Analysis of crystalline and solution states of ligand-free spermidine *N*-acetyltransferase (SpeG) from *Escherichia coli*

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Spermidine *N*-acetyltransferase (SpeG) transfers an acetyl group from acetyl-coenzyme A to an N-terminal amino group of intracellular spermidine. This acetylation inactivates spermidine, reducing the polyamine toxicity that tends to occur under certain chemical and physical stresses. The structure of the SpeG protein from *Vibrio cholerae* has been characterized: while the monomer possesses a structural fold similar to those of other Gcn5-related *N*-acetyltransferase superfamily members, its dodecameric structure remains exceptional. In this paper, structural analyses of SpeG isolated from *Escherichia coli* are described. Like *V. cholerae* SpeG, *E. coli* SpeG forms dodecamers, as revealed by two crystal structures of the ligand-free *E. coli* SpeG dodecamer determined at 1.75 and 2.9 Å resolution. Although both *V. cholerae* SpeG and *E. coli* SpeG can adopt an asymmetric open dodecameric state, solution analysis showed that the oligomeric composition of ligand-free *E. coli* SpeG differs from that of ligand-free *V. cholerae* SpeG. Based on these data, it is proposed that the equilibrium balance of SpeG oligomers in the absence of ligands differs from one species to another and thus might be important for SpeG function.

1. Introduction

Polyamines are positively charged linear molecules containing flexible aliphatic chains with two or more amino groups (Schneider & Wendisch, 2011). Polyamines are widely distributed inside cells and perform diverse functions. For example, polyamines alter chromatin structure, participate in gene transcription and translation, stabilize DNA and RNA, and influence signal transduction, cell growth and proliferation, migration, membrane stability, ion channel function and receptor–ligand interactions (Abraham, 1968; Igarashi & Kashiwagi, 2000; Miller-Fleming *et al.*, 2015; Michael, 2016; Shah & Swiatlo, 2008; Schuber, 1989). Other reports have highlighted the importance of polyamines in toxin activity, the formation of biofilms, protection against oxidative and acid stresses, carcinogenesis owing to bacteria, the production of bacteriocins and escape from phagolysosomes (Babbar & Gerner, 2011; Pan *et al.*, 2006; Shah *et al.*, 2011; Rider *et al.*, 2007; Wortham *et al.*, 2007). Because they play critical roles in the proper functioning of cells, high levels of polyamines, such as spermidine, lead to toxicity and thus to inhibition of growth (He *et al.*, 1993). To overcome this toxicity, bacterial cells

tightly regulate the concentration of intracellular polyamines by controlling their synthesis, degradation, import and export (Shah & Swiatlo, 2008).

A key polyamine catabolic enzyme in *Escherichia coli* is spermidine *N*-acetyltransferase (SpeG), which acetylates spermidine, transforming it into a less toxic form that can be easily exported from or kept within the cell (Fukuchi *et al.*, 1994; Ignatenko *et al.*, 1996; Kakegawa *et al.*, 1991). *E. coli* SpeG (SpeG_*Ec*) can catalyze the acetylation of both the N¹ and N⁸ ends of spermidine using acetyl-coenzyme A (acCoA) as the acetyl donor (Fukuchi *et al.*, 1994). Multiple studies have indicated that the acetylation of spermidine by SpeG_*Ec* is required to avoid spermidine toxicity under diverse stressful conditions, including cold shock, heat shock, ethanol treatment and alkaline shift (Carper *et al.*, 1991; Fukuchi *et al.*, 1995; Limsuwun & Jones, 2000; Matsui *et al.*, 1982).

Biochemical analyses have shown that SpeG_*Ec* can acetylate both spermidine and spermine *in vitro* (Fukuchi *et al.*, 1994). *E. coli* can synthesize spermidine *de novo* and thus it is a natural substrate of SpeG_*Ec*. In contrast, *E. coli* cannot synthesize spermine (Shah & Swiatlo, 2008). However, *E. coli* can transport exogenous spermine across its inner membrane using the PotDABC system (Kakegawa *et al.*, 1991; Sugiyama *et al.*, 1996). The role of spermine in *E. coli* is not well understood, although it has been reported to support ribosomal function (Xaplanteris *et al.*, 2005).

The structure of SpeG from *Vibrio cholerae* (SpeG_*Vc*) has been extensively studied (Filippova, Kuhn *et al.*, 2015; Filippova, Wiegand *et al.*, 2015). Numerous crystal structures of SpeG_*Vc* have been deposited in the Protein Data Bank (PDB; Berman *et al.*, 2000), including structures in the ligand-free state and structures in complex with polyamines (spermidine or spermine) and/or cofactors (acCoA or CoA) (Filippova, Kuhn *et al.*, 2015). The structure of SpeG_*Ec* in complex with spermidine and CoA has been described (Sugiyama *et al.*, 2016), and structures of ligand-free SpeG from *Yersinia pestis* (PDB entries 6d72 and 5wif; Center for Structural Genomics of Infectious Diseases, unpublished work), *Staphylococcus aureus* (PDB entry 5ix3; P. Srivastava, Y. Khandokar & J. Forwood, unpublished work) and *Coxiella burnetii* (PDB entry 3tht; Franklin *et al.*, 2015) have been deposited and are available in the PDB. Together, these data show that the SpeG monomer possesses an α/β architecture similar to the structures of known acetyltransferases from the Gcn5-related *N*-acetyltransferase (GNAT) superfamily (Vetting *et al.*, 2005; Dyda *et al.*, 2000). Biochemical and structural analyses of SpeG_*Vc* and SpeG_*Ec* indicated that the dodecamer is the biologically relevant unit (Filippova, Wiegand *et al.*, 2015; Sugiyama *et al.*, 2016). Structures of SpeG_*Vc* in complex with polyamines (spermidine or spermine) revealed that SpeG has a previously unknown (amongst GNAT family members) allosteric polyamine-binding site; thus, SpeG can bind the same molecule (polyamine) in both its allosteric and its substrate-binding pockets (Filippova, Kuhn *et al.*, 2015). Published data revealed that ligand-free SpeG_*Vc* exists in solution as an equilibrium of an asymmetric open dodecamer with other homo-oligomers, especially tetramers

Table 1

X-ray data-collection, structure-determination and refinement statistics.

Values in parentheses are for the last shell.

SpeG_ <i>Ec</i> structure	6cy6	4r9m
Crystal parameters		
Resolution (Å)	30.0–1.75 (1.78–1.75)	30.0–2.90 (2.95–2.90)
Space group	<i>P</i> 6 ₂₂	<i>P</i> 4 ₂ 2 ₁ 2
<i>a</i> , <i>b</i> , <i>c</i> (Å)	107.45, 107.45, 65.02	110.92, 110.92, 108.50
α , β , γ (°)	90, 90, 120	90, 90, 90
Matthews coefficient (Å ³ Da ^{−1})	2.47	2.26
Solvent content (%)	50.3	45.5
Data collection		
Completeness (%)	100 (100)	99.0 (98.2)
No. of unique reflections	2293	15432
$\langle I/\sigma(I) \rangle$	51.1 (4.3)	32.3 (2.6)
<i>R</i> _{merge} (%)	0.05 (0.60)	0.06 (0.66)
Multiplicity	12.8 (13.0)	7.8 (8.0)
Wilson <i>B</i> factor (Å ²)	26.1	77.3
Refinement		
<i>R</i> / <i>R</i> _{free} (%)	15.4/20.1	17.6/25.9
R.m.s.d., bond lengths (Å)	0.021	0.014
R.m.s.d., bond angles (°)	2.1	1.8
Average <i>B</i> value (Å ²)	31.4	83.4
No. of molecules in asymmetric unit	1	3
No. of atoms		
Protein	1422	4179
Water molecules	105	21
Ramachandran analysis†		
Favored (%/No.)	99.5/186	92.4/447
Allowed (%/No.)	0.5/1	7.4/36
Outliers (%/No.)	—	0.2/1

† Defined by the validation program *MolProbity*.

and dimers (Filippova, Wiegand *et al.*, 2015). The addition of either spermidine or spermine to SpeG_*Vc* shifts this oligomeric state equilibrium towards dodecamers in a closed state with 62 symmetry (Filippova, Wiegand *et al.*, 2015).

Here, we present two previously uncharacterized crystal structures of SpeG_*Ec* in its ligand-free form. Structural and solution analyses by SEC-MALS, SAXS and negative-staining EM show that ligand-free SpeG_*Ec* forms closed symmetric dodecamers in the crystal and, similar to SpeG_*Vc*, forms open asymmetric dodecamers in solution together with other homo-oligomers. A comparative analysis of solution states between SpeG_*Ec* and SpeG_*Vc* in their ligand-free forms suggests that the open dodecamer is conserved among SpeG homologs, while the equilibrium balance of oligomeric states differs. Therefore, we hypothesize that the oligomeric composition of ligand-free SpeG might be functionally important.

2. Materials and methods

2.1. Protein production

The SpeG-encoding gene from *E. coli* strain K-12 substr. MG1655 (gi:16129542; residues 2–186; molecular weight 21.9 kDa) was cloned into the pMCSG7 vector with an N-terminal 6×His tag followed by a TEV cleavage site. The SpeG_*Ec* clone was transformed into competent *E. coli* BL21(DE3) cells and grown in Terrific Broth medium in the

Table 2

Sample details, data-collection parameters, structural parameters and molecular-mass determination of SpeG_Ec in solution by SAXS.

SpeG concentration (mg ml ^{−1})	0.65	1.7	3.9	7.5
Sample details				
Organism	<i>E. coli</i>			
Source	<i>E. coli</i> expressed			
NCBI reference sequence ID	NC_000913.3 (1–186)			
Extinction coefficient [<i>A</i> ₂₈₀ , 0.1%(<i>w/v</i>)]	1.006			
Partial specific volume (cm ³ g ^{−1})	0.74			
Partial contrast from sequence and solvent constituents, $\rho_{\text{protein}} - \rho_{\text{solvent}}$ (10 ¹⁰ cm ^{−2})	2.79 (12.28–9.48)			
Data-collection parameters				
Beam size at sample (μm)	266 × 266			
Wavelength (Å)	1.2398			
<i>q</i> -range (Å ^{−1})	0.0013–0.48			
Detector	Rayonix MX170HS and LX170HS			
Absolute scaling method	Comparison with Milli-Q water scattering in the same cell			
Normalization	To transmitted intensity by beam-stop counter			
Monitoring for radiation damage	X-ray dose maintained below 210 Gy, frame-by-frame comparison			
Detector distance (m)	8.5 and 1.0			
Exposure time per image (s)	8			
Sample volume (μl)	250			
Sample configuration	1.4 mm path-length capillary-flow cell at 4 μl s ^{−1}			
Data-collection temperature (K)	295			
Structural parameters from Guinier plot				
<i>I</i> (0) (cm ^{−1})	0.12	0.14	0.15	0.15
<i>R</i> _g (Å)	42.1	43.1	43.2	42.0
<i>q</i> _{min} (Å ^{−1})	0.0014	0.0014	0.0014	0.0014
<i>qR</i> _g max (<i>q</i> _{min} = 0.0082 Å ^{−1})	1.3	1.3	1.3	1.3
Structural parameters from <i>P</i> (<i>r</i>) plot				
<i>I</i> (0) (cm ^{−1})	0.118	0.142	0.150	0.155
<i>R</i> _g (Å)	42.7	42.9	42.7	42.1
<i>D</i> _{max} (Å)	115.7	131.5	126.6	120.8
Porod volume estimate (nm ³)	293	339	359	371
Volume of correlation (Å ³)	345	1090	2070	2410
Molecular-mass determination				
Molecular mass <i>M</i> _r [<i>V</i> _c ² /(0.1231 <i>R</i> _g)] (kDa)	253	283	290	300
Calculated monomeric <i>M</i> _r from sequence (kDa)	21.9			

presence of 50 µg ml⁻¹ kanamycin and 100 µg ml⁻¹ ampicillin at 37°C. Expression was induced with 0.5 mM IPTG when the optical density of the culture reached ~0.6–0.8 and was followed by incubation overnight at 25°C. After centrifugation, the cell pellets were harvested and lysed in buffer consisting of 1.5 mM magnesium acetate, 1 mM calcium chloride, 250 mM sodium chloride, 3.25 mM citric acid, 100 mM ammonium sulfate, 40 mM disodium phosphate, 5% glycerol, 5 mM imidazole, 5 mM β-mercaptoethanol (BME), 0.08% *n*-dodecyl-β-maltoside (DDM), 1 mM phenylmethylsulfonyl fluoride (PMSF), 20 µM leupeptin. After sonication, the cleared lysate was loaded onto 5 ml HisTrap FF Ni-NTA column and washed with binding buffer consisting of 10 mM Tris-HCl, 500 mM sodium chloride, 5 mM BME pH 8.3. The SpeG_Ec protein was washed with binding buffer containing 25 mM imidazole and eluted with binding buffer containing 500 mM imidazole. To improve the purity, SpeG_Ec was additionally purified by size-exclusion chromatography on a HiLoad 26/60 Superdex 200 column with binding buffer. The purity of the expressed SpeG_Ec protein was analyzed by polyacrylamide gel electrophoresis.

2.2. Crystallization

Crystals of SpeG_Ec were obtained by the sitting-drop vapor-diffusion method at 19°C. Crystallization was carried

out with a Phoenix robotic system (Art Robbins Instruments) using precipitant solutions from The CompAS Suite crystal screen (Qiagen) dispensed at a 1:1 protein:precipitant solution ratio in a total drop volume of 2 µl. The protein solution consisted of 7.6 mg ml⁻¹ SpeG_Ec in binding buffer. Crystals of SpeG_Ec with a tetragonal form were grown in a condition consisting of 3 M sodium formate. Crystals of SpeG_Ec with a hexagonal form were obtained in crystallization conditions consisting of 0.2 M ammonium phosphate monobasic, 0.1 M Tris, 50% (v/v) 2-methyl-2,4-pentanediol (MPD) pH 8.5. In the case of the crystal with the hexagonal form, the SpeG_Ec protein solution was incubated with 5 mM acetylserine for 30 min to obtain a complex with the product. Prior to data collection, crystals of SpeG_Ec were soaked in mother liquor (for the hexagonal crystal form) or cryoprotection solution with 25% MPD (for the tetragonal crystal form). Crystals were flash-cooled in liquid nitrogen for data collection.

2.3. Structure determination and refinement

Native data sets were collected from SpeG_Ec crystals on the Life Sciences Collaborative Access Team (LS-CAT) beamlines 21-ID-F and 21-ID-G at Argonne National Laboratory, Argonne, Illinois, USA at 100 K. The data sets were indexed, integrated and scaled using HKL-3000 (Minor *et al.*, 2006). The structures were solved by the molecular-

replacement method in *Phaser* (McCoy *et al.*, 2007), which is integrated into the *CCP4* program suite (Winn *et al.*, 2011), using the *SpeG_Vc* structure in the ligand-free form (PDB entry 5cnp; Filippova, Wiegand *et al.*, 2015) as the starting model. Structures were refined by *REFMAC* (Murshudov *et al.*, 2011). Manual correction of protein main chains, side chains, turns, gaps and water molecules was performed in *Coot* (Emsley *et al.*, 2010; Emsley & Cowtan, 2004). Refined structures were validated with the PDB validation server (<https://validate.rcsb-2.wwpdb.org>) and *MolProbity* (Chen *et al.*, 2010; Davis *et al.*, 2007). Graphical structure representations were generated in *PyMOL* v.2.2.0 and *CCP4mg* (McNicholas *et al.*, 2011). The data-collection, structure-determination and refinement statistics for *SpeG_Ec* structures in the ligand-free state are summarized in Table 1. The coordinates and the

structure factors of the refined structures have been deposited in the PDB (Berman *et al.*, 2000) as entries 6cy6 and 4r9m.

2.4. Size-exclusion chromatography–multi-angle light scattering (SEC-MALS)

SEC-MALS analysis of *SpeG_Ec* in the ligand-free state was performed using the instrumentation described previously (Filippova, Wiegand *et al.*, 2015). The sample solution (300 μ l) consisted of 3 mg ml⁻¹ *SpeG_Ec* in loading buffer composed of 10 mM Tris–HCl, 500 mM sodium chloride, 5 mM BME pH 8.3. SEC-MALS data were collected and analyzed at 22°C with a flow rate of 0.5 ml min⁻¹. Bovine serum albumin (BSA; Sigma–Aldrich, St Louis, Missouri, USA) was used as a reference standard. Absolute molecular weight was calculated in *ASTRA* 5 (Wyatt Technology).

2.5. Small-angle X-ray scattering (SAXS)

Sample preparation, SAXS data collection and analysis of ligand-free *SpeG_Ec* in solution were performed as described previously (Filippova, Wiegand *et al.*, 2015) on the DND (DuPont–Northwestern–Dow) Collaborative Access Team beamline (5-ID-D) at the Advanced Photon Source at Argonne National Laboratory, Argonne, Illinois, USA. The *SpeG_Ec* protein was measured at concentrations of 0.65, 1.7, 3.9 and 7.5 mg ml⁻¹ in 10 mM Tris–HCl, 150 mM sodium chloride, 0.5 mM tris(2-carboxyethyl)phosphine (TCEP) pH 8.3. The data were azimuthally averaged using *GSAS-II* (Toby & Von Dreele, 2013) and analyzed using the *ATSAS* program suite (Franke *et al.*, 2017) and *Scatter* (Förster *et al.*, 2010). The SAXS scattering curves from the *SpeG* oligomeric models were calculated using *CRY SOL* (Svergun *et al.*, 1995). The volume fractions of closed dodecamer, open dodecamer,

Figure 1
Crystal structure of *SpeG_Ec* in the ligand-free state. (a) Ribbon representation of the *SpeG_Ec* monomer with bound magnesium ion (red ball) created from the 4r9m structure. (b) Ribbon representation of the *SpeG_Ec* monomer with bound chloride ion (green ball), sodium ions (blue balls) and small solvent molecules TRS and MPD (stick models in yellow) created from the 6cy6 structure. The secondary-structure elements are labeled in (a) and (b). (c) Superposition of *SpeG_Ec* monomers in the ligand-free state from the 4r9m and 6cy6 structures.

dodecamer in an intermediate state, decamer, octamer, hexamer, tetramer, dimer and monomer of SpeG were calculated using *OLIGOMER* (Franke *et al.*, 2017; Supplementary Table S1). The SpeG oligomeric models were part of the crystal structures (PDB entries 4r9m, 5cnp and 4ygo). SAXS data analysis was performed following the published guidelines (Trehwella *et al.*, 2017). Sample details, data-collection parameters, structural parameters and molecular-mass determinations are summarized in Table 2.

2.6. Negative-staining electron microscopy (EM)

Negative-staining EM analysis of SpeG_Ec in its ligand-free state was performed as described previously (Filippova, Wiegand *et al.*, 2015). 4 μ l of SpeG_Ec at a concentration of 0.004 mg ml⁻¹ in 10 mM HEPES, 100 mM sodium chloride pH 8.3 was applied to a glow-discharged carbon-coated copper grid followed by washing in two separate 50 μ l drops of water and two drops of 2% uranyl acetate for 15 s in each drop. Grids were examined at room temperature using a Jeol JEM 1400 transmission electron microscope. The images were analyzed in the *ImageJ* program.

3. Results and discussion

3.1. Structure of SpeG_Ec in a ligand-free form

Two crystal structures of SpeG_Ec in its ligand-free form were determined at 1.75 and 2.9 Å resolution by the molecular-replacement method in space groups *P*622 (PDB entry 6cy6) and *P*₄₂₁₂ (PDB entry 4r9m), respectively. The asymmetric unit of the SpeG_Ec protein in space groups *P*622 and *P*₄₂₁₂ contained one and three SpeG_Ec monomers, respectively. Structural and sequence analyses showed that the SpeG_Ec monomer has the classical α/β GNAT protein fold (Vetting *et al.*, 2005) with a central seven-stranded β -sheet (β 1, residues 7–11; β 2, 58–64; β 3, 67–78; β 4, 83–90; β 5, 119–125; β 6, 141–151; β 7, 156–165) surrounded by five α -helices (α 1, 14–21; α 2,

41–50; α 3, 99–113; α 4, 129–137; α 5, 166–171) and one short 3_{10} -helical segment (α' 1, 92–94; Fig. 1a). Residues 5–173 were present in the 6cy6 structure. Within this structure, a chloride ion, eight sodium ions, one TRS (Tris) and six MPD molecules interacting with different residues on the surface of the SpeG_Ec monomer were identified (Fig. 1b). Residues 5–172 (chain A), 5–172 (chain B) and 1–172 (chain C) were present in the 4r9m structure. A portion of the loop (residues 24–29) between helices α 1 and α 2 was disordered in all monomers of the 4r9m structure (Fig. 1a). This loop is important for binding the ligands of SpeG and moves upon either polyamine binding at the allosteric polyamine-binding site or acCoA binding at the active site (Filippova, Kuhn *et al.*, 2015). Residues 1–4 of the N-terminus in chain C of the 4r9m structure protruded towards the solvent, making contacts with active-site residues of the symmetry-related monomer (Figs. 1a and 2b). Additionally, the 4r9m structure contained a magnesium ion that interacts with Glu35 and Glu76 in chain A (Fig. 1a).

The monomers in the 4r9m structure were similar and superimposed well with root-mean-square deviations (r.m.s.d.s) of $\sim$ 0.36–0.45 Å over 164 C α atoms, while the monomer in the 6cy6 structure superimposed with the monomers of the 4r9m structure with r.m.s.d.s of $\sim$ 0.81–0.94 Å over 164 C α atoms. Superposition of monomers from the 6cy6 and 4r9m structures revealed a conformational difference in the position of the loop between α 1 and α 2 (residues 24–39) at the allosteric polyamine-binding site, in the position of the loop between β 4 and α 3 (residues 90–98) and in helix α 4 at the cofactor-binding site in the 6cy6 structure (Fig. 1c). The shift of the loop between helices α 1 and α 2 involved the remodeling of secondary-structure elements (Fig. 1c). Two short β -strands (β' 1 and β' 2) located on the loop in the 4r9m structure transformed into one short helix (α' 2) in the 6cy6 structure. Amongst GNATs, the loop between β 4 and α 3 and helix α 4 are known to support distinctive acCoA-binding interactions with active-site residues (Dyda *et al.*, 2000). The differences in the 6cy6 structure could be attributed to the binding of small

Figure 2
Dodecameric structure and SEC-MALS analysis of SpeG_Ec. (a, b) Ribbon representation of the SpeG_Ec dodecamer [front (a) and side (b) view] created from the 4r9m structure. The GNAT dimer composed of two SpeG_Ec monomers (in gray and golden yellow) related by a twofold rotation axis is shown in (b). (c) The SEC-MALS elution profile of SpeG_Ec in the absence of ligand is shown in red. The molecular-mass distribution of the sample is shown as a bold solid line. The SEC-MALS elution profile of BSA, which was used as a reference sample, is shown in blue.

Figure 3 SAXS analysis of SpeG_Ec in the absence of ligand. (a–d) SAXS experimental scattering curves and curves calculated from SpeG structural models with *OLIGOMER* are shown in black and red, respectively. The upper plot shows $\log I(q)$ versus q with a Guinier plot (inset). The lower plot shows the error-weighted residual difference $\Delta\sigma$ versus q . (e) Dimensionless Kratky plot of SpeG_Ec at concentrations of 0.65 mg ml⁻¹ (red dotted line), 1.7 mg ml⁻¹ (green), 3.9 mg ml⁻¹ (blue) and 7.5 mg ml⁻¹ (black). (f) Distance distribution function for SpeG_Ec in the absence of ligand at concentrations of 0.65, 1.7, 3.9 and 7.5 mg ml⁻¹ (from bottom to top).

solvent molecules/ions, the presence of acetylated polyamine in the protein solution during crystallization and/or different crystal-packing modes.

In both of the ligand-free SpeG_*Ec* structures described above, the monomer(s) formed a dodecamer (Fig. 2*a*) similar to our previously described SpeG_*Vc* dodecamer (Filippova, Kuhn *et al.*, 2015). The SpeG_*Ec* monomer within the dodecamer had identical structural features compared with the SpeG_*Vc* monomer in its ligand-free state (Filippova, Kuhn *et al.*, 2015), with similar positions of α -helices, β -strands and interconnecting loops between the monomers (r.m.s.d. of ~ 0.4 Å over 167 C α atoms). In the dodecamer, the monomers from two hexamers related by a crystallographic twofold rotation axis formed a distinctive GNAT dimer (Fig. 2*b*; Dyda *et al.*, 2000). Therefore, six similar dimers form the closed dodecamer in SpeG structures.

3.2. Oligomeric state of ligand-free SpeG_*Ec* in solution

SEC-MALS analysis suggests that ligand-free SpeG_*Ec* has a dodecameric state in solution with an observed molecular weight (MW) of ~ 270 kDa (the theoretical MW of the SpeG_*Ec* dodecamer is ~ 263 kDa; Fig. 2*c*). To determine whether SpeG_*Ec* exists in an equilibrium of homo-oligomeric states and adopts the asymmetric open dodecamer in the absence of substrate, as we previously reported for SpeG_*Vc* (Filippova, Wiegand *et al.*, 2015), we performed SAXS (Fig. 3)

and negative-staining EM analysis (Supplementary Fig. S1). SAXS experimental curves of SpeG_*Ec* at different protein concentrations were compared with calculated scattering profiles derived from known SpeG crystal structures, including structures of the SpeG closed dodecamer, open dodecamer, dodecamer in an intermediate state, decamer, octamer, hexamer, tetramer, dimer and monomer (Fig. 3). This comparison revealed that SpeG_*Ec*, in the absence of substrate, exists in solution as closed and open dodecamers, with a small fraction of particles in lower order homo-oligomers such as hexamers and tetramers (Figs. 3 and 4). The formation of SpeG_*Ec* tetramers at low protein concentration agrees with previously reported data showing that the native SpeG_*Ec* enzyme may have a tetrameric state in solution (Fukuchi *et al.*, 1994). The proportion of dodecamers in the closed state, dodecamers in the open state, hexamers and tetramers varied in a concentration-dependent manner (Fig. 4). SAXS data showed that SpeG_*Ec* dodecamers in the open and closed states were significantly preferred over smaller oligomers (hexamers and tetramers; Figs. 2*c* and 4*a*). The fraction of closed dodecamers increased with protein concentration, while the reverse was true for open SpeG_*Ec* dodecamers. The existence of both closed and open SpeG_*Ec* dodecamers in solution, as previously reported for SpeG_*Vc*, was supported by negative-staining EM (Supplementary Fig. S1). The combined data suggest that the open asymmetric dodecamer is conserved amongst SpeG homologs.

Figure 4

Oligomer composition of SpeG in solution. (a, b) SAXS-based analysis of the distribution of SpeG_*Ec* (a) and SpeG_*Vc* (b) oligomeric states versus protein concentration in a ligand-free solution. (c) Ribbon diagrams of SpeG homo-oligomeric states.

3.3. Comparison of the oligomeric states of ligand-free SpeG_Ec and Spe_Vc in solution

Based on our previously published SAXS data, SpeG_Vc in the absence of ligand exists in solution as open asymmetric dodecamers, tetramers and dimers (Figs. 4*b* and 4*c*). At similar protein concentrations, SAXS analysis showed that SpeG_Ec forms both open and closed dodecamers, hexamers and tetramers, while it does not form dimers (Fig. 4*a*).

An accompanying SEC-MALS analysis showed that in the absence of polyamine or other ligands, the molecular-mass distribution of SpeG_Ec particles does not change and demonstrates a linear molecular-mass distribution (Fig. 2*c*), which was not the case for SpeG_Vc. The SEC-MALS data show that the MW of SpeG_Vc is ~220 kDa and suggest that larger protein dodecamers co-elute significantly with smaller oligomers (Filippova, Wiegand *et al.*, 2015). The SEC-MALS analysis of SpeG_Ec is in good agreement with the SAXS data and indicates that SpeG_Ec favors a dodecameric state in solution.

4. Conclusions

Our combined data suggest that ligand-free SpeG_Ec has a more stable dodecameric state in solution compared with SpeG_Vc. We find that SpeG_Ec in its ligand-free state exists as a closed dodecamer in the crystalline state, but can adopt both open and closed dodecamers in solution in equilibrium with other homo-oligomers, as reported for SpeG_Vc. However, the equilibrium balance of the homo-oligomers of SpeG_Ec differs from that of SpeG_Vc. This may impact the function of SpeG and its sensitivity to polyamine concentrations. The differences in oligomeric composition may allow SpeG to maintain a 'perfect balance' of polyamines in different bacteria that exist in different environments.

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