The sbcC and sbcD genes mediate palindrome inviability in *Escherichia coli*. The sbcC operon has been cloned into the plasmid pTrc99A under the control of the strong trc promoter and introduced into a strain carrying a chromosomal deletion of *sbcCD*. The SbcC and SbcD polypeptides were overexpressed to 6% of total cell protein, and both polypeptides copurified in a four-step purification procedure. Purified SbcCD is a processive double-strand exonuclease that has an absolute requirement for Mn$^{2+}$ and uses ATP as a preferred energy source. Gel filtration chromatography and sedimentation equilibrium analyses were used to show that the SbcC and SbcD polypeptides dissociate at some stage after purification and that this dissociation is reversed by the addition of Mn$^{2+}$. We demonstrate that SbcD has the potential to form a secondary structural motif found in a number of protein phosphatases and suggest that it is a metalloprotein that contains the catalytic center of the SbcCD exonuclease.

Inverted repeat DNA (palindromic DNA) provides a source of genetic instability in the genome of various prokaryotes (1) and eukaryotes (2–5), presumably because it has the potential to adopt hairpin and cruciform secondary structures that can perturb various biological processes. For example, DNA replication (6–8), mismatch repair (9, 10), and DNA methylation (11).

The metabolism of palindromic DNA is best understood in *Escherichia coli*. Replicons that contain long DNA palindromes (greater than 150–200 base pairs of total length) suffer two fates when introduced into wild-type cells. They are either not recovered (inviability) or recovered with evidence of deletion in and around the palindrome (instability). However, strains of *E. coli* exist in which replicons containing long DNA palindromes can be propagated. These strains carry mutations in either the *sbcC* or *sbcD* genes (12, 13). Both genes have been mapped, cloned, and sequenced, and shown to be transcribed from a common promoter (14). The *sbcD* gene encodes a 44.7-kDa polypeptide and contains the conserved sequence DXHX$_2$GDXXDX$_6$GNH(D/E) (n = −25) found in the serine/threonine phosphatases and other phosphoesterases (15, 16).

The yeast recombination/repair proteins, RAD52 of *S. cerevisiae* (17) and MRE11 of *S. cerevisiae* (18), and the human equivalent of MRE11 (19) also contain D$_{44.7}$-kDa polypeptide and contains the conserved sequence from a common promoter (14). The human $MRE11$ (19) also contains the conserved sequence

1 The abbreviations used are: SMC, structural maintenance of chromosomes; ATP-$S$, adenosine 5′-[γ-thio] triphosphate; IPTG, isopropyl-1-thio-β-D-galactopyranoside; PAGE, polyacrylamide gel electrophoresis; FPLC, fast protein liquid chromatography; HPLC, high pressure liquid chromatography; kb, kilobase pair(s); BSA, bovine serum albumin.
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The SbcCD protein used for gel filtration was 2 mg/ml was applied to a protein assay kit (Bio-Rad) with BSA as a standard. The molar concentration of SbcCD was calculated assuming a stoichiometry of SbcC:SbcD = 1:1.

DNA Substrates—Double-strand pUC19 DNA (New England Biolabs) was linearized with EcoRI (Boehringer Mannheim) and end-labeled at the 3′ end using [α-32P]dATP (Amersham Corp., 300 Ci/mmol) and Klenow enzyme (New England Biolabs). This resulted in a substrate with a specific activity of 3.3 × 106 cpm/μg of total nucleic acid. Uniformly 32P-labeled double-strand DNA was synthesized using the polymerase chain reaction in a 100-μl reaction containing: 10 mM Tris, pH 9.0, 1.5 mM MgCl2, 50 mM KCl, 200 μM each of dATP, dCTP, dGTP, and dTTP, 20 μCi of [α-32P]dCTP (Amersham Corp., 3000 Ci/mmol), 100 pmol of each primer, 30 ng of template DNA, and 5 units of Taq DNA polymerase (Boehringer Mannheim). The primers were 19 bases long with 5′ ends corresponding to nucleotides 596 and 413 of pUC19, and the temperature of pUC19 (2686 base pairs) linearized with EcoRI. The reaction mix was incubated for 3 min at 94°C, 45 s at 58°C, and 3 min at 72°C followed by 25 cycles of 1 min at 94°C, 45 s at 58°C, and 3 min at 72°C and a final 10 min at 72°C. Amplification of template DNA yielded a product of 2686 base pairs. Reaction products were resolved on a 1% agarose gel to remove contaminating DNA, the band corresponding in size to the expected product was excised and purified with a QiAquick gel extraction kit (Qiagen Inc.). This yielded a substrate with a specific activity of 4 × 106 cpm/μg of total nucleic acid.

Standard Assay for Double-strand Exonuclease Activity—Protein was incubated with DNA (7.5 nM double-strand ends) for 30 min at 37°C in a reaction mix containing 5 mM Mg2+, 1 mM ATP, 25 mM Tris, pH 7.5, 1.25 mM dithiothreitol, 2% glycerol, and 100 μg/ml BSA. Reactions were terminated by adding an equal volume of DNA gel loading buffer containing 50 μg EDTA. Reaction products were resolved on agarose gels that were dried onto Whatman DE81 paper before exposure to a storage phosphor screen and quantification using a Molecular Dynamics PhosphorImager.

Determination of Vm and Km(app)—0.5 μM SbcCD (Fraction V) was incubated for 30 min at 37°C in a reaction mix containing: 5 mM Mg2+, 1 mM ATP, 25 mM Tris, pH 7.5, 1.25 mM dithiothreitol, 2% glycerol, and 100 μg/ml BSA. Reactions were terminated by adding an equal volume of DNA gel loading buffer containing 50 μg EDTA. Reaction products were resolved on agarose gels that were dried onto Whatman DE81 paper before exposure to a storage phosphor screen and quantification using a Molecular Dynamics PhosphorImager.

**Assay for SbcCD Processivity**—7.5 nM (double-strand ends) of uniformly labeled pUC19 DNA was incubated at 37°C with 1 nM SbcCD (Fraction V) in a 140-μl standard SbcCD reaction mixture. 18-μl samples were removed at various times (0, 2, 5, 10, 20, 40, and 60 min), and
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RESULTS

Overexpression of SbcC and SbcD—The sbcCD operon of E. coli was subcloned into the plasmid pTrc99A placing the sbcC and sbcD genes under the control of the trc promoter and the lacIq repressor (Fig. 1A). The resulting plasmid, pDL761, was then introduced into the E. coli strain DL733, which carries a chromosomal deletion of the sbcC and sbcD genes under the control of the IPTG-inducible trc promoter (Fig. 1B). Following the addition of IPTG, samples were taken every 1.5 h. Overproduction of the 44.7-kDa SbcC polypeptide was obvious after 7.5 h of IPTG induction (Fig. 1B, lane c), and maximal expression was reached after 6 h (Fig. 1B, lane f) with SbcC expressed to 6% of total protein (judged by protein densitometry; data not shown). A band corresponding in size to the 44.7-kDa SbcD polypeptide was also apparent at 1.5 h (Fig. 1B, lane c), again with maximal levels being reached after 6 h of induction (Fig. 1B, lane f). This was most likely to be SbcD; however, the 38.6-kDa E. coli lacIq repressor co-migrates with SbcD on 12% polyacrylamide gels (determined by amino-terminal sequencing; data not shown), making it difficult to quantify levels of SbcD. This was most obvious when DL776 was grown for 7.5 h in the presence and the absence of IPTG; levels of induction were similar in both (Fig. 1B, lanes g and h). Levels of SbcD were assumed to be at least the same as SbcC because sbcD is transcribed before sbcC (14).

Purification of SbcCD Protein—SDS-PAGE and ATP-dependent double-strand exonuclease activity (28) were used to monitor the purification of SbcCD protein. A summary of the purification procedure and the results obtained are shown in Table I and Fig. 2. 20 g of wet weight of IPTG-induced DL776 cells were thawed overnight at 4 °C then suspended in 60 ml of ice-cold sucrose cell buffer containing 0.1 mM benzamidine and 1 mM phenylmethylsulfonyl fluoride. Cells were then placed in an ice bath and sonicated 20 × 30 s (for a tip with a 1-cm diameter) at maximum output using an MSE sonicator. The lysate was centrifuged at 20,000 rpm for 2 h in a Sorval SS-34 rotor to remove cellular debris. This and all subsequent steps were performed at 4 °C.

To remove nucleic acid, polyethyleneimine (10%, pH 7.5) was added dropwise to 74 ml of clarified cell extract to give a final concentration of 0.075%. After stirring for 15 min, the pellet was removed by centrifugation (15 min at 15,000 rpm). 70 ml (23 mg/ml) of the resulting supernatant, referred to as Fraction I (Fig. 2, lane b), was applied at 48 ml/h to a column (2.6 × 30 cm, 160-ml bed volume) containing DEAE-Sepharose Fast Flow (Pharmacia) equilibrated in buffer A, 5 mM Mn2⁺. After a further 30 min of stirring, the pellet collected after centrifugation (20 min at 20,000 rpm) was resuspended in 10 ml of buffer A, 5 mM Mn2⁺ to become Fraction III (10 ml, 11.17 mg/ml) (see Fig. 2, lane d). This ammonium sulfate precipitate was then applied at 30 ml/h to a Sephacryl S500 (Pharmacia) gel filtration column (100 × 2.6 cm, 530 ml of bed volume) equilibrated in buffer A, 5 mM Mn2⁺. Protein was eluted at 30 ml/h, and 5-ml fractions were collected and assayed by SDS-PAGE. Those containing the peak of SbcCD protein (eluting between 200 and 225 mM NaCl) were then pooled to constitute Fraction II (48 ml, 4.25 mg/ml; see Fig. 2, lane c).

Solid ammonium sulfate was then added to Fraction II, with constant stirring, to 30% saturation. After a further 30 min of stirring, the pellet collected after centrifugation (20 min at 20,000 rpm) was resuspended in 10 ml of buffer A, 5 mM Mn2⁺ to become Fraction III (10 ml, 11.17 mg/ml) (see Fig. 2, lane d). This ammonium sulfate precipitate was then applied at 30 ml/h to a Sephacryl S500 (Pharmacia) gel filtration column (100 × 2.6 cm, 530 ml of bed volume) equilibrated in buffer A, 5 mM Mn2⁺. Protein was eluted at 30 ml/h, and 5-ml fractions were collected. The peak of SbcCD protein was identified using SDS-PAGE and ATP-dependent double-strand exonuclease activity (28) were used to monitor the purification of SbcCD protein. A summary of the purification procedure and the results obtained are shown in Table I and Fig. 2. 20 g of wet weight of IPTG-induced DL776 cells were thawed overnight at 4 °C then suspended in 60 ml of ice-cold sucrose cell buffer containing 0.1 mM benzamidine and 1 mM phenylmethylsulfonyl fluoride. Cells were then placed in an ice bath and sonicated 20 × 30 s (for a tip with a 1-cm diameter) at maximum output using an MSE sonicator. The lysate was centrifuged at 20,000 rpm for 2 h in a Sorval SS-34 rotor to remove cellular debris. This and all subsequent steps were performed at 4 °C.

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### Table I

| Fraction       | Total protein (mg) | Total activity (units) | Specific activity (units/mg protein) | Purification factor | Recovery (%) |
|----------------|--------------------|------------------------|--------------------------------------|---------------------|--------------|
| I Cell extract | 1726               | 2307                   | 1.33                                 | 1                   | 100          |
| II DEAE       | 204                | 1133                   | 5.5                                  | 4.1                 | 49           |
| III Ammonium sulphate | 112   | 1108                   | 9.9                                  | 7.4                 | 48           |
| IV Sephacryl S500 | 37     | 379                    | 10.2                                 | 7.7                 | 16.4         |
| V Hydroxyapatite | 2.53   | 27                     | 10.7                                 | 8                   | 1.2          |

a Determined by the Bio-Rad protein assay using BSA as a standard.

b Units were determined under standard reaction conditions (see “Experimental Procedures”). A unit was defined as the amount of protein that catalyses the formation of 1 μmol of product/min.

c Only 8.86% of Fraction IV was applied to the hydroxyapatite column. Figures were adjusted accordingly.

IV (45 ml, 0.82 mg/ml; see Fig. 2, lane e). Although it contained faint traces of a 87-kDa contaminating band, it was 98% homogeneous and considered to be suitable for physical analysis. Therefore 2-ml aliquots were frozen at −80 °C until required.

To obtain SbcCD suitable for biochemical analysis (free of the 87-kDa contaminating protein), 3.28 mg (4 ml) of Fraction IV was dialyzed twice against 2 liters of buffer P and applied at 30 ml/h to a 5-ml prepacked hydroxyapatite column (Econo-Pac 10/100, Bio-Rad) equilibrated in the same buffer. Unbound protein was removed by washing the column with 15 column volumes of buffer P containing 10–600 mM phosphate. The first peak (eluting between 130 and 250 mM phosphate) was free of contaminating protein and was therefore pooled and dialyzed twice against 2 liters of buffer A before concentrating with a Microsep 10-kDa centrifugal concentrator (Flowgen). This concentrate was called Fraction V (0.64 mg/ml) and contained 224 μg of apparently homogeneous SbcCD protein in 350 μl of buffer A (Fig. 2, lane f). 20-μl aliquots of Fraction V were frozen in liquid nitrogen and stored at −80 °C until required.

Amino-terminal microsequencing was used to verify that the polypeptides purified were SbcC and SbcD. The sequence of eight amino acids from the amino terminus of the putative SbcD polypeptide was MRILHTSD, which is in complete agreement with that predicted (14). The published amino acid sequence of the six residues at the amino terminus of SbcC is MKILSL (14), and the sequence determined for the putative polypeptide was MGILSL.

Biochemical Properties of Purified SbcCD—To determine the reaction requirements for the double-strand DNA exonuclease activity of SbcCD, purified protein (Fraction V) was assayed under various reaction conditions. Standard SbcCD reaction conditions (29) were used with any alterations indicated in the text. A time course showing the amount of limit product obtained when 0.5 mg SbcCD was incubated with 32P end-labeled pUC19 DNA is shown in Fig. 3A. Approximately 25% of the 32P-label appears as product at 30 min, and this was chosen as the standard reaction time (Fig. 3B).

SbcCD had an absolute requirement for Mn2+ as a divalent cation (Table II). Activity was stimulated to a much lesser extent by Cu2+ but not Co2+, Fe3+, Zn2+, Mg2+, Cu2+, or Ca2+ (Table II). No activity was detected in the presence of Mg2+ (up to 50 mM; data not shown). In the presence of 1 mM ATP, Mn2+ stimulated activity at concentrations of 2 mM and above. However, in the presence of 2.5 mM ATP, activity was not seen until the Mn2+ concentration was 3 mM or above (Fig. 4A).

SbcCD required a nucleoside triphosphate co-factor for activity (Table II). In the presence of 5 mM Mn2+, maximum activity was observed with 1–4 mM ATP. Higher concentrations of ATP, 5–10 mM, were inhibitory (Fig. 4B). When SbcCD activity was assayed with increasing concentrations of ATP in the presence of 10 mM Mn2+, no such inhibition was observed (Fig. 4B). This inhibition and the lag seen in the stimulation of SbcCD activity by increasing the concentration of ATP from 1 to 2.5 mM (Fig. 4A) most likely reflect the chelation of free Mn2+ by ATP. ATPγS and GTP exerted a slight stimulatory effect on SbcCD activity, whereas the other nucleotides tested had no effect (Table II).

SbcCD was optimally active in 50 mM NaCl; however, it became increasingly sensitive to NaCl at concentrations greater than 100 mM (Fig. 4C). SbcCD was functional from pH 7 to 9.5. Acidic pH was extremely inhibitory, whereas alkaline pH was less so (data not shown). The more physiological pH of 7.5 was chosen for use in assays.

Mechanism of SbcCD Action—In an effort to determine if SbcCD acts in a catalytic or stoichiometric fashion, increasing concentrations of 32P end-labeled DNA were incubated with 0.25, 0.5, or 1 nM SbcCD (Fraction V), and the Vmax and apparent Km for double-strand DNA ends were calculated. Fig. 5A and Table III show that Vmax increases linearly with increasing SbcCD protein concentration. This indicates that SbcCD acts in a catalytic manner. The apparent Km of SbcCD for double-strand ends also increases (Fig. 5A and Table III). km should remain constant with increasing protein concentration, suggesting that some factor in the standard reaction mixture is limiting.
In the course of these studies it was noticed that a number of substrate molecules always escaped degradation, except when incubated with high concentrations of SbcCD (data not shown). This suggested that SbcCD acts processively. To determine whether this was so, 1 nM SbcCD (Fraction V) was incubated with uniformly labeled DNA, and reaction products were analyzed at various time points over a 60-min period. In parallel, SbcCD was incubated with unlabeled DNA and then challenged at 3 min with uniformly labeled DNA (Fig. 5 B). When labeled DNA was added as a challenge, it was not degraded at the same rate seen when SbcCD was incubated with labeled DNA alone. This experiment suggests that very little free SbcCD is available 3 min into a 60-min reaction and that SbcCD acts processively.

Physical Analysis of SbcCD—An estimate of the relative molecular mass of SbcCD was made by applying 400 µg of purified protein (Fraction IV, prepared in the absence of Mn²⁺)
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A

FIG. 5. Mechanism of SbcCD action. A, 0.25 (○), 0.5 (○), or 1 nM (×) SbcCD incubated with increasing concentrations of DNA. Assays were performed as in the legend to Table II with the concentration of DNA indicated. Data were fitted using GRAPHT software. B, SbcCD processivity determination. SbcCD was incubated with uniformly 32P-labeled DNA in the absence of Mn2+ and SbcC or incubated with unlabeled DNA and then challenged with uniformly 32P-labeled DNA after 3 min (○). Samples were taken at the times indicated.

B

TABLE III

| SbcCD | Vm | K_{m(app)} |
|-------|----|------------|
| μM   | fmol min⁻¹ | μM         |
| 0.25  | 0.50 ± 0.03 | 0.27 ± 0.14 |
| 0.5   | 1.02 ± 0.04 | 0.86 ± 0.16 |
| 1.0   | 2.95 ± 0.14 | 3.50 ± 0.43 |

Fig. 6. FPLC gel filtration analysis of SbcCD. SbcCD protein was applied to a Superose 6 FPLC column as described under "Experimental Procedures." A, elution profile of SbcCD, no Mn2+. Elution positions of molecular mass standards are also shown. B, determination of the relative molecular mass of SbcC and SbcD by comparison with molecular mass standards.

and Sbc did not co-migrate (Fig. 7B). This suggested that Mn2+ promotes interaction between SbcC and SbcD. When Mn2+ was present the molar ratios of SbcC:SbcD in fractions 12, 13, and 14 (Fig. 7A) were 1.3, 0.9, and 1.1, respectively (judged by densitometry), suggesting a ratio of 1:1 in these peak fractions. In the absence of Mn2+ the molar ratios of SbcC:SbcD in fractions 12, 13, and 14 (Fig. 7B) were 6.8, 4.3, and 2.9, respectively.

Sedimentation equilibrium analysis (provided as a service by the United Kingdom National Center for Molecular Hydrodynamics, University of Leicester) was used to gain an absolute estimate of the native molecular mass of SbcCD (Fraction IV, purified in the absence of Mn2+). In the absence of Mn2+, a species with a molecular mass of 720 kDa was detected. In the presence of Mn2+ a species with a molecular mass of 1210 kDa was observed. Sedimentation equilibrium analysis gives an estimate of mass independent of protein shape; therefore association was taking place in both the presence and the absence of Mn2+.

Prediction of SbcD Secondary Structure—SbcD contains the conserved phosphoesterase signature sequence DXHGXGDXXXGNNH(D/E) (n = 25) found in a family of phosphoesterases (15, 16). Several protein phosphatase members of this family have had their x-ray structures solved (33–35). At the active site of these enzymes two metal ions are co-ordinated by a β-a-β-a-β (β = β sheet, α = α helix) secondary structure motif. The conserved elements of the phosphoesterase signature sequence map to the carboxyl termini of the β sheets within this structure. i.e. β-DXH-a-β-GDXD-a-β-GNH(D/E). A secondary structure prediction was generated to determine if SbcD had the potential to form a β-a-β-a-β secondary structure in the...
region of the conserved phosphoesterase signature sequence. The results are shown in Fig. 8. The conserved phosphoesterase motifs were found in regions predicted to form loops next to sequences that have the potential to form a βαβαβ secondary structure. In addition, the conserved phosphoesterase motifs lie adjacent to the carboxyl termini of the β-sheets within this βαβαβ structure. These data indicate that SbcD has the potential to form a metal co-ordinating unit like that which exists within the protein phosphatases.

DISCUSSION

An *E. coli* strain carrying a chromosomal deletion of the *sbcCD* operon was constructed and used to overexpress the SbcC and SbcD polypeptides from a plasmid containing the *sbcC* and *sbcD* genes under the control of the strong trc promoter. Following 6 h of IPTG induction, both polypeptides were co-expressed to approximately 6% of total cell protein. A purification scheme was devised that yielded 2.53 mg of soluble SbcCD protein from 20 g (wet weight) of cells. The SbcC and SbcD polypeptides copurified through three chromatographic steps and an ammonium sulfate precipitation step. A significant reduction in yield had to be accepted due to the presence of an 87-kDa contaminating protein (Table I and Fig. 2).

The double-strand exonuclease activity of SbcCD has an absolute requirement for Mn²⁺ ions, other divalent metal ions tested failed to stimulate activity (Table II). SbcCD is most active under conditions of high free Mn²⁺ concentration (Fig. 4, A and B). SbcD contains the phosphoesterase signature sequence and has the potential to form a βαβαβ secondary structure in and around this conserved sequence (Fig. 8). Many members of this class of protein require transition metals (including Mn²⁺) for activity (15). For example, the recombinant catalytic subunit of protein phosphatase 1 (from rabbit) is produced as an inactive enzyme in *E. coli* that can only be activated by Mn²⁺. However, it has recently been demonstrated that a combination of Fe²⁺ and Zn²⁺ (but not the individual metal ions) can significantly activate this enzyme (36). These observations suggest that SbcD may also be a metalloprotein capable of co-ordinating two metal ions via the βαβαβ secondary structure. A two-metal catalytic mechanism has previously been proposed for the 3' to 5' exonuclease activity of DNA polymerase I (37).

SbcCD has a requirement for a nucleoside triphosphate co-factor (Table II). Stimulation was only seen when the concentration of ATP was lower than that of Mn²⁺ (Fig. 4, A and B). This is reminiscent of the *E. coli* RecBCD enzyme whose non-specific nuclease activities are more active when the concentration of Mg²⁺ ions is greater than that of ATP (38, 39). SbcC possesses the ATP-A and ATP-B nucleotide binding motifs; therefore it is likely to be the component of the SbcCD protein that interacts with ATP. To test whether ATP hydrolysis was required for activity, nuclease assays were performed in the presence of the nonhydrolyzable analog ATPγS. The activity in the presence of 1 mM ATPγS was 30% of that in the presence of 1 mM ATP, suggesting that hydrolysis is not required for activity. The RAD50 protein only binds double-strand DNA in the

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**Fig. 8.** Secondary structure prediction of SbcD. A secondary structure prediction showing the first 120 amino acids of SbcD. Amino acids (AA) are indicated by their single letter abbreviations and numbers. SEC, secondary structure prediction; H, helix; E, extended sheet; blank, other (loop); REL, reliability index of prediction (0–9). The conserved motifs of the phosphoesterase signature sequence are underlined.
presence of an adenosine triphosphate at concentrations of 2–5 mM. High level ATPase activity is not associated with this protein (40). The DNA-binding protein MukB only binds ATP and GTP at concentrations > 0.1 mM in the presence of Zn2+. No ATPase or GTPase activity has been detected for MukB (24).

SbcCD acts catalytically and processively (Fig. 5, A and B). In the experiment that demonstrates that SbcCD acts catalytically, $K_{\text{cat(app)}}$ varies with protein concentration (Table III), suggesting that a factor in the reaction is limiting.

SbcC and SbcD co-eluted throughout purification. It was surprising to find that purified SbcC and SbcD, at a concentration of 2 mg/ml, did not co-migrate when applied to a gel filtration column (Figs. 6A and 7B). In the final stage of purification, protein was applied to a hydroxyapatite column at 0.82 mg/ml, yet both polypeptides still co-eluted (Fig. 2). Therefore the dissociation of SbcC and SbcD that was observed was not due to a concentration effect alone. Human protein phosphatase 1, when purified from native sources, does not require metal ions for activity yet is converted into a metal ion-dependent form after long term storage. This suggests that residues in the vicinity of the active site alter in conformation, and then metal ions are released (Ref. 35 and references therein). Perhaps similar changes result in the dissociation of the SbcCD complex.

FPLC gel filtration analysis revealed that SbcC and SbcD have relative molecular masses of 1150 and 85 kDa, respectively (Fig. 6B). This suggests that in the peak fraction SbcD exists as a dimer and SbcC exists as a higher order multimer. Mn2+ ions, in addition to stimulating the double-strand exonuclease activity of SbcCD, also promoted an interaction between SbcCD complex in the presence of Mn2+.

SbcC (712.2 kDa). The molecular mass of 1210 kDa for the SbcC (24). This work is a first step in understanding the mechanism of SbcCD action. The SbcC and SbcD polypeptides interact to form a high molecular weight complex in the presence of man- ganese ions. Together, in the presence of ATP, both polypeptides function as a processive double-strand exonuclease. SbcC is an SMC family member, and a number of these proteins were isolated as or have been shown to be DNA-binding proteins. SbcD is a member of a group of proteins that have in common the ability to hydrolyze phosphoester bonds (15). We suggest that SbcC is the main DNA-binding subunit of the SbcCD protein (which is activated by ATP-binding or hydrolysis) and that SbcD is a metalloprotein that contains the catalytic center for the hydrolysis of DNA. Exactly how both polypeptides interact with each other and palindromic DNA remains to be investigated.

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