The Chromosomal Protein Sso7d of the Crenarchaeon *Sulfolobus solfataricus* Rescues Aggregated Proteins in an ATP Hydrolysis-dependent Manner*

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In this work, we show that the nonspecific DNA-binding protein Sso7d from the crenarcheon *Sulfolobus solfataricus* displays a cation-dependent ATPase activity with a pH optimum around neutrality and a temperature optimum of 70 °C. Measurements of tryptophan fluorescence and experiments that used 1-anilinonaphthalene-8-sulfonic acid as probe demonstrated that ATP hydrolysis induces a conformational change in the molecule and that the binding of the nucleotide triggers the ATP hydrolysis-induced conformation of the protein to return to the native conformation. We found that Sso7d rescues previously aggregated proteins in an ATP hydrolysis-dependent manner; the native conformation of Sso7d forms a complex with the aggregates, while the ATP hydrolysis-induced conformation is incapable of this interaction. Sso7d is believed to be the first protein isolated from an archaeon capable of rescuing aggregates.

Archaea are microorganisms that are distinct from bacteria and eukarya in the tree of life and mostly thrive in extreme environments (1). The Euryarchaeota branch of the Archaea kingdom includes methanogens and halophiles, while most thermoacidophilic species belong to the Crenarchaeota branch. Crenarchaea, considered the most ancient living cells, have peculiar metabolic pathways and genetics, many of their vital processes still awaiting a clear understanding.

The small, basic, nonspecific DNA-binding proteins of *Sulfolobales* crenarchaea have high sequence identity among them and lack obvious similarity to any other known protein; their tertiary structure (2–6) is very different from that of histones and was found to be similar to the "chromo domain" (7) and SH3 domains (8) involved in protein-protein interactions. The definition of the biological role(s) played by these novel proteins is hampered by the poor knowledge of many DNA-related events in *Sulfolobales* and by the lack of molecular tools to obtain targeted mutants in these microorganisms. In *in vitro* approaches, Sso7d from *Sulfolobus solfataricus*, the best-studied protein of the family, increases the melting temperature of DNA (2), promotes the annealing of complementary DNA strands (9), and induces negative supercoiling (10) and a kink associated with unwinding in oligonucleotides (4, 5).

In this paper, we show that Sso7d has an associated ATPase activity that drives the cycling of the molecule between conformational states. We demonstrate that Sso7d rescues aggregated proteins in the presence of ATP hydrolysis. The native conformation of Sso7d binds to the aggregates, while the ATP hydrolysis-induced conformation is incapable of interacting with the aggregated proteins. Sso7d is the only protein present in a *S. solfataricus* crude extract that has disaggregating activity, and the possible significance of this finding is discussed.

EXPERIMENTAL PROCEDURES

Materials—Malic enzyme from chicken liver (29 units/mg), lysozyme from chicken egg white (183 units/mg), NADP, and adenosine and guanosine nucleotides were purchased from Sigma. Recombinant β-glycosidase of *S. solfataricus* (Ssβgly)[1] (13 units/mg) was obtained as described by Moracci *et al*. (11). ANS was from Aldrich. [γ-³²P]ATP (3,000 Ci/mmol) was from Amersham Pharmacia Biotech. The other chemicals were of the highest grade available.

Miscellaneous Methods—Protein concentration was determined by Bradford assay (12) using bovine serum albumin as the standard. The concentration of Sso7d solutions was estimated spectrophotometrically using the extinction coefficient reported by Baumann *et al*. (2). The concentration of ANS solutions was determined using a millimolar extinction coefficient of 5 OD at 350 nm. SDS-PAGE (15% acrylamide) was carried out according to Laemmli (13). Nondenaturing PAGE (10% acrylamide) was carried out according to Davis (14).

Cell Growth and Purification of Sso7d—Cells of *S. solfataricus* strain MT-4 were grown aerobically at 87 °C to the late exponential phase (about 0.8 OD at 600 nm) in DSM 182 culture medium. Cells harvested by centrifugation at 4000 × *g* for 15 min (6 g from a 4-liter culture) underwent freeze-thawing twice; were added to 6 g of sand and 2 ml of 50 mM Tris-HCl, pH 8.4, containing 0.2 M NaCl, 5% glycerol; and homogenized in an Omni mixer. The homogenate was centrifuged at 4,000 × *g* for 20 min at 4 °C to remove the sand; the supernatant was ultracentrifuged at 160,000 × *g* for 90 min at 4 °C, and the residue was discarded. The crude extract (about 175 mg) was aliquoted and stored at −20 °C. The crude extract (25 mg) was loaded onto a Superdex 75 High Load column (2.6 × 60 cm; Amersham Pharmacia Biotech), which was eluted with 10 mM Tris-HCl, pH 8.4, containing 0.2 M NaCl (buffer A) at a flow rate of 2 ml/min; the fractions containing Sso7d (6 mg) were pooled, concentrated by polyethylene glycol 6,000, and rechromatographed on a Superdex 75 High Load column (1.6 × 60 cm; Amersham Pharmacia Biotech), which was eluted with buffer A at a flow rate of 0.8 ml/min. The peak containing Sso7d (870 μg) was dialyzed against 10 mM Tris-HCl, pH 8.4, and concentrated by a Savant vacuum centrifuge. Freshly prepared Sso7d was used in all of the experiments described in this paper.

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[1] The abbreviations used are: Ssβgly, β-glycosidase from *S. solfataricus*; ANS, 1-anilinonaphthalene-8-sulfonic acid; PAGE, polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography; MgATP, 5 mM MgCl₂, 2 mM ATP; MgAMP-PNP, 5 mM MgCl₂, 2 mM 5′-adenylyl-β,γ-imidodiphosphate; Sso7d^ATP, the ATP hydrolysis-induced conformation of Sso7d.

FIG. 1. **Electrophoretic analysis of Sso7d samples and Sso7d-associated ATPase activity.** *A*, SDS-PAGE (*left panel*) and nondenaturing PAGE (*right panel*) analyses of Sso7d samples. The *lanes* were loaded with 10 μg of protein; after the electrophoresis, the gels were silver-stained. The migration of the molecular weight markers is indicated. *B*, 50 μg of pure Sso7d were loaded onto a Superdex 75 High Load gel filtration column (Amersham Pharmacia Biotech; 1×30 cm; eluent 10 mM Tris-HCl, pH 8.4, 0.2 M NaCl; flow rate of 0.3 ml/min; fraction volume of 0.6 ml) (*left panel*) or a Matrex Gel Red A affinity column (Amicon; 1×7 cm; eluent 0–0.4 M NaCl in 10 mM Tris-HCl pH 8.4; flow rate of 0.25 ml/min; fraction volume of 3.8 ml) (*right panel*). The column fractions were assayed for ATPase activity as described under "Experimental Procedures."
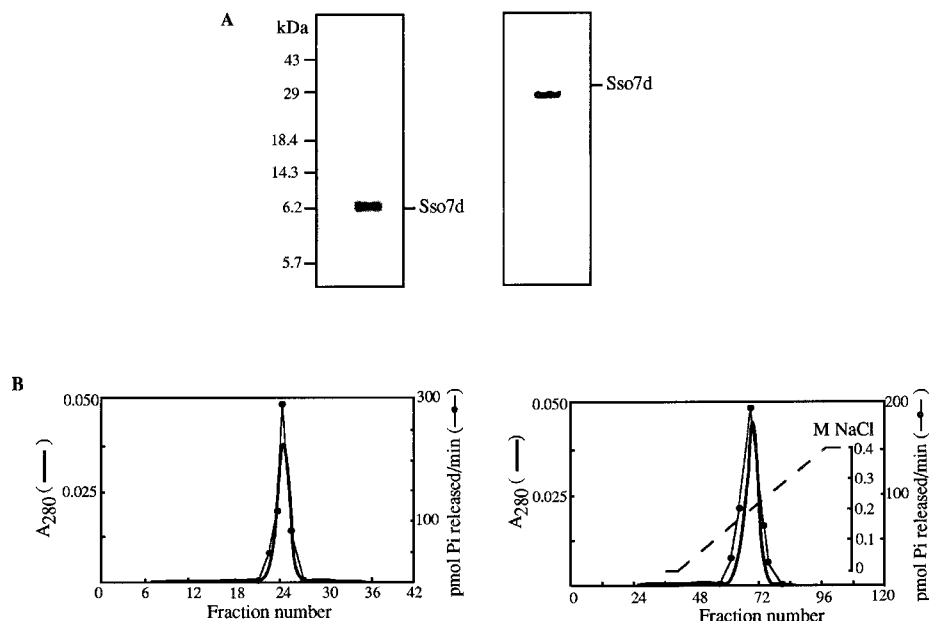
Analysis of the Nucleotide Content of Sso7d—Nucleotide standards were loaded onto a C_{18} reverse phase HPLC column (Vydac; 0.46×25 cm; eluent 50 mM sodium phosphate, pH 7.5; flow rate of 0.5 ml/min; detection at 254 nm) and quantified by integrating the nucleotide peak area. Purified Sso7d (10 μg) was incubated in 1 M $HClO_4$ (100-μl final volume) for 30 min at -20 °C and then centrifuged at $20,000 \times g$ for 30 min. The supernatant was neutralized with NaOH, clarified by a further centrifugation, and analyzed for nucleotide content as described above.

Fluorescence Measurements—Two 100-μg samples of Sso7d were incubated for 10 min at 70 °C, respectively, in the presence of MgATP and in the presence of MgAMP-PNP and then loaded onto a Superdex 75 High Load column (Amersham Pharmacia Biotech; 1×30 cm; eluent 10 mM Tris-HCl, pH 7.5, 0.2 M NaCl; flow rate of 0.3 ml/min) to remove the nucleotide excess. The protein samples recovered from the columns and a sample of native Sso7d were analyzed for tryptophan fluorescence at 3 μM final protein concentration (the excitation wavelength was 295 nm and the emission was recorded between 310 and 410 nm) and for ANS fluorescence at 10 μM protein concentration (the excitation wavelength was 350 nm, and the emission was recorded between 400 and 600 nm; 50 μM ANS concentration) using a Perkin-Elmer Spectrofluorimeter model LS 50B at 25 °C.

Enzymatic Assays—In standard conditions, the ATPase activity of Sso7d was assayed in mixtures containing 2 mM ATP, 15 μCi of [γ-^{32}P]ATP, 5 mM $MgCl_2$, 10 μg of pure protein, in 50 mM sodium phosphate, pH 7.5 (150-μl final volume). After a 5-min incubation at 70 °C, a 25-μl aliquot was drawn from the assay mixture; added to 0.5 ml of a suspension containing 50 mM HCl, 5 mM H_3PO_4, 7% activated charcoal; and centrifuged at $4,000 \times g$ for 20 min. The radioactivity of the supernatant was counted on a 100-μl aliquot. In rate calculations, the amount of spontaneous ATP hydrolysis in the absence of Sso7d has been corrected for.

The enzymes were assayed at 25 °C (lysozyme and malic enzyme) or at 60 °C (Ssβgly) by a Cary 1E Varian recording spectrophotometer equipped with a thermostated cell compartment. Each activity assay was performed in duplicate. The assay mixture for lysozyme consisted of 1 ml of a fresh suspension of lyophilized *Escherichia coli* cells in 50 mM Tris-HCl pH 7.4; 1 unit was min^{-1} required for an absorbance decrease of 0.1 OD at 350 nm. Malic enzyme was assayed in 20 mM Tris-HCl, pH 7.5, 0.05 mM NADP, 1 mM $MgCl_2$, 1 mM L-malate (1-ml final volume). Ssβgly was assayed in 50 mM sodium phosphate, pH 7.0, 5 mM 4-nitrophenyl-β-D-glucopyranoside (1-ml final volume).

Preparation of Protein Aggregates—Thermal aggregates of lysozyme, malic enzyme, and Ssβgly were prepared as follows. Solutions of 10 mM Tris-HCl, pH 7.5, containing 0.2 mg/ml lysozyme or 0.07 mg/ml malic enzyme and a solution of 10 mM sodium phosphate, pH 7.0, containing 0.2 mg/ml Ssβgly were incubated, respectively, at 80 °C for 1.5 h, at 60 °C for 40 min, and at 90 °C for 1.5 h. For each incubation, the precipitates were pelleted by centrifugation at $20,000 \times g$ for 20 min. Molecular sieving chromatographies of the suspended pellets on a Su-

perose 6 column (Amersham Pharmacia Biotech; 1×70 cm; eluent 10 mM Tris-HCl, pH 7.5, 0.2 M NaCl for lysozyme and malic enzyme or 0.1 M sodium phosphate, pH 7.0, 0.1 M NaCl for Ssβgly; flow rate of 12 ml/h) yielded aggregates in the ranges 150–500 kDa (lysozyme), 450–1000 kDa (malic enzyme), and 450–1500 (Ssβgly), which were used in the experiments described. Lysozyme aggregates that formed during refolding were prepared as follows. The protein (500 μg in 1 ml) was denatured by a 5-h incubation at room temperature in 4 M guanidinium hydrochloride plus 50 mM 2-mercaptoethanol and then diluted 5-fold in 10 mM Tris-HCl, pH 7.5, at a final protein concentration of 6.9 μM and incubated at 50 °C; after 1.5 h, the precipitates were pelleted by centrifugation at $20,000 \times g$ for 20 min. Molecular sieving chromatography of the suspended pellets (conditions as above) yielded aggregates in the range 200–500 kDa, which were used in the experiments described.

RESULTS

Purification of the Protein and Its ATPase Activity—Sso7d was purified from crude extracts of *S. solfataricus* using a chromatographic procedure that exploits its small size. The Sso7d sample utilized in this study showed one band on overloaded denaturing and nondenaturing gels that were silverstained (Fig. 1*A*); a single symmetrical peak (monitoring at 280 and 214 nm) was detected when Sso7d samples were chromatographed onto reverse phase HPLC column (not shown). The Sso7d concentrations calculated using the extinction coefficient reported by Baumann *et al.* (2) were in agreement with those determined using the Bradford method. Sequence analysis confirmed the presence of Sso7d in solution.

The analysis of the nucleotide content of pure samples by HPLC showed that neither adenosine nor guanosine nucleotides are associated with Sso7d (not shown). It has been demonstrated that Sso7d *in vitro* binds to various polynucleotides and monodinucleosides (2). We found that Sso7d hydrolyzed ATP but did not carry out the hydrolysis of GTP, CTP, or UTP. Fig. 1*B* shows that a peak of ATPase activity co-eluted with the ultraviolet absorption peak upon loading Sso7d samples on different resins (details in the legend to Fig. 1). Notably, most of the protocols described in the literature for the purification of the chromosomal proteins from *Sulfolobales* implicate acidmediated extractions of DNA-bound proteins and cationic resins that exploit the basic character of the proteins. Early results from our laboratory showed that Sso7d samples lose ATPase activity following these procedures.

We provide a characterization of the ATPase activity of Sso7d. The hydrolysis of ATP by Sso7d required the presence of
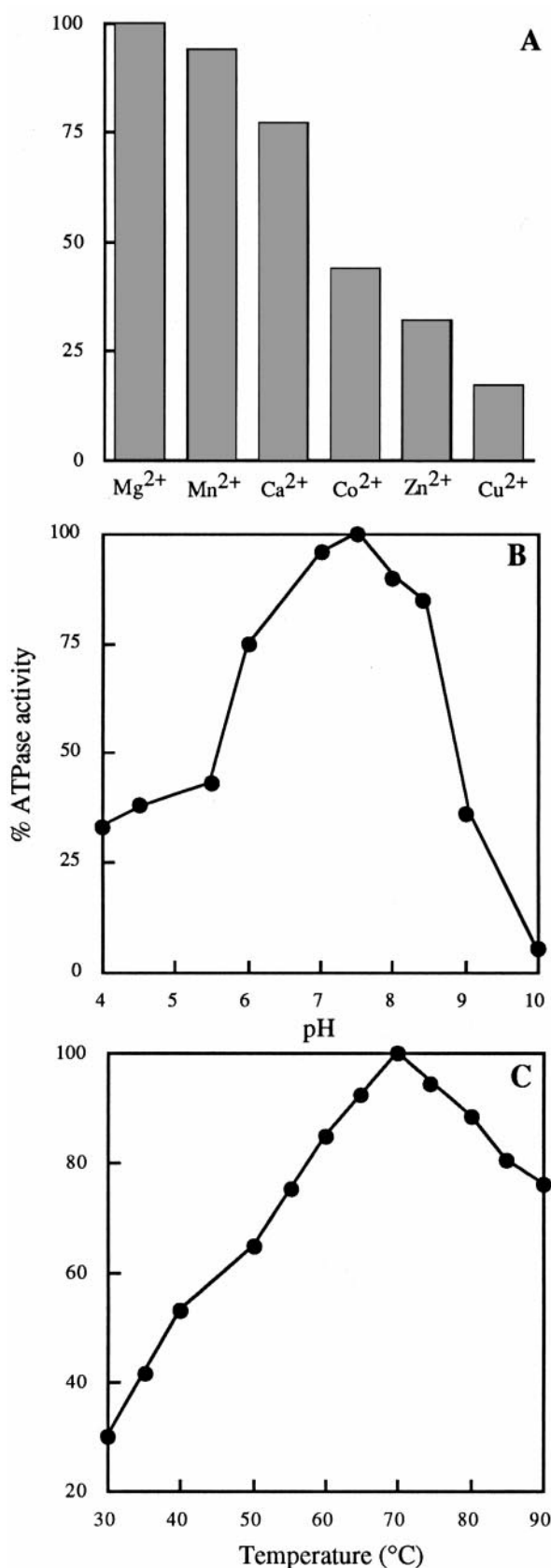
FIG. 2. **The ATPase activity of Sso7d.** The assays were performed under standard conditions (see "Experimental Procedures") except for the ion at 5 mM (*A*), the pH value (50 mM sodium acetate for pH 4–5.5, 50 mM sodium phosphate for pH 6–8, 50 mM Tris-HCl for pH 8.4, 50 mM glycine-NaOH for pH 9 and pH 10) (*B*), and the temperature (*C*). The activity assayed under standard conditions was 13.6 pmol of P_i released/min/μg, which was taken as 100%.

TABLE I
Effects of some reagents on the ATPase activity of Sso7d

Sso7d (10 μg) was preincubated at 37 °C for 15 min in the presence of the indicated reagent and then assayed for ATPase activity as described under "Experimental Procedures."

Reagent	Concentration	Residual activity[a]
	mM	*%*
None		100
NaN$_3$	5	100
NaVO$_3$	0.5	92
	1	66
	2	30
NaNO$_3$	50	80
	70	67
	90	58
	100	10
EDTA	50	90
	100	50
	200	0
CDTA	50	95
	100	55
	200	0

[a] The control activity in the absence of any reagent was 13.6 pmol of P_i/min/μg, which was taken as 100%.

divalent metal ions; various cations supported nucleotide hydrolysis, Mg^{2+} giving the highest rate of hydrolysis (Fig. 2*A*). When assayed in the pH range 4–10, the Sso7d-catalyzed hydrolysis of ATP displayed a maximum around the neutrality (Fig. 2*B*). Assays performed in the temperature range 30–90 °C showed that the optimal temperature was 70 °C (Fig. 2*C*); the hydrolysis of ATP was linear for up to 30 min at this temperature. Freshly purified Sso7d hydrolyzed ATP with a K_m of 0.2 mM and a V_{max} of 13.6 pmol of P_i released/min/μg (Mg^{2+}, pH 7.5, 70 °C), corresponding to a turnover number of 0.095 min^{-1}. In activity inhibition experiments, ATP hydrolysis was inhibited by vanadate, sodium nitrate, EDTA, and CDTA and was unaffected by azide (Table I).

As already pointed out (15), Sso7d presents the sequence GKTGRG (residues 38–43), which resembles the glycine-rich motif G*XX*G*X*G of the ATPase domains of the eukaryotic chaperone hsp90 (16) and type II DNA topoisomerases and MutL DNA mismatch repair proteins (17). Moreover, the sequence GKT (residues 38–40) identifies the nucleotide-binding site of many ATPases and kinases (18) and the ATP-dependent chaperones hsp60, hsp70, and hsp100/Clp (19, 20). The hypothetical nucleotide-binding site of Sso7d is located in a loop that protrudes from the compact structure of the molecule, which is not part of its DNA-binding surface (4). Indeed, we found that Sso7d in complex with DNA also catalyzes the hydrolysis of ATP. Mutational studies are under way in our laboratory to identify the residues responsible for the ATPase activity of Sso7d.

ATPase-dependent Cycling of Sso7d between Conformational States—We wondered whether the conformation of Sso7d is influenced by ATP hydrolysis or binding. The presence of Trp[23] enabled us to perform intrinsic fluorescence experiments. The tryptophan emission spectrum of native Sso7d displayed a maximum around 355 nm (Fig. 3*A*, *solid line*), in accordance with other authors (21). A Sso7d sample that was incubated in the presence of hydrolyzable ATP (MgATP) gave a spectrum different from that of native Sso7d (Fig. 3*A*, *dashed line*). A Sso7d sample that was incubated in the presence of a nonhydrolyzable ATP analog (MgAMP-PNP) gave a spectrum almost identical to that of the native protein (Fig. 3*A*, *dotted line*).

The fluorescence of ANS increases with a concomitant blue shift of the emission maximum on binding to hydrophobic molecules; this fact makes ANS a sensitive probe for distinguishing protein molecules on account of their surface hydrophobicity
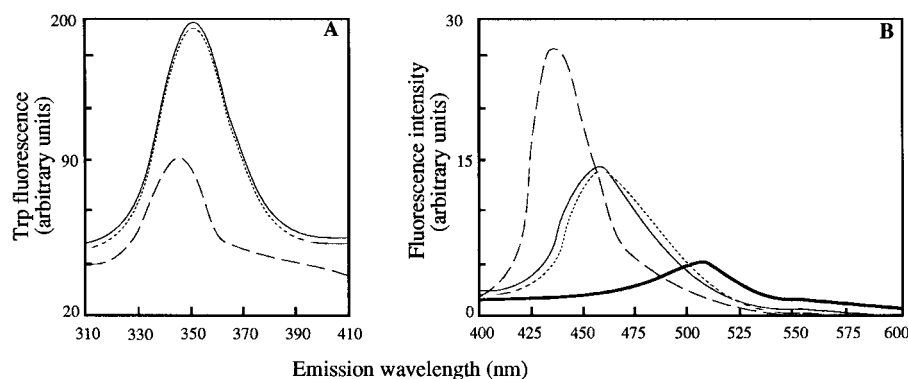
FIG. 3. **Effects of ATP hydrolysis and binding on the conformation of Sso7d.** The experimental details are under "Experimental Procedures." *A*, tryptophan fluorescence emission spectra of native Sso7d (*solid line*), Sso7d after incubation in the presence of MgATP (*dashed line*), and Sso7d after incubation in the presence of MgAMP-PNP (*dotted line*). *B*, ANS fluorescence emission spectra of the probe alone (*thick solid line*), added to native Sso7d (*thin solid line*), added to Sso7d that was incubated in the presence of MgATP (*dashed line*), and added to Sso7d that was incubated in the presence of MgAMP-PNP (*dotted line*).

(22). The spectrum of ANS added to native Sso7d (Fig. 3*B*, *thin solid line*) was different from that of ANS added to Sso7d that was incubated in the presence of MgATP (Fig. 3*B*, *dashed line*) and almost identical to the spectrum of ANS added to Sso7d that was incubated in the presence of MgAMP-PNP (Fig. 3*B*, *dotted line*).

These experiments taken together indicate that the hydrolysis of ATP induces a conformational rearrangement in the Sso7d molecule (we indicate this conformation as Sso7d^{ATP}), while the binding of the nucleotide does not have any detectable effect on the conformation of Sso7d. No conformational changes were induced by ADP, GTP, CTP, or UTP.

We found that a sample of Sso7d^{ATP} that was incubated in the presence of MgAMP-PNP behaved as native Sso7d in the measurements of tryptophan and ANS fluorescence. Thus, the binding of the nucleotide triggers Sso7d^{ATP} to return to the native protein conformation.

Sso7d-promoted Rescue of Aggregated Proteins in the Presence of ATP Hydrolysis—Lysozyme (14.4 kDa) and chicken malic enzyme (a 260-kDa oligomer of 65-kDa subunits) irreversibly inactivate by aggregation upon heating (23). Aggregates with apparent sizes in the ranges 150–500 kDa (lysozyme) and 450–1000 kDa (malic enzyme) were obtained as described under "Experimental Procedures." Twenty micrograms of inactive aggregates were incubated for 30 min at 37 °C in the presence of increasing amounts of Sso7d alone or plus MgAMP-PNP; no lytic activity (Fig. 4*A*, *circles* and *squares*) or malic enzyme activity (Fig. 4*B*, *circles* and *squares*) were assayed on aliquots withdrawn from each mixture. The aggregated proteins did regain their enzymatic activities when incubated in the presence of Sso7d plus MgATP; in particular, 40% of lytic activity (Fig. 4*A*, *triangles*) and 60% of malic enzyme activity (Fig. 4*B*, *triangles*) were restored, respectively, by 15 and 10 μg of Sso7d. We verified that these activity regains did not increase upon prolonged incubation time (see *insets*).

Inactive aggregates form during the refolding of chemically unfolded lysozyme (24). Aggregates of this kind with apparent sizes in the range 200–500 kDa were obtained as described under "Experimental Procedures," and the effects of Sso7d were tested as described above. Again, Sso7d alone (Fig. 4*C*, *circles*) or plus MgAMP-PNP (*squares*) did not cause any reactivation, while 15 μg of Sso7d plus MgATP rescued 40% of lytic activity (*triangles*) within the incubation time (see *inset*).

We verified that no enzymatic activity was regained when aggregated lysozyme and malic enzyme were incubated in the presence of buffer alone or bovine serum albumin plus MgATP (not shown). These experiments together show that Sso7d res-

cues inactive aggregates in the presence of ATP hydrolysis.

The activity regains from lysozyme and malic enzyme aggregates did not increase when the ATP concentration in the reaction mixtures was increased. We hypothesized that the low extent of ATP hydrolysis catalyzed by Sso7d at 37 °C (Fig. 2*C*) could be the cause for the partial regains of activity. Given the lability of lysozyme and malic enzyme above 37 °C, we used the β-glycosidase of the same *S. solfataricus* (Ssβgly) to perform the Sso7d-mediated disaggregating reaction at high temperature. Ssβgly, a 240-kDa oligomer of 60-kDa subunits, undergoes irreversible inactivation by aggregation upon heating at 90 °C, but it resists prolonged exposures at 70 °C (11). Thermal aggregates of Ssβgly with apparent sizes in the range 450–1500 kDa were obtained as described under "Experimental Procedures." When 20 μg of Ssβgly aggregates were incubated at 70 °C in the presence of 5 μg of Sso7d plus MgATP, the Ssβgly activity was fully regained within 10 min of incubation.

We further demonstrated that Sso7d is the only protein endowed with disaggregating activity present in *S. solfataricus*. An aliquot of crude extract was fractionated onto a Superose 6 gel filtration column, and each column fraction was assayed for the ability to rescue aggregated proteins; only the fractions containing Sso7d were positive in the assay.

ATPase-regulated Interaction between Sso7d and the Aggregates—We expanded on the Sso7d-dependent reactivation of aggregated proteins employing Ssβgly. The molecular sieving chromatography for 180 μg of Ssβgly aggregates that were incubated for 10 min at 70 °C in the presence of buffer alone yielded inactive molecules in the range 450–1500 kDa (Fig. 5 *left panel*, *run a*). The chromatography for aggregates that were incubated in the presence of 20 μg of Sso7d plus MgATP (*run b*) yielded the Sso7d peak and a peak at 240 kDa (accounting for about 180 μg) that displayed the specific activity of native Ssβgly and showed a 60-kDa band upon SDS-PAGE (Fig. 5, *right panel*, *lane 1*); *i.e.* the chromatography separated Sso7d from native Ssβgly, the product of the disaggregating reaction. Sso7d that was recovered from the column also rescued the aggregated proteins.

The chromatography for Ssβgly aggregates that were incubated with Sso7d alone (Fig. 5, *run c*) yielded inactive aggregates and no free Sso7d; in this case, SDS-PAGE analysis showed the co-elution of the Sso7d molecule with the aggregates (*lane 2*). The chromatography for Ssβgly aggregates that were incubated with Sso7d plus MgAMP-PNP yielded a profile comparable with that of *run c* (not shown in the figure). These findings show that the dissolution of the aggregates requires the hydrolysis of ATP but that the interaction of Sso7d with the aggregates occurs in the absence of the nucleotide as well as
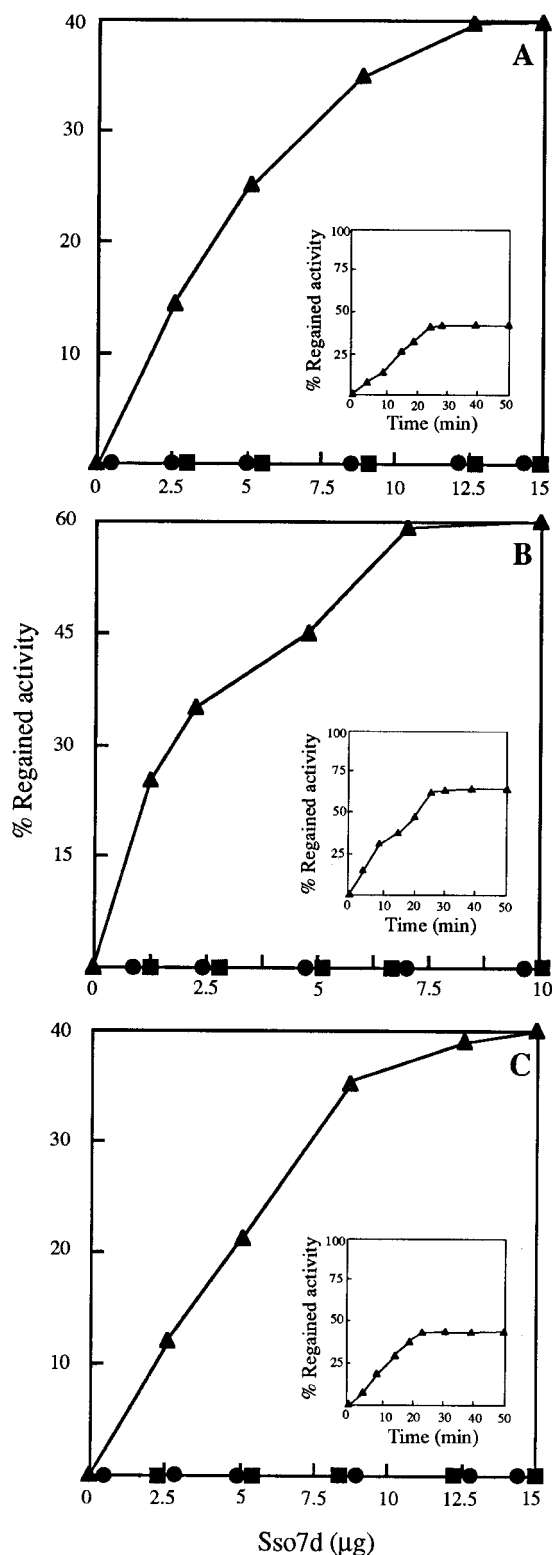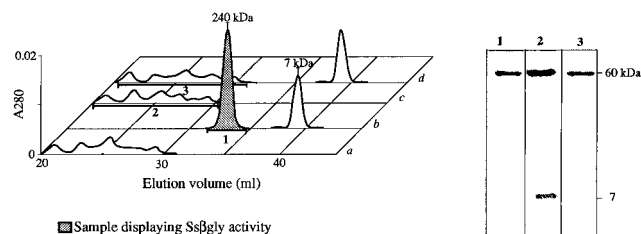
FIG. 5. **Analysis of the interaction of Sso7d with the aggregates.** *Left,* a Superose 6 gel filtration column (Amersham Pharmacia Biotech; 1×70 cm; flow rate of 12 ml/h; eluent 0.1 M sodium phosphate, pH 7.0, 0.1 M NaCl) was separately loaded with 180 μg of Ssβgly aggregates (in 500 μl of 50 mM sodium phosphate, pH 7.0), which were incubated for 10 min at 70 °C with buffer only (*run a*), with 20 μg of Sso7d plus MgATP (*run b*), with 20 μg of Sso7d (*run c*), or with 20 μg of Sso7d^{ATP} (*run d*). The peaks of the nucleotide are omitted. The Ssβgly activity was assayed as described under "Experimental Procedures." *Right,* SDS-PAGE analysis of samples from the columns (5 μg/*lane*). *Lane 1,* sample 1 from *run b*; *lane 2,* sample 2 from *run c*; *lane 3,* sample 3 from *run d*. After the electrophoresis, the gel was silver-stained.

upon ATP binding. The Sso7d-aggregate complexes that were recovered from the column yielded native Ssβgly when supplemented with MgATP, as expected.

We wondered whether the conformational change that Sso7d undergoes upon ATP hydrolysis influences its ability to interact with the aggregates. The chromatography for Ssβgly aggregates that were incubated with Sso7d^{ATP} separated the Sso7d molecule from the aggregates (*run d* and *lane 3*). Thus, the ATP hydrolysis-induced conformational change of Sso7d renders it unable to interact with the aggregates.

The molecular mechanism of the Sso7d-mediated rescue of aggregates is speculative. The cycling of Sso7d between a native conformation that binds to the aggregates and an ATP hydrolysis-induced conformation that is incapable of interacting with the aggregates could mean that rounds of protein binding and releasing are possible over the folding pathway. As regards the intermediates of the reaction, we were unsuccessful in our attempts to detect the binding of Sso7d to protein states other than aggregates during the ATP-driven reactions. Since Sso7d does not renature unfolded proteins,[2] it is unlikely that such structures are intermediates in the Sso7d-assisted pathway from aggregated proteins.

DISCUSSION

Sso7d is a novel protein whose physiological role is still unknown. In this work, we show that Sso7d has an ATPase activity that drives a conformational cycle in the molecule and that Sso7d rescues aggregated proteins in the presence of ATP hydrolysis.

Sso7d is the first protein able to renature protein aggregates to be described from an archaeon. In eukarya and bacteria, the chaperones of the hsp70 and hsp100 families (whose counterparts have not yet been found in crenarchaea) and of the hsp60 family rescue aggregated proteins in an ATP hydrolysis-dependent reaction whose molecular details are still unknown (see Ref. 25 and references therein). hsp70 and hsp100 chaperones dissolve and reactivate the protein complexes that form before or during initiation of DNA replication, thus enabling this event (26–28). It is tempting to speculate that the disaggregating activity of Sso7d may be involved in its hypothesized ability to regulate replication in *Sulfolobus*. hsp70 and hsp100 chaperones are also thought to rescue proteins that have aggregated upon a stress (see Ref. 25 and references therein). Here we report that Sso7d, an abundant protein that is not induced by heat shock

FIG. 4. **Sso7d-dependent reactivations of aggregated lysozyme and malic enzyme.** Solutions (1-ml final volume in 10 mM Tris-HCl, pH 7.5) containing 20 μg of aggregates (*A* and *B*, respectively, lysozyme and malic enzyme aggregates formed upon heating; *C*, lysozyme aggregates formed during refolding) and the indicated amounts of Sso7d were incubated for 30 min at 37 °C with no nucleotide (*circles*), in the presence of MgAMP-PNP (*squares*), or in the presence of MgATP (*triangles*). The lytic activity and the malic enzyme activity were assayed as described under "Experimental Procedures" on aliquots withdrawn from each solution; the activity regains were calculated as percentages with respect to the specific activity of the native enzymes. *Inset,* the activity regains *versus* the incubation time for the solutions containing 15 μg of Sso7d plus MgATP (*A* and *C*) and 10 μg of Sso7d plus MgATP (*B*).

[2] A. Guagliardi, L. Cerchia, M. Moracci, and M. Rossi, unpublished results.

(29), is the only molecule among those present in an *S. solfataricus* crude extract that rescues aggregated proteins; accordingly, the hsp60 chaperone of *S. solfataricus* does not renature aggregated proteins (23, 24). Hence, a role for Sso7d in the archaeal mechanisms of protein homeostasis cannot be ruled out. Identifying the natural substrate(s) of the disaggregating activity of Sso7d could provide clues to its biology.

This paper provides evidence that Sso7d has a protein binding activity. Biochemical assays showed that DNA and the protein aggregates compete for the binding to Sso7d and that Sso7d in complex with DNA lacks disaggregating activity.[2] These findings could mirror a regulatory mechanism of Sso7d function. The ATP-dependent chromosomal protein RecA plays multiple roles in the cell thanks to its various activities (pairing and strand exchange of DNA molecules and a "chaperone" activity in promoting the autodegradation of repressor proteins). The functional domains of RecA are concentrated in one part of the protein; the DNA-binding site overlaps with the repressor protein LexA-binding site for a regulation of the protein activities (reviewed in Ref. 30).

Sso7d is not a conventional chaperone, but it has some features in common with hsp60 and hsp70 chaperones, the best known ATP-dependent chaperones (reviewed in Ref. 31). First, it has in common the intrinsic ATPase activity; the turnover number for the ATPase activity of Sso7d falls within the range of values reported for hsp70 chaperones. Second, it has regulation of the affinity for the protein substrate by nucleotide-induced conformational changes. In the absence of nucleotide or in the presence of ATP binding, hsp60 and hsp70 exist in a "high affinity" conformation for the protein; following hydrolysis of ATP, these chaperones adopt a "low affinity" conformation for the protein. Finally, Sso7d has the ATPase-driven conformational cycling between functionally distinct states. Sso7d could represent a simple model to study the mechanisms of protein renaturation from aggregates. The applied perspectives of such research deal with the rescue of biological activity from inclusion bodies, inactive precipitates that often accumulate in host cells upon the overexpression of foreign proteins.

<div align="center">REFERENCES</div>

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