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Review

Protein–lipid interactions of bacteriophage M13 major coat protein

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Abstract

During the past years, remarkable progress has been made in our understanding of the replication cycle of bacteriophage M13 and the molecular details that enable phage proteins to navigate in the complex environment of the host cell. With new developments in molecular membrane biology in combination with spectroscopic techniques, we are now in a position to ask how phages carry out this delicate process on a molecular level, and what sort of protein–lipid and protein–protein interactions are involved. In this review we will focus on the molecular details of the protein–protein and protein–lipid interactions of the major coat protein (gp8) that may play a role during the infection of *Escherichia coli* by bacteriophage M13.

Keywords: Phage particle disassembly; Protein topology; Protein anchoring; Site-directed labelling; Spectroscopy; Protein–lipid interaction; Protein–protein interaction

1. Introduction

It is well established that the composition of biological macromolecule defines its structure and, ultimately through the motion of specific molecular groups, its function. The biological membrane with its myriad of different chemical structures, proteins and lipids being the most important constituents, is an excellent example of this principle. The function of a biological membrane can be quite easily perturbed by interaction with viruses, amphiphilic agents, peptides, or organic solvents [1–4]. Viruses, in general, use the physico-chemical properties of the membrane for two fundamentally different purposes. During the initial stages of the infection they transfer genetic material inside the host cells with a minimum of perturbation of the membrane structure. During later stages of the infection, however, they tend to maximally perturb the membrane structure in order to efficiently and quickly release the progeny [5]. For both processes they utilise the biophysical properties of the viral and host proteins embedded in the membrane.

By comparison with other viruses, filamentous phages are remarkable for their sophisticated use of the physico-chemical properties of the host membrane during the disassembly and assembly process [6]. Filamentous phages do not disturb the host cell to a great extent and the infected cells continue to grow and divide [7,8]. The only substantial effect on the host is an increase in generation time. During disassembly the coat proteins are inserted and stored in the host membrane. Newly synthesised coat proteins are also inserted in the membrane, but only up to the level that minimally affects the biological function of the membrane. To avoid critical concentrations the coat proteins leave the host membrane and assemble in a coat around the viral DNA. For a detailed description of the filamentous bacteriophages the reader is advised to consult excellent reviews that have been published during the past years [8–13].

With new developments in molecular membrane biology in combination with spectroscopic techniques, we are now in a position to ask how phages carry out this delicate process on a molecular level, and what sort of protein–lipid and protein–protein interactions are involved. In this review we will focus on the molecular details of the protein–protein and protein–lipid interactions of the major coat protein (gp8) that may play a role during the infection of *Escherichia coli* by bacteriophage M13.

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2. Structure of bacteriophage M13

Bacteriophage M13 is probably one of the most thoroughly studied filamentous phages by various biophysical techniques. The structure of the virion protein sheet was determined by X-ray fibre diffraction [14–16]. Bacteriophage M13 particles are 7 nm in diameter. The length is dependent on the length of the enclosed genome and is for the wild type approximately 900 nm. The viral particle is composed of a single-stranded circular DNA molecule that is encapsulated in a long cylindrical protein coat. The protein coat is composed of about 2800 copies of the major coat protein (gp8). At both termini there are five copies of each of the two minor coat proteins, gp7 and gp9 at one end and gp3 and gp6 at the other end (see Fig. 1) [17].

The major coat protein is a small protein with a molecular weight of about 5240 Da and forms a 1.5–2.0-nm-thick flexible cylindrical shell. It is composed of three specific domains: a hydrophobic core, an acidic N-terminal, and a basic C-terminal part (see the primary sequence in Fig. 2) [18–20]. In the phage particle the major coat protein is largely α-helical with four to five flexible unstructured amino acid residues in the N terminus. The helical lattice positions of the neighbouring N-terminal arms are about 2.7 nm apart. This space can be filled with extra amino acids genetically added to the N terminus of the major coat protein (up to 10 amino acids can be added) [21]. This has been exploited in the peptide display technology where a payload of small peptides is attached at each N terminus of the major coat protein without significantly impairing the viability of the phage.

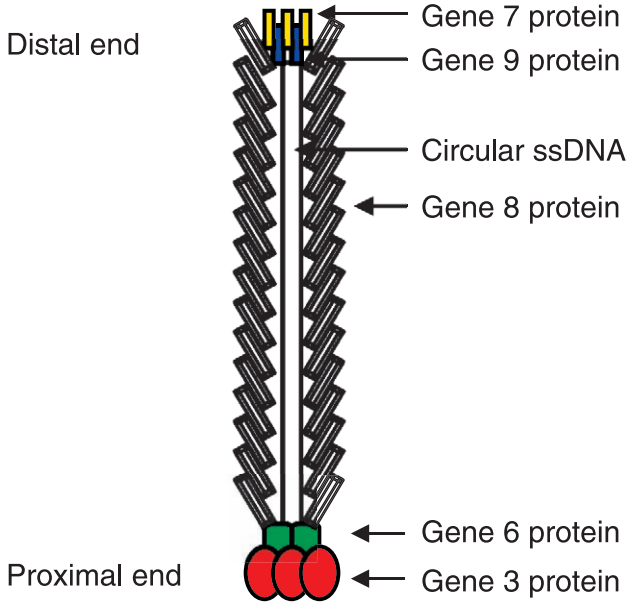

Fig. 1. Schematic illustration of a bacteriophage M13 filament. The body of the phage particle is composed of the major coat protein gp8 (about 2800 copies). Five copies of gp3 and gp6 at the proximal end and five copies of gp7 and gp9 at the distal end cover the two ends of the phage particle.

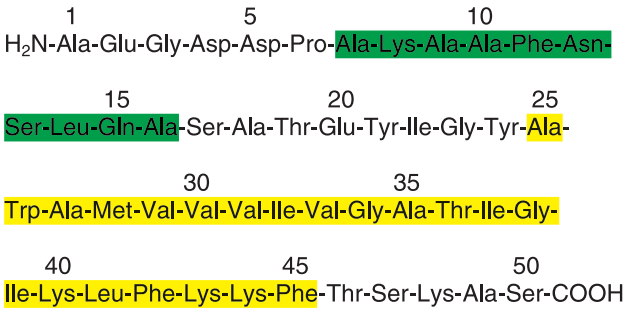

Fig. 2. Primary structure of M13 major coat protein. The α-helical segments that are observed for the protein in the membrane-bound form are highlighted in green for the amphipathic N-terminal helix and yellow for the hydrophobic transmembrane helix.

The protein shell reflects the major coat protein structure and can be considered to be composed of three sections: (i) the acidic N-terminal part is exposed to the surface of the virus and gives the virus a low isoelectric point; (ii) the interior of the shell is composed of hydrophobic amino acid residues; (iii) the basic C-terminal part of the coat cylinder interacts with the viral DNA [22]. The interactions between the coat and the DNA are nonspecific. The charge per unit length on the negatively charged DNA molecule is about equal in magnitude, but opposite in sign to that on the interior of the positively charged protein sheet. The major coat protein molecules are arranged around the DNA with a fivefold rotation axis and a twofold screw axis with a pitch of about 3.2 nm.

An arbitrary subunit indexed $i=0$ in the phage particle has neighbours along the helical array defined by the Fibonacci sequence (see Fig. 3). This means the arbitrary subunit $i=0$ has axial and tangential contacts with 10 subunits: $i\pm1$, $i\pm5$, $i\pm6$, $i\pm11$, and $i\pm17$ [23]. Of those the contacts in the direction 0–6 and 0–11 are of special importance because they comprise the nearest neighbour interactions between amino acid residues of the two subunits. The distance between the axes of neighbouring subunits is similar in both the 0–6 and 0–11 directions. However, the crossing angle in the 0–11 direction is negative, while in the 0–6 direction the two backbones are nearly parallel [16]. Because of this situation, the noncovalent interactions between proteins in the 0–6 direction are relatively stronger than between other subunits. This can be nicely demonstrated if one calculates the closest neighbouring interactions (assuming a cut-off distance of 0.5 nm) between amino acid residues of protein $i=0$ and amino acid residues of the neighbouring protein obtained from X-ray fibre diffraction co-ordinates [16,24]. For the direction 0–6, one gets 41 amino acid residues in contact between the two subunits. For instance, the bulky residue Trp^{26} of subunit $i=0$ is in contact with Gly^{34}, Ala^{35}, Gly^{38}, Ile^{39} and Phe^{42} of the subunit $i=6$. The corresponding number of contacts between the amino acid residues in the direction 0–11 is 29, in the 0–5 direction 10, in 0–17 direction 5. In the 0–1 direction there is only

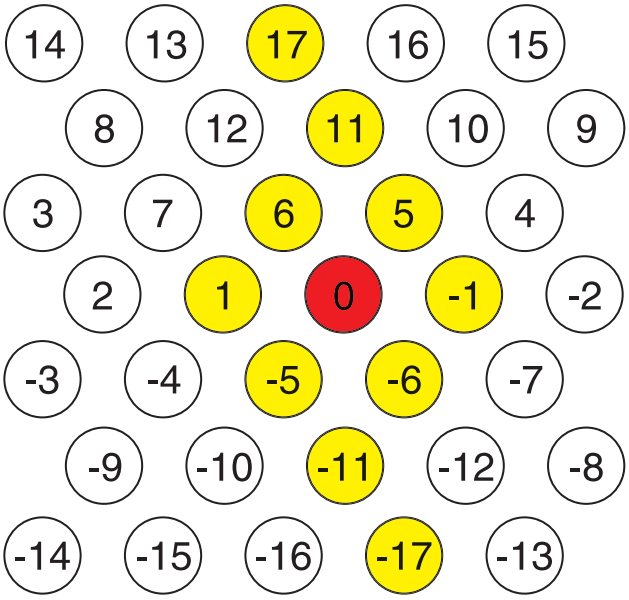

Fig. 3. A schematic representation of the M13 major coat proteins in the phage particle according to the model proposed by Marvin et al. [16] and Papavoine et al. [24]. The numbered circles give the relative positions of the coat proteins subunits on an opened and flattened protein coat cylinder. Each layer consists of five protein molecules corresponding to a row. The following layer is shifted 1.6 nm along the direction of the helix axis. The residue in red is designated by $i=0$ and has axial and tangential interactions with 10 neighbouring proteins at positions ± 1, ± 5, ± 6, ± 11, ± 17, which are shown in yellow.

one contact point between the two neighbouring helices [16,24]. An additional feature that locks the subunits in the phage coat is the interaction of phenylalanine amino residues on different subunits. The Phe^{11} residue on subunit $i=-17$ intercalates between the Phe^{42} and Phe^{45} residues on subunit $i=0$ [16]. The subunit interlocking via aromatic stacking is important for phage integrity. No viable phage particles are produced if the phenylalanine residues are replaced for nonaromatic residues.

3. Entry of the major coat protein in the host membrane

The major coat protein enters the host membrane via two fundamentally different mechanisms either as a mature parental coat or as a newly synthesised coat protein. The mature parental coat protein enters the membrane during the phage infectious entry when it is solubilised in the host cytoplasmic membrane [25]. By using radiolabelled phages, it can be shown that no parental major coat protein is inserted in the cytoplasmic membrane when TolQ, -R and -A proteins are absent as a consequence of a missing interaction of gp3 with these proteins [26].

Alternatively, the major coat protein can be incorporated in the membrane as a newly synthesised coat protein with an additional signal sequence consisting of 23 amino acid residues [27,28]. Charged residues and their interactions with the lipids [29–31] affect the membrane insertion of the newly synthesised precursor coat protein. After being inserted the signal sequence is cleaved off by the bacterial leader peptidase. Insertion of the major coat protein in the membrane does not require the bacterial Sec translocation machinery. However, it has been shown recently that protein YidC, which co-operates with Sec translocase, is involved in the procoat insertion in the inner host membrane [32]. Regardless of the mechanism of insertion, the final structure of the protein is the same: the hydrophobic part of the coat protein spans the plasma membrane, with the negatively charged N terminus located outside in the periplasm and the positively charged hydrophilic C terminus inside in the cytoplasm [33].

Although the phage coat is solubilised in the host membrane during the in vivo infection process, it is surprisingly resistant in vitro to the addition of phospholipids to a phage solution. Similarly it is insensitive to high or low pH, and high or low salt concentrations [16]. Under certain nonphysiological conditions the phage particle can, however, exist in alternative I and S forms [34–38]. For instance, on exposure to a water–chloroform interface, bacteriophage M13 contracts into a hollow spherical particle, the so-called S form. The contraction can be slowed down at low temperatures, and then intermediate I forms can be obtained. Because of the enhanced solubility of the I and S forms by lipids as compared to the filaments, it was suggested that these forms mimic intermediates that occur during the infection process [37–39]. The structure of the coat protein upon conversion from filaments to the I and S forms is intermediate relative to the structure of the coat protein in the phage and membrane; i.e. the amount of α-helix is reduced relative to the situation of the protein in the phage-bound form, but it is still higher than in the membrane bound form [40,41]. On the other hand, strong detergents such as SDS and CTAB can completely disrupt the tight phage structure [41]. Detergents have been used extensively for phage disruption studies as well in protocols to reconstitute the major coat protein into lipid bilayers [42,43].

4. Watching phage particle disassembly using site-directed labelling

Crystallographic techniques and NMR have been shown to be useful methods for obtaining detailed structural information of the major coat protein in the phage particle and in detergent systems, respectively. However, much less is known about the structural intermediates and the initial, or catalytic events that trigger structural changes during phage disassembly. Site-directed spin labelling and fluorescence labelling provide a means to investigate these problems. The general strategy of site-directed labelling is to introduce a reporter nitroxide or fluorescence label at a selected site along the protein using site-directed mutagenesis, and take advantage of the wealth of information in the electron spin

resonance (ESR) and fluorescence spectra [44–47]. For example, in the ESR spectral line shape detailed information about the rate of motion, anisotropy, and amplitude can be obtained. In addition, the ability of ESR spectroscopy to detect tertiary contact sites through the side-chain mobility is an extremely useful tool. This provides a means for monitoring reorganisations in the tertiary structure that accompany phage disassembly. The major coat protein of wild-type bacteriophage M13 does not contain a suitable labelling site for specific labelling. In order to create such a specific site in the major coat protein a single cysteine residue can be introduced [47]. The highly reactive SH group of the cysteine residue can then be used either for spin or fluorescence labelling.

Although it is generally accepted that in vivo the major coat proteins are stripped off from the entering DNA molecule in the lipid bilayer, it is possible to mimic the local events during disassembly by adding detergents. Using the site-directed spin labelling approach in combination with phage disruption by detergents, intermediate phage structures have been examined that may represent different stages of the phage disruption process [22,43]. When detergents disrupt phage particles, the system passes from a range in which phage particles saturated with detergent coexist with detergent in solution, to a range in which the coat proteins are solubilised in detergent micelles. Because inter-chain contacts and distances are altered during the disruption, the mobility of the side chains of the protein changes and consequently the mobility of the spin label changes as well. With this approach it was possible to detect the first molecular events during detergent-induced phage disruption. For instance, the spin-labelled major coat protein mutants V31C and G38C were selected, because these sites are located in the hydrophobic part of the protein and could be labelled in situ [22]. Therefore, these sites are good reporter locations for monitoring phage integrity. At the initial stages of the disruption, at sub-solubilising detergent concentrations, the detergent molecules wedge into the intact phage structure, breaking the original protein–protein interactions. This is reflected by an increased motional freedom of the spin label [22].

An additional support for a breaking of the original protein–protein contacts at sub-solubilisation detergent concentrations comes from CD spectroscopy studies. The CD spectrum of the intact phage is dominated by an anomalous negative ellipticity at 222 nm. This anomaly in the CD spectrum has been attributed to a strong absorption of the chromophore oscillator coupling between Trp^{26} and Phe^{45} of neighbouring protein subunits in the phage particle [48]. When at sub-solubilising concentrations the detergent molecules wedge into the phage particle, this coupled oscillator system breaks down, thereby decreasing the anomalous negative ellipticity in CD spectrum at 222 nm.

At solubilising detergent concentrations the outer hyperfine splitting and the amount of strongly immobilised component in the ESR spectra decrease dramatically. This is consistent with a complete disruption of the phage particle and a solubilisation of the major coat protein into the detergent micelles [22]. In vivo the major coat proteins of the phage particle are solubilised in the membrane upon phage disassembly, and no detergent-like molecules are involved. It is, however, likely that the solubilisation process by the lipids will be similarly fast and efficient once the minor coat proteins are stripped off from the entering phage particle. However, the mechanism by which the minor coat proteins are stripped off from the phage particle is not known.

5. Structural rearrangements of the major coat protein during infectious entry

Upon reconstitution in the membrane the secondary structure of the major coat protein is thought to change dramatically. Unfortunately a detailed atomic structure of the coat protein in the phospholipid membranes cannot be obtained by solution NMR or X-ray crystallography. Structural rearrangements were studied, however, indirectly by applying different spectroscopic techniques, such as solid-state NMR, ESR, FTIR, Raman, UV resonance Raman and Polarised Raman microspectroscopy, fluorescence, and CD spectroscopy [49–61]. The structure of the major coat

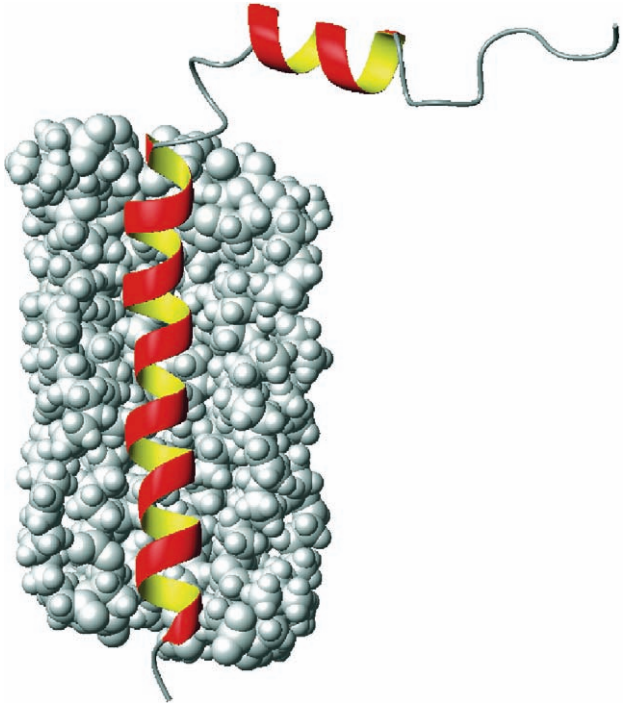

Fig. 4. Model of M13 major coat protein and first-shell lipid structures illustrating the secondary structure of the protein in a phospholipid bilayer. The model is optimised by molecular mechanics for best match with the experimental constraints form ESR data on spin-label mobility and membrane location of site-directed maleimide-labelled M13 coat protein [46,76]. The figure was based on Ref. [63] and was made with MOLMOL [100]. Courtesy: Denys Bashtovyy.

protein was determined either in the phage particle or when reconstituted in a model membrane environment.

Spectroscopic and other experiments in vitro on the major coat protein in lipid bilayers, lipid vesicles, detergents, and other organic solvents converge to support the view that the single slightly curved helix of the coat protein in the phage particle is decomposed into two α-helices that are roughly perpendicular to each other (see Fig. 4) [13,60,62,63]. In the membrane-bound form the N terminus (amino acids 1–5) is flexible and extends in the periplasm. The amino acids 7–16 form an amphipathic α-helix. The amino acids 17–24 form a loop region and connect the amphipathic helix with a transmembrane helix. The transmembrane helix extends from amino acid residues 25–45. The remaining amino acids 46–50 form a flexible C terminus that extends in the cytoplasmic membrane (see Fig. 2) [24,62,64–68].

6. Topology of the major coat protein in the lipid bilayer

Solid-state NMR spectroscopy and site-directed labelling enable the determination of the major coat protein location in the membrane. The location of the major coat protein in the membrane is schematically depicted in Fig. 5. Both the charged N terminus and C terminus extend into the polar lipid headgroup [64,69]. The results of solid-state NMR suggest that the N-terminal amphipathic part of the protein lies almost parallel to the surface of the membrane [62]. However, site-directed fluorescence and ESR experiments indicate that the N-terminal amphipathic helix is slightly tilted and may be in a more extended configuration [70,71]. Furthermore, depending on the conditions applied, the N terminus is not firmly attached to the membrane, but can move away from the membrane surface into solution. Solution NMR backbone relaxation studies indicate the importance of the hinge region, which enables flexibility between the amphipathic and transmembrane helix [24,68,72–74]. The location of the amphipathic helix appears to be dependent on the specific amino acid composition, the presence of specific membrane anchors, and the nature of the flanking protein domains. This will be discussed in detail below.

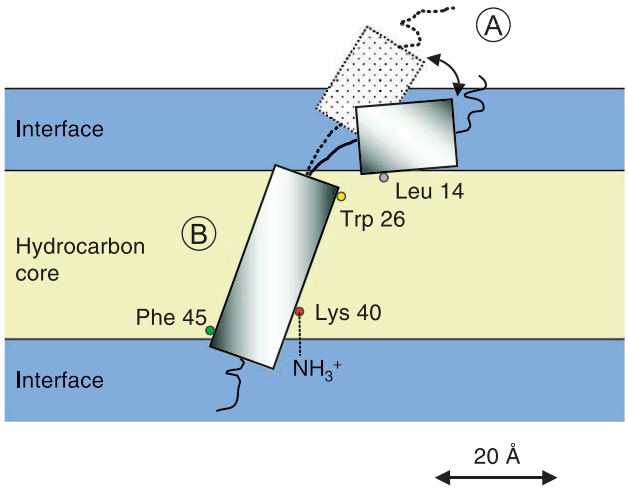

Fig. 5. Location of the membrane anchors of the M13 major coat protein. The two boxed regions represent the amphipathic helix (A) aligned along the membrane surface and transmembrane helix (B). A more extended and contracted configuration of the amphipathic helix is depicted. The dynamics of the formation and closing of the loop determines the configurational fluctuations of the protein. Only the locations of the amino acid residues that are most important for the anchoring of the major coat protein are indicated in the transmembrane and amphipathic helix: Leu^{14} (grey), Trp^{26} (yellow), Lys^{40} (red), and Phe^{45} (green).

As is suggested by solid-state NMR, the transmembrane helix is tilted by $20 \pm 10°$ with respect to the lipid bilayer normal [75]. The relative position of the transmembrane helix in the lipid bilayer has been studied by site-directed labelling [47,76]. Using progressive-saturation ESR spectroscopy in dioleoylphosphatidylcholine (DOPC) bilayers, and by applying oxygen and Ni^{2+} ions as paramagnetic relaxation agents preferentially confined to the hydrophobic and aqueous region, respectively, it was possible to precisely locate the position of the labelled part of the protein in the lipid bilayer. From these results it is clear that the section of the protein around position Thr^{36} is located in the middle of the membrane, and that the amino acid residues 25 and 46 are located close to the polar headgroup region of the lipids. Such a topology has been confirmed by fluorescent studies of AEDANS-labelled cysteine mutants reconstituted in mixed phospholipid bilayers consisting of DOPC and dioleoylphosphatidylglycerol (DOPG). Furthermore, it is in accordance with the transmembrane helix being fully embedded in the hydrophobic membrane [47]. The hinge region that connects the amphipathic and transmembrane helix appears to be fully embedded in the membrane as well [70].

7. Anchoring of the major coat protein to the lipid bilayer

It is essential for the major coat protein to remain in a stable thermodynamical association with the lipids once it is inserted in the membrane. Because it is a small protein it could be easily lost from the membrane, if it would not be anchored well enough. In addition, it should be in the membrane in a correct conformation and orientation in order to be able to participate in the phage assembly that takes place in the membrane. In general, anchoring of the protein to the membrane is accomplished by a combination of different mechanisms: (i) hydrophobic interactions between the hydrophobic domains of the protein and the acyl chains of the lipids; (ii) electrostatic interactions between the charged amino acid residues (i.e. lysines) of the protein and corresponding charges on the phospholipid headgroups; (iii) interactions of a polar part of aromatic amino acids (i.e. tyrosine and tryptophan) of the protein

with the phospholipid headgroups at the membrane–water interface, and hydrophobic part of these amino acid residues with lipid acyl chains. The hydrophobic transmembrane part and the amphiphatic helix are the most important anchors of the coat protein. In addition there are specific aromatic and charged amino acid residues in the structure of the coat protein that fine-tune the association of the protein to the membrane (see Fig. 5).

The amphipathic helix is anchored in the membrane primarily by the hydrophobic amino acid residues (i.e. Phe^{11} and Leu^{14}). When these two amino acid residues are replaced for alanine residues, the N-terminal arm moves to a more extended configuration into the aqueous solution [70,71]. The opposite is found when more hydrophobic amino acid residues are introduced in the N-terminal amphipathic helix. In this case the amphipathic helix is brought closer to the hydrophobic core of the membrane. Since the N-terminal part of the coat protein is composed of both hydrophobic and hydrophilic amino acid residues, it is clear that the topology of the N terminus is determined primarily by its physico-chemical properties. In this respect, it is interesting to note that the major coat protein of other filamentous phages, i.e. fd with a similar primary sequence and Ike, Pf1 and Pf3, which lack primary sequence homology with the M13 major coat protein, displays a similar topology of the N terminus [62,77–79]. Although chemically different, N-terminal parts show comparable physico-chemical properties (i.e. hydrophobicity and hydrophilicity) and consequently a similar topology in a membrane environment.

The N terminus of the coat protein can be forced into a more extended conformation also when the lipid to protein ratio is decreased [70]. This allows less space for the N terminus at the surface of the lipid bilayer. This lifting effect of the N terminus at an increased local protein density may have important consequences for the protein during the phage assembly process.

The transmembrane part of the coat protein is, due to its hydrophobic character, comfortably anchored in the membrane. Nevertheless, several additional anchoring elements can be identified along the transmembrane helix [80]. At the N-terminal part of the transmembrane helix, a tryptophan residue, which is a known anchor, is located at the hydrophobic/hydrophilic interface. However, when the thickness of the lipid bilayer is increased from 2.0 nm in dimyristoleoylphosphatidylcholine (DMPC) lipid bilayers to 3.1 nm in dieicosenoylphosphatidylcholine (DeiPC) lipid bilayers, the tryptophan sinks to a more hydrophobic environment, as suggested by the decreased emission maximum of the tryptophan fluorescence [80]. If a mutant coat protein with three extra alanine residues in the transmembrane part of the coat protein is inserted in DOPC, the tryptophan moves out of the membrane to a more hydrophilic environment [80]. This indicates that the tryptophan residue itself is only a weak membrane anchor. Depending on the situation, the tryptophan residue moves in or out of the membrane (see Fig. 6).

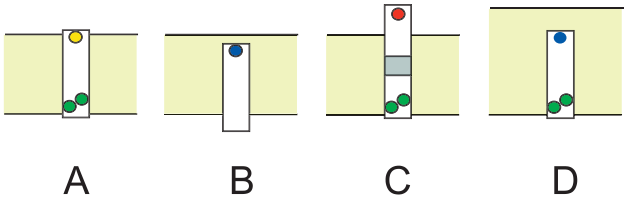

Fig. 6. Position readjustment of the transmembrane helix. (A) In the transmembrane helix in DOPC the amino acid residues Trp^{26} (yellow), Phe^{42}, and Phe^{45} (both in green) are close to the lipid–water interface (indicated by horizontal lines). (B) A mutation that removes both Phe^{42} and Phe^{45} brings Trp^{26} in a more hydrophobic environment. This causes a blue shift of the emission maximum of the Trp fluorescence and is indicated by a blue colour. (C) Insertion of three alanines (indicated in grey) brings Trp^{26} in a more hydrophilic environment. This causes a red shift of the emission maximum of the Trp fluorescence and is indicated by a red colour. (D) The transmembrane helix in DeiPC, which has an increased bilayer thickness as compared to DOPC, brings Trp^{26} in a more hydrophobic environment.

On the other hand, the two phenylalanines and a number of lysines at the C terminus of the major coat protein provide a stronger anchoring mechanism. When a fluorescence or spin label was attached to the hydrophobic/hydrophilic interface at the C-terminal part of the transmembrane helix, it preferred to stay at the interface despite increasing or decreasing the hydrophobic thickness of the lipid bilayer, or changing the hydrophobic length of the protein [80]. If both phenylalanine residues were replaced by alanines the anchoring effect was gone and the C-terminal part of the protein moved into the water phase, as suggested by a red shift of the AEDANS emission maximum and a blue shift of the tryptophan residue [80] as indicated in Fig. 6.

Apart from the two phenylalanine anchors in the C terminus of the major coat protein, there are also three lysine residues that may be important in transmembrane helix anchoring. Due to their deep burial in the lipid bilayer the lysine residues 40, 43, and 44 have their α-carbons in the hydrophobic interior of the lipid bilayer, while by a snorkelling effect the ε-amino groups are located in the polar head-group region of the membrane [76,80]. The lysine residues tend to counterbalance the effect of the phenylalanine residues, which pull protein in the hydrophobic membrane interior. When this happens, the ε-amino groups of the lysine residues will be in an unfavourable environment and stop the inward helix movement. The opposite effect is taking place when the protein moves to the water phase. In this case, the hydrophobic phenylalanines are in a thermodynamically unfavourable environment and stop the outward movement of the protein. If the two phenylalanines are replaced by alanines, there is no counterbalancing force and the C-terminal end of the transmembrane helix will be shifted to the hydrophilic environment. The shift of the transmembrane helix along the normal of the membrane can therefore be visualised by a motion of a float in a rough lipid sea.

Since the transmembrane helix is connected via the hinge to an amphipathic helix, it may be expected that the inward

and outward movement of the transmembrane helix and the membrane location of the amphipathic helix are coupled. Therefore, the loop region is an important factor in the anchoring interactions of the major coat protein in the membrane. It possibly acts as a spring connecting the two helices. This suggests that the only real change during the insertion of the coat protein in the membrane is a structural backbone rearrangement of the loop region. The dynamics of the formation and closing of the loop therefore determines the configurational fluctuations of the membrane-bound state of the protein (see Fig. 5).

8. Reversible aggregation schemes of the major coat protein

During the life cycle of the filamentous phage the aggregational state of the major coat protein changes repeatedly. In the intact phage particle the coat protein is aggregated in a helical array as described in detail in the section about the structure of the phage [13,16,17]. During the disruption of the phage particle, the aggregational status of the major coat protein changes. In disruption studies of phage particles in SDS and cholate detergents with spin-labelled M13 bacteriophage particles a structural dimer unit was observed [81]. A structural dimer is a reminiscent structure of the phage coat with two monomers parallel, but slid with respect to each other for about 1.6 nm. The two monomers in the structural dimer are removed together from the phage particle upon detergent solubilisation. During cholate disruption of the phage the amphiphiles first disrupt the weak hydrophobic forces that hold the phage together, in the $i=0$ to $i=1$, 5, 11, and 17 direction, while strong hydrophobic forces between subunit $i=0$ and $i=6$ are initially not affected. The structural dimer is a stable aggregate that cannot be dissociated by phospholipids, but its dimeric state is affected by higher pH values. At pH 10.0, structural dimer appeared to be irreversibly disrupted, and the two monomers become in line with each other [81].

The aggregational state of the major coat protein in a membrane environment is less clear. It is generally accepted that the major coat protein is evolutionary designed to aggregate. Nevertheless, a monomeric state of the major coat protein was proposed in a membrane environment [82]. This has been also suggested from SDS-PAGE experiments [81,83–86]. There is, however, an evidence that protein monomers can reversibly aggregate and form transient dimers, tetramers, and higher oligomers [42,84]. Thus, it is conceivable that the major coat protein exists in the lipid bilayer in a range of aggregational states depending on the history of the coat protein preparation, detergent or lipid composition, lipid to protein ratio, pH, ionic strength, and temperature.

The aggregational state can also change uniquely upon different types of mutations or locations of the mutation [84,85,87]. In vitro, aggregation behavior is further complicated by protein tendency to orient upon the reconstitution in the membrane in two ways instead of one observed in vivo. The data on the lateral diffusion of the coat protein in the plane of the membrane could not also unambiguously resolve the aggregational status of the coat protein in the membrane environment [81,88,89]. It has been demonstrated by fluorescence photobleaching experiments that above the DMPC phase transition the lateral diffusion coefficient D of the coat protein ranges from 10^{-9} to less than 5×10^{-12} cm^2/s, the later being comparable to the lateral diffusion of lipids. Such a wide distribution of diffusional coefficients suggests that the protein appears to be nonuniformly distributed as a result of either protein aggregation or phase separation into protein-rich and protein-poor regions [88].

In addition, depending on the sample preparation, two specific protein conformations have been observed in detergents and membranes: a predominantly α-helical conformation, where the protein has an ability to undergo a reversible aggregation, and a β-polymeric state, where the protein with predominantly β-sheet conformation is irreversibly aggregated [90,91]. The current view is that the β-polymeric state is an artefact, which could be interpreted as a denatured form of the coat protein, because it forms in an irreversible way, unable to convert back to the original α-helical conformation found in the virus particle [6].

Clearly the coat protein has the ability to adopt its conformation, which allows the protein to exist in distinctly different environments, such as the phage filament, the I-form phage, the S-form phage, and the membrane-bound form. This is possible because of the amphipathic nature of the coat protein so that it can have both hydrophobic and hydrophilic interactions with its environment. This property gives the protein a large conformational space that allows

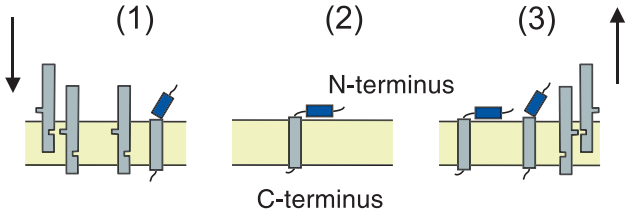

Fig. 7. Model of the structural and aggregational changes of the major coat protein during disassembly and assembly of the phage particle. (1) During the disassembly process of the phage particle the major coat solubilise as a structural dimer. Knobs and holes represent protein–protein interactions in the phage particle. The two horizontal lines indicate the hydrogen carbon core of the lipid bilayer. (2) When reconstituted in the lipid bilayer as a monomer, the major coat protein undergoes a major structural change from a single slightly curved continuous helix in the phage particle to a transmembrane helix and an amphipathic helix. The C and N terminii are not fully structured. (3) During the assembly of the new phage particle, the major coat protein interacts with its amphipathic helix with the already assembled proteins. Virus particle extrusion and addition of new coat proteins are regulated in such a way that the extruding phage moves 1.6 nm out of the plane of the membrane before the next coat protein is added. This allows an optimal side-chain interlocking between neighbouring proteins in the virus particle.

very flexible protein aggregational schemes. A schematic diagram of the main structural and aggregational changes of the major coat protein during phage disassembly or assembly and in the membrane-bound form is shown in Fig. 7.

9. Exit of the major coat protein from the membrane

In vitro, the major coat protein is incapable of spontaneous combining with the DNA to give the regular viral pattern in a solution. However, it can interact with the DNA during membrane-bound assembly [92–94]. This ATP-driven process is mediated by several phage-encoded proteins (i.e. gp1 and gp4) and host proteins (i.e. thioredoxin) that constitute the membrane-bound assembly site. In order to participate in the phage assembly process, the major coat protein must exit from the membrane and lift its anchors that keep it in a stable thermodynamical interaction with the membrane. In addition, the lysine residues of the major coat protein must be exposed for interaction with the viral DNA. There seems to be no interaction between the C terminus of the coat protein in the lipid bilayer and the DNA prior to the assembly of the phage particle [46]. This is explained by a lack of interactions between the lysine ε-amino group and the phosphates of the DNA due to the interactions of ε-amino group with the omnipresent phospholipid phosphates. It is, however, not known how the major coat protein gets rid of the lipids during the assembly, which would allow the interaction with the DNA.

The membrane-spanning region of the major coat protein is assumed to interact with the gp1. This interaction can facilitate the transfer of the coat protein from a membrane to a proteinous surrounding in the assembly site [12,86]. During the initiation of the assembly process the major coat protein interacts with the membrane-bound gp9 and gp7 proteins [95], and a 32-base-pair palindrome-packaging signal of the viral DNA [8]. Using the similarities in structure and sequence elements, a model has been constructed that assumes that the first five major coat proteins (gp8) have a structure that is similar to the structure of the protein in the membrane [17]. These five major coat proteins interact with the homologue parts of the gp9 and gp7 proteins. The major coat protein first interacts with the gp9 protein, which is a small largely hydrophobic protein with charges in the C terminus that interact with the DNA. The major coat protein then interacts with the gp7 protein. In order to provide a stable viral cap, the major coat protein must cover the exposed hydrophobic structure of both gp9 and gp7 proteins. The first 15 coat proteins assembled (i.e. five copies of gp9, gp7, and gp8) make up the initiation complex. It is suggested that this complex is a preformed membrane-bound structure at the inner host membrane, although its presence could not be demonstrated in vivo [95].

Upon addition of the next major coat proteins, the body of the virus particle is growing and protruding from the membrane, via a pore, formed by a homo-multimer of gp4 in the outer membrane [96]. The major coat proteins that form the body of the virus particle must go through substantial structural rearrangements. From a two-helix conformation in the lipid bilayer the protein must go to a single-helix conformation in the virus particle. It is anticipated that the major coat protein that is approaching the assembly site first specifically interacts with gp1 and subsequently binds via specific amino acid residue interactions to already assembled proteins. After being hooked, the protein interacts with already assembled proteins as well as with the DNA via the lysine residues. Protein packing is facilitated by small amino residues, which promote the close approach of α-helices [97]. However, no details are known. When one protein is assembled a new can be added. For this to happen the phage must be extruded for 1.6 nm along the normal to the membrane. It takes four successive steps for a protein to be completely incorporated into the phage particle [16,17,24]. In the first step, the subunit with index $i=0$ interacts with the subunit $i=-1$; in the next step, it interacts with subunits $i=-5$ and $i=-6$. In the third step, interaction takes place with subunit $i=-11$, and in the final step, the phenylalanine residues become interlocked with the phenylalanine residue in subunit $i=-17$.

As the end of the viral DNA is reached, gp6 protein successfully competes with the major coat protein for the binding to the elongating virus particle [17]. The terminal major coat proteins at the proximal ends of the virion are exposed to the solvent much more than the major coat proteins that form the body of the virus particle. This potentially unstable situation can be relieved by interaction of the major coat protein with the amphipathic part of the gp6 protein. After gp6 is attached the gp3 protein molecules are added. Since the structure of the gp3 proteins appears to be rather flexible, it may contribute to the breaking away of the virus particle from the membrane without rupturing the membrane and a collapse of membrane potential [98,99]. This process completes the extraordinary journey of the major coat protein. The major coat protein being integrated in the phage particle is now ready to go through another cycle of bacteriophage infection.

10. Conclusions

During the past years, remarkable progress has been made in our understanding of the replication cycle of bacteriophage M13 and the molecular details that enable phage proteins to navigate in the complex environment of the host cell. From biophysical work it follows that the relatively simple phage proteins have a complex set of protein–protein, protein–DNA, and protein–lipid interactions. Individual amino acid residues or domains of amino acid residues with similar physico-chemical characteristics specifically provide these interactions. It is interesting to observe that a small protein, such as the major coat protein

of bacteriophage M13, can profoundly change its conformation and structure to accommodate constraints imposed by distinctly different environments (i.e. phage filament, I-forms, S-forms, and cytoplasmic membrane). Due to its multiple-task character, it is likely that for a given interaction (i.e. protein–lipid, protein–protein, or protein–DNA) a better protein could be designed than the existing major coat protein. However, by keeping in mind that for an efficient macromolecular assembly different and sometimes contradictory requirements are encountered, the major coat protein apparently represents an optimal co-evolutionary design.

From the work on M13 bacteriophage presented here, it is demonstrated that the phage can serve as a challenging model system for understanding macromolecular assemblies. The work is relevant for the field of membrane biology and biophysics as well, since it uses the biological membrane as a platform to examine and unravel complex molecular interactions. Furthermore, it contributes to our understanding of the mechanisms of transport of macromolecules across the biological membrane, which is of high importance for optimising the overexpression and excretion of proteins across the membrane in biotechnology applications.

The major coat protein of bacteriophage M13 is probably one of the smallest proteins of its kind, serving as an example of the utmost genetic, biochemical, and biophysical economy of viruses in the living world. This is most clearly exemplified by the small size of the protein, its very efficient transcriptional and translational regulation and recycling of parental major coat proteins between the phage particle and the membrane, its ability to recruit host proteins for membrane insertion, and, probably most important of all, its capability of forming and releasing protein–protein, protein–DNA, and protein–lipid assemblies.

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