

Nucleotide and negatively charged lipid-dependent vesicle aggregation caused by SecA

Evidence that SecA contains two lipid-binding sites

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Received 7 July 1993; revised version received 2 August 1993

SecA which is an overall acidic protein was found to induce an increase in the turbidity of a solution of vesicles consisting of negatively charged phospholipids. This increase was found to be due to an aggregation of the vesicles mediated by SecA. The SecA-mediated vesicle aggregation was not found for zwitterionic 1,2-dioleoyl-*sn*-glycero-3-phosphocholine and showed a large dependence on both temperature and ionic strength. Furthermore it was shown that ATP and to a lesser extent ADP+P_i were able to reduce the SecA-mediated vesicle aggregation, while no effect could be seen for a non-hydrolysable ATP analog AMP-PNP. Using the steady state fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene present in 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol vesicles we could show that SecA inserts in the bilayer. Monolayer studies confirmed that SecA is able to cause close contact between two membranes and gave a direct insight into the different types of lipid–protein interactions involved. From our results we propose that the SecA monomer possesses two lipid-binding sites which in the functional dimer conformation are responsible for the SecA-mediated vesicle aggregation.

Protein translocation; SecA; Vesicle aggregation; Model membrane; Lipid packing

1. INTRODUCTION

The SecA protein plays a central role in the translocation of preproteins across the inner membrane of *E. coli*. It is believed to couple the hydrolysis of ATP to the translocation of a precursor [1]. Furthermore its interaction with several components involved in the translocation process has been reported, the SecB protein [2], the SecY protein [3], and the negatively charged lipids [4–6]. That this interaction of SecA with negatively charged lipids is also important *in vivo* is indicated by the study of Kusters et al. [7] who found an increase in the amount of SecA upon depletion of the negatively charged lipids. We have previously reported that SecA can insert into a phospholipid monolayer [6], this insertion was enhanced by negatively charged lipids and modulated by binding and hydrolysis of ATP. These data resulted in a binding, insertion, deinsertion model by which SecA may facilitate the translocation of precursor proteins upon binding and hydrolysis of ATP. Recently Ulbrandt et al. showed that SecA could insert into the bilayer of lipid vesicles as shown by fluores-

cence quenching experiments on the intrinsic tryptophan fluorescence of SecA [8].

Upon studying the interaction of SecA with lipid vesicles we observed under certain conditions an increase in the turbidity of the vesicle solution upon addition of SecA. We now report on this increase in turbidity, which is due to SecA-induced vesicle aggregation. This vesicle aggregation was greatly dependent on the presence of negatively charged lipids and modulated by ATP binding and ATP hydrolysis, and is best explained by the existence of two lipid-binding sites on the SecA monomer.

2. MATERIALS AND METHODS

2.1. Materials

SecA was purified as described and stored as a 3 mg/ml stock solution in 50 mM Tris-HCl, pH 7.6, 10% (w/v) glycerol, 1 mM dithiothreitol at –80 °C [6]. Protein concentrations were assayed according to Bradford (1976) using bovine serum albumin as a standard. 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol (DOPG) were purchased from Avanti Polar Lipids. ¹⁴C-labeled 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol was synthesised as described [9]. Phospholipid was determined as inorganic phosphate after destruction with perchloric acid [10]. Large unilamellar vesicles (LUVs) with an estimated diameter of 400 nm were made by extrusion in 50 mM Tris-HCl, pH 7.6, 100 mM NaCl [11]. PhoE-signalpeptide was obtained and treated as described [12]. Proteinase K was purchased from Boehringer. AMP-PNP, ADP and ATP were purchased from Sigma. All other chemicals were of analytical grade or better.

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Abbreviations: pmsf, phenylmethylsulfonylfluoride; LUV, large unilamellar vesicle; DPH, 1,6-diphenyl-1,3,5-hexatriene; DOPG, 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine.

2.2. Turbidity measurements

SecA (30 μ g) was added to 1 ml of buffer (50 mM Tris-HCl, pH 7.6, 100 mM NaCl) containing the indicated amount of LUVs under continuous stirring with a magnetic bar. Nucleotides and $MgCl_2$ (final concentrations for both, 2 mM) were added to the vesicle solution before SecA addition. The increase in turbidity in a total volume of 1 ml was measured with a Hitachi U3200 spectrophotometer at 400 nm using a 10 mm cuvet. Unless stated otherwise the measurements were performed at room temperature.

2.3. Carboxyfluorescein (CF) leakage assay

The leakage assay was essentially performed as described [13] following the same protocol as was used for the turbidity measurements.

2.4. Steady-state fluorescence anisotropy

The determination of the fluorescence anisotropy was essentially performed as described [14]. 1,6-diphenyl-1,3,5-hexatriene (DPH) was added to 25 μ M DOPG LUVs in a 1:250 molar ratio. DPH fluorescence was measured on a SPF 500 C spectrophotometer (SLM instruments Inc., Urbana, IL, USA), with an excitation wavelength of 360 nm and an emission wavelength of 430 nm. Emission and excitation slit widths were 5 nm. SecA or the signal peptide of prePhoE were titrated to a DPH-containing lipid solution and the anisotropy ratio (A) of each sample was calculated according to the equation.

$$A = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + 2I_{\perp})^{-1}$$

Samples were continuously stirred and measured at room temperature. Each presented ratio is the average of at least four different experiments.

2.5. Monolayer experiments

Monolayer surface pressure and surface radioactivity were measured simultaneously at 25°C as described [6]. The teflon dish had a volume of 12 ml and a surface area of 20.25 cm². The subphase buffers (50 mM Tris-HCl, pH 7.6, supplemented with either 100 mM or 500 mM NaCl) were filtered through a 22 μ m pore filter and degassed prior to use. In a typical experiment 36 μ g SecA was injected in the subphase under a DOPG monolayer at an initial surface pressure of 25 mN/m. After 30 min the excess of SecA present in the subphase was washed away with 10 times the subphase volume. This was followed by the addition of 1 μ mol of ¹⁴C-labeled DOPG LUVs (500 dpm/nmol). After 40 min proteinase K (240 μ g) was added and the measurements were prolonged for another 10 minutes.

3. RESULTS AND DISCUSSION

3.1. SecA causes vesicle aggregation

Addition of SecA to a solution of DOPG vesicles (125 μ M lipid) causes a marked and rapid increase in the turbidity of the solution (Fig. 1). This increase was proportional to the SecA concentration and had a maximum at a concentration of 30 μ g/ml while addition of SecA to the buffer solution without vesicles did not result in an increase in the absorbance at 400 nm (data not shown). The increase in turbidity could in principle arise from either vesicle aggregation or vesicle fusion. To discriminate between these possibilities proteinase K (PK) was added to the solution shortly after the increase in turbidity had reached its maximum value. This resulted in a rapid decrease in the absorbance at 400 nm to near starting level (Fig. 1). Apparently proteolytic digestion of SecA causes the turbidity increase to disappear which strongly argues in favour of SecA-mediated

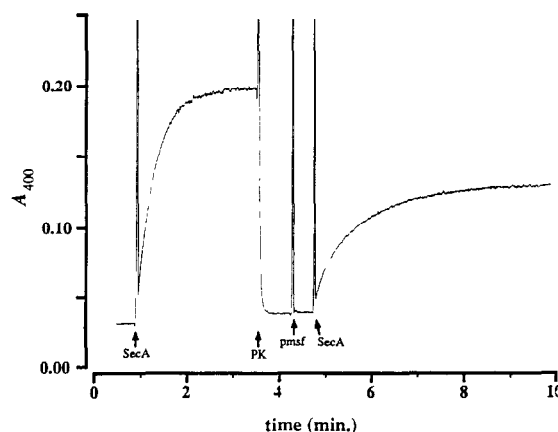


Fig. 1. SecA induced increase of the turbidity of a DOPG vesicle solution. SecA (30 μ g) was added to a DOPG vesicle solution (125 μ M). The increase in the turbidity was followed in time at a wavelength of 400 nm. SecA (30 μ g), Proteinase K (PK, 30 μ g) and pmsf (1.5 mM final concentration) were added at the indicated time points.

vesicle aggregation. To test whether the vesicles were still sensitive to SecA we subsequently added the protease inhibitor pmsf and SecA, which indeed resulted in a second increase in the turbidity to about 50% of the initial value. The observation that the increase is only 50% of the initial value can be explained by the occupation of binding sites on the vesicles by the peptides resulting from the SecA degradation by the protease.

Possible vesicle leakage due to interaction of SecA with the DOPG vesicles was tested using the carboxyfluorescein leakage assay. No increase in the carboxyfluorescein fluorescence could be observed due to SecA addition (data not shown). This demonstrates that the membrane barrier remains intact upon the interaction of SecA with DOPG vesicles as was also observed by Ulbrandt et al. using a different leakage protocol [8].

3.2. Characterisation of the SecA-mediated vesicle aggregation

Lill et al. [4] have shown that SecA rapidly (within 5 min) loses its ATPase activity when incubated in the presence of negatively charged lipids at 40°C. Also the SecA-mediated DOPG vesicle aggregation shows a strong temperature dependency, as shown by a steep decrease of the turbidity increase around 40°C (Fig. 2A). Apparently the conformational change of SecA reflected in the loss of ATPase activity makes it impossible for SecA to cause vesicle aggregation. Ulbrandt et al. [8] showed that SecA in presence of vesicles containing negatively charged lipids displayed another conformational transition around 24°C as was measured by the intrinsic tryptophan fluorescence of SecA. We observed no abrupt change in the SecA-mediated aggregation of DOPG vesicles in the range of 10–30°C. Apparently this conformational change has no influence on the SecA-mediated vesicle aggregation.

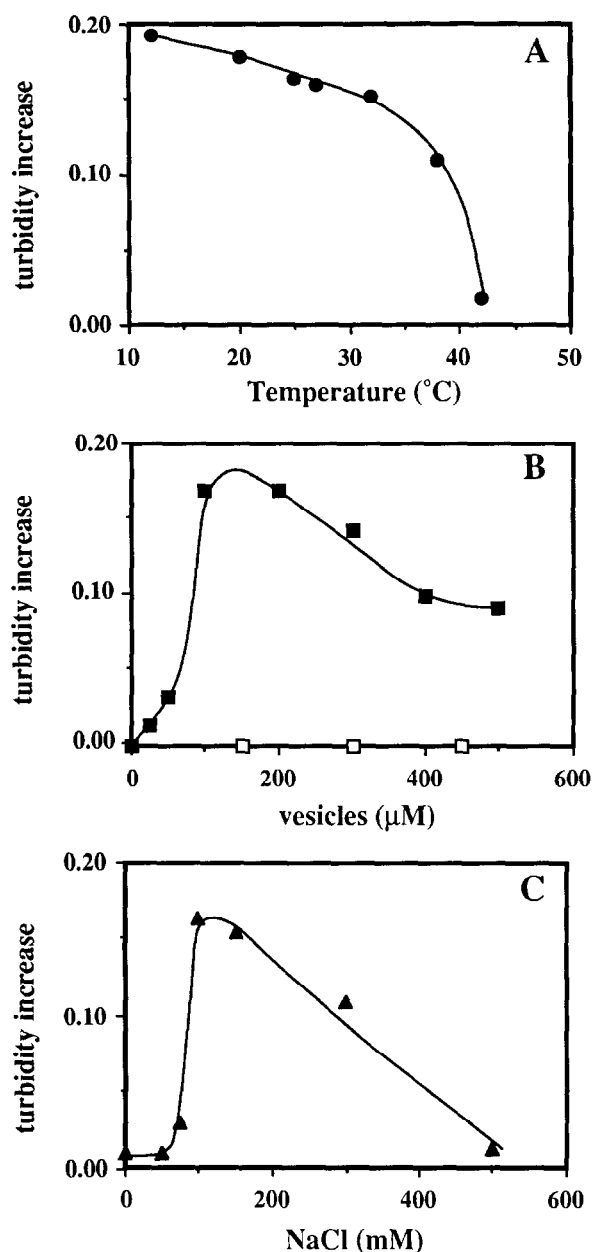


Fig. 2. Characterisation of the SecA-mediated vesicle aggregation. (A) The temperature dependency of the SecA-mediated vesicle aggregation. SecA was added from a stock solution stored on ice to a solution containing 100 μ M DOPG vesicles and the maximal increase in turbidity was determined at the indicated temperature. (B) The SecA-mediated vesicle aggregation as a function of the vesicle concentration. 30 μ g SecA was added to solutions containing indicated amounts of DOPG vesicles (filled squares) or DOPC vesicles (open squares) and the maximal increase in turbidity was determined. (C) The SecA-mediated vesicle aggregation as a function of the ionic strength. 30 μ g SecA was added to a 100 μ M DOPG vesicle solution containing an indicated amount of NaCl and the maximal increase in turbidity was determined. In this case the LUVs were made using a buffer with the same NaCl concentration used for the measurements.

Several studies on the ATPase activity, the protease resistance and the monolayer insertion have revealed that the SecA protein has a preferential interaction with

negatively charged lipids [4–6]. We tested whether the SecA-mediated vesicle aggregation also showed specificity for negatively charged lipids. Fig. 2B shows that indeed the increase of the turbidity caused by SecA can only be detected with negatively charged DOPG vesicles (closed symbols). No increase in the turbidity could be observed with various concentrations of zwitterionic DOPC vesicles (open symbols), demonstrating the negatively charged lipid specificity of this effect. The increase in turbidity shows a substantial increase in the 0–100 μ M range, and is maximal at 150 μ M. At this concentration of vesicles the SecA/lipid molar ratio is 1:400, corresponding to a SecA/vesicle ratio of approximately 1500:1, taken into account that 80% of the phospholipids in the 400 nm LUVs is present in the outermost bilayer [15]. Higher lipid concentrations tend to reduce the turbidity increase probably due to dilution of SecA at the membrane surface.

This preferential interaction of the highly negatively charged SecA protein with negatively charged lipid vesicles can be most easily explained by the presence of positive domains on the SecA molecule. Since SecA causes vesicle aggregation, two lipid-binding domains must be present to cause inter-bilayer contact. Assuming that SecA is active as a dimer [16,17] at least two possible explanations for this effect can be provided. Either the two monomers of SecA in the dimer each possess one lipid-binding site which have different orientations with respect to the membrane such that two vesicles can be bound simultaneously by the dimer, or the two monomers in the dimer have the same orientation towards the membrane but possess two lipid-binding sites per monomer.

Insight into these possibilities can be obtained by studying the ionic strength dependency of the turbidity increase. Fig. 2C shows that the SecA-mediated DOPG vesicle aggregation is highly dependent on the ionic strength of the medium. At low ionic strength no turbidity increase could be observed, followed by a steep increase in turbidity which was maximal at 100 mM NaCl. Such behaviour is expected to be due to an effect of NaCl on the structure of SecA and not due to an effect on the lipid–water interface. This view is supported by the observation that Protease K treatment of SecA at low ionic strength (0 mM NaCl) resulted in a protease-resistant fragment of SecA of approximately 20 kDa which was absent at an ionic strength of 100 mM (data not shown). Further increase of the salt concentration resulted in a decrease in vesicle aggregation such that at an ionic strength of 500 mM virtually no turbidity increase could be observed anymore. This result indicates that the SecA-mediated vesicle aggregation is caused by a predominant electrostatic interaction of SecA with the DOPG vesicles. This behaviour contrasts that of the insertion of SecA in a DOPG monolayer which could not be inhibited by an ionic strength as high as 1.5 M NaCl [6].

Together these results suggest that there are two different binding sites on the monomers in the SecA dimer. One binding site leading to insertion of SecA in the membrane (as revealed by the monolayer experiments) causing the interaction of SecA with this membrane to be more hydrophobic thus insensitive to high ionic strength. The other binding site being responsible for the SecA-mediated vesicle aggregation. This site interacts with the bilayer primarily in an electrostatic manner which can be overcome at high ionic strength.

3.3. SecA causes close contact between a DOPG monolayer and DOPG vesicles

The existence of two lipid-binding sites on the SecA molecule can be directly tested using the monolayer technique and lipid vesicles in the subphase [18]. Fig. 3 shows that injection of SecA underneath a DOPG monolayer results in an increase in surface pressure at subphase salt concentrations of both 100 and 500 mM, in agreement with previous observations [6]. After washing the subphase, injection of radiolabeled DOPG vesicles resulted in a small increase in surface pressure which also occurred upon injection of the vesicles in the absence of SecA (data not shown). The surface radioactivity tracing shows that SecA caused a massive accumulation of vesicles at the interface which effect was more pronounced for the low salt condition. Subsequent addition of proteinase K resulted in an immediate reduction of the surface radioactivity to a level which corresponds to that observed in the absence of SecA (data not shown). This experiment shows that the vesicle-monolayer system behaves very similar to the bulk vesicle system in terms of inter-membrane contact formation, and directly visualizes at the same time the striking different salt dependency for monolayer insertion and inter-membrane contact formation, explaining the existence of two lipid binding sites on the SecA protein. The result furthermore shows that SecA does not cause significant flow of lipids between the two model membranes because this would have resulted in a large increase in surface pressure. It also shows that SecA stably inserts in the monolayer because no major decrease in surface pressure is observed upon proteinase K treatment.

3.4. Effect of Nucleotides on the SecA-mediated vesicle aggregation

ATP binding and hydrolysis by SecA is shown to facilitate protein transport in vitro [1]. The insertion and binding of SecA (in)to DOPG monolayers was also effected by nucleotides [6]. We examined whether the different nucleotides used in the monolayer study also influenced the SecA-mediated DOPG vesicle aggregation. The addition of the nucleotides and $MgCl_2$ itself had no effect on the turbidity of the DOPG vesicle solution (data not shown). Table I shows that ATP causes a reduction of 50% of the increase in turbidity compared

Table I

The effect of nucleotides on the SecA-mediated vesicle aggregation

Nucleotide added ^a	SecA mediated vesicle aggregation (in%) ^b	SecA monolayer insertion (in%) ^c
ATP	49 ± 2	47 ± 6
ADP	73 ± 1	50 ± 6
AMP-PNP	94 ± 2	92 ± 2

^a All nucleotides were tested at a concentration of 2 mM in presence of 2 mM $MgCl_2$.

^b The maximal turbidity increase in absence of nucleotides was taken as 100%.

^c Adapted from [6]. The increase in surface pressure of a DOPG monolayer due to SecA insertion in absence of nucleotides was taken as 100%.

to the increase in turbidity in the absence of nucleotides (which was taken as the 100% value). Comparing the values for the SecA-mediated vesicle aggregation to the values for the SecA monolayer insertion (adapted from [6]), shows that the effects of the different nucleotides are quite similar for both experimental systems. These data support the conclusions made in [6], that the SecA conformation is effected by binding of the different nucleotides. Since ATP lowered the binding of SecA to the DOPG monolayer while in presence of $ADP+P_i$ the binding of SecA was not effected [6], the lower SecA-mediated vesicle aggregation in presence of ATP compared to ADP is best explained by a overall reduced binding of SecA to the DOPG vesicles. From these experiments it is not possible to say which lipid binding site of SecA is effected by ATP.

3.5. Effect of SecA on microviscosity in DOPG vesicles

To analyse whether SecA could effect acylchain packing upon interaction with the bilayer we used the steady-state fluorescence anisotropy of DPH embedded in the DOPG vesicles as a monitor of the order of the fatty acyl chains of the phospholipids [19,20]. Since a turbidity increase could potentially interfere with the fluorescence measurements we chose a vesicle concentration of 25 μM in these experiments. At this concentration of vesicles only a small increase in the turbidity could be detected (Fig. 2A), which did not interfere with the fluorescence measurements. As is shown by Fig. 4 (open circles), SecA causes an increase in the anisotropy which can be interpreted [14] as insertion of SecA into the acylchain region of the bilayer. This insertion was inhibited by $ADP+P_i$ (2 mM), since no increase in fluorescence anisotropy could be detected (Fig. 4, closed circles). In this case $ADP+P_i$ were chosen, since the measurements lasted 20 minutes, in which time interval a considerable amount of ATP would have been hydrolysed by the SecA protein. As reference compound PhOE-signalpeptide was added to the DPH containing DOPG vesicles. From the literature it is known that the

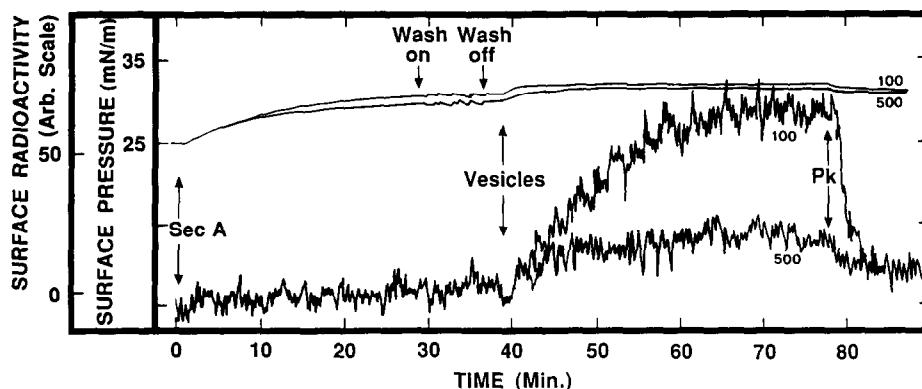


Fig. 3. SecA causes close contact between a DOPG monolayer and DOPG vesicles which is dependent on the ionic strength of the subphase. Experimental details are given in section 2. The experiments done in presence of 100 mM or 500 mM NaCl are labeled 100 and 500, respectively. The upper two tracings are those of the surface pressure, the lower two tracings are those of the surface radioactivity. Arrowheads mark the different events of SecA addition (SecA), washing the subphase (Wash on & off), addition of radiolabeled vesicles (vesicles) and the addition of proteinase K (PK).

signal peptide of PhoE inserts into model phospholipid membranes [12]. This can also be seen in Fig. 4 where the PhoE-signalpeptide caused an increase of the fluorescence anisotropy, similar to earlier results with the signal peptide of preOmpA [14]. That this increase in anisotropy is higher than in the case of SecA may be explained by a deeper insertion of the PhoE-signalpeptide compared to the insertion of SecA.

4. CONCLUDING REMARKS

The above results led us to propose that the SecA monomer contains two lipid-binding sites which are different in nature. These two binding sites are probably positive domains existing within the overall negatively charged SecA molecule, explaining in this way the preferential interaction of SecA with negatively charged lipids. One of the candidates for these positive domains is the region of the first 25 amino acids of the N terminus having an excess of 7 positive residues [21]. The other candidate could be the extreme C terminus where there is a domain of 4 positive residues [21]. One of these binding sites should lead to a more hydrophobic interaction of SecA with the membrane. Calculation of the hydrophobic moment of the first 12 N-terminal residues of SecA according to Eisenberg et al. [22] showed that this peptide can be considered as surface seeking with a hydrophobic moment of 0.49. Therefore we propose that the first 25 amino acids of SecA are responsible for the hydrophobic character of the interaction of SecA with membranes containing negatively charged lipids. Consequently the C-terminal domain is proposed to be involved in a primarily electrostatic interaction with the anionic lipids.

The importance of a second lipid binding site on the SecA molecule in protein translocation is not clear but could contribute to the efficiency of protein translocation as proposed in the following scheme which follows

the previous proposed model for a nucleotide mediated membrane binding cycle of SecA [6]. Assume that SecA first binds to the precursor-SecB complex in the cytosol [23] in which step the N terminus of SecA becomes shielded from interaction with the membrane lipids. The SecA molecule in the complex then can either dock on SecY/E in the inner membrane or interact first with the lipid phase of the membrane with its second lipid binding site followed by two-dimensional diffusion of the complex to SecY/E. This latter process can be expected to give a more efficient delivery of the precursor to the translocation machinery. Upon the assembly of the translocation complex (Translocase) SecA binds ATP and as a result undergoes a conformational change unshielding the N terminus allowing it to mediate the insertion of SecA in the membrane, thereby initiating the translocation of the precursor. Due to hydrolysis of the

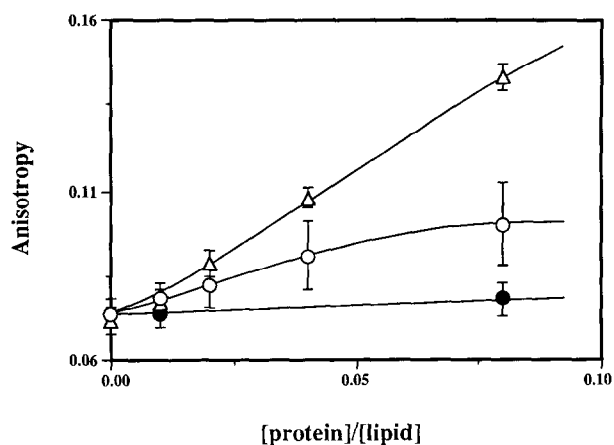


Fig. 4. Steady-state fluorescence anisotropy of DPH as a function of SecA and PhoE signalpeptide concentration. The lipid (DOPG) concentration for all experiments was 25 μ M. SecA (open circles); SecA in presence of ADP+P_i and MgCl₂ (2 mM, filled circles); PhoE signal peptide (open triangles)

bound ATP SecA again changes its conformation re-shielding the N terminus and dissociating from the precursor followed by deinsertion of SecA and dissociation from the membrane leading to completion of the cycle.

Our proposal for the second lipid-binding site to be present at the extreme C terminus does not necessarily contradict the results of Matsuyama et al. [24] who showed that removal of 70 amino acids of the C terminus did not lead to loss of translocation activity *in vitro*. However, the differences may not appear in the experimental setup used by these authors, in which an excess of vesicles was present. Furthermore the loss of a binding site may have its influences on the kinetics of translocation rather than the overall translocation after 15 minutes.

Acknowledgements We like to thank Dr. R.A. Demel for his assistance with the monolayer experiments. This work was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organisation for Scientific Research (NWO)

REFERENCES

- [1] Schiebel, E., Driessen, A.J.M., Hartl, F.U. and Wickner, W. (1989) *Cell* 64, 927-939.
- [2] Hartl, F.U., Lecker, S., Schiebel, E., Hendrick, J.P. and Wickner, W. (1990) *Cell* 63, 269-279.
- [3] Lill, R., Cunningham, K., Brundage, L.A., Ito, K., Oliver, D. and Wickner, W. (1989) *EMBO J.* 8, 961-966.
- [4] Lill, R., Dowhan, W. and Wickner, W. (1990) *Cell* 60, 271-280.
- [5] Shinkai, A., Mei, L.H., Tokuda, H. and Mizushima, S. (1991) *J. Biol. Chem.* 266, 5827-5833.
- [6] Breukink, E., Demel, R.A., de Korte-Kool, G. and de Kruijff, B. (1992) *Biochemistry* 31, 1119-1124.
- [7] Kusters, R., Huijbregts, R. and de Kruijff, B. (1992) *FEBS Lett.* 308, 97-100.
- [8] Ulbrandt, N.D., London, E. and Oliver, D.B. (1992) *J. Biol. Chem.* 267, 15184-15192.
- [9] Kusters, R., Dowhan, W. and de Kruijff, B. (1991) *J. Biol. Chem.* 266, 8659-8662.
- [10] Rouser, G., Fleischer, S. and Yamamoto, A. (1975) *Lipids* 5, 494-496.
- [11] de Kroon, A.I.P.M., Vogt, B., van 't Hof, R., de Kruijff, B. and de Gier, J. (1991) *Biophys. J.* 60, 525-537.
- [12] Killian, J.A., Keller, R.C.A., Struyvé, M., de Kroon, A.I.P.M., Tommassen, J. and de Kruijff, B. (1990) *Biochemistry* 29, 8131-8137.
- [13] Fabrie, C.H.J.P., de Kruijff, B. and de Gier, J. (1990) *Biochim. Biophys. Acta* 1024, 380-384.
- [14] Hoyt, D.W. and Gierasch, L.M. (1991) *Biochemistry* 30, 10155-10163.
- [15] Tournois, H., Fabrie, C.H.J.P., Burger, K.N.J., Mandersloot, J., Hilgers, P., van Dalen, H., de Gier, J. and de Kruijff, B. (1990) *Biochemistry* 29, 8297-8307.
- [16] Shinkai, A., Akita, M., Matsuyama, S. and Mizushima, S. (1990) *Biochem. Biophys. Res. Commun.* 172, 1217-1223.
- [17] Cabelli, R.J., Dolan, K.M., Quan, L. and Oliver, D.B. (1992) *J. Biol. Chem.* 267, 24420-24427.
- [18] Demel, R.A., Jordi, W., Lambrechts, H., van Damme, H., Hovius, R. and de Kruijff, B. (1989) *J. Biol. Chem.* 264, 3988-3997.
- [19] Lakowicz, J.R. (1983) *Principles of Fluorescence Spectroscopy*, pp. 112-150, Plenum Press, New York.
- [20] Lentz, B. (1989) *Chem. Phys. Lipids* 50, 171-190.
- [21] Schmidt, M. G., Rollo, E. E., Grodberg, J. and Oliver, D. B. (1988) *J. Bacteriol.* 170, 3404-3414.
- [22] Eisenberg, D., Schwarz, E., Komaromy, M. and Wall, R. (1984) *J. Mol. Biol.* 179, 125-142.
- [23] Mizushima, S., Tokuda, H. and Matsuyama, S. (1992) in: *Membrane Biogenesis and Protein Targeting* (Neupert, W. and Lill, R., Eds.) *New Comprehensive Biochemistry*, Vol. 22, pp. 21-32. Elsevier, Amsterdam.
- [24] Matsuyama, S., Kimura, E. and Mizushima, S. (1990) *J. Biol. Chem.* 265, 8760-8765.