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Anionic Phospholipids Are Essential for α-Helix Formation of the Signal Peptide of prePhoE upon Interaction with Phospholipid Vesicles

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Received August 26, 1991; Revised Manuscript Received November 8, 1991

ABSTRACT: The conformational consequences of the interaction of the PhoE signal peptide with bilayers of different types of phospholipids was investigated using circular dichroism. It was found that interaction of the signal peptide with anionic phospholipid vesicles of dioleoylphosphatidylglycerol and dioleoylphosphatidylserine results in induction of high amounts of α-helical structure of 70% and 57%, respectively. Upon addition of the signal peptide to cardiolipin vesicles, less but still significant α-helical structure was induced (29%). In contrast, no α-helix formation was observed upon the interaction of the signal peptide with zwitterionic dioleoylphosphatidylcholine vesicles. In bilayers of dioleoylphosphatidylcholine with dioleoylphosphatidylglycerol, it was shown that in the presence of 100 mM NaCl a minimum amount of 50% of negatively charged lipid was required for induction of the maximal percentage of α-helix, whereas in the absence of salt a minimum amount of 35% of negatively charged lipid was necessary. Induction of α-helix structure appeared to be correlated with functionality, since, in a less functional analogue of the PhoE signal peptide, the PhoE-[Asp$^{-19,20}$] signal peptide, less α-helix was induced than in the wild-type PhoE signal peptide. It is proposed that the interaction with anionic phospholipids is essential for a functional conformation of the PhoE signal sequence during protein translocation.

Proteins synthesized in the cytoplasm of *Escherichia coli* cells but destined for the periplasmic space or outer membrane have to cross the plasma membrane. In this translocation process, proteinaceous components (Oliver & Beckwith, 1981; Collier et al., 1988; Bieker et al., 1990) as well as phospholipids (De Vrije et al., 1988; Lill et al., 1990; De Vrije et al., 1990; Kusters et al., 1991) are involved. Proteins which are exported from the cytoplasm of *E. coli* are synthesized as precursor

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0006-2960/92/0431-1672$03.00/0

proteins with an N-terminal extension, the signal sequence (Oliver & Beckwith, 1982; Gierasch et al., 1989), which is the most general requirement for the export of proteins from both eukaryotic and prokaryotic cells. Although signal sequences show little sequence homology (Watson, 1984; Von Heijne, 1985), they all consist of a positively charged N-terminal region, followed by a stretch of 8–15 hydrophobic amino acids and a more polar C-terminal region, containing the cleavage site (Von Heijne, 1985). This suggests that overall characteristics such as hydrophobicity and conformation are important factors for their function.

In vivo and in vitro studies established that hydrophobicity is an important requirement for a functional signal sequence (Briggs et al., 1985; Kendall et al., 1986; Bird et al., 1990; Chou & Kendall, 1990). In addition, in vivo studies with functional and nonfunctional sequences suggested an important role for the conformational flexibility of the signal sequence and its propensity to form an α-helix (Rosenblatt et al., 1980; Engelman & Steitz, 1981; Emr et al., 1983; Bankaitis et al., 1984). Conformational studies with the isolated signal peptide analogues of functional and nonfunctional precursors gave further indications that the ability to adopt α-helical structure is essential for translocation (Briggs & Gierasch, 1984; McKnight et al., (1989).

The signal sequence may be involved in various steps of the secretion process [reviewed by Gierasch (1989)]. In eukaryotic systems, it is now well documented that the signal sequence is involved in recognition with the signal recognition particle (SRP) and the membrane-associated signal sequence receptor (Rapoport et al., 1990). Whether similar components are involved in protein translocation in *E. coli* is unknown yet [for a current discussion on this topic, see Bassford et al. (1991)]. Therefore, it is possible that in *E. coli* the signal sequence is directly involved in membrane binding of the precursor by electrostatic interactions between its positively charged N-terminus and anionic membrane lipids and that it is this binding which initiates membrane translocation of the precursor. This would also explain the observation that anionic phospholipids are essential for efficient translocation (De Vrije et al., 1988; Kusters et al., 1991). In agreement with this possibility, biophysical studies revealed a strong interaction between isolated signal peptides and lipids in model membrane systems (Shinnar et al., 1984; Briggs et al., 1985, 1986; Nagaraj et al., 1987; Killian et al., 1990b; McKnight et al., 1991). For the synthetic signal peptide analogue of the *E. coli* outer membrane protein PhoE, it was shown that the interaction was much stronger with anionic phospholipids than with zwitterionic phospholipids (Batenburg et al., 1988a,b; Demel et al., 1990; Killian et al., 1990a). Moreover, circular dichroism studies with detergents showed an α-helical structure for the PhoE signal peptide in a negatively charged detergent (SDS) and β-sheet structure in a neutral detergent (Lubrol), suggesting also a lipid specificity for the conformation of the signal peptide (Batenburg et al., 1988a).

Knowledge about the lipid dependency of the conformational behavior of the signal peptide will not only provide further insight into the nature of the signal peptide–lipid interaction but, since the ability to adopt α-helical structure is important for translocation (Briggs & Gierasch, 1984), it could also contribute to an understanding of the molecular mechanism of protein translocation. The inner membranes of the *E. coli* strain SD12 consist of about 20% the phosphatidylglycerol (PG),[1] 75% phosphatidylethanolamine (PE), and 4% cardiolipin (Kusters et al., 1991). Therefore, in this study the conformation of the signal peptide of prePhoE is investigated using mainly phosphatidylglycerol (PG), the most abundant anionic phospholipid, and phosphatidylcholine (PC), as an alternative for the zwitterionic phosphatidylethanolamine (PE). It will be demonstrated that induction of α-helix in the signal sequence of PhoE after interaction with phospholipid vesicles is dependent upon the anionic phospholipid content. The results will be discussed in relation to the translocation process and will be compared with conformational studies on signal peptides described in the literature.

Materials and Methods

Synthetic Signal Peptides. The PhoE signal peptide and the PhoE-[$Asp^{19,20}$] signal peptide analogue in which the lysine residues are replaced by aspartic acid residues were prepared by solid-phase synthesis by D. Olshevski (University of California, San Diego) and are not *N*-formylated. The amino acid composition of both peptides was confirmed by amino acid analysis on a Kontron Liquimat III amino acid analyzer. HPLC profiles, performed as described previously (Batenburg et al., 1988a) indicated a >95% purity of the signal peptides.

Phospholipids. Cardiolipin was isolated from bovine heart and purified according to Smaal et al. (1985). 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol (DOPG), and 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (DOPS) were obtained according to established methods (Van Deenen & De Haas, 1964; Comfurius & Zwaal, 1977) and purified as described (Geurts van Kessel et al., 1981). *E. coli* cardiolipin was purchased from Avanti Polar Lipids, Inc. (Alabama).

Sample Preparation. Stock solutions of peptides (3.1 mM) in trifluoroethanol (TFE) were freshly prepared after first dissolving the peptides in trifluoroacetic acid (Killian et al., 1990a). Small unilamellar lipid vesicles (SUV) with a phospholipid phosphorus content of 9 mM were prepared by hydrating a dry lipid film in a 100 mM NaCl, 10 mM PIPES, and 2 mM EDTA buffer at pH 7.4 (unless stated otherwise), followed by 10 × 30 s of sonication under N_2 with an input power of 50 W using a Branson B12 sonifier with a 0.5-in. flat tip, while cooling on ice. Titanium particles and any residual multilamellar structures were removed by a 15-min 30000*g* centrifugation at 4 °C. The phospholipid concentration in the supernatant was determined according to Rouser et al. (1970) and was at all times at least 90% of the concentration based on the stock solutions. Cardiolipin concentrations are expressed on the basis of their phosphorus content.

Large unilamellar vesicles (LUV) were prepared by hydrating dry lipid films, which then were frozen and thawed 10 times and passed subsequently through 400- and 200-nm pore size polycarbonate filters as described (Hope et al., 1985). The peptide was added at room temperature and under vigorous vortexing to lipid dispersions which were diluted with buffer to the desired concentrations. The samples were directly used for the circular dichroism (CD) measurements.

Circular Dichroism Experiments. All measurements were performed at room temperature on a Jasco J600 spectropo-

[1] Abbreviations: CD, circular dichroism; CL, cardiolipin; DOPC, dioleoylphosphatidylcholine; DOPG, dioleoylphosphatidylglycerol; DOPS, dioleoylphosphatidylserine; EDTA, ethylenediaminetetraacetic acid; HPLC, high-performance liquid chromatography; LUV, large unilamellar vesicles; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PIPES, 1,4-piperazinediethanesulfonic acid; RMS, root mean square; SUV, small unilamellar vesicles; TFA, trifluoroacetic acid; TFE, trifluoroethanol.

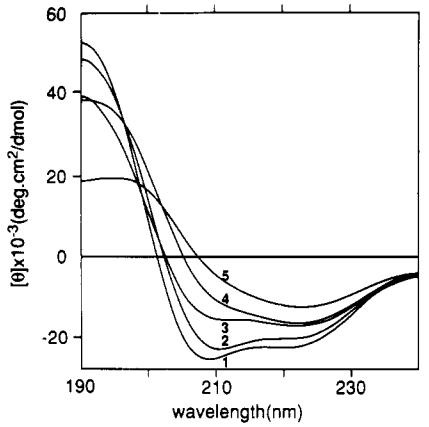

FIGURE 1: CD spectra of the PhoE signal peptide upon interaction with varying amounts of DOPG SUV. The lipid to peptide ratios depicted here are 100:1 (1), 40:1 (2), 20:1 (3), 12.5:1 (4), and 6.25:1 (5).

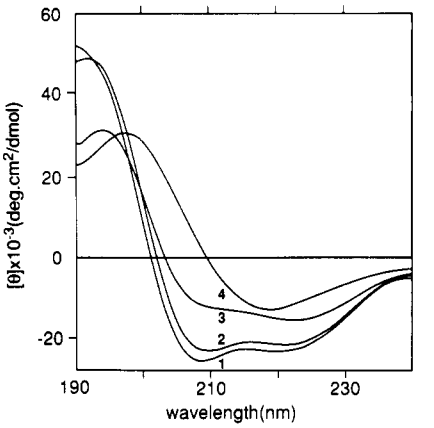

FIGURE 2: CD spectra demonstrating the lipid specificity of the induction of secondary structure after interaction of PhoE signal peptide with phospholipid vesicles. The PhoE signal peptide was added in a lipid to peptide molar ratio of 72:1 to SUV of DOPG (1), DOPS (2), *E. coli* CL (3), and DOPC (4).

Table I: Secondary Structure of the PhoE Signal Peptide (WT) and PhoE-[Asp$^{-19,20}$] (ASP) upon Interaction with Different Lipid Vesicles

lipid	peptide	polylysine[a] [θ] 222[b]				
		α	β	T	R	α
DOPG	WT	70	15	4	11	67
DOPS	WT	57	33	7	3	58
E. coli CL	WT	29	58	11	2	41
DOPC	WT	1[c]	54	0	45	35
DOPG/DOPC (45:55 mol/mol)	WT	35	61	3	1	50
DOPG	ASP	23	53	3	21	38
DOPG/DOPC (45:55 mol/mol)	ASP	6	84	0	10	27

[a] Reference spectra are essentially from Greenfield and Fasman (1969) (see Materials and Methods). [b] The ellipticity at 222 nm has been used as a rough measure for the relative helicity, for which θ_{222} = 36 300 deg·cm^2/dmol was taken as 100% α-helix (Hodges et al., 1988). It should be noted that at low α-helicity this method becomes very inaccurate. [c] The corresponding RMS value was 17.

larimeter, interfaced to a Laser 386 computer, using a 0.1-mm cell with a resolution of 0.1 nm and a scan speed of 10 nm. The final concentration of signal peptide was 125 μM in all experiments. All spectra were baseline corrected by subtraction of spectra of protein-free samples and smoothed. The spectra were the average of four consecutive scans measured from 240 to 190 nm.

The CD spectra were analyzed for contributions from the secondary structure by fitting the experimental data to poly(L-lysine) reference spectra for α-helix, β-sheet, and random coil (Greenfield & Fasman, 1969) and to the β-turn as described by Bolotina et al. (1980). This set of reference spectra gave the lowest RMS values as defined by Brahms and Brahms (1980) which were, unless indicated otherwise, lower than 10.

RESULTS

The conformational behavior of the PhoE signal peptide upon interaction with PG vesicles was investigated as a function of the lipid/peptide ratio using circular dichroism (CD). The results are shown in Figure 1. At a molar ratio of lipid to peptide of 100:1 the CD spectrum exhibits two minima at 208 and 222 nm and a cross point at 202 nm, which is indicative for an α-helical conformation. The amount of α-helix was calculated to be 70% (Table I). Upon lowering the lipid to peptide ratio to 60:1, no changes in the CD pattern were observed (data not shown). Further decreasing the lipid concentration leads to a decrease in intensity and a shift of the cross point from 202 nm up to 208 nm, indicative for a transition from α-helical conformation to β-structure. At a ratio below 10:1 almost no α-helix is present anymore and a typical β-sheet spectrum is observed with one minimum at 218 nm and a cross point at about 208 nm. The decrease in spectral intensity upon increasing the lipid/peptide ratio as well as the lack of an isodichroic point suggest that upon conversion from α-helix to β-structure aggregation of the peptide also occurs. This lipid/peptide ratio dependency of the secondary structure of the signal peptide was not influenced by decreasing (25 μM) or increasing (200 μM) the absolute amounts of signal peptide (data not shown).

In order to test the lipid specificity of the induction of secondary structure in the signal peptide, a comparison was made between zwitterionic and anionic phospholipids. Figure 2 shows CD spectra obtained upon interaction of the signal peptide with the anionic phospholipids DOPG, DOPS, and *E. coli* CL and the zwitterionic phospholipid DOPC at a molar ratio of 72:1. For both DOPG and DOPS, a spectrum with two minima at 208 and 222 nm and a cross point at about 202 nm is observed, and the percentage of α-helix is calculated to be 70% and 57%, respectively (Table I). In contrast, upon interaction with the zwitterionic phospholipid DOPC, the CD spectrum exhibits only one minimum at 218 nm and a cross point at about 208 nm, indicating a high amount of β-structure. With the negatively charged *E. coli* cardiolipin, substantial helical structure is induced (29%, see Table I) but less than upon interaction of the signal peptide with DOPG and DOPS. This could either indicate a headgroup specificity of the interaction, or it could be due to a difference in acyl chain composition, since PG and PS contain oleic acid only and the cardiolipin isolated from *E. coli* contains several unsaturated fatty acids and also a large amount of saturated fatty acids (Burnell et al., 1980). In order to investigate this latter possibility, we next studied the interaction of the signal peptide with cardiolipin isolated from bovine heart, which has a much more unsaturated fatty acid composition (Smaal et al., 1985). Since both cardiolipin samples gave identical results (not shown), this indicates that the observed difference in α-helical content in the signal peptide upon interaction with cardiolipin and the other anionic phospholipids is most probably due to headgroup specificity of the interaction.

Previously, it was shown that a reduction of the anionic lipid content in *E. coli* membranes drastically diminishes translocation of PhoE (De Vrije et al., 1988; Kusters et al., 1991). Therefore, we thought it of interest to investigate the influence of a varying anionic phospholipid content on the interaction

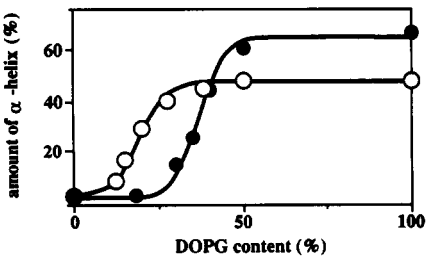

FIGURE 3: Amount of α-helical structure in the PhoE signal peptide as a function of anionic phospholipid content. The PhoE signal peptide was added to DOPC SUV with varying DOPG content in the presence (●) or absence (○) of 100 mM NaCl.

of the signal peptide with phospholipid vesicles, using SUV composed of DOPC and DOPG. In the presence of 100 mM NaCl, an induction of α-helix is observed which is dependent on the amount of DOPG present, as shown in Figure 3. When the DOPG content is decreased from 100% DOPG down to 50%, no change in α-helical content in the signal peptide is observed. Upon further lowering the DOPG content, however, the amount of helical structure decreases, and below 25% DOPG it is virtually zero. While in pure DOPG the CD spectrum of the peptide was unchanged even after more than 6 h, it was found that the conformation of the signal peptide in the region 40–30% DOPG was not stable in time. Already within 5 min a decrease in the α-helical content was observed. We have no good explanation for this phenomenon. Therefore all percentages α-helix calculated in Figure 3 are from spectra of one scan, recorded immediately after addition of the signal peptide.

Monolayer studies demonstrated a marked difference in the interaction of signal peptide with phospholipid monolayers in the presence or absence of 100 mM NaCl (Demel et al., 1990). In order to investigate whether the ionic strength also affects the conformation of the signal peptide, the same CD experiments were performed in the absence of salt. Figure 3 shows that the α-helical content is now unchanged from 100% DOPG down to 35%, although the absolute amounts of α-helix (50%) are lower than in the presence of salt (70%). Upon further lowering the amount of DOPG, the amount of helical structure decreases and is virtually zero below 10%. While the conformation of the signal peptide was stable at all percentages DOPG above 20%, a time-dependent decrease in the α-helical content was observed in the region 20–12.5% DOPG. Thus, Figure 3 clearly demonstrates that, for the induction of α-helical conformation in the signal peptide, a certain minimum amount of anionic phospholipid is required, which is dependent upon the presence of salt.

To see whether this induction of α-helical conformation is related to functionality, the signal peptide analogue of a less functional precursor was also studied (Bosch et al., 1989), the PhoE-[$Asp^{-19,20}$] signal peptide. Both the PhoE- and the PhoE-[$Asp^{-19,20}$] signal peptide were added to 45% DOPG SUV in the presence of 100 mM NaCl. CD spectra are shown in Figure 4. The mutant signal peptide clearly contains a lower amount of α-helix than the wild-type signal peptide (6% and 35%, respectively, see Table I). Qualitatively similar behavior was observed in the absence of salt (data not shown) and at 100% DOPG (see Table I). Both peptides behaved identically in TFE, showing a high amount α-helical content. Upon titration with increasing amounts of MeOH, in which solvent the peptide adopts a β-structure (Batenburg et al., 1988a), the helical structure was retained longer in the case of the PhoE-[$Asp^{-19,20}$] signal peptide (data not shown), indicating a larger helix stability. Therefore, the difference in

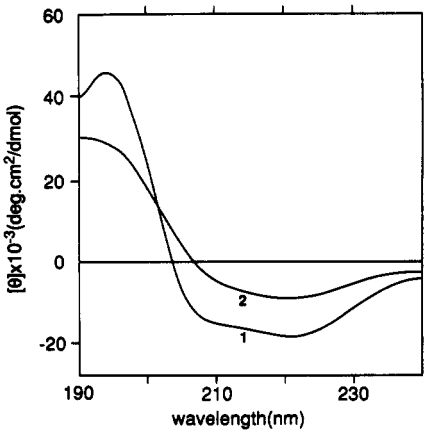

FIGURE 4: CD spectra of the PhoE signal peptide (1) and the PhoE-[$Asp^{-19,20}$] signal peptide (2) upon interaction with DOPG/DOPC SUV (45:55 mol/mol) at a 72:1 molar ratio of lipid to peptide and in the presence of 100 mM NaCl.

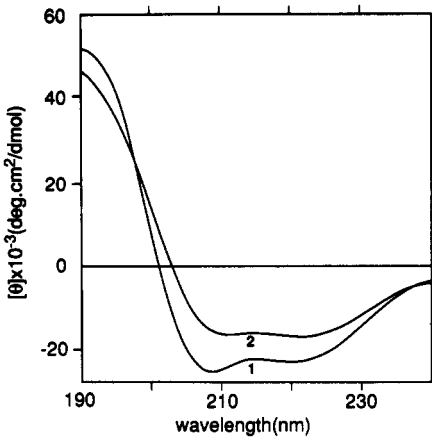

FIGURE 5: CD spectra of the PhoE signal peptide upon interaction with SUV (1) and LUV (2) of 100% DOPG at a 72:1 molar ratio of lipid to peptide and in the presence of 100 mM NaCl.

interaction of both peptides with phospholipids cannot be due to differences in conformational preference of the isolated peptides.

The interaction of signal peptide with lipids was investigated using SUV instead of LUV to reduce the possibility of scattering artifacts. To check whether the high curvature of the SUV influences the interaction of the signal peptide with the lipid vesicles, we compared the conformation of the signal peptide upon addition to DOPG SUV and DOPG LUV. The line shape of the spectra indicates that there is a small decrease in the α-helical content in the case of LUV as compared to SUV (Figure 5). This was also observed under other experimental conditions, such as with other concentrations of signal peptide or using buffer with or without salt (data not shown). We conclude that the curvature gives rise to minor differences in conformation of the signal peptide upon interaction with LUV and SUV.

Discussion

Both a functional signal sequence and anionic phospholipids are important for efficient translocation of precursor proteins across the *E. coli* inner membrane (De Vrije et al., 1990). Furthermore, the ability of the signal sequence to adopt an α-helical conformation has been proposed to be essential for a functional signal sequence (Briggs & Gierasch, 1984; McKnight et al., 1989). In this study, we investigated the possible correlation between the appearance of helical structure

in the signal peptide of PhoE and the interaction of the signal peptide with anionic phospholipids.

The results clearly show that the signal peptide can adopt α-helical structure upon interaction with anionic phospholipids, but not when added to the zwitterionic PC. This lipid specificity corresponds well with the results of monolayer and fluorescence studies, which revealed a strong and preferential interaction of the signal peptide with anionic phospholipids (Batenburg et al., 1988a,b; Demel et al., 1990; Killian et al., 1990a). The signal peptide was also found to interact with zwitterionic phospholipids, but to a lower extent, and to penetrate less deeply into the hydrophobic core of the membrane than with anionic phospholipids (Demel et al., 1990; Killian et al., 1990a).

In bilayers composed of PC and PG, it was found that below a certain amount of DOPG less α-helix is induced in the signal peptide. The amount of DOPG necessary for maximal α-helix formation was lower in the absence than in the presence of 100 mM NaCl. A similar result was obtained previously from monolayer experiments (Demel et al., 1990) where the maximal pressure increase upon interaction with monolayers was reached at lower DOPG content in the absence of NaCl. A possible explanation is that the peptide itself behaves differently in the absence and presence of salt, as indicated by tryptophan-fluorescence measurements (Killian et al., 1990a).

Although a large number of conformational studies with signal peptides have been published [for a review, see Gierasch (1989)] up until now this has not resulted in a consistent picture. While a large number of studies indicated the importance for a functional signal sequence to adopt an α-helical structure (Gierasch, 1989), other studies proposed that it is the ability to adopt a β-sheet conformation that is of functional importance (Harris et al., 1984; Reddy & Nagaraj, 1989). This apparent controversy can easily be explained by differences in the systems or peptides used. The present study has clearly shown that experimental conditions like concentration of the peptide, the lipid/peptide ratio, the use of zwitterionic or anionic phospholipids, and the presence or absence of salt are important in determining the final conformation. Thus, when using a low lipid to peptide ratio or when using DOPC vesicles to mimic the biological membrane, it is very well possible that a β-sheet conformation is observed, regardless of the functionality of the signal peptide and its propensity to form α-helical structure.

The less functional PhoE-[$Asp^{-19,20}$] signal peptide induces less α-helical structure than the functional wild-type PhoE signal peptide. Since the conformational behavior of both peptides was identical in TFE, the difference in α-helical content has to be a consequence of less or different interaction of the PhoE-[$Asp^{-19,20}$] with phospholipid vesicles. This corresponds well with results from monolayer and NMR studies (Demel et al., 1990; Killian et al., 1990b.) and could give an explanation for its less functional properties in the translocation process.

The scheme now emerging from this and previous studies [for review, see De Vrije et al., (1990)] on the interaction of the PhoE signal peptide with model membranes is that the signal peptide binds preferentially to anionic phospholipids present in the membrane and that upon interaction with these phospholipids the signal peptide adopts an α-helical structure and inserts into the hydrophobic core of the bilayer. Since for efficient translocation of prePhoE in *E. coli* an anionic phospholipid dependence is also observed (De Vrije et al., 1988; Kusters et al., 1991), this suggests that similar signal sequence–phospholipid interactions may be involved in translocation. In agreement with this possibility, monolayer and fluorescence studies using phospholipid extracts of inner membrane vesicles from a PG-depleted *E. coli* strain (HD3122) and a wild-type strain (SD12) demonstrated a correlation between the in vitro translocation efficiency of prePhoE across the membranes of the vesicles and the interaction of the signal peptide with the corresponding lipid extracts (Demel et al., 1990; Killian et al., 1990a). Furthermore, in the present study as well as in the previous studies (Demel et al., 1990; Killian et al., (1990b), a marked correlation was found between the decreased ability of a less functional signal peptide to interact with phospholipids as compared to the wild-type signal peptide and the decreased translocation efficiency of the corresponding precursor mutant. Altogether, this strongly suggests that the signal sequence–anionic phospholipid interaction plays a role in the initial step of the translocation of a precursor protein over the membrane. Whether or not the signal sequence indeed interacts with the anionic phospholipids during the translocation process is still unknown and is under current investigation.

ACKNOWLEDGMENTS

We are grateful to H. van Dalen for the synthesis and purification of the phospholipids.

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C-Terminal Cysteines of Tn*501* Mercuric Ion Reductase†

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Received July 29, 1991; Revised Manuscript Received October 31, 1991

ABSTRACT: Mercuric ion reductase (MerA) catalyzes the reduction of Hg(II) to Hg(0) as the last step in the bacterial mercury detoxification pathway. A member of the flavin disulfide oxidoreductase family, MerA contains an FAD prosthetic group and redox-active disulfide in its active site. However, the presence of these two moieties is not sufficient for catalytic Hg(II) reduction, as other enzyme family members are potently inhibited by mercurials. We have previously identified a second pair of active site cysteines (Cys_{558} Cys_{559} in the Tn*501* enzyme) unique to MerA, that are essential for high levels of mercuric ion reductase activity [Moore, M. J., & Walsh, C. T. (1989) *Biochemistry 28*, 1183; Miller, S. M., et al. (1989) *Biochemistry 28*, 1194]. In this paper, we have examined the individual roles of Cys_{558} and Cys_{559} by site-directed mutagenesis of each to alanine. Phenotypic analysis indicates that both *merA* mutations result in a total disruption of the Hg(II) detoxification pathway in vivo, while characterization of the purified mutant enzymes in vitro shows each to have differential effects on catalytic function. Compared to wild-type enzyme, the C558A mutant shows a 20-fold reduction in k_{cat} and a 10-fold increase in K_m, for an overall decrease in catalytic efficiency of 200-fold in k_{cat}/K_m. In contrast, mutation of Cys_{559} to alanine results in less than a 2-fold reduction in k_{cat} and an increase in K_m of only 4-5-fold for an overall decrease in catalytic efficiency of only ca. 10-fold in vitro. From these results, it appears that Cys_{558} plays a more important role in forming the reducible complex with Hg(II), while both Cys_{558} and Cys_{559} seem to be involved in efficient scavenging (i.e., tight binding) of Hg(II).

The extraordinarily high affinity of mercuric ions for thiols, and thus proteins, renders them exceedingly toxic to living systems. Since several hundred million tons of mercurials are dispersed throughout the biosphere, it is not surprising that microorganisms have evolved a variety of means for their detoxification [for review see Foster (1987), Summers (1986), and Summers and Silver (1978)]. By far the most common resistance mechanism is a strategy of reductive biotransformation. In this system, Hg(II) is scavenged from the immediate microenvironment and transported into the cytoplasm by the sequential action of two gene products, MerP and

† This work was supported in part by NIH Grants GM-21643 (C. T.W.) and GM-11106 (awarded to Dr. Vincent Massey at the University of Michigan).
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0006-2960/92/0431-1677$03.00/0