PROTEIN—LIPID INTERACTIONS

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A. Watts (Ed.), *Protein–Lipid Interactions*

CHAPTER 8

Lipid–protein interactions involved in bacteriophage M13 infection

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Abbreviations

DMPC	1,2-dimyristoyl-*sn*-glycero-3-phosphocholine	DOPE	1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine
DMPG	1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol	DOPG	1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol
DOPC	1,2-dioleoyl-*sn*-glycero-3-phosphocholine	ESR	Electron spin resonance
		L/P	Lipid to protein molar ratio
		NMR	Nuclear magnetic resonance

1. Introduction

A challenging question in biology is the way viruses and bacteriophages utilize the properties of the plasma membrane of biological cells to pass this cell barrier. The plasma membrane consists of many different species, such as phospholipids and protein molecules that interact mutually. To pass a plasma membrane, a virus utilizes the receptor properties of the membrane, and triggers a sequence of processes enabling it to pass its genetic information on to the host cell, which will then start to produce new copies of the virus. The large number of possible mechanisms has been only partly elucidated on a detailed biochemical and biophysical level, involving the role of the major coat proteins of filamentous bacteriophages in the plasma membrane.

Among the coat proteins of bacteriophages, the major coat protein of bacteriophage M13, that is the product of gene VIII, has been studied in several laboratories. The membrane-bound state of this protein has been characterized using biochemical and biophysical techniques that have resolved the structural and functional properties of the protein incorporated into model membrane

systems. It is found that membrane-bound M13 coat protein appears in two different states, denoted as α-helical protein (or α-oligomers) and β-polymeric protein, respectively. Based on its chemical and physical properties, the α-helical form is supposed to be the putative native state of the coat protein during virus infection. The β-polymeric form is an unnatural state of the protein that occurs in lipid model membranes.

In a previous article, we have compared the properties of the α-helical protein and the β-polymeric membrane-bound forms of the coat protein in detail, describing the spectroscopic experiments that have been carried out on reconstituted lipid–protein systems [1]. The present article will mainly deal with the α-helical form of M13 major coat protein and its properties in relation to the in vivo biological processes.

2. *The M13 virion and its reproductive life cycle*

2.1. M13 bacteriophage

M13 bacteriophage and the closely related phages f1 and fd are *Escherichia coli* specific filamentous phages belonging to the genus Inovirus. The virion consists of a circular single-stranded DNA molecule of about 6408 nucleotides (coding for 10 identified proteins) encapsulated in a long cylindrical protein coat. The protein coat is composed of about 2700–3000 copies of the major coat protein, the processed product of gene VIII. Apart from this abundant coat protein, the virions contain at one end 3–5 copies of the adsorption protein (the product of gene III) together with some copies of the bacteriophage particle stabilizing gene VI protein. The opposite end of the virion is composed of about 5 copies each of a complex of the protein products of gene VII and gene IX. Detailed descriptions of the viral particle can be found in the excellent and extensive reviews by Rasched and Oberer (1986) [2] and Model and Russel (1988) [3].

2.2. Reproductive cycle

The reproductive life cycle of bacteriophage M13 includes the adsorption of the virions at the F-pili of the host *Escherichia coli* cells specifically mediated by the viral adsorption protein (gene III protein). The viral particle is brought to the cell surface, by a mechanism that is yet not well understood. The gene III protein is thought to play a key role in facilitating the entry of the viral DNA into the cell by the formation of a pore, enabling passing through both the outer cell envelope and the cytoplasmic or inner membrane [4]. During the infectious entry of the virus the hydrophobic gene VI protein, which functions as a sort of

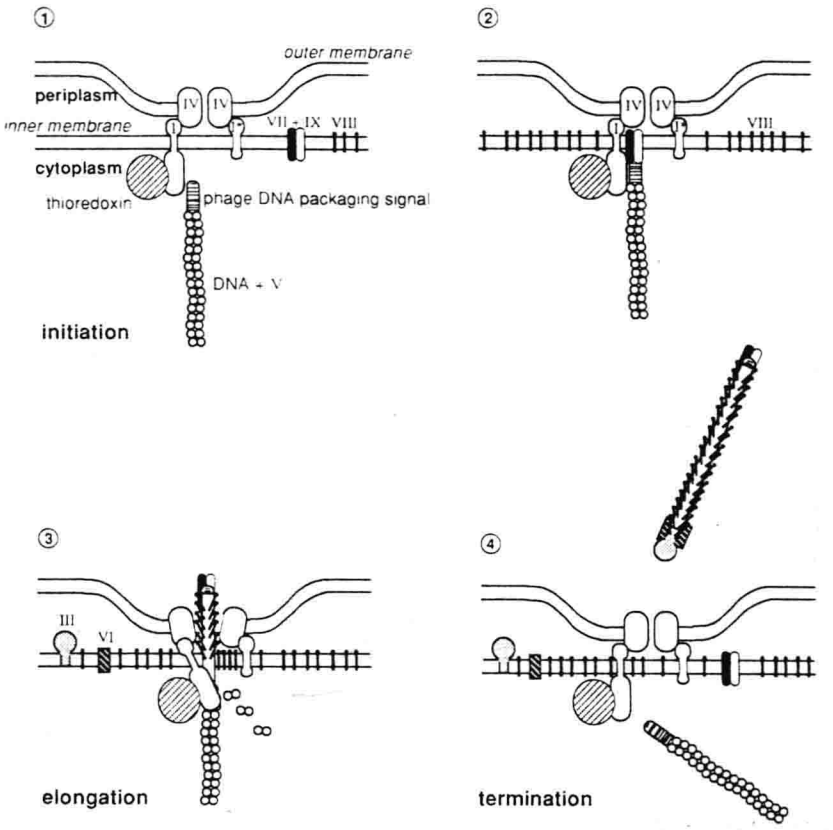

Fig. 1. Schematic illustration of the assembly site of bacteriophage M13. In the assembly site the gene IV proteins are thought to constitute an extrusion channel through the outer membrane. The gene I and I* proteins are assumed to form an extrusion channel in the inner membrane. The viral protein coded by genes VII and IX and at least one host protein (thioredoxin) are necessary for the initiation of the viral assembly process, in which the viral DNA and the abundant gene VIII (major) coat proteins are assembled and extruded without lysis of the host cell. The gene V protein is released as dimers in the cell. Assembly of the virus is terminated with the proteins coded by genes III and VI. Steps 1 and 2 show the initiation of the assembly of the phage, in step 3 elongation takes place, and in step 4 the assembly is completed. After Russel [7] and Guy-Caffet et al. [9].

sealing agent to stabilize the phage particle, is lost from the virion, resulting in a destabilized nucleo protein particle. The major coat protein of this destabilized nucleo protein particle is stripped off from the DNA and is meanwhile dissolved in the inner membrane. The DNA is released in the cytoplasm and will start the synthesis of new DNA and proteins using the host cell machinery. Newly synthesized coat proteins are inserted into the inner membrane prior to being used in the assembly process.

Two other viral proteins (the products of genes I and IV) play a functional and structural role in the formation of membrane-bound assembly sites, resembling adhesion zones between the inner and outer membrane [5,6]. In such an assembly site, which is schematically shown in Fig. 1 [7–9], the gene IV proteins are thought to constitute the part of an extrusion channel passing the outer membrane [8]. The gene I protein as well as an in-frame translated C-terminal part of the gene I (denoted as gene I* protein) are both associated with the inner membrane, and are assumed to constitute together the part of the extrusion channel passing the inner membrane [9]. The gene I protein interacts with the packaging signal of the cytosolic DNA–gene V protein complex, thereby probably initiating the viral assembly process in which the viral gene V protein on the viral DNA is replaced by the major coat protein. At least one host protein (thioredoxin) is thought to have a catalytic role in this process [10]. During assembly the viral particles are extruded without lysis of the host cell.

The complete structure of the assembly site, including the associations between the gene I and gene I* proteins in the inner membrane and the gene IV proteins in the outer membrane, remains, however, to be elucidated. Also, the mechanisms that prevent leakage of the host cell contents and loss in membrane potential have not yet been described in detail. Excellent reviews with emphasis on the assembly process that deal with these complex subjects have appeared recently [2,3,7,11].

2.3. Biological questions

In our research, we have aimed at understanding the molecular properties of the membrane-bound bacteriophage disassembly and assembly processes, based on the following questions: (1) How does the phage succeed in its reproductive cycle, using the host cell machinery without causing severe damage? (2) What are the specific functions of the viral and host proteins involved? (3) What kind of protein–protein interactions and/or protein conformational changes play an initial or catalytic role in (dis)assembly, and (4) What is the role of the phospholipids involved?

These questions have been approached by studying the structural and functional behaviour of the major coat protein of bacteriophage M13 and related phages, when incorporated into model membrane systems. Various spectroscopic techniques have been applied that provided detailed information about these reconstituted systems, whereas biochemical separation techniques allowed to purify and characterize these systems. In the following sections, we will first discuss the role of M13 coat protein in the infection process (section 3), leading to conditions to reconstitute the protein in its native state into model membrane systems (sections 4 and 5). Finally, the structure of the coat protein and protein–lipid interactions will be discussed in sections 6 and 7, respectively.

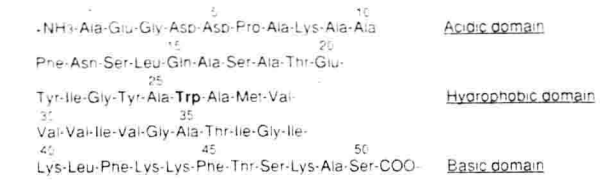

Fig. 2. Amino acid sequence of M13 coat protein. Three domains are indicated: an acidic N-terminus of 20 residues, a hydrophobic domain of 19 residues and a basic C-terminus of 11 residues. The single tryptophan that has been used for time-resolved fluorescence studies is at position 26.

3. *The major coat protein during the infection process*

3.1. *M13 major coat protein*

The major coat protein with a molecular weight of 5240 Da is composed of three specific domains (Fig. 2): a hydrophobic core of 19 amino acids is flanked by an acidic N-terminal part and a basic C-terminal part. During infection the major coat protein is involved in four processes located at the *Escherichia coli* inner or cytoplasmic membrane: (1) the parental coat proteins are inserted into the membrane while the viral DNA is released in the cytoplasm [12], (2) newly synthesized coat proteins are inserted into the membrane and processed by a leader peptidase [13,14], (3) parental as well as newly synthesized and processed coat proteins are stored oriented and transmembrane at high local levels [15–17], and (4) parental as well as newly synthesized coat proteins are assembled around the viral DNA during the combined assembly–extrusion process at membrane-bound assembly sites [6,18–20].

3.2. *Effects of phospholipids*

The phospholipid composition of the inner membrane of *E. coli*, which is about 70% phosphatidylethanolamine, 25% phosphatidylglycerol and 5% cardiolipin [21,22], is suitable to accommodate the major coat protein of the bacteriophage as well as many other bacterial proteins in a proper way. During the normal development of the infection process in case of a wild type bacteriophage, the phospholipid composition of the cell envelope is only little affected in favour of the charged phospholipids phosphatidylglycerol and especially cardiolipin [23–25]. However, as has been shown for abortively infected cells employing various amber mutants of the bacteriophage (and thereby blocking the assembly–extrusion process), accumulation of high amounts of the major coat protein in the inner membrane resulted in significantly increased levels of cardiolipin and phosphatidylglycerol, and a compensating

decline in phosphatidylethanolamine [21,24,25]. This strongly suggests a role of phosphatidylglycerol and cardiolipin in conserving the functional state of the membrane-bound coat protein as well as the proper functioning of the cytoplasmic membrane at these stressed low L/P ratios in vivo.

4. *Reconstitution of M13 coat protein*

4.1. *General principles*

To be able to perform advanced biochemical and biophysical techniques on membrane proteins, it is necessary to have a well-defined lipid–protein model systems that enables detailed studies on the molecular level of lipid–protein interactions. Of course, the biochemical integrity of such lipid–protein model systems is essential, and it should be carefully checked whether the functional properties of the protein are conserved. From the literature it is clear that, in general, reconstituting proteins in lipid membranes involves many difficulties, such as proper handling of the proteins with respect to solvation during isolation, purification, and reconstitution. The conditions in vitro during and after reconstitution of the M13 coat protein should reflect the in vivo situation as much as possible with respect to L/P ratio, lipid composition of the membrane, orientation of the proteins in the membrane, physiological condition (such as ionic strength, pH, temperature) and, if possible, functional activity [26–29].

Due to the amphiphilic character of the M13 coat protein, a detergent has to be applied to transfer the protein from the phage into a lipid system. Although such a reconstitution procedure enables the incorporation of the protein into the bilayers, it will generally result in a random orientation of the protein in the lipid system, because the conditions do not favour a specific orientation. This is in contrast with the situation encountered in vivo, where the N-terminal part is directed outward in the periplasmic space and the C-terminal part is in the cytoplasm [16,17]. Up until now this problem has not been solved, and all reconstitution experiments in the literature have been carried out on randomly oriented coat proteins.

4.2. *Basic conformations of transmembrane domains*

The basic conformations that can be expected for transmembrane domains of proteins are an α-helix or β-sheet [30,31]. To stabilize these structures, most hydrogen bonds must be satisfied between the protein strands. This requirement can be met by the formation of intra-chain hydrogen bonds resulting in an α-helix or inter-chain hydrogen bonds resulting in a β-sheet (see Fig. 3). For a single α-helix 21 amino acid residues are required to traverse the 3.2 nm

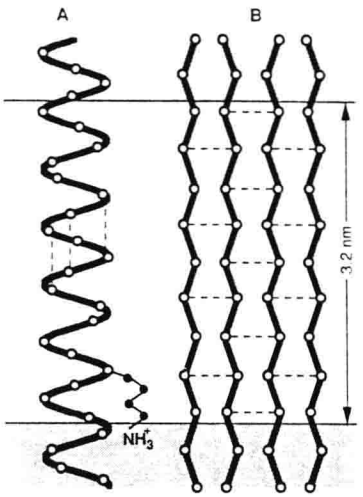

Fig. 3. Basic conformations of an integral protein to penetrate and traverse the hydrophobic core of a phospholipid bilayer. (A) α-helix, (B) β-sheet. The open circles schematically represent the C_α–CO–NH moiety of the polypeptide backbone. Within the hydrophobic core the amino acid side chains must be hydrophobic in character. In general, charged side chains are prohibited in the hydrophobic core, except near the core surface where a residue may have its charged group outside, as illustrated for a lysyl residue. The dashed lines schematically indicate intra-molecular (A) or inter-molecular (B) hydrogen bonds. The phospholipid headgroup region is shaded. After Tanford and Reynolds [30].

hydrocarbon layer. In the case of a β-sheet, at least two antiparallel strands of 9 residues per strand will be needed to span the hydrophobic membrane part [30]. In the case of the α-helix, the intra-molecular hydrogen bonds are directed perpendicular to the membrane surface, whereas for the β-sheet the inter-molecular hydrogen bonds are in the plane of the membrane. It should be noted that the maximum number of hydrogen bonds is formed when the protein is structured in an α-helix, whereas only half of the maximum number of hydrogen bonds is present in two β-structured strands. Therefore the most stable hydrophobic transmembrane structure will be an α-helix. A β-structure is only stabilized when as many hydrogen bonds as possible are formed, resulting in huge β-pleated sheet structures, or cylindrical β-barrel formation [1].

The major coat proteins of Ff bacteriophages have been studied widely by biochemical and biophysical methods, but unfortunately these have often been reconstituted with less attention to the reconstitution conditions and biochemical integrity of the proteins. Various artificial lipid–protein systems have been used due to different protein isolation procedures, reconstitution methods, and amphiphiles applied. Recently, it has been found that for coat proteins associated in vitro with amphiphilic agents, special care should be taken with respect to the α-helical and β-polymeric states that the coat protein exhibits in these systems.

These conformations, which have been recently reviewed [1], will be described in more detail in the following section. Especially the presence of one of these two states of the coat protein (or worse: a mixture) is in general poorly described. Therefore, the results described in the literature are often incomplete and can be incomparable or even contradictory.

5. *The in vitro membrane-bound state of M13 coat protein*

5.1. *The α-helical and β-polymeric state*

The in vitro membrane-bound M13 coat protein can adopt two distinct states: the α-helical and β-polymeric state. Previously, the α-helical state of M13 coat protein has been characterized using circular dichroism and laser Raman spectroscopy, revealing an overall conformation containing about 50% α-helix and 30% β-structure [32–39]. Fourier transform infrared and two-dimensional NMR spectroscopy have been applied recently, indicating a higher content of α-helix and lack of β-sheet structures [40,41]. In the literature, there is good agreement about the conformation of the hydrophobic transmembrane part of the protein, being an α-helix, and it has been proposed that the C-and N-terminal regions also contain a substantial amount of helical structure, similarly as has been found for the coat protein of the related phage Pf1 [42]. A dimeric form of M13 coat protein solubilized in detergents has been suggested [34,40,43–48]. However, based on recent studies, it can be assumed that the aggregational entity of α-helical M13 coat protein in micellar systems and phospholipid bilayers is monomeric [49]. The monomers have an ability to undergo a reversible aggregation, depending on the conditions [38].

M13 coat protein in the β-polymeric state is strongly aggregated in an irreversible way, and the corresponding conformation is characterized by a high amount of anti-parallel β-sheet (about 70%) and a lack of α-helix [1,33,34,36,38,41]. When reconstituted in the lipid bilayer, the α-helical coat protein can undergo an aggregation related conformational change in which the α-helical conformation is changed into a β-conformation. This process is accompanied by a strong aggregation of the protein [38]. Some aspects of this β-polymerization process have been described by Spruijt and Hemminga [49]. Protein in the β-polymeric state is characterized by an extended intermolecular hydrogen bond network in the plane of the membrane (see Fig. 3) [1].

5.2. *Putative in vivo state of M13 coat protein*

The in vivo state of the membrane-bound M13 coat protein has not been elucidated in a direct way. The major coat protein is a structural protein, not

exhibiting any enzymatic activity to monitor its functional activity. Therefore the presumable biological native state of the membrane-bound form of the coat protein has to be deduced from other clues. For a number of reasons the α-helical state is thought to be the functional membrane-bound state: (1) The coat protein when associated with DNA in the virion is almost completely α-helical, as established by X-ray diffraction techniques [50,51], circular dichroism [32,33,36,52,53] and laser Raman spectroscopy [37,54]. The parental coat protein can be re-used in viral assembly after storage in the host cytoplasmic membrane during the infection process [18–20]. Therefore, from a thermodynamic point of view it is not likely that this protein will completely change its conformation during the disassembly in the membrane, since it has to regain its conformation upon assembly. (2) Insertion of the procoat of M13 into the cytoplasmic membrane, its subsequent processing by a leader peptidase [55], and the exchange of gene V on the viral DNA by the coat protein [56] are assumed to occur with single monomeric coat protein molecules. (3) In agreement with other known proteins that have transmembrane domains embedded in the bacterial cytoplasmic membranes, an α-helical conformation is most likely to occur in the hydrophobic protein domain that contains 19 amino acid residues (see Fig. 2) [31,57–60]. (4) The α-helical coat protein appears to be stable in amphiphilic media that closely mimic the in vivo situation with respect to phospholipid headgroups, length and degree of unsaturation of acyl chains as well as the ionic strength of the aqueous environment [38,49]. The stability of the α-helical state is also supported by molecular dynamics calculations, since no inter-molecular hydrogen bonds will be formed [61]. (5) Finally, it should be mentioned that the β-polymeric state of the coat protein can be excluded from participation in the infection process, since it is formed in an irreversible way [34,38], never being able to reconvert into an α-helical conformation again.

5.3. Reconstitution procedures

After knowing the presumable in vivo state of the coat protein, a suitable reconstitution procedure has to be used to maintain the coat protein in its native state when it is taken from the bacteriophage and is incorporated into lipid systems. The isolation and purification of the coat protein from the bacteriophage particle before reconstitution requires the use of detergents and therefore restricts the number of reconstitution methods that can be used. In our approach, we have chosen for a detergent dialysis method resulting in lipid–protein systems organized in a unilamellar vesicle structure. Sodium cholate is the most suitable amphiphilic intermediate, because of its high critical micelle concentration and low aggregation number [62]. This allows almost complete removal of the detergent during dialysis [38,63]. The reconstitution conditions have been optimized by monitoring the aggregational and conformational properties of

the coat protein using circular dichroism and high-performance size-exclusion chromatography [38,49]. It has been observed that the α-helical state of the coat protein is strongly stabilized by an increased ionic strength of the buffer. Without salt the α-helical state is maintained only in lipid systems containing at least one unsaturated acyl chain and then only at high L/P ratios [49].

In conclusion, complete conservation of the α-helical state of the coat protein after reconstitution into phospholipid bilayers has been observed in the situation that the transmembrane domain of the coat protein matches the thickness of the hydrophobic interior of the membrane, and β-polymerization is prevented [1,38,49]. The process of β-polymerization is suggested to be induced by the oppositely charged hydrophilic protein termini [38,49]. Therefore, the reconstituted protein molecules should be kept separated either by a large protein–protein distance (i.e. high L/P ratios), or by shielding the electrostatic interactions. An effective shielding is provided by charged phospholipids, or an increased ionic strength of the medium. It should be noted that in vivo the electrostatic interactions are repulsive, since the protein is parallel oriented in the membrane. This effect will be an additional factor to prevent the protein from forming inter-molecular anti-parallel β-structures in vivo. Based on the arguments presented here, there is good evidence that M13 coat protein in the α-helical form is the biological active form in membrane-associated processes.

6. *The coat protein structure*

6.1. Secondary structure – transmembrane helix

A study of the secondary structure of the M13 coat protein in the α-helical form has been carried out by Sanders et al. [41] using circular dichroism in combination with Raman and Fourier transform infrared spectroscopy. The overall conformation of the M13 coat protein has been determined from a quantitative computer analysis of the secondary structure data. As a result of the high amount of α-helix observed, it is suggested that the coat protein is predominantly in an α-helical conformation, in the transmembrane part as well as in the C- and N-terminal regions [41].

The time-resolved fluorescence and anisotropy decay of the single tryptophan of α-helical M13 coat protein incorporated into phospholipid bilayers has been studied by Sanders et al. [64]. The L/P ratio was varied from 10 to 100, where the protein is monomeric [49]. It is found that the aggregation state of the protein does not affect the tryptophan environment and local protein structure. This finding excludes the interpretation of tryptophan fluorescence given by Johnson and Hudson [65] that the coat protein is a dimer in the lipid bilayer.

High-resolution and solid state NMR experiments on coat proteins in detergent micelles and phospholipid bilayers, respectively, support these conclusions. For M13 coat protein and the coat proteins of the related fd and Pf1 bacteriophages it is found that the backbone of the hydrophobic transmembrane amino acid residues is rigid on the picosecond to microsecond time scale and has a stable helical secondary structure, whereas the N- and C-terminal regions show an increasing mobility towards the ends of the polypeptide chain [40,42,47,48,66–80]. This suggests a stable protein core with a progressive increase in amplitude or frequency of motions as the ends of the protein molecule are approached.

6.2. Secondary structure – the terminal parts

The most detailed structure of membrane-bound M13 coat protein follows from recent NMR studies of the protein solubilized in perdeuterated sodium dodecyl sulphate. Henry and Sykes [40] propose that micellar-bound M13 coat protein consists of two α-helices linked by a short region of uncertain conformation. The longer transmembrane helix extends through much of the hydrophobic section and basic region of the protein, ending near the C-terminus. This part of the molecule is very stable. The N-terminal helix is suggested to reside outside the micelle and appears to be more structurally labile as compared to the helix that extends through the hydrophobic section. The amide exchange rates in the N-terminal region are quite fast, and this region appears to be in a state of relatively rapid dynamic flux. The very ends of the polypeptide chain are disordered. The mobile amino acids in these regions have been identified as alanine 1, glutamine 2, glycine 3, aspartic acid 4 in the N-terminal region (see Fig. 1) and lysine 48, alanine 49, and serine 50 in the C-terminal region. Shon et al. [42] previously proposed a similar model for the 46-residue coat protein of the related phage Pf1 solubilized in dodecylphosphocholine micelles: Two helices (residues 6–13 and 19–42) are connected by a short loop. Solid-state NMR experiments on Pf1 coat protein reconstituted into phospholipid bilayers enabled to determine the orientation of the N-terminal helix, which is found to be parallel to the surface of the membrane bilayer.

6.3. The N-terminal helix

For M13 coat protein, the relative orientation of the two helices of M13 coat protein is not known, but it is possible that the N-terminal helix, which has an amphipathic nature, is similarly associated with the membrane surface [40]. An N-terminal helix parallel to the membrane surface is expected to have a strong steric effect on the aggregation of the transmembrane helix at increasing protein concentrations. In fact, it can be considered as a large "knob" on one

end of the transmembrane helix. It has been suggested that the hydrophilic termini initiate the artificial β-polymerization process [49], and a perpendicular orientation of the N-terminus would facilitate this process. This indicates that the terminal regions are important in protein–protein interactions. A parallel orientation of a helical N-terminal region along the membrane would increase the apparent diameter of the membrane-bound coat protein, thereby also increasing the number of annular or boundary phospholipid molecules at the lipid–protein interface and providing a larger action region for interactions. It can be estimated that the area, which can be occupied by a parallel N-terminal domain, is about 1.5 nm. This corresponds to about three shells of surrounding phospholipid molecules. This would thus more strongly influence the properties of the phospholipids in the bilayer than in case of a straight cylinderlike protein molecule.

Spectroscopic experiments carried out on reconstituted lipid–protein systems, however, do not give evidence for such a strong deviation from a cylindrical shape of the M13 coat protein [1,64,81–83]. It may be possible that the orientation of the N-terminal part of M13 coat protein changes from parallel to perpendicular to the membrane surface, when going from high to low L/P. An extended helix structure could be the conformation prior to assembly, since the coat protein in the intact virus has an extended, slightly bent, helical structure [84]. Such a conformational change could only involve a few amino acids that connect the two helices. This may be difficult to observe, for example, with secondary structure determination methods. In conclusion, it can be said that the orientation of the N-terminal part of reconstituted M13 coat protein with respect to the bilayer surface remains to be solved.

6.4. Molecular dynamics

The method of molecular dynamics has been used to provide information about the stability, energy and secondary structure of M13 protein in phospholipid systems [61]. A starting configuration consisting of an ideal α-helix monomer and dimer has been used to observe the stability of this system. Fig. 4 shows stereo-pictures of the α-helix monomer and dimer after a 100 ps molecular dynamics simulation. A bending of the molecule occurs near glycine 38 (see Fig. 2). A similar bending of an α-helix has been reported for glycophorin [85]. The variation of the root-mean-square fluctuations of the Cα-atoms of the monomeric structure with position along the polypeptide chain shows a relatively large fluctuation at both the C- and N-terminal protein parts. This is in agreement with NMR studies [69,70,86]. The α-helix monomer has the lowest energy, which is reduced by 46 kJ/mol for the dimer. This means that α-helices have a tendency to aggregate, but a low one. The short time scales studied in molecular dynamics

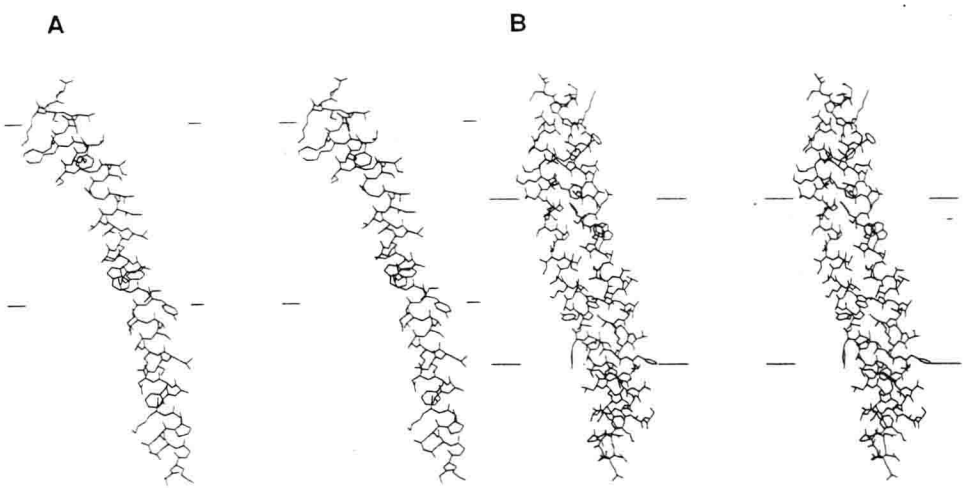

Fig. 4. Stereo-pictures of the α-helix monomer (A) and anti-parallel dimer (B) after a 100 ps MD simulation. The borders indicate the hydrophobic membrane core. The membrane thickness is 3.2 nm [61].

simulations (100 ps), however, do not allow to draw conclusions about knob-into-hole packing, anchor principles, and orientation of the N-terminal region.

7. *Lipid order and dynamics in reconstituted systems*

7.1. *Protein packing in the bilayer*

When incorporated into a membrane, the hydrophobic transmembrane domain of α-helical M13 coat protein (see Fig. 2) that ranges from tyrosine 21 to isoleucine 39 (a stretch of 19 amino acid residues), will be structured in an α-helical conformation. It is also possible that the hydrophobic amino acids leucine 41 and phenylalanine 42 are buried in the hydrophobic region of the membrane, the side-chain of lysine 40 sticking out into the aqueous phase, as is schematically illustrated in Fig. 3. This would then result in a hydrophobic stretch of 22 amino acids [1]. Either of these helices will nicely match the thickness of the membrane bilayer. The packing of the coat protein in the lipid bilayer may be based on an anchor or float principle of suitable amino acid residues, a float being a dynamic form of the anchor. Strong lipid anchors (or floats) may be provided by the flexible aromatic side chains of the amino acids that are buried in the hydrophobic region of the membrane, close to the lipid/water interface. This could be a common property of membrane proteins, since at the lipid/water

interface often aromatic amino acid residues are found [87–90]. In the same way, the charged amino acid residues of M13 coat protein (i.e. glutamic acid 20 and lysine 40, see Fig. 2) could act as hydrophilic anchors.

7.2. Protein aggregation

The aggregation of α-helical coat protein is mainly based on weak interactions of the hydrophobic amino acid side chains. M13 coat protein does not have a strong tendency to aggregate, as has been found from molecular dynamics calculations [61] and high-performance size-exclusion chromatography experiments [38,49]. In the literature, models for helix-to-helix packing have been assumed in which the ridges and grooves on the helix surface intercalate (ridges-into-grooves packing) or in which the packing is achieved by requiring a knob (side chain) on the surface of one protein to match a hole (space between side chains) on the surface of the other (knobs-into-holes packing) [91–94]. Dunker and Jones [91] proposed a knobs-into-holes packing for coat proteins from filamentous bacteriophages incorporated into lipid membranes. The coat proteins are thought to be aggregated into bundles of α-helices with hydrophobic exteriors and with hydrogen bonds between the side chains of one α-helix and the side chains of its knobs-into-holes packed neighbours. Although not yet experimentally or theoretically verified, the principles of knobs-into-holes packing or ridges-into-grooves packing could be important for the relative two-dimensional orientation and lateral packing of M13 coat protein molecules reconstituted in phospholipid systems, especially at a low L/P ratio in vitro, or prior to phage assembly in vivo. Also the presence of knobs and holes on the protein surface could affect the conformations of the surrounding phospholipids [81].

7.3. Model for protein–lipid interaction

On the basis of spectroscopic studies, a schematic model of M13 coat protein in the α-helical form in a membrane has been derived (Fig. 5A) [82]. It is suggested that the bulk lipids (1) can easily exchange with the boundary lipids (2). For comparison, a schematic illustration of M13 protein in the β-polymeric form in phospholipid bilayers is shown in Fig. 5B [82]. The aggregated protein is assumed to be in an extended β-structure, thereby increasing the membrane thickness by forming non-bilayer structures at the interface of the protein and lipids to match the hydrophobic region. The induced additional lipid structure is proposed to consist of hexagonal tubes of inverted lipids in the membrane along the protein aggregates. Lipids in the rod-like lipid structure (lipid molecule 3, Fig. 5B) can not exchange with the bulk lipids (lipid molecule 1, Fig. 5B), because the flip-flop rates are slow [95]. They will therefore be trapped, in

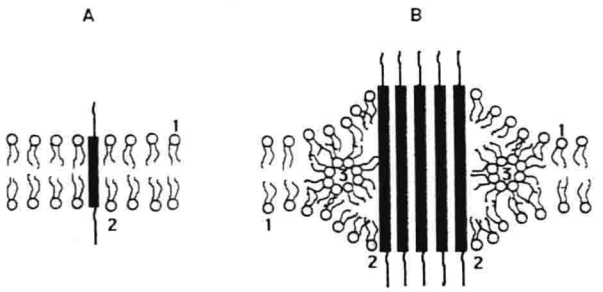

Fig. 5. A schematic model for the interaction of the M13 coat protein, in (A) the α-helical state and (B) the β-polymeric state, with phospholipid bilayers. The hydrophobic transmembrane region of the protein is represented as a black bar. Bulk lipids (1) are in fast or intermediate exchange with boundary lipids (2). Lipids trapped in rodlike structures (3) are formed to match the hydrophobic part of the stretched β-polymeric protein with that of the lipids. In (B), a cross-section through a β-sheet aggregate is shown [82].

agreement with the ESR and NMR experiments [82, 96, 97]. In addition to lipids in the inverted rod-like structures (lipid molecule 3, Fig. 5B), there is a fraction of lipids in contact with the protein, but not present in the inverted rod-like structures. This fraction of lipids will behave as boundary lipids (lipid molecule 2, Fig. 5B), which can exchange with the bulk lipids (lipid molecule 1, Fig. 5B). These lipids will give a "normal" boundary behaviour, similar as found for M13 coat protein in the α-helical form (Fig. 5A).

Because a transmembrane α-helical cylinder has about the same cross-sectional area as a lipid molecule, a relatively small effect on the motional properties and orientational order of the lipids will result [64, 81–83]. At low L/P ratios, two protein monomers start to share lipid molecules and this may finally result in an immobilization of the lipid and protein molecules [38,49,98]. M13 coat protein provides a rigid hydrophobic surface for the surrounding acyl chains of the phospholipid molecules [81]. It has been assumed previously that immobilization of lipids by proteins originates from the rigidity of the protein backbone of the membrane bound domain of the protein [99,100]. The results obtained for M13 coat protein indicate that not only the rigidity of the transmembrane part of the protein is important, but also the size of the hydrophobic surface of the protein region. Clearly, a single α-helical M13 coat protein is too small to strongly immobilize the phospholipid molecules, and the protein has relatively minor influences on the surrounding lipid matrix. In this respect the effect of M13 coat protein resembles that observed upon introducing rigid amphipathic molecules, such as cholesterol, into lipid bilayers [101–103].

Phospholipid model membranes containing various amounts of M13 coat protein in the α-helical form have been studied using time-resolved fluorescence

with the fluorescent probe diphenylhexatrienyl (DPH) propionic acid [81]. This probe is a rigid molecule due to the presence of conjugated bonds in the molecule. It is found that the order of the lipid bilayers, as sensed by the probe, increases upon incorporation of the M13 coat protein. This is in contrast with results from ^{2}H-NMR spectroscopy of bilayers of chain-labelled 1,2-[11,11-$^2H_2$]-DOPC [82], which indicate that on the ^{2}H-NMR time scale the order in the hydrophobic part of the bilayer is decreased in the presence of α-helical M13 coat protein. This difference may be interpreted as arising from bulky amino acid side chains of M13 coat protein in the hydrophobic region. These groups will provide "holes" in between that will be sensed by the flexible acyl chains, resulting in an decreased order if more M13 coat protein is introduced into the phospholipid system. However, the rigid fluorescent probe will only experience the average protein surface and sense an increased order. Bulky side chains may be provided by the aromatic amino acids of tyrosine 21 and 24, tryptophan 25, and phenylalanine 42 [81]. As has been discussed above, bulky side chains and holes between them may also affect the association of the transmembrane part of the protein.

7.4. The phospholipid headgroup region

The headgroups of the phospholipids are not strongly affected by α-helical M13 coat protein as has been found from ^{31}P-NMR [82]. However, ^{2}H-NMR experiments on headgroup labelled DOPC-d_4 and DOPE-d_4 show that the torsion angles within the headgroup are changed upon introduction of M13 coat protein, due to the fact that it acts as a positively charged molecule at the membrane surface [82]. However, these changes are small as compared to those reported for positive amphiphiles introduced into a lipid membrane [104]. This suggests a larger distance between the charges and the headgroup or a different distribution of charges at the surface of the lipid bilayer in the case of the M13 coat protein, as compared to the charges induced by amphiphiles. The positive charges introduced by the coat protein at the membrane surface have been assigned to its positively charged lysine residues [83]. Lysine 40 is situated at the C-terminal end of the hydrophobic part of the protein (see Fig. 2), probably close to the membrane bilayer surface. Also the lysine residues 43 and 44 are in the vicinity of the bilayer surface. Due to the dialysis procedure in preparing the lipid protein systems, the protein is randomly inserted in the membrane, giving a completely symmetric bilayer with one net positive charge at both membrane surfaces.