Amersham Biosciences). The fractions from the high and low salt peaks were pooled separately; dialyzed against 20 mM HEPES, 200 mM NaCl, 2 mM EDTA, 1 mM DTT, 20% glycerol; and kept on ice for up to 2 weeks.

**Molecular Weight of MukB**—The size of the purified MukB was measured using a combination of sucrose density gradient centrifugation and gel filtration chromatography. The size markers used for centrifugation and gel filtration were as follows: thyroglobulin (19.2 S, 8.6 nm), apoferritin (17.6 S, 6.2 nm), β-amyrase (8.9 S, 4.8 nm), alcohol dehydrogenase (7.3 S, 4.6 nm), bovine serum albumin (4.3 S, 3.6 nm), and carbonic anhydrase (2.8 S). MukB migrated as a single peak on the sucrose gradient and Sephacryl S500 column (data not shown). The Stokes radius of MukB was estimated as 9.1 nm, and the sedimentation coefficient was 8.6 S (data not shown). These values are in very good agreement with the previous measurements performed for MukB in cell extracts (30). The molecular mass of MukB was calculated as 330 kDa, and the form factor was 2.0.

**Sucrose Density Gradient Centrifugation**—30 μg of MukB was mixed with 30 μg each of thyroglobulin (Amersham Biosciences), apoferritin, β-amyrase, yeast alcohol dehydrogenase, bovine serum albumin, and carbonic anhydrase (Sigma) in 20 mM HEPES, 200 mM NaCl, 10% glycerol, 2 mM EDTA, 1 mM DTT. The protein mixture was layered on top of a 10–40% sucrose gradient at a 70% sucrose cushion in the same buffer and centrifuged at 45,000 rpm at 4 °C for 12 h.

**DNA Reshaping**—DNA knotting was done essentially as described in Ref. 17. The indicated amounts of MukB were added to the mixture containing 10 ng (3.5 fmol) of singly nicked pBR322 DNA in 10 μl of Reaction Buffer (20 mM HEPES, pH 7.7, 40 mM NaCl, 2 mM MgCl2, 7% glycerol, 1 mM DTT) containing 1 ng/ml bovine serum albumin and, where indicated, 1 mM MgATP. The reaction mixture was supplemented with 3.5 fmol of phage T2 topoisomerase and incubated at 37 °C for 40 min. The reactions were quenched with Stop Buffer (50 mM Tris-HCl, 0.5% SDS, 20 mM EDTA, 200 mM NaCl, 0.5 mM/mg proteinase K) plus 10 μg/ml yeast tRNA and incubated at 50 °C for 40 min. Following phenol/chloroform extraction and ethanol precipitation, the DNA samples were resolved using gel electrophoresis through 0.8% agarose gels in 0.75× TBE for 39 h at 3 V/cm. The DNA was then visualized using Southern blot hybridization and quantified using a Storm PhosphorImager.

For the supercoiling reaction, 10 ng of relaxed pBR322 DNA was treated with either 35 fmol of wheat germ or E. coli topoisomerase I in the presence or absence of MukB in 10 μl of Reaction Buffer. Following deproteinization, the DNA was analyzed using gel electrophoresis through 4% agarose gels in 1× TAE (40 mM Tris acetate, pH 8.3, 1 mM EDTA) at 4 °C for 22 h at 3 V/cm followed by Southern blot hybridization. For the time course of DNA supercoiling, DNA topoisomerase was added prior to the addition of condensin.

For gel shift experiments, 10 ng of DNA in Reaction Buffer was mixed with the indicated amounts of MukB and incubated for 30 min at 37 °C. The reaction mixtures were then placed on ice and analyzed by gel electrophoresis through a 0.7% agarose gel in 1× TAE (40 mM Tris acetate, pH 8.3, for 12 h to resolve pBR322 DNA) or 2.5 h (for 443-bp DNA) at 4 °C. To visualize DNA, the gels were stained with SYBR Gold (Molecular Probes, Inc., Eugene, OR).

**ATPase Rate**—1 μg of MukB was added to Reaction Buffer (10-μl final volume) supplemented with 1 mg/ml bovine serum albumin, 0.1 mM MgATP, 0.1 μCi of [γ-32P]ATP, and, where indicated, 200 ng of pBR322 or phage φX174 DNA. Reaction mixtures were incubated at 37 °C up to 1 h. 1-μl aliquots were removed every 20 min, mixed with 10 μl of Stop Buffer, incubated for 40 min at 50 °C, and resolved by thin layer chromatography using polyethyleneimine-cellulose plates (J. T. Baker Inc.).

**Two-dimensional Gel Electrophoresis**—DNA was first electrophoresed through a 1.1% agarose gel in 1× TAE for 22 h at 3 V/cm. The gel was then turned by 90°, soaked for 3 h in TAE supplemented with 0.8 μg/ml chloroquine, and further electrophoresed for 18 h at 2 V/cm.

**Electron Microscopy**—40 ng of singly nicked pBR322 DNA were incubated with 5.8 pmol of MukB in 20 μl under the same conditions that were used for the knotting assay. The reaction was terminated by the addition of 80 μl of Stop Buffer. Following deproteinization, we combined DNA from five separate reactions, denatured it in the presence of glyoxal, coated it with ReCa protein in the absence of ATP, and examined it by electron microscopy as described in Ref. 35.

**RESULTS**

**MukB Elutes from Heparin-Sepharose as Two Peaks**—We initially purified MukB using the C-terminally placed His10 tag. That preparation of MukB, however, contained numerous contaminants, including nucleases. We therefore introduced the His16 C-terminal tag onto MukB. MukB-His16 fusion protein was overproduced from the pBB10 plasmid, which carries mukB-His10 under the control of the arabinose inducible promoter pBAD. The protein encoded on the

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2 The abbreviations used are: DTT, dithiothreitol; ACP, acyl carrier protein; AMPPNP, S-adenosyl-β,γ-imidodiphosphate.
plasmid was functionally active, since the plasmid suppressed the temperature sensitivity, anucleate cell formation and chromosome decondensation of mukB null mutant GC7528 (data not shown). Following purification by nickel-chelate chromatography, MukB was 95% pure. To make sure that no nucleic acids carried over with MukB, we next applied the protein to a heparin column (Heparin I). MukB eluted as a broad peak from this column (Fig. 1A). The high salt fractions of the peak (marked with the black bar in Fig. 1A) were pooled and analyzed further. The purified MukB was found to be a dimer (see "Materials and Methods").
Several additional species could be found in our main preparation of MukB (Fig. 1B). There was ~3% acyl carrier protein (ACP), which translates into about 1 ACP per MukB dimer. The 8.8-kDa ACP migrates through SDS-PAGE with an anomalous mobility that depends on the length of the attached lipids (36). Copurification of ACP and MukB has been reported earlier (28, 37). The significance of this interaction remains unknown.

We also found several high molecular weight species that migrate more slowly than MukB on SDS-PAGE (Fig. 1B, X). These species are likely to be the cross-linked oligomers of MukB, since they appear equally abundant, about 2% of total protein, when visualized by Coomassie Blue staining and immunoblotting using anti-MukB antibody, and they are resistant to boiling in the presence of SDS and β-mercaptoethanol (Fig. 1B). The cross-linking most probably happened on the nickel column. Similar cross-linking has been reported earlier for other proteins (38).

Two unidentified proteins, p30 and p24, were removed in the next round of heparin chromatography (Fig. 1C). These proteins comprised less than 1% of total protein and did not co-migrate with MukB or DNA reshaping activities during gel filtration or heparin chromatography (see below).

Finally, we observed a variety of proteolytic fragments of MukB (marked F in Fig. 1B). The degradation of MukB was significantly less pronounced when the protein was coated with SDS at room temperature rather than at 100 °C (Fig. 1B). We detected no other contaminants in excess of 0.2% (Fig. 1B).

MukB eluted as a broad peak from the first heparin column (Fig. 1A). This raised the possibility that MukB exists as a mixture of two different populations. We indeed found two separate peaks when we applied the Heparin I pool of MukB (black bar in Fig. 1A) to a HiTrap heparin column (Heparin II) and eluted it with a gradient of NaCl (Fig. 1, A and C). ACP copurified exclusively with the low salt fraction of MukB, indicating that the two peaks represent two different conformations of MukB. Furthermore, only one of the two protein populations was efficient in DNA reshaping (see below). The more active, high salt fraction of MukB was devoid of any detectable contaminants. This preparation (fractions 22–24 in Fig. 1C) was used below to characterize the topological activities of the protein.

It was not immediately clear why the separation between the two peaks of MukB improved during the second run of the heparin column (Fig. 1A). Perhaps this difference can be attributed to the properties of heparin-agarose supplied by different manufacturers (see “Materials and Methods”). Alternatively, the variable elution of MukB could reflect conformational instability of the protein. In agreement with the latter interpretation, we again found two peaks (with similar partitioning between the high and low salt fractions) when we applied the low salt half of the Heparin I peak to the Heparin II column (data not shown).

MukB Is a Slow ATPase—To characterize the ATPase activity of MukB, we used the Heparin I pool (black bar in Fig. 1A) of the protein that was additionally purified by gel filtration through Sephacryl S300. This additional purification step removed the contaminating ATPase activity from our main preparation of the protein (see below).

MukB was a slow ATPase. At the optimal salt conditions, one dimer of MukB hydrolyzed 0.2 ATPs/min. This is perhaps the lowest ATPase rate reported so far for the SMC proteins and is comparable with that found for the Walker A mutants of SMCs (25). The ATPase activity of MukB declined at 10 mM MgCl₂ and above 100 mM NaCl and was not affected by the presence of single-stranded or double-stranded DNA (Fig. 2). We found virtually the same rates of ATP hydrolysis when we further fractionated MukB into the high and low salt peaks using heparin chromatography (Fig. 2B). We again found no effect of DNA on the rates of ATP hydrolysis (data not shown). The salt conditions that resulted in the highest ATPase rate, 2 mM MgCl₂, 40 mM NaCl, were selected to assay for DNA reshaping.

MukB Binds Linear and Circular DNA—10 ng of MukB was incubated with increasing amounts of MukB in our standard reaction buffer (see “Materials and Methods”) for 30 min at 37 °C, the reactions were quenched by placing the tubes on ice, and the mixtures were resolved by agarose gel electrophoresis. The protein-bound DNA migrated as a smear through the gel, with the average mobility of the smear decreasing at higher concentrations of the protein (Fig. 3). The same gel shift pattern, at similar concentrations of the protein, was reported for MukB (28) and other SMC proteins (19, 21, 39) and is consistent with the view that numerous condensins bind the same DNA molecule. In agreement with this interpretation, discrete bands of gel-shifted DNA could often be observed at low protein concentration (Fig. 3, A and D).

The high salt fraction of MukB was binding linear and circular DNA with similar affinities (Fig. 3A). Over several experiments, the difference in the apparent dissociation constants for binding to linear and circular, supercoiled pBR322 DNA was always less than a factor of 2 (data not shown). Such a small difference, if any, does not immediately support the topological entrapment mechanism proposed for cohesins (9). Rather, it can be readily explained by the higher local concentration of DNA segments next to each other typical for supercoiled DNA (40). Furthermore, the 10-fold shorter, 443-bp linear DNA required only 4-fold more MukB for complete gel shift (Fig. 3A). The low salt fraction of MukB showed similar DNA binding properties, albeit with a 3-fold lower affinity (Fig. 3B).

The binding of MukB to either linear (data not shown) or supercoiled (Fig. 3C) DNA was not affected by the presence of ATP and was only marginally weakened when we replaced magnesium with EDTA in our reaction mixtures (Fig. 3C). Thus, neither ATP nor magnesium is required for DNA binding.
Similar to the B. subtilis SMC protein (25), MukB was binding to the phage single-stranded DNA much better than to double stranded DNA (Fig. 3D). However, we did not observe any gel shift for the 60-nucleotide single-stranded DNA oligonucleotides (data not shown). Thus, the high affinity of MukB for the phage DNA might reflect its interaction with the secondary structure elements, which are abundant in long single-stranded DNAs.

**Chiral Knotting by MukB**—Treatment of nicked circular DNA with phage T2 topoisomerase in the presence of MukB resulted in knotting of the DNA substrate, whereas the level of DNA catenanes increased only modestly (Fig. 4A). In contrast, a similar assay performed on cohesins leads to extensive DNA catenation, leaving virtually no unlinked DNA circles (20). Thus, MukB promotes intramolecular DNA condensation and therefore can be qualified as a condensin. No knotting or catenation was detected for the low salt fraction of MukB (data not shown).

As found for other condensins, the majority of generated knots were the three-noded knots, trefoils. In addition, we found a fair amount of four- and five-noded knots. We found using electron microscopy (35)
that the vast majority of the generated knots were chiral. Of 38 analyzed trefoils, 37 were right-handed (Fig. 4C). This pattern of DNA knotting is consistent with the model that was originally proposed to explain the predominant formation of trefoil knots by the frog condensin (17). In this model, condensins stabilize large right-handed loops in DNA. A single strand transport between two such loops generates either a three- or four-noded knot. The proportion of three- and four-noded knots is expected to vary depending on spatial arrangement of the loops (1). The five-noded knots were proposed to result from two rounds of strand transport (17). In full accord with this model, we found an increase in the abundance of five-noded but not four-noded knots when we doubled the amount of topoisomerase-2 in the reaction (data not shown).

It is noteworthy that fewer knots were produced at high concentrations of MukB (Fig. 4A). This was probably caused by extensive binding of the proteins to DNA, which restricts topoisomerase access to DNA. In agreement with this view, we found that relaxation rates by type-1 or type-2 topoisomerase declined dramatically in the presence of MukBEF (data not shown).

Right-handed Knotting Is Paralleled by Left-handed Supercoiling—Formation of right-handed knots by 13 S condensin was accompanied by the generation of net positive supercoiling (17). Because the knots produced by MukB were right-handed, we expected that the protein would stabilize net positive supercoiling in DNA. We found net negative supercoiling instead.

In this assay, relaxed DNA is treated with a type-1 topoisomerase in the presence of condensin. If condensin introduces positive supercoiling into DNA, the generated negative compensatory supercoils can be removed by either type-1A or type-1B topoisomerase (41–43). The net change in supercoiling is then detected by gel electrophoresis. If condensin stabilizes negative supercoils, only type-1B topoisomerase would be able to change DNA supercoiling. As shown in Fig. 4B, relaxed DNA acquired several extra supercoils upon treatment with wheat germ topoisomerase I, a type-1B enzyme, in the presence of MukB. We observed no change in supercoiling when we used E. coli topoisomerase I, a type-1A enzyme in reaction (data not shown). Thus, the generated net supercoiling was negative. We confirmed this result using two-dimensional gel electrophoresis (Fig. 4D). Similar to the knotting reaction, the low salt MukB did not support any supercoiling (data not shown).

**ATP Is Not Needed for DNA Reshaping**—The purified MukB was a very slow ATPase. The low level of ATPase activity is consistent with the
view that ATP plays a role of a conformational switch rather than the energy source for DNA reshaping. If this conclusion holds true for MukB, no effect of ATP on DNA reshaping would be expected.

Fig. 4E shows a time course of supercoiling reaction carried out in the presence or absence of ATP or its nonhydrolyzable analog AMPPNP. In all cases, reaction is almost complete within 2 min, and no changes can be detected after the 20-min time point. In all cases, the same degree of supercoiling is achieved. Quantification of the time courses showed that approximately the same fraction of DNA (between 35 and 45%) became supercoiled under all tested conditions (data not shown). Furthermore, we found efficient supercoiling by MukB in the absence of magnesium (Fig. 4F). Since the Mg$^{2+}$ ion is required for ATP hydrolysis, this result demonstrates that ATP is not needed for DNA supercoiling by MukB.

Cofractionation of MukB and Its Activities—DNA binding and reshaping required a large excess of MukB over DNA. About 100 molecules of dimeric MukB for every DNA were needed for optimal knotting and supercoiling (Fig. 4) or for a complete gel shift of supercoiled DNA (Fig. 3). Although our preparation of MukB appeared to be sufficient pure (Fig. 1, B and C), it was not impossible that a small contaminating protein is responsible for DNA binding and reconfiguration. To address this possibility, we determined whether the activities of MukB coelute with the protein during chromatographic separation.

All DNA reshaping activities were found exclusively in the MukB fractions eluted from the heparin column. Both fractions of MukB formed a stable complex with supercoiled DNA that remained largely in the wells in the gel shift assay (Fig. 1D). When smaller aliquots of the fractions were tested for the gel shift of linear DNA, however, the high salt peak of MukB appeared more active (Fig. 1E). Note, for example, a more pronounced gel shift for fraction 24 than for fraction 18 (Fig. 1E) despite the fact that the MukB concentration is higher in fraction 18. This result is in accord with the finding that the high salt MukB has severalfold greater affinity for both linear and supercoiled DNA than the low salt peak (Fig. 3).

In agreement with the emerging pattern, we found that both DNA supercoiling (Fig. 1F) and knotting (Fig. 1G) comigrate with the high salt peak of MukB. Virtually no supercoiling or knotting was detected in the peak fraction of the low salt MukB, fraction 18, whereas even small amounts of the high salt MukB (e.g. fraction 26) were efficient in DNA reshaping.

The peaks of DNA supercoiling and knotting only roughly coincided with the peak of MukB. Note, for example, the lower knotting for the peak fraction 22 compared with fractions 20 and 24. This suggested that high concentrations of MukB could inhibit the reactions, similar to what we observed earlier for the high salt preparation of the protein (Fig. 4). To verify this interpretation, we next carried out DNA knotting reactions using several dilutions of fractions 21 to 26 and quantified the extent of knotting in the reactions. The fraction of trefoils found in these reactions is plotted in Fig. 1H as a function of the amount of MukB used in the reaction. For all tested fractions, whether from the left-hand or the right-hand side of the high salt peak, the dependence of knotting on the amount of MukB follows a smooth line. This dependence is very similar to that found for the loaded, Heparin I preparation of MukB (Fig. 1H). If anything, the high salt MukB was more active in DNA knotting than the unfraccionated protein. Similar nonmonotonic dependence was obtained for the pooled high salt fractions of MukB; however, the pooled protein had a greater specific activity for unknown reasons. We conclude that DNA reshaping is a property of the high salt fraction of MukB.

We found similar cofractionation of MukB and its activities during gel filtration through Sephacryl S300. MukB eluted as a single peak close to the void volume of the column (Fig. 5A). Both DNA binding and supercoiling could be found only in the fractions that contained MukB,

![Image](https://example.com/image.png)
and the peak of the activities coincided with the peak of the protein (Fig. 5, B and D). The ATPase activity, however, showed two distinct peaks. The ATPase activity that comigrated with MukB was not affected by the presence of DNA, whereas the contaminating ATPase activity was stimulated by double-stranded and single-stranded DNA (Fig. 5C). The contaminating ATPase activity was probably associated with p30 protein (Fig. 1B), since the two comigrated on gel filtration and heparin columns (Fig. 5, A and C, data not shown). Given the low rate of ATP hydrolysis, however, another, undetected protein could be responsible for the contaminating ATPase activity.

The Activity of MukB Is Reversibly Attenuated during Gel Filtration—Our finding of two peaks of MukB with distinct DNA reshaping properties suggested that the protein could adopt two different conformations. In tentative support of this view was the finding that acyl carrier protein comigrates only with the inactive peak (Fig. 1C). Furthermore, we noticed that the specific activity of MukB declines following gel filtration (data not shown), indicating that the conformational transition of MukB can spontaneously occur during chromatographic separation. Alternatively, the inactive protein could be misfolded. To distinguish between these two possibilities, we next quantified the activity of MukB after the protein was first subjected to gel filtration through Sephacryl S300 and then resolved by heparin chromatography. For this experiment, we used the preparation of MukB that was enriched in the high salt fraction of the protein. This preparation was obtained by pooling fractions more conservatively during Heparin I chromatography (gray bar in Fig. 1A).

As shown in Fig. 6A, the activity of MukB indeed declined after gel filtration. About 8-fold more protein is needed now to produce the same amount of ACP remained virtually the same (data not shown). It is also difficult to imagine another, undetected inhibitor that would be enriched in the MukB fractions after gel filtration. It seems more likely therefore that MukB undergoes conformational transitions during gel filtration and heparin chromatography.

DISCUSSION

We report here that MukB can reconfigure double-stranded DNA in vitro. The activities found for MukB are highly reminiscent of eukaryotic condensins. MukB promoted formation of knots of very distinctive topology in nicked circular DNA. DNA knotting was paralleled by DNA supercoiling, which was detected in an assay coupled to a type-I topoisomerase. MukB also formed a stable complex with circular and linear DNA at the same range of protein concentrations that was needed for knotting and supercoiling. Finally, all DNA reshaping activities of MukB required vast excess of protein over DNA. The highest level of knots and supercoils was observed at approximately one MukB dimer for every 50 bp of DNA (Fig. 4). This feature, again, is typical for condensins. For example, one 13 S condensin for every 50 bp of DNA was needed to observe efficient knotting and supercoiling (17). Similarly, the S. cerevisiae SMC2-SMC4 complex was used at one protein per 20-bp DNA to observe the widening in topoisomer distribution (24). We conclude therefore that MukB acts as a condensin in E. coli.

DNA condensation in vitro was accompanied by an increase in DNA knotting in a type-2 topoisomerase coupled assay. The pattern of generated knots is often informative about the structure of a protein-DNA complex (44). For a example, if a condensing agent merely increases the frequency of random collisions between DNA segments, an increase in the amount of all possible knots would be expected (45). SMC proteins produce knots of a very distinctive pattern. The major products are right-handed trefoils, whereas two rounds of strand transport can produce a five-noded torus knot (17). The knots that we found for MukB fit this pattern precisely. The majority of knots were positive trefoils, whereas five- but not four-noded knots were increasingly formed at higher levels of topoisomerase, when multiple strand transport events become more likely. We conclude that, similar to 13 S condensins, MukB stabilizes large right-handed loops in DNA.

Several features of MukB-induced DNA reshaping were distinct for our protein. First, the net supercoiling generated in the reaction was negative; the opposite would be expected, given the positive handedness of the stabilized loops. Although knots and supercoils had opposite chirality, their formation paralleled each other. About the same amount of condensin was needed to yield the optimal level of knots and supercoils (Fig. 4). Both reactions occurred with the high salt but not the low salt fractions of MukB eluted from the heparin column (Fig. 1). Both reactions were attenuated by gel filtration (Fig. 6; data not shown). Thus, the generated knots and supercoils are two separate topological attributes of the same DNA reshaping event. This result is not a paradox, since the supercoiling recovered in the topoisomerase-I-based assay sums up the contributions from writhe, both global and local, and twist. Apparently, the right-handed looping is accompanied by an even greater DNA untwisting or left-handed wrapping around the protein.

Second, trefoils were not the only knots produced. At high topoisomerase/DNA molar ratios, the enzyme itself could distort the DNA and thereby alter the knotting pattern. We found, however, an appreciable amount of 4-, 5-, and 6-noded knots, even at low levels of topoisomerase. These knots therefore appear because of MukB. Remarkably, the solenoidal loop model proposed for 13 S condensin can be easily adapted to incorporate this distinctive feature of MukB. As illustrated in Fig. 7, the type of created knots depends on how MukB-stabilized loops

FIGURE 5. MukB undergoes reversible changes during chromatographic separation. MukB was passed through the Sephacryl S300 column, the peak fractions (fractions 7–9 in Fig. 5) were pooled and subjected to heparin chromatography. The high salt peak eluted from the heparin column was dialyzed as in Fig. 1D and tested for DNA supercoiling (A) or the binding to linear pBR322 DNA (B) alongside the pooled fractions obtained after gel filtration and the starting pool of MukB.
that the role of MukBEF in condensation is more structural than catalytic and is consistent with the finding that MukB is a very slow ATPase.

We found that MukB elutes from the heparin column as two distinct peaks. This phenomenon has not been previously reported for SMC proteins. The two forms of MukB had different affinity for acyl carrier protein, suggesting that these represent two conformations of MukB. Only one of the proteins was active in DNA binding and reshaping. This observation brings up the possibility that the conformational transition in MukB is related to its DNA condensation-decondensation cycle. It is noteworthy in this respect that the two forms of MukB are revealed somehow precluded in the MukB-DNA complex. Perhaps protein-protein collisions between the DNA loops. The knotting frequency would be reduced if the knots can be obtained if further spinning is allowed. That complex knots were not observed in our experiments indicates that such spinning is somehow precluded in the MukB-DNA complex. Perhaps protein-protein interactions between adjacent MukBs maintain the regularity of this structure.

Third, DNA supercoiling by MukB did not require ATP. This feature makes MukB, again, unlike 13 S condensin but more similar to the yeast SMC2-SMC4 complex. The result is somewhat perplexing, since mutations within ATPase site inactivate MukB in vivo. Perhaps ATP hydrolysis is needed for the disassembly of MukB-DNA complex, or maybe it modulates the interaction with other, as yet unidentified, components of the chromosome segregation machinery. In either case, one thing is apparent. The generated knots and supercoils arise from some static structure imposed onto DNA by MukB. This result supports the view that the role of MukBEF in condensation is more structural than catalytic and is consistent with the finding that MukB is a very slow ATPase.

How could MukBEF condense chromosomes in vivo? Obviously, this cannot occur exactly the same way as in our topological assays. We observe increased knotting (which cannot happen unless DNA is compacted) only at high protein concentration, when the entire DNA is covered by MukBEF and other proteins have limited access to DNA. If nothing else, the relatively low copy number of MukB (between 150 and 400 per cell by different estimates) would preclude it from binding along the entire chromosome. It would be wasteful, however, in an evolutionary sense, if knotting had nothing to do with the physiological mechanism of chromosome condensation. Why would MukBEF possess two separate activities, knotting-related and knotting-unrelated, for DNA compaction? And why would knotting be so highly conserved between the organisms?

Two mechanistic features seem primary to the knotting reaction of MukBEF. First, the protein can stabilize solenoidal loops in DNA. Second, these loops are arranged in space to form a more or less regular macromolecular structure (see discussion above and Fig. 7). Furthermore, MukBEF was recently shown to form macromolecular structures even without DNA (46). These properties make MukBEF a promising candidate for the role of the chromatin scaffold protein. Perhaps the association-dissociation of DNA loop bound MukBEFs is the mechanism that controls the extent of chromatin condensation.

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