Distinct Membrane Binding Properties of N- and C-terminal Domains of Escherichia coli SecA ATPase*

Vesna Dapic and Donald Oliver‡

From the Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, Connecticut 06459

SecA is a motor protein that drives protein translocation at the Escherichia coli translocon. SecA membrane binding has been shown to occur with high affinity at SecYE and low affinity at anionic phospholipids. To dissect SecA-membrane interaction with reference to SecA structure, the membrane binding properties of N- and C-terminal SecA domains, denoted SecA-N664 and SecA-619C, respectively, were characterized. Remarkably, only SecA-N664 bound to the membrane with high affinity, whereas SecA-619C bound with low affinity in a nonsaturable manner through partitioning with phospholipids. Moreover, SecA-N664 and SecA-619C associated with each other to reconstitute wild type binding affinity. Corroborative results were also obtained from membrane binding competition and subcellular fractionation studies along with binding studies to membranes prepared from strains overproducing SecYE protein. Together, these findings indicate that the specific interaction of SecA with SecYE occurs through its N-terminal domain and that the C-terminal domain, although important in SecA membrane cycling at a later stage of translocation, appears to initially assist SecA membrane binding by interaction with phospholipids. These results provide the first evidence for distinct membrane binding characteristics of the two SecA primary domains and their importance for optimal binding activity, and they are significant for understanding SecA dynamics at the translocon.

*This work was supported by Grant GM42033 from the National Institutes of Health (to D. O.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 860-685-3556; Fax: 860-685-2141; E-mail: doliver@wesleyan.edu.

SecA is one of the central components of Escherichia coli preprotein translocase, a multimeric, membrane-associated protein complex that secretes proteins across the inner membrane. A combination of genetic and biochemical approaches has greatly advanced our understanding of the basic components and mechanism of protein translocation (1, 2). Translocon consists of obligatory conserved and accessory components (reviewed in Ref. 3). The conserved elements of this molecular machine are SecA, the translocation ATPase that binds preproteins and facilitates protein translocation through conformational cycling promoted by its nucleotide binding domains (4–11), and SecYE, the proposed protein-translocation channel (12–14). SecA insertion into and retraction out of the membrane at SecYE is coupled to the stepwise translocation of preproteins (15, 16). SecDF and SecG proteins, the accessory components of translocon, are essential only at low temperature (17, 18). Both of these proteins are believed to enhance the basic function of the translocon by promoting the SecA insertion and retraction cycle at SecYE (15, 19–23).

Although the overall composition and function of translocon has been defined, the details of the basic reaction cycle are not clear. For example, the importance of soluble and phospholipid-bound pools of SecA relative to the SecYE-bound pool for the initial preprotein recognition step has not been defined, and differences have been reported for in vivo and in vitro studies (24–26). Furthermore, although SecA membrane cycling has generally been accepted, different views exist about the specificities; there is a report that protein translocation can occur from membrane-inserted SecA that does not undergo repetitive cycles of insertion and retraction (27). There is also a recent finding that proton-motive force can stimulate deinsertion of SecA from the membrane, raising the question of whether it also serves as an alternative energy source for the late stage of protein translocation as originally proposed (28, 29).

One approach to dissecting the reaction cycle of a complex biochemical system is to create altered proteins that allow characterization of functional sites of interaction or identification of important biochemical intermediates. This approach has recently provided new information about translocon structure and function (30–32). Such an approach seems particularly appropriate for the large multifunctional SecA protein that plays a dynamic role in the translocon reaction cycle. Previous studies have shown that SecA contains both high and low affinity nucleotide-binding domains, a preprotein-binding site, a SecY-binding site, and a region that interacts with SecB and phospholipids (8, 9, 25, 33–35). Beyond localization of the various ligand-binding sites on SecA, it is important to clarify the dynamics of SecA conformational states and its relation to the translocation process. For instance, the 30-kDa C-terminal region of SecA undergoes membrane cycling at SecYE, where in the membrane-inserted state the C terminus is peripherally accessible (15, 36, 37). However, because the C terminus contains the SecB- and phospholipid-binding sites, it must have been oriented toward the cytoplasm and/or inner membrane prior to insertion.

To clarify the initial membrane binding step of SecA with reference to SecA structure, we have genetically engineered two SecA truncates, SecA-N664 and SecA-619C, that correspond approximately to previously described 65-kDa N-terminal and 30-kDa C-terminal proteolytic fragments of SecA (denoted N and C domain, respectively (38, 39). We have characterized the membrane binding properties of these recombinant proteins. The results indicate that the specific interaction of SecA with the translocon occurs through its N domain and that the C domain, although important in SecA membrane cycling at a later stage of translocation, appears to initially increase its membrane binding by interaction with phospholipids.

25000

This paper is available on line at http://www.jbc.org
The abbreviations used are: IMV, inverted membrane vesicles; IPTG, isopropyl-β-D-thiogalactopyranoside.

EXPERIMENTAL PROCEDURES

**SecA Membrane Binding**

**RESULTS**

**Construction and Purification of N- and C-terminal SecA Truncates—**Proteolysis studies indicate that SecA can be divided into primary N and C domains of 65 and 30 kDa, respectively, (38, 39). The 65-kDa N domain contains the high and low affinity nucleotide-binding domains of SecA flanking its proposed preprotein-binding site (8, 9, 33), whereas the 30-kDa C domain contains the region that undergoes membrane cycling as well as binding of SecB and phospholipids (15, 25, 35). We created T7 expression plasmids that produce similar N- and C-terminal SecA truncates (Fig. 1). The N-terminal truncate comprises residues 1–664 of the primary structure (molecular mass = ~75 kDa) and is referred to as SecA-N664 (34). The C-terminal truncate contains residues 619–901 (molecular mass = ~32 kDa) and is referred to as SecA-619C. Both proteins were stably produced in vivo (see Fig. 7A below), where they lacked protein translocation activity as assessed by the inability to complement the growth defect of the secA13(Am) supF(Ts) mutant BL21.19 at 42 °C. They also showed protein secretion defects in this strain background at 42 °C when chromosomally derived SecA was no longer synthesized (results not shown).

To characterize the membrane binding properties of SecA-N664 and SecA-619C, the proteins were purified from BL21.19

**FIG. 1.** Map of SecA domains. A schematic representation of SecA-N664 and SecA-619C is shown with reference to the known ligand-binding regions of SecA protein. NBD1 and NBD2 are low affinity and high affinity nucleotide binding domains, respectively.

---

1 The abbreviations used are: IMV, inverted membrane vesicles; IPTG, isopropyl-β-D-thiogalactopyranoside.
SecA Membrane Binding

A

200
116
97
66
45
30

SecA
SecA-N664
SecA-619C

B

150 kDa
67 kDa
(ADH)
(BSA)

O.D. at 280 nm (Arbitrary scale)

ELUTION VOLUME (ml)

12
16
20

SecA-N664

ELUTION VOLUME (ml)

12
20

C

67 kDa
(ADH)
29 kDa
(carboxy anhydride)

O.D. at 280 nm (Arbitrary scale)

ELUTION VOLUME (ml)

11
14.5
18

SecA-619C

ELUTION VOLUME (ml)

11
18

D

150 kDa
(ADH)

O.D. at 280 nm (Arbitrary scale)

ELUTION VOLUME (ml)

9
11
13

SecA-N664
SecA-619C

ELUTION VOLUME (ml)

9
13
containing the appropriate T7 expression plasmid. SecA-N664 was purified similarly to SecA by its affinity for Cibacron Blue, which binds to nucleotide-binding proteins (Fig. 2A). SecA-619C was engineered to contain a His tag at its N terminus, allowing for rapid affinity purification on a nickel column. The ATPase activities of the purified proteins were quantified as described previously (9). SecA normally possesses a low endogenous ATPase activity that is stimulated modestly by binding IMV (termed membrane ATPase activity) and a robust ATPase activity that is induced by binding IMV and an export-competent preprotein (termed translocation ATPase activity) (5). SecA-N664 possessed 2-fold higher endogenous ATPase activity and half of the normal level of membrane ATPase activity compared with SecA protein, but it lacked measurable translocation ATPase activity (data not shown). The membrane ATPase activity of SecA-N664, albeit lower then for SecA, suggests that this protein can still interact with SecYE because SecA membrane ATPase activity as opposed to its lipid ATPase activity requires the presence of SecYE protein (7, 43). SecA-619C lacked any detectable ATPase activity, consistent with the absence of a nucleotide-binding domain in this protein (9).

The oligomeric state of the two SecA truncates was characterized by gel filtration studies. Both SecA-N664 and SecA-619C eluted with an apparent molecular size of 65 kDa (Fig. 2, B and C); this is consistent with a monomer and dimer subunit structure, respectively. These results are in agreement with a previous study demonstrating that the C-terminal region of SecA is necessary and sufficient for dimerization, and that similar N- and C-terminal SecA truncates existed as monomer and dimer, respectively (52, 53). The observed leading shoulder in the SecA-N664 elution profile may be due to different protein conformational states, or, alternatively, it may reflect heterogeneous...
ity at the primary structural level because of proteolytic degradation at the N terminus of SecA as observed previously (54).

Finally, when SecA-N664 and SecA-619C were mixed in a 1:1 molar ratio and analyzed by gel filtration, they eluted at a position similar to SecA protein (Fig. 2D). This indicated that SecA-N664 and SecA-619C were capable of association, consistent with a recently published result with similar SecA truncates (53).

Binding of SecA Truncates to IMV—Previous studies have established that SecA dimer binds with high affinity to SecYE \((K_d = 40 \text{ nM})\) and with low affinity to phospholipids \((6, 7, 55)\). To determine the membrane binding properties of SecA-N664 and SecA-619C, we measured their binding to IMV utilizing established methods \((6)\). The binding analysis showed that SecA-N664 bound to IMV with a \(K_d\) of 270 nM, which was approximately 4-fold higher than that of SecA protein \((K_d = 75 \text{ nM})\) (Fig. 3, A and B). This result may be due to the monomeric state of SecA-N664 or, alternatively, to the missing C-terminal region, which could contribute to binding affinity in either a direct or indirect fashion. The membrane binding density of SecA-N664 was also higher than that of SecA protein \((1490 \text{ pmol/mg membrane protein versus } 652 \text{ pmol/mg membrane protein, respectively})\), because at this lower binding affinity, phospholipid binding contributed significantly to the binding density. Binding of SecA-619C to IMV was found to be nonspecific and nonsaturable, consistent with phospholipid association (Fig. 3C). SecA-619C binding was described by a partition coefficient \((k_p = 0.47 \pm 0.01)\), and this value was obtained from the slope of the binding isotherm (Fig. 3C).

To determine whether SecA-N664 binds to IMV through the same type of receptor as wild type SecA protein as well as to confirm our results with SecA-619C, we performed competition binding studies. Membrane binding assays were performed with increasing amounts of SecA-N664 or SecA-619C and a constant amount of \(^{125}\text{I}-\text{SecA}\) and IMV. SecA-N664 competed with the binding of \(^{125}\text{I}-\text{SecA}\), and its \(K_I\) was approximately 5-fold higher than that of SecA protein \((922 \text{ nM versus } 173.4 \text{ nM, respectively; Fig. 4, A and B})\). By contrast, SecA-619C was an ineffective competitor even at a 100-fold molar excess over \(^{125}\text{I}-\text{SecA}\) (Fig. 4C). These results are consistent with the direct binding studies shown above (Fig. 3).

In addition to competition binding experiments, the specificity of SecA binding was also confirmed by performing membrane binding assays utilizing IMV made from a strain that overproduced SecYEG protein. These IMV had approximately five times more SecY protein than normal IMV as determined by Western blot analysis of the purified IMV utilizing different

**Fig. 4.** Competition binding assays. Binding of 130 nM \(^{125}\text{I}-\text{SecA}\) to urea-treated IMV was competed with increasing amounts of unlabeled SecA (A), SecA-N664 (B), or SecA-619C (C) as described under “Experimental Procedures.” Binding of unlabeled competitor is expressed relative to the total binding of \(^{125}\text{I}-\text{SecA}\) in the absence of competitor.

**Fig. 5.** SecA membrane binding assays with IMV from SecYEG-overproducing strains. A, urea-treated IMV were prepared from CK1801 (pSE420secYEG) that was grown without (- IPTG) or with (+ IPTG) IPTG induction as described under “Experimental Procedures,” and their SecY content was analyzed by Western blotting as described previously (34). 20 \(\mu\)g of total membrane protein was analyzed. Samples were incubated for 5 min at 100 °C (+ boiled sample) or 23 °C (- boiled sample). SecY aggregates when heated at 100 °C in the presence of SDS \((12)\). B, binding of \(^{125}\text{I}-\text{Labeled SecA or }^{125}\text{I}-\text{labeled SecA-N664 to urea-treated IMV from CK1801 (pSE420secYEG) was determined as described under “Experimental Procedures.”}

**SecA Membrane Binding**
Reconstitution of SecA membrane binding. A, binding of an equimolar mixture of SecA-N664 and 125I-SecA-619C to urea-treated IMV was determined as described under “Experimental Procedures.” The inset shows a Scatchard plot of the binding data. B, competition binding assay identical to Fig. 4 except an equimolar mixture of unlabeled SecA-N664 and SecA-619C was used as competitor.

Reconstitution of Membrane Binding Activity with a Mixture of SecA-N664 and SecA-619C—Because both SecA truncates possessed some membrane-binding/partitioning activity that was not equivalent to wild type SecA, we decided to test whether a mixture of proteins was capable of reconstituting normal SecA membrane binding activity. An equimolar mixture of purified SecA-N664 and 125I-SecA-619C was allowed to reassociate and tested for binding to IMV. The binding affinity observed in this case ($K_d = 110 \pm 10$ nM) was similar to SecA (Fig. 6A), indicating that effective reconstitution of normal membrane binding activity had occurred. Furthermore, the SecA-N664/SecA-619C mixture competed the binding of 125I-SecA as efficiently as SecA protein ($K_i = 106 \pm 3.8$ nM; compare Fig. 4A with Fig. 6B). These data indicate that N- and C-terminal truncates of SecA have distinct roles in membrane binding and that wild type membrane binding activity can be reconstituted when suitable portions of SecA are present.

Subcellular Localization of SecA-N664 and SecA-619C—To investigate the membrane binding properties of SecA-N664 and SecA-619C within cells, we performed subcellular fractionation experiments. Consistent with previous results SecA protein was present in both cytoplasmic and membrane fractions, where both peripheral and integral membrane forms of SecA were detected as defined by extraction with 0.1 M sodium carbonate, pH 11 (51, 56) (Fig. 7A). The distribution of the cytosolic protein 6-phosphogluconate dehydrogenase and the integral membrane protein OmpA confirmed the quality of the fractionation procedure, and similar results were obtained for these control proteins for all strains tested. SecA-N664 and SecA-619C fractionated differently than wild type SecA. Whereas SecA-N664 was enriched in the integral membrane fraction at the expense of the peripheral membrane fraction, SecA-619C showed the opposite pattern; its membrane pool consisted almost exclusively of the peripheral membrane form with almost no integral membrane form present. This basic fractionation pattern did not change when cells were induced with IPTG, suggesting that the overall level of SecA did not effect its subcellular distribution as assessed by this method. These results suggest that SecA-619C is defective in its integral membrane binding property, consistent with its inability to associate with SecYE protein in vitro. The larger pool of integral membrane-associated SecA-N664 compared with wild type SecA protein may be due to a limitation of this fractionation procedure, which is unable to distinguish between authentic membrane association and protein aggregation. Therefore, to solely examine membrane-associated protein, we prepared sucrose gradient-purified IMV from these strains and determined their SecA content by Western blotting. Previous studies have shown that this membrane purification procedure removes lipid-bound SecA leaving only SecYE-associated SecA protein (57). SecA-N664 was present at a reduced level in IMV compared with wild type SecA (Fig. 7B), consistent with our in vitro binding studies. By contrast, SecA-619C was not detected in IMV, consistent with its peripheral membrane location and failure to bind SecYE in vitro.
interaction of SecA with SecYE in native membranes as well as to localize the SecYE-binding determinant for this primary interaction, we characterized the membrane binding activity of two SecA truncates, SecA-N664 and SecA-619C, that correspond to primary structural domains of SecA. Our initial bias favored the notion that the SecYE-binding determinant might lie within SecA-619C based on the previous ligand blotting study (34) and the report that the 30-kDa C domain of SecA undergoes membrane cycling at SecYE where it is largely shielded from phospholipids in the membrane-inserted state (15, 58, 59). However, contrary to this expectation, we observed that SecA-N664 contained most of the SecYE-specific binding activity of SecA protein. This result was confirmed by both direct and competition binding studies as well as by utilizing IMV with an increased SecYE content. They were also supported by subcellular fractionation and IMV localization studies. By contrast, we were unable to detect any saturable binding activity for SecA-619C, whose phospholipid binding activity was described by a partition function. Localization studies revealed that SecA-619C was peripherally associated with the membrane and that essentially no SecA-619C was present in IMV. The membrane binding characteristics of SecA-619C are consistent with the presence of a phospholipid-binding site within the 70 C-terminal amino acid residues of SecA (35), as well as the observation that SecA insertion into model membranes containing anionic phospholipids utilizes its C domain (55, 60).

The reduced membrane binding affinity of SecA-N664 (~25% of wild type) may arise from its monomeric state (normally the C domain ensures dimerization of the N domain). Alternatively, it may reflect a requirement for the C domain to contribute some specific binding energy either directly or indirectly (e.g. via an allosteric effect) to the N domain. The latter alternative is suggested by the observation that depletion of anionic phospholipids resulted in loss of the high affinity membrane binding component of SecA (61), although a defect in SecYE conformation caused by the altered lipid composition was not excluded in this case. The ability of SecA-N664 and SecA-619C to associate with each other and reconstitute wild type SecYE binding activity indicates that our results reflect the probable subdivision of the membrane binding properties of SecA protein. This conclusion is also supported by a recent study, which showed that similar recombinant 65-kDa N and 30-kDa C domains were capable of reconstituting a limited number of biochemical activities of SecA protein (53).

The observation that the SecYE-binding determinant is located primarily on SecA-N664, whereas SecA-619C contains a lipid-binding determinant, presents some intriguing considerations for SecA structural organization and function. By having ATP, preprotein, and translocon binding activities within a single 65-kDa structural module, the different functions of SecA are more readily integrated. Thus preprotein binding can be sensitive to both nucleotide- and SecYE-bound states of SecA, a conclusion which is also supported by a recent study, which showed that similar recombinant 65-kDa N and 30-kDa C domains were capable of reconstituting the limited number of biochemical activities of SecA protein (53).

The observation that the SecYE-binding determinant is located primarily on SecA-N664, whereas SecA-619C contains a lipid-binding determinant, presents some intriguing considerations for SecA structural organization and function. By having ATP, preprotein, and translocon binding activities within a single 65-kDa structural module, the different functions of SecA are more readily integrated. Thus preprotein binding can be sensitive to both nucleotide- and SecYE-bound states of SecA. A recent study supports the importance of this line of thinking. It was found that prlA (secY) alleles increased the affinity of SecA for SecYE, and in so doing they largely prevented the ATP-dependent dissociation of the SecA-SecY-preprotein ternary complex that appears to be the biochemical basis for the signal peptide proof-reading activity of the translocon (30, 62).

The 30-kDa C domain appears to be unusually dynamic in its function. The existence of SecB- and phospholipid-binding sites on this domain demonstrate that it must function on the cis side of the membrane (25, 35), but its exposure to the trans side of the membrane after preprotein- and ATP-driven SecA membrane insertion (15, 36, 37) suggests an important topological
reorientation of this domain during the translocation cycle. These observations along with our results suggest that the C domain initially associates with the membrane through interaction with phospholipids, but after SecYE recognition by the N domain and under the influence of other translocation ligands, it associates with the translocon. By having little or no affinity for the peripheral surface of the SecYE protein, the C domain may achieve the mobility necessary to undergo membrane cycling.

The structural subdivision between the N and C domains of SecA suggests a likely scenario for SecA-translocon association. It seems probable that after SecA synthesis, the C domain would initially bind to the membrane through association with phospholipids and by a two-dimensional diffusion process locate its receptor utilizing the N domain. Binding of the N domain to the translocon would trigger conformational changes allowing association of the C domain and give rise to full SecA binding activity. This proposal is consistent with studies demonstrating that depletion of anionic phospholipids resulted in vivo and in vitro defects in SecA-membrane binding and in vitro protein translocation that could be suppressed by high levels of SecA protein (56, 61, 63). The affinity of SecA for SecYE and the existence of a lipid pool of SecA may be important parameters that allow the translocon ready but not exclusive access to SecA. This strategy may be necessary in light of recent reports indicating that a SecA-free translocon is required for biogenesis of certain integral membrane proteins that utilize the signal recognition particle targeting system (64, 65).

Our study represents the first systematic analysis of the membrane binding properties of the two SecA domains, and it opens up a number of interesting new questions concerning SecA mechanism. What is the ultimate structural basis for N domain recognition of SecYE protein? What properties of the C domain contribute to its eventual interaction with the translocon and its membrane cycling capability? How are the activities of the N and C domains coordinated during the translocation cycle? Additional structural and biochemical studies defining the interaction of N and C domains with model membranes and proteoliposomes reconstituted with SecYE protein in the presence of ATP and preprotein are needed to obtain a detailed picture of this complex system.

Acknowledgments—We are grateful to Robert Cone (University of Connecticut Health Center) for use of the γ-counter, Marcel Schmidt for help with fast protein liquid chromatography analysis, and Visvanathan Ramamurthy for construction of pSE42vecYEg. We thank Irina Rusu for enthusiastic support and assistance with the analysis of binding data and Eillka Weber-Ban, Lauralynn Kourtz, and Ryszard Michalczyzk for comments, discussions, and critical reading of the manuscript.

REFERENCES

1. Danese, P., and Silhavy, T. (1998) Annu. Rev. Genet. 32, 59–94
2. Driessen, A. J. M., Bekker, P., and van der Wolk, J. (1998) Curr. Opin. Microbiol. 1, 216–222
3. Pohlschroder, M., Prinz, W. A., Hartmann, E., and Beckwith, J. (1997) Cell 91, 563–566
4. Cabelli, R. J., Chen, L., Tai, P. C., and Oliver, D. B. (1988) Cell 55, 683–692
5. Lill, R., Cunningham, K., Brundage, L. A., Ko, K., Oliver, D., and Wickner, W. (1990) EMBO J. 9, 13898–13904
6. Hartl, F.-U., Schiebel, E., Hendrick, J. P., and Wickner, W. (1990) J. Biol. Chem. 216, 7297–7304
7. Pohlschroder, M., Prinz, W. A., Hartmann, E., and Beckwith, J. (1997) EMBO J. 16, 3272–3277
8. Nishiyama, K.-I., Hanada, M., and Tokuda, H. (1994) EMBO J. 13, 2327–2337
9. Nishiyama, K.-I., Suzuki, T., and Tokuda, H. (1996) Cell 85, 71–81
10. Cunningham, K., Rice, M., Moore, K., Wickner, W., and Kuhle, H. (1996) J. Biol. Chem. 271, 13898–13904
11. Chun, S. Y., and Randall, L. L. (1994) J. Biol. Chem. 269, 4197–4203
12. Fekkes, P. van der Does, C., and Driessen, A. (1997) EMBO J. 16, 6105–6113
13. Behrmann, M., Koch, H.-G., Hengelag, T., Wieseler, B., Hofschulte, H., and Muller, M. (1999) J. Biol. Chem. 274, 13898–13904
14. Chen, X., Xu, H., and Tai, P. (1996) J. Biol. Chem. 271, 29686–29706
15. Schiebel, E., Driessen, A. J. M., Hartl, F.-U., and Wickner, W. (1991) Cell 64, 857–869
16. Nishiyama, K., Fukuda, A., Morita, K., and Tokuda, H. (1999) EMBO J. 18, 1049–1058
17. van der Wolk, J., Fekkes, P., Boorsma, A., Huije, J., Silhavy, T., and Driessen, A. (1998) EMBO J. 17, 3631–3639
18. Chun, S. Y., and Randall, L. L. (1994) J. Biol. Chem. 269, 4197–4203
19. Fekkes, P. van der Does, C., and Driessen, A. (1997) J. Biol. Chem. 272, 3438–3444
20. Matsuyama, S., Kimura, E., and Mizushima, S. (1990) J. Biol. Chem. 265, 8760–8765
21. Matsumoto, G., Mori, H., and Ito, K. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 11302–11306
22. Breukink, E., Brouwer, N., van Raalte, A., Mizushima, S., Tommassen, J., and de Kruijff, B. (1995) J. Biol. Chem. 270, 7992–7997
23. Dikalov, S., and Oliver, D. (1996) FEBS Lett. 385, 4807–4817
24. Winzor, D., and Sawyer, W. (1995) Quantitative Characterization of Ligand Binding, Wiley-Liss, New York
25. Cheng, Y., and Prusoff, W. (1973) Biochem. Pharmacol. 22, 3099–3108
26. Miller, J. H. (1972) Experiments in Molecular Genetics, pp. 431–435, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
27. Karamanou, S., Vrontou, E., Slanidis, G., Baud, C., Roos, T., Kuhn, A., Politou, A., and Economou, A. (1999) J. Biol. Chem. 274, 554–561
28. brett, J., Demel, R. A., de Korte-Kool, G., and de Kruijff, B. (1992) Biochemistry 31, 1111–1119
29. Nakamura, K., Fujita, M., and Kakegawa, S. (1998) Biochem. Biophys. Res. Commun. 259, 90–95
30. Hirosawa, M., and Tommassen, J. (1996) Biochem. Biophys. Res. Commun. 229, 2163–2173
31. Scotti, P., Vallet, Q., and Economou, A. (1999) J. Biol. Chem. 274, 29683–29688