SUPPLEMENTARY INFORMATION

STABILIZATION OF SECA ATPASE BY THE PRIMARY CYTOPLASMIC SALT OF *ESCHERICHIA COLI*

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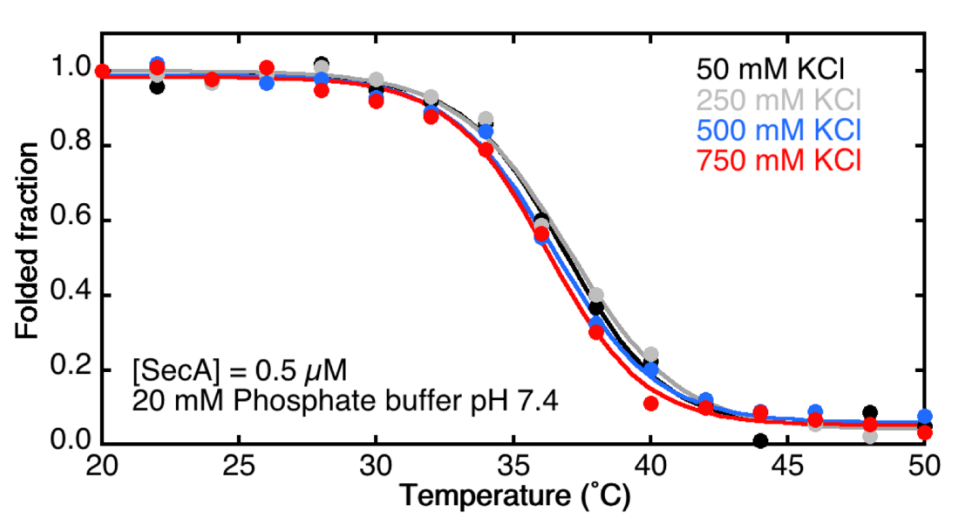

Figure S1. High concentrations of KCl do not stabilize SecA. The graph shows changes in tertiary structure monitored by tryptophan fluorescence as a function of the temperature. The folded fraction was determined from the Trp fluorescence at 340 nm, normalized by setting the intensities of the native (20˚C) and fully unfolded SecA (60˚C) to be 1 and 0, respectively, after proper correction for the temperature dependence of the fluorescence intensity. See Figure 1 for other details.

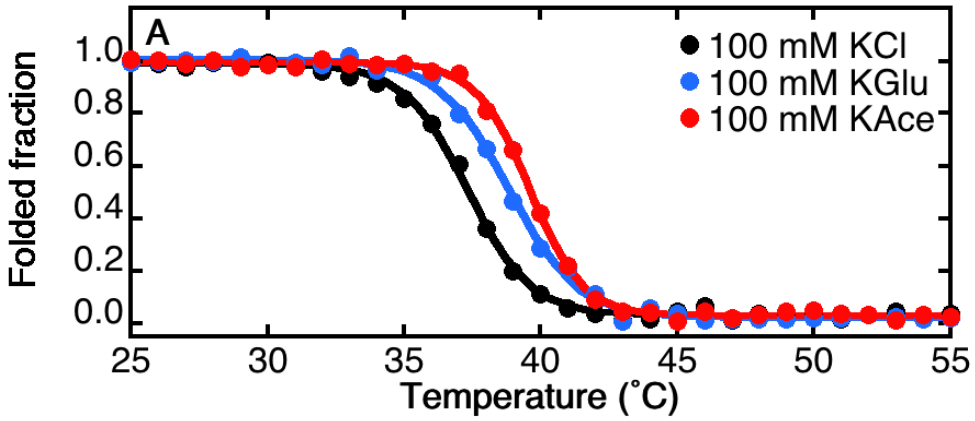

Figure S2. Potassium acetate stabilizes SecA. The graph shows changes in tertiary structure monitored by tryptophan fluorescence as a function of the temperature. The folded fraction was determined from the Trp fluorescence at 340 nm, normalized by setting the intensities of the native (20˚C) and fully unfolded SecA (60˚C) to be 1 and 0, respectively, after proper correction for the temperature dependence of the fluorescence intensity.

Table S1 – Thermal transition temperatures of SecA determined by tryptophan fluorescence and circular dichroism spectroscopy[1]

Electrolyte composition[2]	Fluorescence Transition temperature (˚C)	Circular Dichroism Transition Temperature (˚C)
50 mM KCl	37.7±0.4	43.6±0.5
+ 100 mM KGlu	38.8±0.6	44.9±0.4
+ 200 mM KGlu	40.3±0.7	46.8±0.7
+ 300 mM KGlu	42.0±0.8	47.9±0.6

1. The transition temperatures are defined as the denaturation mid-points in Figure 1 determined from three separate experiments at each salt concentration. Data shown are means and standard deviations of the three data sets obtained for each condition.
2. All solutions contained with 25 mM phospate buffer (pH 7.4) and 50 mM KCl.

Materials and Methods

SecA Protein production. Sec A was obtained from *E. coli* BL21 coco cells carrying the *secA* gene with a C-terminal His_6-tag under the control of the T5 promotor. Cells were grown in LB medium at 37°C with constant shaking. Log-phase cultures (OD 0.8) were stimulated with IPTG (1 mM) for 2 hours at 30°C. Cells were then harvested by centrifugation at 4,000 rpm for 15 minutes and the resulting pellet was stored at −20°C until needed. All protein purification steps and centrifugations are performed at 4˚C.

SecA Protein purification. Bacterial pellets were solubilized in 48 mL of Buffer A (20 mM phosphate buffer pH 7.4, 10 mM imidazole and 50 mM KCl) for 10 minutes at room temperature. Protease inhibitor cocktail (Roche) was added before the cell suspension was passed through a French Pressure Cell (SLM-Amico) at 10,000 lb/in^2. The suspension was then centrifuged at 13,000 g for 15 minutes to pellet the membrane fraction and the supernatant was loaded onto a Talon-Resin (1.5x5 cm) previously equilibrated with 25 mL of Buffer A). His-tagged SecA protein was eluted with Buffer B (20 mM phosphate buffer pH 7.4, 500 mM imidazole and 50 mM KCl). Two mL fractions were collected, and the protein profile analyzed using SDS-PAGE. Fractions containing 100 kDa SecA were then pooled and concentrated a centrifugal filter device with a cutoff of 50 kDa until reaching a volume of 2.5 mL or less. The

resulting sample was then loaded onto a PD-10 column previously equilibrated with Buffer C (20 mM phosphate buffer pH 7.4 and 50 mM KCl) and SecA was eluted following the manufacturer protocol. The protein concentration was estimated using the BioRad Assay using BSA as a reference.

Sample preparation. Samples were prepared by dilution from stock solutions (1 M Potassium chloride, 1 M Potassium glutamate, 1 M Potassium Acetate) and pH adjusted to a value of 7.4)

Tryptophan fluorescence. Fluorescence spectra were recorded using a SLM 8000c spectrophotometer (Urbana, FL). SecA (final dimeric concentration of 5 µM) was prepared in 20 mM Phosphate Buffer pH 7.4, 1 mM EDTA, 2 mM $MgCl_2$, 1 mM DTT, and 50 mM KCl. For the thermal unfolding of SecA, the Trp fluorescence intensities, excited at 295 nm (1 nm slit) and measured at 340 nm (4 nm slit) were collected in 2˚C increments (5 minutes). The same conditions were used for recording scans in buffer alone, which were then subtracted from the appropriate peptide spectra. Three or more data sets were collected for all experiments.

*Circular dichroism spectroscop*y. CD measurements were performed with a JASCO J-810 spectropolarimeter, using a homodimer protein concentration of 1 µM and a 0.1 cm cell path length. The buffer was 20 mM phosphate buffer pH 7.4, 2 mM $MgCl_2$, 1 mM DTT, 50 mM KF, and either 100, 200, or 300 mM KGlu. Spectra were acquired at temperature ranging from 20 to 60˚C in 2˚C increments (5 minutes) at a scan speed of 20 nm min^{-1}, with a 0.2 nm data pitch, using a 1 nm bandwidth and a 4s digital integration time. The spectra were averaged after four accumulations and corrected by subtraction of the buffer spectrum obtained under the same conditions.

Size-exclusion chromatography. SecA samples (5 µM) were prepared in 20 mM phosphate buffer pH 7.4, 0.5 mM DTT, 2 mM $MgCl_2$ and increasing concentrations (100 to 500 mM) of either KCl or potassium glutamate, and incubated 30 minutes at room temperature or at 37˚C. Filtered samples were then loaded (100 µL at 5 µM) loaded onto a Superdex 200 increase 10/300GL previously equilibrated in the adequate buffer. SecA was then eluted at a flow rate of 0.5 mL/min. and the optical absorbance at 280 nm was used to monitor the elution of the protein. To investigate the stability of the protein at 37˚C, the column was stored in an in-house incubator and the temperature was set at 37˚C. Molecular standards were used to calibrate the column: 440 Kda = Ferritin, 158 kDa = Aldolase and 75 kDa = Conalbumin.

ATPase assays. SecA (final dimeric concentration of 5 µM) was prepared in 20 mM Hepes-KOH pH 7.4, 30 mM NH_4Cl, 0.5 mM $MgOAc_2$, 1 mM EDTA, 2 mM $MgCl_2$, and 1 mM DTT in the absence of other ligands (endogenous ATPase) and incubated for 1 hour at temperatures ranging from 22 to 60˚C. The reaction was then initiated by ATP (final concentration of 4 mM) for 15 minutes. The ATP hydrolysis was determined by measuring the release of inorganic phosphate using the malachite green assay[1] with the modifications described previously[2].

MalE and preMalE protein production. MalE and preMal protein were obtained from *E. coli* BL21 cells carrying the either the pET5_A14EMal (preMalE) or the pET5_MalE plasmid were grown in LB medium at 37°C with constant shaking. Log cultures (OD 0.8) were stimulated with

IPTG (20 µM) for one hour at 37°C. Cells were then harvested by centrifugation at 4,000 rpm for 15 minutes and the resulting pellet stored at −20°C until needed. All protein purification steps and centrifugations were performed at 4°C.

MalE and preMalE protein purification. Bacterial pellets were solubilized in 48 mL of Buffer D (20 mM phosphate buffer pH 7.4, 10 mM imidazole, 50 mM KCl and 3 M GdmCl) for 10 minutes at room temperature. The cell suspension was passed through a French Pressure Cell (SLM-Amico) at 10,000 lb/in^2 and then then centrifuged at 13,000g for 15 minutes to pellet the membrane fraction. The supernatant was loaded onto a Talon-Resin (1.5x5 cm) previously equilibrated with 25 mL of Buffer D. His-tagged SecA protein was eluted with Buffer E (20 mM phosphate buffer pH 7.4, 500 mM imidazole, 50 mM KCl, and 3 M GdmCl). Two mL fractions were collected, and the protein profile analyzed using SDS-PAGE. Fractions containing 40 kDa preMalE or MalE were then pooled and concentrated using a centrifugal filter device with a cutoff of 50 kDa until reaching a volume of 2.5 mL or less. The protein concentration was estimated using the BioRad Assay using BSA as a reference.

MalE and preMalE protein renaturation. Guanidine chloride was removed by an overnight dialysis step at 4°C against either buffer F (20 mM phosphate buffer pH 7.4, 50 mM KF, and 200 mM KGlu when noted).

*MalE and preMalE circular dichroism spectroscop*y. CD measurements were performed with a JASCO J-810 spectropolarimeter, using a protein concentration of 1 µM and a 0.1 cm cell path length. The final buffer was 20 mM phosphate buffer pH 7.4, 50 mM KF and 200 mM KGlu when noted. Spectra were acquired at temperature ranging from 30 to 80°C in 1°C increments (2 minutes) at a scan speed of 60 nm min^{-1}, with a 0.2 nm data pitch, using a 2 nm bandwidth and a digital integration time of 4s. The spectra were averaged after five accumulations and corrected for buffer absorption by subtraction of the buffer spectrum obtained under the same conditions.

References

1. Lanzetta PA, Alvarez LJ, Reinach PS, Candia OA. An improved assay for nanomole amounts of inorganic phosphate. Analytical Biochemistry 1979;100:95-97.

2. Mitchell C, Oliver D. Two distinct ATP-binding domains are needed to promote protein export by Escherichia coli SecA ATPase. Molecular Microbiology 1993;10:483-497.