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Biophysical Characterization of the Influence of Salt on Tetrameric SecB

Carien Dekker,* Bogos Agianian,† Martin Weik,† Giuseppe Zaccai,‡§ Jan Kroon,† Piet Gros,† and Ben de Kruijff*

*Department Biochemistry of Membranes, Institute of Biomembranes, Bijvoet Center for Biomolecular Research, Utrecht University, Padualaan 8, NL-3584 CH Utrecht, The Netherlands; †Department Crystal and Structural Chemistry, Bijvoet Center for Biomolecular Research, Utrecht University, Padualaan 8, NL-3584 CH Utrecht, The Netherlands; ‡Institut Laue Langevin, F-38042 Grenoble Cedex 9, France; §Institut de Biologie Structurale CEA-CNRS, F-38027 Grenoble Cedex 1, France

ABSTRACT SecB is a tetrameric chaperone, with a monomeric molecular mass of 17 kDa, that is involved in protein translocation in *Escherichia coli*. It has been hypothesized that SecB undergoes a conformational change as a function of the salt concentration. To gain more insight into the salt-dependent behavior of SecB, we studied the protein in solution by dynamic light scattering, size exclusion chromatography, analytical ultracentrifugation, and small angle neutron scattering. The results clearly demonstrate the large influence of the salt concentration on the behavior of SecB. At high salt concentration, SecB is a non-spherical protein with a radius of gyration of 3.4 nm. At low salt concentration the hydrodynamic radius of the protein is apparently decreased, whereas the ratio of the frictional coefficients is increased. The protein solution behaves in a non-ideal way at low salt concentrations, as was shown by the analytical ultracentrifugation data and a pronounced interparticle effect observed by small angle neutron scattering. A possible explanation is a change in surface charge distribution dependent on the salt concentration in the solvent. We summarize our data in a model for the salt-dependent conformation of tetrameric SecB.

INTRODUCTION

SecB is a cytosolic chaperone of *Escherichia coli* (Kumamoto, 1991). It is involved in protein translocation via the Sec-machinery (Driessen et al., 1998). SecB is able to keep precursor proteins translocation competent, while preventing them from aggregation (Breukink et al., 1992b; Collier et al., 1988; Kumamoto, 1990). The affinity of SecB for the ATPase SecA assures the targeting of precursor proteins to the multi-subunit translocation complex at the inner membrane (Breukink et al., 1995; Fekkes et al., 1998). SecB is highly specific toward a subset of precursor proteins that do not share a common recognition sequence (Randall and Hardy, 1995). Rather, a nine-residue pattern of aromatic and positively charged residues seems sufficient to bind to SecB (Knoblauch et al., 1999). The high substrate specificity is then most likely achieved by the ability of the chaperone to bind at multiple sites on the precursor, establishing tight interactions (Randall et al., 1998). Hardly anything is known about the tertiary or quaternary structure of SecB. SecB, with a monomeric mass of 17 kDa (Weiss et al., 1988), is active as a tetramer (Kumamoto et al., 1989). The tetramer binds in a one to one stoichiometry to substrates (Randall et al., 1998). Size exclusion chromatography (Kumamoto et al., 1989) and dynamic light scattering experiments (den Blaauwen et al., 1997) have shown a higher apparent molecular weight than expected based on the amino acid sequence. This revealed either that tetrameric SecB is non-globular, or that its subunits are loosely packed. The latter could result in a spherical but hollow tetramer. Shape asymmetry of the tetramer has been shown by anisotropy spectroscopy (den Blaauwen et al., 1997). We have crystallized the protein (Dekker et al., 1999), but its three-dimensional structure has not yet been solved.

It has been shown that the C-terminus of SecB can be cleaved at low salt conditions, whereas it is protease resistant at high salt concentrations (Randall, 1992). Furthermore, the affinity of SecB for a hydrophobic probe was increased upon increasing the salt concentration. This led to the hypothesis of a salt-dependent conformational change of the protein. Dynamic light scattering (DLS) experiments have also indicated that the hydrodynamic radius of the oligomer is dependent on the ionic concentration (den Blaauwen et al., 1997). It has been suggested that tetrameric SecB is a dimer of dimers, based on dissociation into dimers during electrospray ionisation mass spectrometry (ESI-MS) experiments (Smith et al., 1996). Recently, mutational studies together with size exclusion chromatography have revealed an altered equilibrium between dimeric and tetrameric SecB; one cysteine-mutant did not form tetramers at all (Muren et al., 1999). Except for the ESI-MS experiments at high pH or high inlet temperature, dissociation into dimers of tetrameric wild-type SecB has never been reported, not even at very low protein concentrations (Schonfeld and Behlke, 1998). Although there is some evidence for conformational variability in the protein, this hypothesis has never been tested by detailed biophysical studies. To gain more insight into the salt-dependent behavior of SecB, we studied the protein by DLS, size exclusion chromatography, analytical ultracentrifugation, and small angle neutron scattering (SANS).

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Address reprint requests to Dr. Carien Dekker National Institute for Medical Research, Div. Parasitology, The Ridgeway, Mill Hill, London, NW7 1AA, United Kingdom. Tel.: 442-0895-93666213; Fax: 442-0891-38593; E-mail: cdekker@nimr.mrc.ac.uk.

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MATERIALS AND METHODS

Materials

Bovine serum albumin (BSA, 98%), and α-lactalbumin were purchased from Sigma (Zwijndrecht, NL), soybean trypsin inhibitor was purchased from Merck (Dorset, UK). SecB was overexpressed and purified as described previously (Dekker et al., 1999). The protein was stored in 5 or 10 mM Tris (pH 7.5) at concentrations ranging from 2 to 10 mg/ml. SecA was purified as described (Breukink et al., 1992a).

DLS experiments

The protein was diluted in buffer to 2.0 mg/ml and passed through a 0.1 μm filter (Whatman, Maidstone, UK). Samples of 12 μl were measured in a DynaPro-801 (ProteinSolutions, Charlottesville, VA) both at 4°C and 20°C. From the measured translational diffusion coefficient D_T, the hydrodynamic radius R_H can be calculated using the Stokes-Einstein relation

$$D_T = k_B T/6\pi\eta R_H \quad (1)$$

with the Boltzmann constant k_B, the temperature T in Kelvin and η being the viscosity of the solvent. Molecular masses are estimated from R_H using an empirically derived relationship between R_H and molecular masses for a number of well-characterized globular proteins.

Values for D_T reported are statistical averages over at least 20 independent measurements

Gel filtration chromatography

All gel filtration experiments were carried out at room temperature on a prepacked Superose 6 10/30 column (Pharmacia, Uppsala, Sweden), calibrated with the specified elution buffer, and a flow rate of 0.1 ml/min. The sample volume applied to the column was 100 μl. The column was calibrated using SecA, BSA, soybean trypsin inhibitor, and α-lactalbumin as molecular weight markers. Protein was detected by absorbance at 220 nm, after correcting for buffer contributions.

Analytical ultracentrifugation

Sedimentation velocity and equilibrium experiments were carried out using a Beckman Optima XL-A equipped with absorbance optics. A 3.0-mm double sector centerpiece was used with protein sample volumes of 45 μl. All experiments were performed at 4°C. Absorbance was measured at 290 nm. Rotor speeds were 8500, 11500, and 15000 rpm in the case of equilibrium sedimentation, and 42000 rpm for the sedimentation velocity experiments. Sedimentation velocity experiments were performed using protein concentrations of 0.1 mg/ml, 0.5 mg/ml, and 1.0 mg/ml at 4°C. Velocity data were analyzed using the program UltraScan (Demeler and Saber, 1998), which determines the sedimentation coefficient s using the second-moment method or the van Holde-Weischet method. The obtained s-values were converted to the commonly reported $s_{20,w}$, corrected for water at 20°C according to

$$S_{20,w} = s(1 - \bar{\nu}\rho)_{20,w}\eta_{T,b}/(1 - \bar{\nu}\rho)_{T,b}\eta_{20,w} \quad (2)$$

with η being the viscosity of water at 20°C, or the viscosity of buffer b at temperature T, $\bar{\nu}$ the partial specific volume of the protein, and ρ the density of the solution. The molecular mass M in $gmol^{-1}$ of the species was calculated by

$$M = {}^0s_{20,w}RT/D(1 - \bar{\nu}\rho_w) \quad (3)$$

where D is the diffusion coefficient, R is the gas constant, and T is the temperature. The ${}^0s_{20,W}$ is now the value of $s_{20,W}$ extrapolated to zero protein concentration.

Sedimentation equilibrium experiments were performed using protein concentrations of 0.2 mg/ml, 0.5 mg/ml, and 1.0 mg/ml at 4°C. The equilibrium data were analyzed with the program NONLIN (Johnson et al., 1981). This program is able to correct for non-ideality by incorporating a value for the second virial coefficient B. The nine data sets from the three concentrations at three speeds were fit globally while keeping the baselines of each curve fixed. Masses were calculated from σ, the reduced molecular weight, using

$$\sigma = M * (1 - \bar{\nu}\rho)\omega^2/RT \quad (4)$$

where ω is the radial speed in rad/s, $\bar{\nu} = 0.72\ cm^3g^{-1}$ for SecB (Schonfeld and Behlke, 1998), and buffer densities as calculated by the program Sednterp (Laue et al., 1992). Knowing the molar mass and the sedimentation coefficient, the frictional coefficient f was calculated from

$$f = M(1 - \bar{\nu}\rho)/{}^0s_{20,w}N_{av} \quad (5)$$

where N_{av} is Avogadro's number

Shape asymmetry can be deduced from the ratio f/f_0, where f_0 is the frictional coefficient of a rigid sphere with the same M and the Stokes radius R_S,

$$R_S = (3M\bar{\nu}/4\pi N_{av})^{1/3} \quad (6)$$

f_0 can be calculated via the Stokes equation

$$f_0 = 6\pi\eta R_S \quad (7)$$

Additional information concerning molecular asymmetry can be deduced from the ratio of the hydrodynamic radius over the Stokes radius, providing a measure of the solvation volume via

$$R_H/R_S = (\bar{\nu} + \delta\bar{\nu}_0/\bar{\nu})^{1/3} \quad (8)$$

where $\bar{\nu}$ is the volume of the solvation shell (Cantor and Schimmel, 1980)

SANS

SANS experiments were performed at the Institut Laue Langevin (ILL), instrument D11, in Grenoble (http://www.ill.fr/). Sample volumes of 150 μl were measured in 0.100 cm path length quartz cuvettes (Hellma, Müllheim, Germany). All measurements were done at room temperature. Heavy water solutions are often used for better signal to noise ratio's in neutron scattering experiments. However, to exclude additional effects due to possibly reduced solubility of the protein in D_2O, all measurements were carried out using H_2O buffered solutions. Data were reduced with the ILL purpose-designed programs RNILS and SPOLLY. Data were analyzed by Guinier-analysis, giving the relation between scattering intensity $I(Q)$ and radius of gyration R_G

$$\ln(I(Q)) = \ln(I(0)) - \tfrac{1}{3}R_G{}^2Q^2 \quad (9)$$

where $Q = 4\pi \sin\theta/\lambda$, 2θ the scattering angle and λ the neutron wavelength. $I(0)$ and $R_G{}^2$ are calculated from $(\ln(I(Q))$ versus Q^2, the approximation is valid for $R_GQ \leq 1$ (e.g., review by Zaccai and Jacrot, 1983). In the case of an ideal monodisperse solution the molar mass can be determined from $I(0)$ and the protein concentration (Jacrot and Zaccai, 1981), whereas the radius of gyration of a single particle is determined from the slope of the Guinier plot. The molar mass M and the radius of gyration are thus determined independently. If there is a repulsive interparticle effect, M and R_G are underestimated from the plot. An attractive interparticle effect leads to higher apparent values for both parameters. Normally, correct values can

be obtained from extrapolation of the measured $I(0)$ and R_G^2 to zero protein concentration (Zaccai and Jacrot, 1983).

RESULTS

To get a first indication of the salt-dependent behavior of SecB in solution, we measured the protein by DLS. We selected two extreme conditions: one in the absence and one in the presence of 100 mM NaCl. The results are shown in Table 1.

In the absence of salt, the measured diffusion coefficient is higher than it is in the presence of salt, corresponding to a smaller radius of hydration. The measured polydispersities are similar in both situations. Samples were also measured at 4°C, resulting in hydrodynamic radii that were in all cases ~0.1 nm larger than at 20°C (results not shown). No significant differences were observed when using 5 mM instead of 10 mM Tris (pH 7.5; results not shown). The molecular mass, as estimated from the hydrodynamic radius, is an average value, assuming a globular protein model, and will be influenced by the heterogeneity of the sample. The difference of one unit in diffusion coefficient between the samples with and without NaCl, as listed in Table 1, might indicate the presence of lower order oligomeric species in the absence of salt. This should then be revealed by size exclusion chromatography. We conducted gel filtration chromatography using a high and a low salt elution buffer. The resulting peak profiles for SecB at both conditions are shown in Fig. 1. The peak for SecB in the presence of salt (Fig. 1 *A*) is symmetrical, whereas in the absence of salt the peak shows a slight shoulder on the right side (Fig. 1 *B*), indicating possible heterogeneity to a minor extent. The elution profiles resulting from the gel filtration experiments are shown as a function of the logarithm of the molecular mass in Fig. 2. The absolute elution volumes varied with the salt concentration. Fig. 2 *A* shows that in the presence of salt SecB elutes as a 100-kDa globular particle. As can be seen from Fig. 2 *B*, SecB is the only protein showing a relative shift in apparent molecular weight, from 100 to 60 kDa, upon changing the elution buffer. Both DLS and gel filtration experiments illustrate the influence of salt on the diffusion coefficient of SecB.

A possible explanation for these observations is a change in molecular mass, which in the case of SecB would indicate a change in oligomeric state. Therefore, we analyzed the protein by analytical ultracentrifugation (AUC) in the presence and absence of salt. The results of second-moment analysis of the sedimentation velocity experiments are shown in Table 2 and Fig. 3. The presence of salt leads to a higher ${}^0s_{20,W}$. As can be concluded from Fig. 3, there is a concentration dependence of the sedimentation coefficients, resulting in lower s-values at higher protein concentrations. The same trend was observed when analyzing the data by the van Holde-Weischet method (results not shown). The concentration dependence of the *s*-values can be due to excluded-volume effects, molecular asymmetry or charge effects. Fig. 3 shows a higher negative slope for the Tris-situation (−1.23) than for the Tris-NaCl situation (−0.99), that cannot be solely explained by volume-exclusion effects. Knowledge of the sedimentation coefficient and the diffusion coefficient, as determined by DLS, allows an estimation of the molecular mass, as is presented in Table 2 as well. The same trend as in Table 1 is observed, namely a lower apparent mass in the absence of salt.

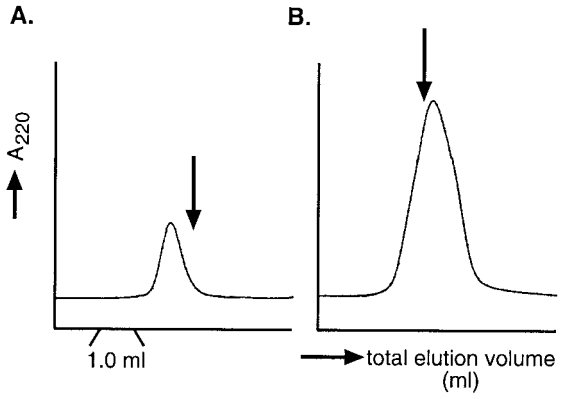

FIGURE 1 Peak profiles in gel filtration chromatographs of 0.2 mg/ml SecB eluted with 10 mM Tris pH 7.5, 100 mM NaCl (*A*); and 0.5 mg/ml SecB eluted with 10 mM Tris pH 7.5 (*B*). The arrows indicate the positions of the peak of monomeric BSA eluted with the corresponding buffers.

More accurate estimates of the molecular mass of the species, irrespective of the shape of the protein, is provided by sedimentation equilibrium experiments, that were done subsequently. In all cases the best fit to the data was obtained by using a single species model. There was no improvement in fit when a monomer-dimer, or monomer-dimer-tetramer model was used. The equilibrium data and the deviations from the global fit are shown in Fig. 4. Fitting of the data obtained at high salt concentration results in a

TABLE 1 Results of dynamic light scattering measurements on SecB

Buffer	D_T* (10^{-7} cm^{2}s^{-1})	R_H† (nm)	Polydispersity (nm)	M (kDa)
10 mM Tris, pH 7.5	5.9 (0.1)	3.6 (0.14)	0.9 (0.06)	66 (6.7)
10 mM Tris, pH 7.5, 100 mM NaCl	4.9 (0.3)	4.3 (0.30)	1.0 (0.25)	99 (17)

SecB concentration was 2 mg/ml, temperature was 20°C.
*Translational diffusion coefficient, standard deviation between brackets.
†Hydrodynamic radius calculated from D_T using Equation 1.

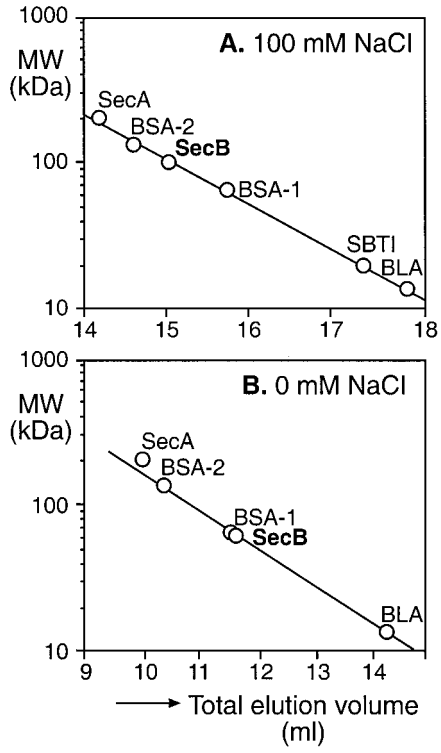

FIGURE 2 Gel filtration analysis of SecB. (*A*) Elution buffer 10 mM Tris pH 7.5, 100 mM NaCl. (*B*) Elution buffer 10 mM Tris pH 7.5. BSA-2, dimeric BSA; BSA-1, monomeric BSA.

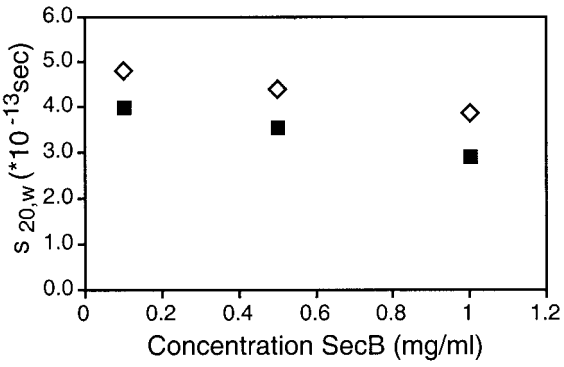

FIGURE 3 Sedimentation coefficients, determined by sedimentation velocity experiments, as a function of protein concentration. SecB in 5 mM Tris pH 7.5 (*solid squares*), and in 5 mM Tris pH 7.5, 100 mM NaCl (*open diamonds*). Error bars are not depicted, because all standard deviations were less than $0.05*10^{-13}$.

good fit with randomly distributed residuals (Fig. 4 *A*). A good fit of the data obtained at low salt concentration, however, was impossible, resulting in obviously non-random deviations from the fit (Fig. 4 *B*). The results of global fitting and the calculated apparent molecular masses are presented in Table 3. The apparent molecular mass as determined for the high salt condition was 70.17 kDa, that is extremely close to the theoretical mass of tetrameric SecB based on its amino acid sequence (68.76 kDa). Global fits having M (Eq. 4) fixed to the theoretical value of the tetramer resulted in a low negative value for B, indicating that the protein is behaving ideal in solution. The results of the fit to the data obtained at low salt concentration are also given in Table 3. Although the apparent molecular mass obtained (45.78 kDa) suggests the presence of dimeric SecB (34.38 kDa in theory), the twofold increase in variance and residuals with respect to the data obtained in the presence of salt, and the non-randomness of the deviations from the fit, indicate that this is not the case. The concomitant high negative value of the virial coefficient obtained when fixing M to the tetramer mass of SecB, as reported in Table 3, is therefore very unreliable. The data suggests non-ideality of SecB in the absence of salt, as was already indicated by the sedimentation velocity data. However, it is not clear that this can be accounted for by a change in virial coefficient between the low and high salt condition. When analyzing the apparent weight averaged molecular masses derived from the different AUC-experiments as a function of concentration or speed, there is no trend observed for the high salt data, whereas there is a concentration dependency observed for the low salt equilibrium data (results not shown). For the latter, the weight averaged masses tend to be lower at higher protein concentration, reinforcing the idea of non-ideal behavior of the protein at low salt concentrations.

TABLE 2 Sedimentation coefficients for SecB

Buffer	${}^0s_{20,W}$ ($*10^{-13}$ sec)*	M (kDa)†
5 mM Tris, pH 7.5	4.1	62.1
5 mM Tris, pH 7.5, 100 mM NaCl	4.9	89.9

*Sedimentation coefficient for water at 20°C, extrapolated to zero protein concentration.
†Molar mass, calculated using Eq. 3 and D_T from Table 1.

Combining the results of DLS and AUC allows the calculation of frictional coefficient ratios and solvated volumes of the protein. These values are listed in Table 4. For both high and low salt conditions, the f/f_0 ratio is >1, indicating that SecB is asymmetric. The solvation volume is much smaller in the absence of salt with respect to the high salt situation.

The AUC-experiments in the absence of salt could not provide reliable mass estimates due to the large non-linearity. To provide an alternative characterization of the oligomeric state at low salt concentrations, we conducted SANS experiments. The Guinier-plots for two typical experiments are shown in Fig. 5. The resulting radius of gyration and the molar mass, based on the extrapolated scattering intensity at zero angle, are given in Table 5. For the protein sample containing 100 mM NaCl, the resulting R_G of 3.4 nm and a molar mass of 82 kDa are fully consistent with the results obtained by gel filtration, DLS and AUC at high ionic strength. However, when salt is absent from the buffer, the

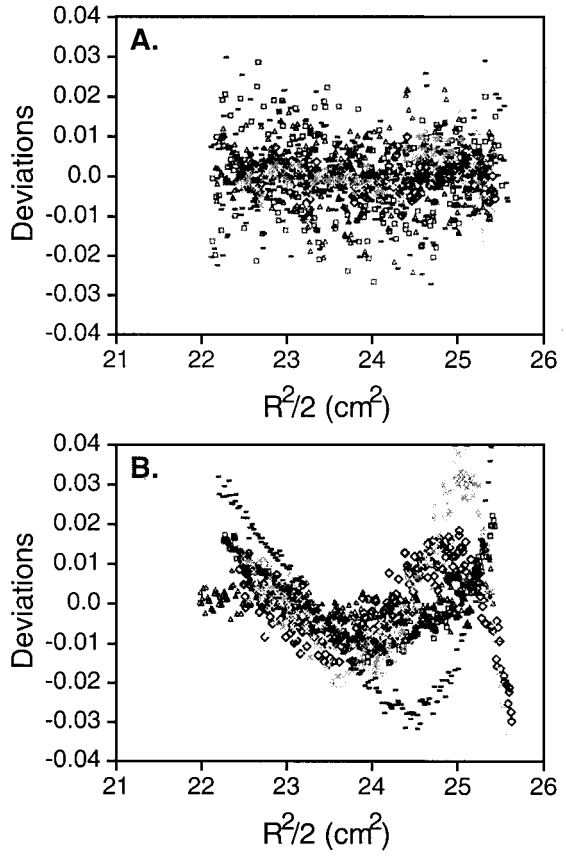

FIGURE 4 Results of sedimentation equilibrium analysis of SecB in 5 mM Tris pH 7.5, 100 mM NaCl (*A*); and in 5 mM Tris pH 7.5 (*B*). Shown are the deviations from the fit, where in both panels the symbols correspond to 1.0 mg/ml protein at 11500 rpm (*open diamonds*); 0.5 mg/ml at 11500 rpm (*open squares*); 0.2 mg/ml at 11500 (*open triangles*); 1.0 mg/ml at 15000 rpm (*cross*); and 0.5 mg/ml at 8500 rpm (minus).

Guinier plot is clearly deviating from the straight line at low Q^2 values (Fig. 5 *B*). Because the deviations are only occurring in the region for very small angles, we estimated

TABLE 3 Results of global fitting of sedimentation equilibrium data

Buffer*	*M* (kDa)	Variance (*10^{-5})	Σ (residuals)^2	B† (ml/mg)
Tris	45.78	23.4	0.475	
	68.76	51.2	1.035	0.243
Tris-NaCl	70.17	6.35	0.149	
	68.76	6.36	0.149	−0.03

Results of global fitting as determined by the program NONLIN using a single species model. Measurements done at 4°C using 0.2, 0.5 and 1.0 mg/ml SecB at 8,500, 11,500, and 15,000 rpm.
*Tris: 5 mM Tris, pH 7.5; Tris-NaCl: 5 mM Tris, pH 7.5, 100 mM NaCl.
†Virial coefficient as reported by NONLIN. This is the colligative virial coefficient multiplied by the molecular weight (Johnson et al., 1981). When no B is reported, the B was not refined. Upon refining B, the reduced molecular weigth σ, and thus *M* (see Eq. 4), was fixed at the value corresponding to the tetrameric state of SecB.

TABLE 4 Shape parameters for SecB

Buffer*	f/f_0†	R_H/R_S‡	$\delta\bar{\nu}_0$ (ml/g)
Tris	1.49	1.34	1.03
Tris-NaCl	1.22	1.60	2.25

*Tris: 5/10 mM Tris, pH 7.5; Tris-NaCl: 5/10 mM Tris, pH 7.5, 100 mM NaCl.
†Ratio of the frictional coefficients, calculated using Eqs. 5 and 7.
‡Calculated using Eq. 8.

the radius of gyration from the remaining part of the data, although this is not strictly within the Guinier region anymore. The resulting value for R_G is 30 Å, which is comparable to the radius of gyration measured at high salt concentration.

However, the extrapolation of the fit to yield the intercept, determining the molar mass, could not be determined accurately because of the deviating data. The deviations from the fit in the Giunier plot are most likely the result of interparticle effects. This effect was most pronounced at the highest protein concentration measured. It decreased with decreasing concentration, but was still present at a concentration as low as 1.4 mg/ml (results not shown), that is the lower limit for neutron scattering experiments. Both in the presence or absence of salt a similar concentration depen-

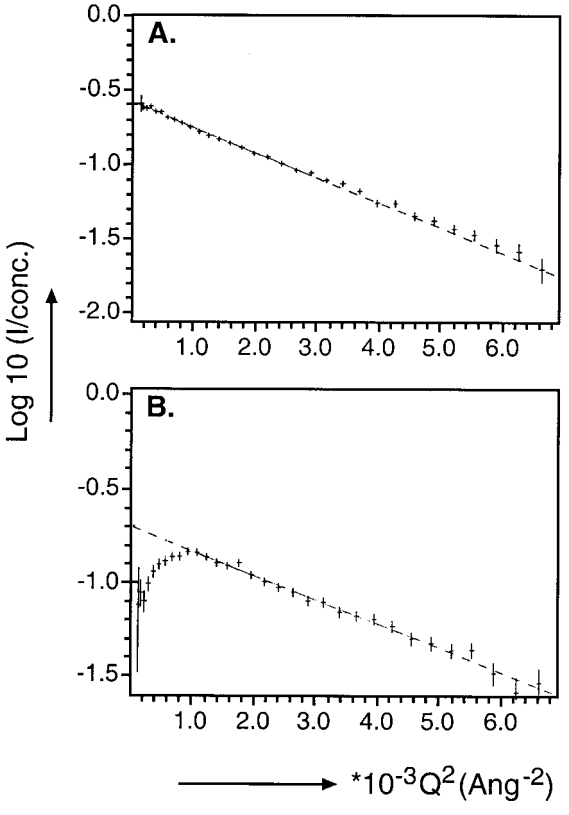

FIGURE 5 Guinier plot for two typical SANS-experiments. (*A*) 4.7 mg/ml SecB in 10 mM Tris pH 7.5, 100 mM NaCl. (*B*) 7.2 mg/ml SecB in 10 mM Tris pH 7.5. The fit is shown as a *dashed line*.

TABLE 5 Results of Guinier analysis of SANS Data

Buffer	Concentration of SecB (mg/ml)	$\langle R_G \rangle$ (nm)*	${}^0R_G$ (nm)†	M‡ (kDa)	Comments
10 mM Tris, pH 7.5	7.16, 3.45, 2.37	2.98 (±0.23)	2.97	47 (±14)	Interparticle effect
10 mM Tris, pH 7.5, 100 mM NaCl	7.55, 4.66	3.36 (±0.59)	3.40	82 (±16)	

*Radius of gyration, averaged value for all concentrations.
†Radius of gyration extrapolated to zero protein concentration.
‡Molar mass as deduced from the extrapolated value of I_0.

dence of R_G was observed (results not shown), leading to a slightly lower R_G with increasing protein concentration.

DISCUSSION

Influence of salt on biophysical parameters

The results obtained by DLS, gel filtration chromatography, AUC, and SANS clearly demonstrate the influence of the salt concentration on the behavior of SecB in solution. At high NaCl concentration, SANS and AUC data show that the protein is a stable tetramer with a radius of gyration of 3.4 nm, and a molar mass of 70.2 g/mol, that fits with the theoretical mass of 68.8 g/mol. There is no detectable fraction of lower or higher order oligomers, nor is there an indication of major aggregation. In the presence of salt the tetramer has a frictional ratio (f/f_0) of 1.22, revealing that tetrameric SecB is non-spherical. A non-spherical tetramer is in agreement with the radius of gyration of 3.4 nm for a 69-kDa protein. The radius of gyration for a globular protein of this mass is much lower, e.g., Hemoglobin of 64 kDa has a radius of gyration of 2.3 nm. Furthermore, non-sphericity explains partly the concentration dependence observed by sedimentation velocity experiments.

In the absence of salt the R_G of 3.0 nm as determined by SANS is a bit smaller than in the presence of salt (3.4 nm), but because of deviations in the Guinier plot we cannot speculate about those small differences. The radii of gyration obtained at high and low salt concentration by SANS are very similar, and are also comparable to the hydrodynamic radii as obtained by DLS. Compared to the salt condition, the apparent hydrodynamic radius of SecB is lower in the absence of salt. Earlier reported data (den Blaauwen et al., 1997) indicated a decrease in apparent hydrodynamic radius upon decreasing the salt concentration, although many more additives were present in those experiments. Our DLS data show a decrease in R_H as well, from 4.3 to 3.6 nm, that is now entirely due to the absence of 100 mM NaCl. In the absence of salt, the sedimentation coefficient of SecB is lower, resulting in a higher ratio of the frictional coefficients of 1.49 compared to the salt condition (1.22). Furthermore, the concentration dependence of the sedimentation coefficients is more pronounced in the absence of salt, suggesting that it is influenced by more factors than solely shape asymmetry or excluded volume effects.

Non-ideality

Both sedimentation velocity and equilibrium experiments indicate that SecB is behaving non-ideally in the absence of salt. Non-ideal behavior thus seems a likely explanation for the bad fit to the equilibrium data obtained at low salt concentrations. Non-ideality can be explained in terms of excluded volume or charge-charge interactions. Although the program we used to analyze the AUC-data is able to account for a certain amount of non-ideality, the non-ideality in the case of SecB in the absence of salt is too large to correct for. The results of SANS show deviations from the straight line in the Guinier plot that clearly demonstrate the non-ideal behavior of the protein in the absence of salt. This aberrant behavior is referred to as interparticle effect. It means that the data can not be interpreted in terms of single particles that do not interact, which is the basic assumption of the Guinier analysis in small angle scattering. The occurrence of such an interaction can again be explained in terms of excluded volume, leading to a non-random distribution of molecules, or in terms of charge-charge interactions. Interparticle effects were observed for SecB even at a concentration as low as 1.4 mg/ml. SecB is highly negatively charged at the pH of study, having a pI = 3.95 to 4.0 (Weiss et al., 1988), and in the absence of salt charge-charge interactions might dominate, leading to the here described interparticle effects.

Because the protein reveals a concentration dependent behavior in the absence as well as in the presence of salt (AUC and SANS), the observed differences cannot be solely explained by a change in virial coefficient. Therefore the assumption of a salt dependent conformational change is the most likely. The occurrence of a conformational change upon changing the ionic strength has been shown by Randall (1992). Such a conformational change offers as well a possible explanation for the differences in hydrodynamic radius as measured by DLS at high and low salt concentration.

The shape parameters reveal that SecB is non-spherical. Non-sphericity may also explain why in the presence of salt the protein elutes on a gel filtration column as a 100-kDa particle. The shift in elution volume of SecB upon lowering the salt concentration is very pronounced. This difference in apparent molecular weight of SecB may have several causes. First, it might reflect a change in true molecular

weight, e.g., by dissociation of the tetramer into lower order oligomers, which is highly unlikely given the results of AUC and SANS. Second, it might be caused by a change in specific affinity of SecB for the column at different ionic strength. For example, when SecB exposes more hydrophobic residues, hydrophobic interactions of the protein with the column support will lead to elution volumes that are too high, and consequently yield molecular masses that are underestimated. This seems not likely in the case of SecB, especially because a decrease in hydrophobic surface area on SecB has been postulated to occur upon lowering the ionic strength (Randall, 1992). Finally, it might reflect a change in conformation, leading to a different retention time. By using other techniques, it has been shown that a conformational change takes place upon increasing the salt concentration (Fasman et al., 1995; Randall, 1992), upon which the SecB C-terminus becomes protease-resistant.

Putative model

Our data show that SecB can exist in two states with distinct biophysical parameters. The ratio of the frictional coefficients for SecB is >1 both in the presence and absence of salt. In the absence of salt this ratio is even larger than in the presence of salt. In contradistinction with this, the ratio of the hydrodynamic radius over the Stokes radius is lower in the absence of salt, implying a smaller solvation volume. In both situations, however, the radius of gyration remains more or less the same. This could be explained as follows: in the presence of salt the protein is allowed to adopt an open or rather swollen conformation with a relatively large hydration shell. In the absence of salt a lack of counter-ions will diminish the need for a large solvation volume. Because it has been reported that SecB decreases its accessible hydrophobic surface upon lowering the ionic strength (Randall, 1992), we can speculate on a model for the salt-dependence of SecB. SecB may consist of four monomers, each having a hydrophobic region (Fig. 6). In the presence of salt, these hydrophobic regions are partly exposed, possibly to become involved in substrate binding (Randall, 1992). The negatively charged regions on the protein are screened by counter-ions. In the absence of counter-ions, the monomers putatively move or rotate with respect to each other, possibly as a consequence of charge proximity. Due to this rotation, the hydrophobic patches are facing each other and hydrophobic interactions result in a more compact tetramer than in the case of electrostatic interactions. In this model, a rotation of the monomers does not significantly affect the radius of gyration of SecB, but decreases the solvation volume while concomitantly increasing the ratio of the frictional coefficient.

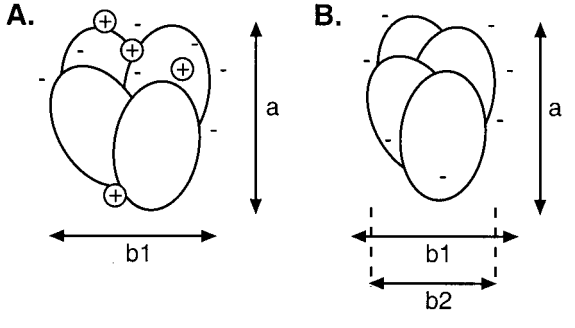

FIGURE 6 Model for the salt dependent conformation of tetrameric SecB. (*A*) At 100 mM NaCl counter-ions are available to allow the protein to adopt a swollen conformation. (*B*) At 0 mM NaCl, hydrophobic stretches facing each other establish tight interactions, resulting in a more compressed tetramer. Because $b2 < b1$, the axial ratio a/b is larger for the 0 mM NaCl situation, resulting in a higher ratio of frictional coefficients, whereas the solvation shell has decreased.

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