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Mutual effects of MinD–membrane interaction: I. Changes in the membrane properties induced by MinD binding

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ABSTRACT

In *Escherichia coli* and other bacteria, MinD, along with MinE and MinC, rapidly oscillates from one pole of the cell to the other controlling the correct placement of the division septum. MinD binds to the membrane through its amphipathic C-terminal α-helix. This binding, promoted by ATP-induced dimerization, may be further enhanced by a consequent attraction of acidic phospholipids and formation of a stable proteolipid domain. In the context of this hypothesis we studied changes in dynamics of a model membrane caused by MinD binding using membrane-embedded fluorescent probes as reporters. A remarkable increase in membrane viscosity and order upon MinD binding to acidic phospholipids was evident from the pyrene and DPH fluorescence changes. This viscosity increase is cooperative with regards to the concentration of MinD-ATP, but not of the ADP form, indicative of dimerization. Moreover, similar changes in the membrane dynamics were demonstrated in the native inverted cytoplasmic membranes of *E. coli*, with a different depth effect. The mobility of pyrene-labeled phosphatidylglycerol indicated formation of acidic phospholipid-enriched domains in a mixed acidic-zwitterionic membrane at specific MinD/phospholipid ratios. A comparison between MinD from *E. coli* and *Neisseria gonorrhea* is also presented.

1. Introduction

In bacteria, cell division is initiated by formation of the so-called Z-ring in the middle of the cell [1]. The Z-ring is formed of protofilaments of the tubuline-like FtsZ protein, and the assembly occurs on the inner surface of the cytoplasmic membrane. To ensure the correct, central positioning of the division ring, two negative regulation systems are required – Min proteins and nucleoid occlusion [2–4]. While the latter prevents Z-ring formation over the not yet segregated nucleoid occupying the central part of the cell volume, Min proteins protect bacteria from formation of nucleoid-less cells ("minicells") by inhibiting a non-central septum placement. The major protein interfering with FtsZ ring assembly is MinC [5] that targets the membrane-associated FtsZ filaments but is not able to bind to the membrane by itself. Its recruitment to the membrane is mediated by another protein, MinD [6–8]. MinD is therefore responsible for both the MinC localization to and distribution pattern on the membrane. MinD is either permanently positioned on the cell poles as in *B. subtilis* [9] or displays a striking oscillatory behavior together with MinE in e.g. *Escherichia coli* [10]. MinD binds to the membrane by a short conserved C-terminal region, which is not structured upon MinD crystallization [11–13], but is presumed to form an amphipathic helix predicted to align parallel to the membrane surface [14–16]. This membrane targeting sequence (MTS) was shown to mediate a direct attachment between MinD and membrane phospholipids [17], maintaining this function when transplanted to other proteins [15]. While the relatively long MTS of MinD from *B. subtilis* ensures the permanent attachment of the protein to the membrane in these species, the shorter MTS of the *E. coli* MinD requires at least dimerization of MinD for binding and underlies its highly dynamic, oscillatory localization [15]. The molecular mechanism of the membrane affinity modulation is based on the ability of MinD-ATP, but not of MinD-ADP, to form dimers (or even oligomers) with higher affinity to the membrane surface and the antagonistic action of MinE, that stimulates the ATPase activity of membrane-bound MinD, promoting detachment [18,19]. This mechanism, together with spontaneous ADP-ATP exchange, appears sufficient to build numerous physical models explaining the pole-to-pole oscillations of MinD-MinE in *E. coli* [20–25].

Amphipathic helices mediate interaction with the membrane of many other amphitropic proteins [26,27]. Two essential features of this interaction are: i) the binding affinity can be modulated both by protein ligands and by membrane lipid composition, and ii) the binding affects both the protein function and the membrane physical properties. The advantage of these features is in providing a spatially

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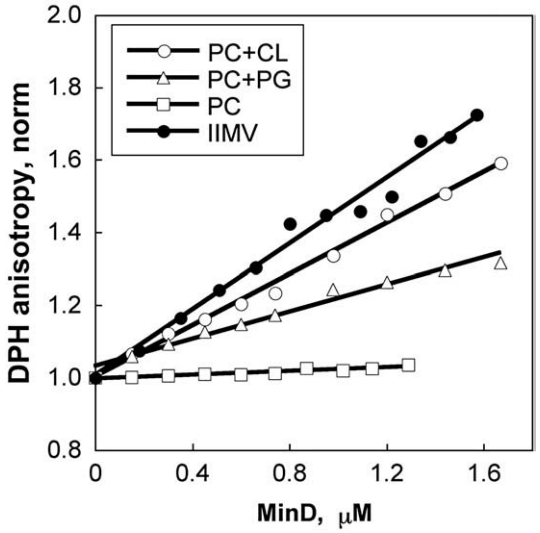

Fig. 1. Dependence of fluorescence anisotropy of DPH on MinD concentration added to liposomes of different phospholipid compositions and to IIMV's. The values are normalized to those in the absence of protein for each type of vesicle. The absolute values of anisotropy in liposomes without MinD were 0.1, 0.08, 0.075 and 0.1 for 100% PC, 40/60% PG/PC, 20/80% CL/PC liposomes and IIMV's, respectively. Membranes (containing 200 μM phospholipids of liposomes and 50 μM of phospholipids for IIMV's) pre-labeled with 5×10^{-7} M DPH (final concentration) were titrated with MinD-ATP to reach the shown concentrations, and fluorescence anisotropy was measured after 10 min equilibration at each concentration.

localized marker for randomly distributed cytoplasmic proteins participating in temporal and spatial regulation mechanisms. *E. coli* MinD displays a preference for anionic phospholipids as shown by in vivo and in vitro studies [15,28]. It was suggested that selection of the cell division site might be regulated by membrane phospholipids composition [28,29]. Existence of membrane heterogeneity or membrane domains is a prerequisite of such regulation. Indications for domains in bacterial membrane were reviewed recently [30]. Most relevant for this consideration are polar localization of cardiolipin (CL) [31,32] explained through an equilibrium mechanism of lipid microphase separation [33], and sequestration of fluorescent acidic and zwitterionic phospholipids into separate pre-existing domains in the bacterial membrane [34]. The combination of the MinD binding dependence on phospholipid composition with the membrane compositional heterogeneity could modulate both spatial and temporal oscillatory characteristics of the protein. On the other hand, putative changes in the membrane physical properties induced by MinD binding may affect other intracellular mechanisms, serving to coordinate between them, e.g. as demonstrated for adjustment of nucleoid morphology and segregation [35].

Part of our working hypothesis is that binding of MinD to the membrane, promoted by the ATP-induced dimerization, is further enhanced by a consequent attraction of acidic phospholipids and formation of a stable proteolipid domain. Furthermore, if an anionic phospholipid domain preexists in a heterogeneous membrane, then high local surface concentration of MinD monomers will encourage protein dimerization and anchoring. The present work tests these assumptions in a model system composed of purified MinD and liposomes of defined composition. Accordingly, the first part of the work is focused on characterization of the changes in the membrane induced by MinD binding as reported by three different membrane-embedded fluorescent probes. In the second part, we compare binding of MinD to the membrane containing a fraction of acidic phospholipids either evenly distributed or concentrated into domains due to phase separation. Moreover, we examine the protein from two different species — rod-shaped *E. coli* and spherical *Neisseria gonorrhoeae* in a quest of generalization of the phenomena under investigation. Actually, MinD's from the two sources are not that different. Having considerably high sequence identity, they both oscillate in *E. coli* and are functionally interchangeable [36]. In view of significant similarity in the phospholipid compositions of the two species [37], we also expect the same behavior of these proteins on the model membrane.

Here we show that binding of MinD causes a remarkable increase in the membrane order and decrease in lateral mobility, with different depth effects in artificial and native (inverted inner membrane vesicles, IIMV's) membranes. We present also indications for domain formation induced by MinD binding at particular fractions of acidic phospholipids in the liposomal membrane.

2. Materials and methods

2.1. Materials

1-Stearoyl-2-Oleoyl-*sn*-Glycero-3-Phosphocholine (SOPC), 1-Stearoyl-2-Oleoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol] (SOPG), CL from heart were purchased from Avanti Polar Lipids, Inc (Alabaster, AL). Chloroform was HPLC-grade. 1,6-diphenylhexatriene (DPH), pyrene and 1-hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycero-3-phosphoglycerol, ammonium salt (Py-PG) were obtained from Molecular Probes (Eugene, OR). All other chemicals were analytical grade. Buffers were prepared in deionized water.

2.2. Expression and purification of MinD

His-tagged MinD was overexpressed in *E. coli* strain WM1682 and purified as described in [28] with modifications. Cells from 1 l of culture were suspended in 25 ml cold lysis buffer containing 50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, 8 M urea, pH 8.0 and broken by sonication. Cell debris was removed by centrifugation at 10,500 ×*g* for 10 min (4 °C). The supernatant was further centrifuged at 120,000 ×*g* for 90 min (4 °C). Nickel-nitrilotriacetic acid Superflow slurry (Qiagen) was added to the supernatant and the mixture was slowly shaken on ice for 1 h. The slurry was poured into a column and washed 4 times with 8 ml washing buffer containing 50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0 and 0.5 mM phenylmethylsulfonyl fluoride. Nickel-nitrilotriacetic beads were shaken in 12 ml lysis buffer containing 250 mM imidazole for 1 h and beads were removed. The supernatant containing the protein was dialysed three times against a 100 fold volume of dialysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20% glycerol, pH 8.0) containing urea in concentrations 4, 2 and 1 M, and then twice against the same buffer without urea. Protein aggregates were removed by ultracentrifugation (300,000 ×g, 10 min) and the soluble protein was stored at −80 °C. In addition, before each experiment thawed protein was again ultracentrifuged. Protein concentration was determined by the Bradford method and purity was evaluated by SDS-PAGE. A comparison between the protein purified with urea and that obtained as described in [28] has confirmed that their basic membrane-binding behavior, such as phospholipid preferences and nucleotide dependence, was indistinguishable (not shown). We have previously verified that His-tag, connected to the N-terminal of MinD through a linker containing the thrombin cleavage cite, does not interfere with MinD–membrane interaction [28]. Note that the His-tag is neither charged at pH 7.5 nor hydrophobic and is spatially well separated from MinD MTS. Therefore, removal of the His-tag by thrombin cleavage is not necessary for proper interaction of MinD with the membrane and was therefore not performed.

2.3. Preparation of Large Unilamellar Vesicles (LUV)

Phospholipids were dissolved in chloroform and dried to a thin layer film under a gentle steam of nitrogen. Dried phospholipids were hydrated in a 25 mM Tris–HCl (pH 7.5) 50 mM KCl buffer above the phase transition temperature for 1 h and then were vigorously vor-