Biophysical Chemistry 41 (1991) 193–202
Elsevier Science Publishers B.V., Amsterdam

BIOCHE 01592

Conformation and aggregation of M13 coat protein studied by molecular dynamics

Johan C. Sanders [a,*], Nico A.J. van Nuland [a], Olle Edholm [b] and Marcus A. Hemminga [a]

[a] *Department of Molecular Physics, Agricultural University, Dreijenlaan 3, 6703 HA Wageningen (Netherlands)*
[b] *Department of Theoretical Physics, Royal Institute of Technology, S-10044 Stockholm 70 (Sweden)*

(Received 21 December, 1990; accepted 28 March, 1991)

Abstract

Molecular dynamics (MD) simulations are performed on M13 coat protein, a small membrane protein for which both α- and β-structures have been suggested. The simulations are started from initial conformations that are either monomers or dimers of α-helices or U-shaped β-sheets. The lipid bilayer is represented by a hydrophobic potential. The results are analyzed in terms of stability, energy and secondary structure. The U-shaped β-structure changes from a planar to a twisted form with larger twist for the monomer than the dimer. The β-sheet is much more flexible than the α-helix as monitored by the root mean square (rms) fluctuations of the C_α atoms. A comparison of the energies after 100 ps MD simulation shows that of the monomers, the α-helix has the lowest energy. The energy difference between α- and β-structures decreases from 266 kJ/mol to 148 kJ/mol, when going from monomers to dimers. It is expected that this difference will decrease with higher aggregation numbers.

Keywords: M13 coat protein; Molecular dynamics simulations; Protein aggregation; α-Helix; β-Sheet

1. Introduction

Molecular dynamics has been shown to be a useful tool in predicting the three-dimensional structure of membrane proteins [1–3]. Here, MD simulations are describe of a small membrane protein, M13 coat protein, for which both α- and β-structures have been suggested [4–6]. The primary structure of the major coat protein of bacteriophage M13 (Reviews: [7–9]) is given in Fig. 1.

NH_3^+-Ala-Glu-Gly-Asp-Asp-Pro-Ala-Lys-Ala-Ala-
Phe-Asn-Ser-Leu-Gln-Ala-Ser-Ala-Thr-Glu- Acidic domain

Tyr-Ile-Gly-Tyr-Ala-Trp-Ala-Met-Val-Val
Val-Ile-Val-Gly-Ala-Thr-Ile-Gly-Ile-Lys- Hydrophobic domain

Leu-Phe-Lys-Lys-Phe-Thr-Ser-Lys-Ala-Ser-COO^- Basic domain

Fig. 1. Primary structure of the M13 coat protein.

Three specific domains can be distinguished: (1) an acidic N-terminus (residues 1–20) containing negatively charged glutamic and aspartic acids, which in the phage is in contact with the solvent; (2) a basic C-terminus (residues 40–50), which

* To whom correspondence should be addressed at Department of Molecular Physics, Agricultural University, P.O. Box 8128, 6700 ET Wageningen, Netherlands.

contains positively charged lysines and interacts with the negatively charged DNA phosphate backbone in the intact virus; and (3) a hydrophobic core (residues 21–39), which has a possible role in the protein–protein interactions in the phage and in the hydrophobic protein–lipid interactions when the coat protein is incorporated into the membrane. Various initial conformations of monomeric and dimeric states of the M13 coat protein are created and MD simulations are performed. These initial conformations include both α-helices and U-shaped β-sheets. The membrane core is modelled by a hydrophobic potential. The MD results are analysed in terms of stability, energies and secondary structure of the various monomer and dimer conformations.

2. Methods

2.1 The initial conformations

There are two major conditions that have to be fulfilled of a reasonable structure of a membrane protein such, as the M13 coat protein: (1) the hydrophobic residues should fit into the 32 Å thick membrane core; and (2) at the same time as many hydrogen bonds as possible should be satisfied in the membrane spanning part. M13 coat protein has a 20 residues long hydropobic sequence (21–39) in the middle, while the ends are hydrophilic. This gives one obvious candidate for the structure, a membrane spanning α-helix (1.5 Å rise per residue). The hydrophilic parts outside the membrane core that stick into the aqueous phase could have a more disordered structure, because it is not necessary to form internal hydrogen bonds there. Since it is not known how to make a possibly disordered structure for the hydrophilic part, a start is made with the whole protein in an α-helical conformation.

Secondly, considering β-structures, which have a rise of about 3.4 Å per residue, it is realized that to fit 20 hydrophobic residues into a membrane core of 32 Å thickness, a reverse turn in the middle of the hydrophobic segment is necessary. This turn was put at Val-30–Val-31, giving a U-shaped structure. That the hydrophilic ends in this case do not match is not serious, since the hydrogen bonds that are not satisfied internally may be formed with water.

To study protein–protein interactions, dimers were constructed. However, there are many possible dimer structures. For the α-helix, we took as main candidate an anti-parallel arrangement with Lys-40 of one helix close to Glu-20 of the other one. This structure has favourable electrostatic interactions.

For a monomeric U-shaped β-structure only about half as many hydrogen bonds are satisfied as for the α-helix. The point with dimeric and polymeric structures would then be to increase the amount of hydrogen bonding. A dimer may then be constructed in six possible ways. The binding of the monomers could either be between two 1–29 strands, two 32–50 strands or between one 1–29 and one 32–50 strand. In all these three cases the strands could either be parallel or anti-parallel. Even with the basic requirement that the hydrophobic part of both monomers should stay within the membrane core, there is freedom to slide the monomers a couple of residues along each other. Two possible structures were chosen, one parallel with the 1–29 strand of one monomer binding to the 32–50 strand of the other one (residue 27 to 32, 25 to 34 and so on) and one with two monomers stacked on top of each other (no hydrogen bonding). For the other possibilities it is noted that the same strands binding parallel to one another would be unfavourable, since then the same residues would face each other and there are some with bulky side groups.

2.2 The calculations

For the EM and MD simulations the GROMOS package was used (W.F. van Gunsteren and H.J.C. Berendsen, BIOMOS B.V., Laboratory for Physical Chemistry, University of Groningen, The Netherlands). The integration of the classical equations of motion was done with a time step of 2 fs using the SHAKE algorithm to constrain the bond lengths [10]. The temperature was kept at 300 K by coupling the kinetic energy of the system to a heat bath with a relaxation time of 100 fs

[11]. The potential energy function for membrane-spanning proteins is given by:

$$V = V_{\text{angle}} + V_{\text{dihedral}} + V_{\text{Hbond}} + V_{\text{Coul}} + V_{\text{LJ}} + V_{\text{hphobic}} \quad [1]$$

For the first five terms expressions and parameters were taken from Van Gunsteren and Karplus [12]. For the nonbonded interactions (Coulomb and Lennard-Jones) a cut-off at 10 Å was used and a neighbour list specifying the interacting atoms was updated every 10 steps. The fractional charges were chosen to give electroneutral residues, including Lys, Glu and Asp. Care was taken to avoid splitting of electroneutral groups by the cut-off. The long-range electrostatic interactions decay as dipole-dipole interactions. For the hydrogen bonds the cut-off was chosen to be 5 Å and the neighbour list was updated every 20 steps. Since the polypeptide atoms may form hydrogen bonds with water molecules, the hydrogen bond potential was switched off outside the membrane.

An external potential, V_{hphobic}, is employed to account for the lipid/water interface. This method and the parameters used, are the same as in Edholm and Jähnig [2]. According to this definition the membrane surfaces specify the hydrophobic core of the membrane. The thickness of a fully hydrated DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) bilayer is 32 Å at 300 K [13]. We thus took the thickness of the hydrophobic core equal to 32 Å and had the hydrophobic force to act over a boundary zone of 2 Å. The initial conformations were generated and positioned with their hydrophobic segments in the membrane.

The initial conformations were energy-minimized for at least 500 steps before starting the MD simulations. The MD simulations were performed on a Cray-XMP supercomputer. A 100 ps run took about 5.5 hours CPU time for the dimers. Stereopictures were produced on an Apollo work station.

The energies obtained from the MD simulations do not include the interaction energy with surrounding lipid and water molecules since the runs are vacuum ones. These excluded interactions are on the average attractive. They are of Lennard-Jones type and electrostatic interactions with the water dipoles. This negative contribution to the energy is proportional to the surface area of the protein. To get comparable energies, therefore, an energy is added that is proportional to the surface area. The proportionality constant is determined from a simulation including lipids, which gives a surface energy −600 kJ/mol for a helix of 23 residues (O. Edholm, unpublished data). The surface areas are calculated using a standard program [14]. Although this term will give large contributions, it should be noted that only the energy difference between the different conformations is important. Therefore, the α-helix monomer (see initial conformations) is used as a reference and the difference in surface energy is added or subtracted for the other conformations.

3. Results

MD simulations were performed on the M13 major coat protein, starting from various initial conditions. All runs reach a stable or at least metastable state after an equilibration period of 40–50 ps (Fig. 2).

Figure 3 shows stereopictures of the M13 coat protein in the α-helix monomer configuration after 100 ps MD simulation. As can be seen, bending occurs near Gly-38. The ϕ and ψ torsion angles near this residue differ from the values of an ideal α-helix, which are −57° and −47°, respectively. Glycine is known as a Chou and Fasman α-helix breaker [15]. In addition bending can only occur to the side which is marked by non-bulky residues such as glycines and alanines. Similar bending of an α-helix has been reported for glycophorin [2]. Therefore, it is believed that the bending at this position is occurring in a deterministic manner.

In solid-state NMR experiments a relatively high flexibility for the termini [16] has been observed. This flexibility is also found in the MD simulations, as can be seen from the rms fluctuation around the average position (Fig. 4) of the C_α atoms. These are for the α-helix monomer

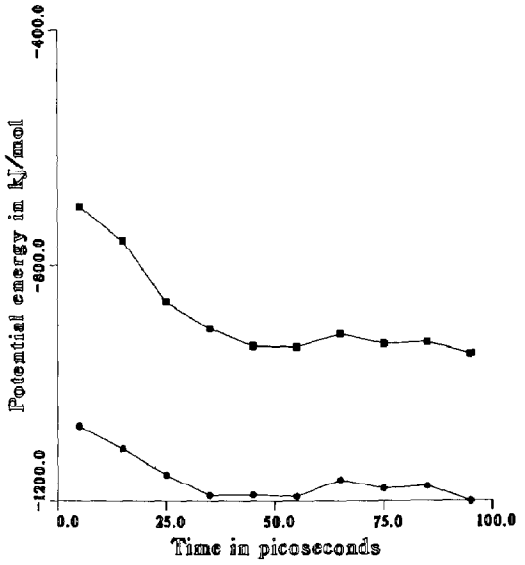

Fig. 2. Temporal variation of the total potential energy averaged over successive 10 ps time intervals for the M13 coat protein in α-helical (●) and U-shaped β-sheet monomer (■) configuration during the 100 ps MD simulations. Membrane thickness 32 Å.

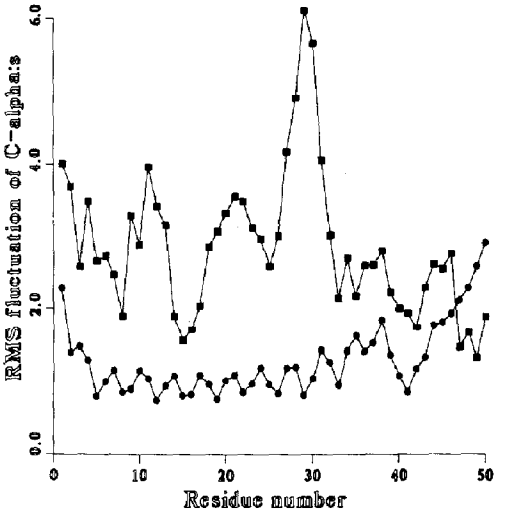

Fig. 4. Variation of the rms positional fluctuations of the C_α atoms along the M13 coat protein chain during the MD simulation. Membrane thickness 32 Å. (■) U shaped β-sheet monomer, and (●) α-helix monomer.

large in the terminal residues in contrast to the middle part, where small rms values are observed. The rms fluctuations are about 1 Å for the inner helical region, which is comparable with other MD simulations [17,2] and with experimental data on crystallized proteins [18].

The ability of the protein to form aggregates of α-helices is tested by running MD starting from a configuration with two α-helices at 10 Å distance. Figure 5 shows stereopictures of the M13 coat

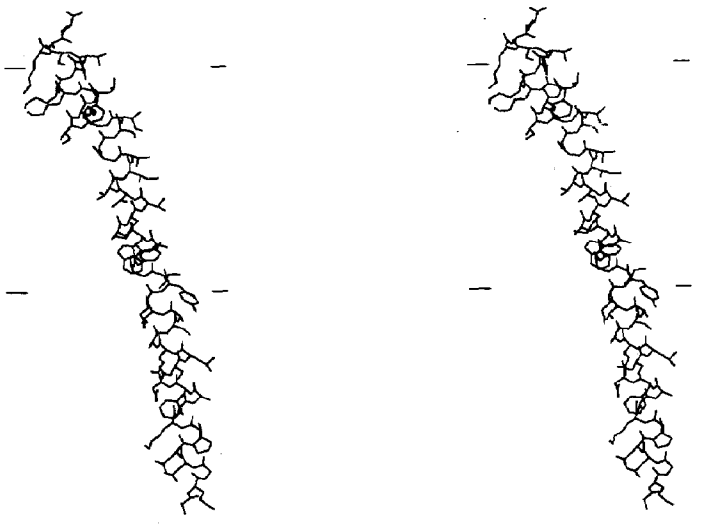

Fig. 3. Stereopictures of the M13 coat protein in the α-helix conformation. The borders represent the hydrophobic membrane core, 100 ps MD structure, membrane thickness 32 Å.

protein α-helix dimer after a 100 ps MD. During the run the molecules become twisted around each other as a result of finding the optimum interaction between the two molecules. The α-helix content of the dimer is higher than that of the monomer (Table 1). In addition the rms fluctuations of the C_α atoms in the C-terminus are smaller for the dimer than for the monomer. This indicates that this part of the molecule is somewhat more rigid as a result of non-bounded interactions between the two molecules, leading to a subsequent stabilization of the α-helix structure.

Figure 6 shows MD structure of the M13 coat protein in the U-shaped β-sheet conformation. Large conformational and orientational changes occur during the 100 ps MD run. While the EM structure still is an anti-parallel β-sheet, with the torsion angles ϕ and ψ equal to $-139°$ and $135°$, respectively, the chains get completely twisted during the MD run. The torsion angles become closer to the values of a twisted sheet, $-90°$ and $105°$. This agrees with results for polyalanine by Chou et al. [19] based on energy minimization studies. The twist is a consequence of sidechain–backbone interactions (intrachain) and interchain interactions. The twist δ per two residues is defined as the angle between the C_i–O and C_{i+2}O vectors. The value is divided by two to get the twist per residue and assigned to residue $i+1$. The average twist of the entire molecule in the U-shaped β-sheet monomer is $21°$ over the last 20 ps excluding the twist of residues at the end (2 and 49) and in the turn (28–31). This average twist value is within the range of observed twists of β-sheets in globular proteins, $0° < \delta < 30°$ [20]. As a result of the twisting the amount of residues within the hydrophobic membrane core is increased by six. Also the residues in the reverse turn are moved further into the hydrophobic membrane core.

The variation of the rms fluctuations of the C_α-atoms with the position along the polypeptide chain is shown in Fig. 4. For the β-structure, there is a pronounced maximum at the reverse turn. The fluctuations are, after the structures have been given proper time to stabilize, almost a factor two larger for the β-sheet than for the α-helix. Since this means that the β-structure takes up a larger volume in phase space, it will have higher entropy that gives a negative contribution to free energy.

The U-shaped β-sheet is not the only possible β structure. An extended β-sheet, cannot be ruled out, although it has a far too long hydrophobic part to fit into an ordinary membrane. It is favoured by the absence of the destablizing re-

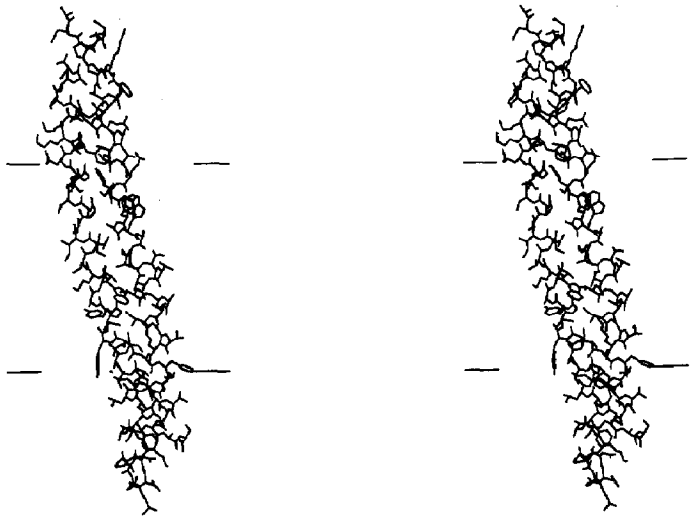

Fig. 5. Stereopictures of the M13 coat protein in the α-helix conformation. The borders represent the hydrophobic membrane core of 32 Å. Dimer 100 ps MD structure.

Table 1

The secondary structure of the individual residues in the different 100 ps MD simulations for the α-helix monomer and dimer and the U-shaped β-sheet monomer and dimer (parallel configuration). Membrane thickness 32 Å. H = α-helix, A = antiparallel β-structure, T = twisted β-structure, R = reverse turn, – = no assigned structure and D = dubious

Residue	α-helix	α-helix		U-shaped	U-shaped β-sheet	
		mol. 1	mol. 2		mol. 1	mol. 2
1 Ala						
2 Glu	–	H	–	T	A	D
3 Gly	H	H	–	–	–	–
4 Asp	H	H	D	T	–	A
5 Asp	H	H	H	A	T	–
6 Pro	H	H	H	–	T	T
7 Ala	H	H	H	T	T	T
8 Lys	H	H	H	D	T	T
9 Ala	H	H	H	–	–	–
10 Ala	H	H	H	T	T	T
11 Phe	H	–	H	–	D	–
12 Asn	H	H	–	T	T	D
13 Ser	H	H	–	T	D	–
14 Leu	H	H	H	D	T	–
15 Gln	H	H	H	D	–	A
16 Ala	H	H	H	T	T	D
17 Ser	H	H	H	T	T	T
18 Ala	H	H	H	A	D	T
19 Thr	H	H	H	T	T	T
20 Glu	H	H	H	T	T	T
21 Tyr	H	H	H	T	T	T
22 Ile	H	H	H	T	A	T
23 Gly	H	H	H	T	–	T
24 Tyr	H	H	H	D	A	T
25 Ala	H	H	H	T	T	T
26 Trp	H	H	H	T	T	T
27 Ala	H	H	H	T	T	T
28 Met	H	H	H	T	T	T
29 Val	H	H	H	R	R	R
30 Val	H	H	H	R	R	R
31 Val	H	H	H	T	T	T
32 Ile	H	H	H	T	T	T
33 Val	H	H	D	T	T	T
34 Gly	H	H	–	T	T	T
35 Ala	H	H	H	T	–	T
36 Thr	H	H	H	T	A	T
37 Ile	–	H	H	D	T	T
38 Gly	–	H	H	T	T	T
39 Ile	H	H	H	T	T	T
40 Lys	H	H	H	T	T	T
41 Leu	H	H	D	A	T	–
42 Phe	H	H	H	T	T	D
43 Lys	H	H	H	T	–	T
44 Lys	H	H	H	T	T	–
45 Phe	–	H	H	A	A	–
46 Thr	–	H	H	T	T	T
47 Ser	D	H	H	T	T	D
48 Lys	D	H	H	D	T	T
49 Ala	–	H	H	–	A	T
50 Ser						

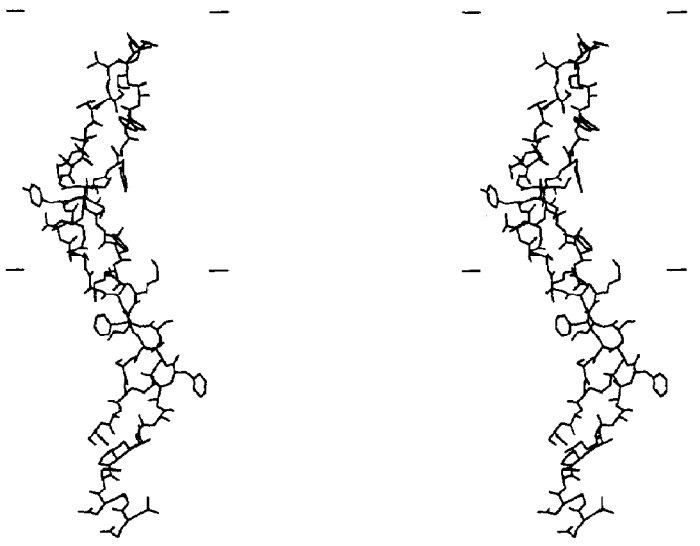

Fig. 6. Stereopictures of the M13 coat protein in the U-shaped β-sheet conformation. The borders represent the hydrophobic membrane core of 32 Å. the 100 ps MD structure.

verse turn. However, it requires a large local change of the bilayer to accommodate the hydrophobic region of the protein. Except for these effects, the results with the U-shaped β-sheet will be comparable to an extended β-sheet.

Figure 7 shows the three-dimensional structure of the U-shaped β-sheet dimer (parallel) after 100 ps MD simulation. The average rms fluctuations of the C_α atoms are about half the size of those of the corresponding monomer (Ta-

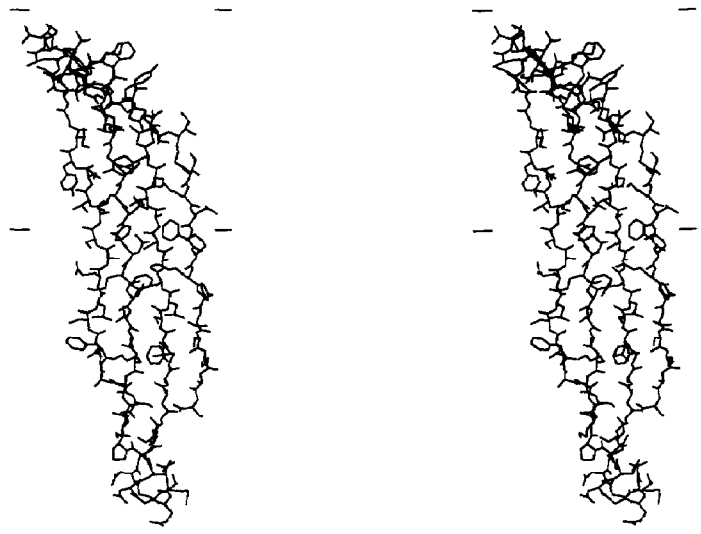

Fig. 7. Stereopictures of the M13 coat protein in the U-shaped β-sheet conformation. The borders represent the hydrophobic membrane core of 32 Å. Dimer 100 ps MD structure.

Table 2

The average rms positional fluctuations for five different 100 ps MD simulations of the M13 coat protein. The values represent the last 50 ps of the MD simulations

Simulation	rsm $(r-\langle r\rangle)$ (Å)
α-Helix	1.76
α-Helix dimer	1.18
U-shaped β-sheet	3.25
U-shaped β-sheet parallel dimer	1.58
U-shaped β-sheet stacked dimer	2.06

ble 2) while their variation with chain position is similar (results not shown). The average twist is 10° for the dimer, which should be compared to 21° for the monomer. This agrees with the general finding that an increasing number of β-strands reduces the twist [19,20].

In Table 1 every residue for the conformations studied is assigned to a secondary structure type: α-helix (H), anti-parallel (A) or twisted (T) β-structure depending on the values of the torsion angles ϕ and ψ. A residue is assigned to a given structure type if its torsion angles fall within a circle of radius 30° from the ideal values of the structure type in the Ramachandran plot. For these ideal values the following values are taken H(−57°, −47°), A(−139°, 135°) and T(−90°, 105°). The places where the α-helices bend are easily discovered from the non-helical assignments. The fraction of α-helix is for the monomer and dimer 80% and 90%, respectively. This shows that the helical structure is roughly stable over 100 ps. The β-structure is almost completely converted into a twisted form after 100 ps MD, as seen from Table 1. The amount of β-structure, anti-parallel and twisted, is after 100 ps about 75% for both monomer and dimer.

In Table 3 some different contributions to the energy from the simulations are listed. For the dimers, the non bonded energy per monomer increases substantially due to the interaction between the two units of the dimer. As seen from this table, the energies of the α-helix monomer and the α-helix dimer differ 46 kJ/mol in favour of the dimer. In the case of the U-shaped β-sheet parallel dimer the energy difference is 164 kJ/mol in favour of the dimer. From simulations, in which dimerization of the U-shaped β-sheet was due to stacked interactions, in which no hydrogen bonding takes place, a clearly smaller decrease of the energy is found (33 kJ/mol).

4. Discussion

In model membranes the M13 coat protein can adopt two different forms [4–6], the α-oligomeric and β-polymeric ones. It is of considerable interest to compare these structures, in particular in relation to their different aggregation behaviour. Recently the usefullness of MD for structure prediction of membrane spanning proteins has been investigated [3]. It was shown that one can distinguish between different packings of helices based on the energies and that the helices spontaneously tilt in the correct way. This motivation us to study the structure of M13 coat protein using MD simulations. Different initial conformations of the M13 coat protein were generated to

Table 3

Contribution of the individual energies in different MD simulations of the M13 coat protein. Values are expressed in kJ/mol

	V_{angle}	V_{dihyd}	V_{Coul}	V_{LJ}	V_{hphob}	V_{Hbond}	V_{tot}	V_{totcpm} [a]
α-Helix	749	520	−1022	−1103	−182	−159	−1197	−1197
α-Helix dimer	1451	1009	−2301	−2730	−224	−350	−3147	−1243
U-shaped β-sheet	718	532	−993	−991	−144	−76	−954	−931
U-shaped β-sheet Parallel dimer	1419	1025	−2283	−2549	−243	−152	−2784	−1095
U-shaped β-sheet Stacked dimer	1428	1045	−2188	−2446	−212	−114	−2487	−964

[a] Energies corrected for the lost on surface area given per subunit incase of a dimer.

compare α- and β-structures and to study the importance of protein/protein interactions for the stability of the various protein conformations.

4.1 Monomeric structures

The α-helix monomer has a lower energy (266 kJ/mol) than the U-shaped β-monomer (see Table 3). There are several origins for this difference. First, since the β-sheet has only half as many hydrogen bonds as the α-helix, the hydrogen bonding energy favours the α-helix. However, this accounts only for about one third of the energy difference. The remaining part comes mainly from non-bonded Lennard–Jones interactions, but there are also smaller contributions of electrostatic and hydrophobic origin. It is noteworthy that bonded interactions (angles and dihedrals) are about the same (even 19 kJ/mol higher for the α-helix), so that strains in these degrees of freedom caused by the reverse turn are unimportant for the energy difference. Both monomers have almost the same surface area, so the relatively uncertain surface energy correction is of small importance.

To compare conformations, the free energy should be used instead of the energy. But to calculate the entropic contribution to the free energy of a certain structure with sufficient accuracy is not possible. The rms fluctuations (Table 2) give an idea about the differences in entropy between the different structures. These are, after that the structures have been given proper time to equilibrate, almost a factor two larger for the β-sheet than for the α-helix. If we assume that the fluctuations are equilibrium ones, this gives rise to an entropy difference in favour of the β-sheet that could be large enough to completely alter the free energy balance between the structures. We do, however, see that dimerization reduces the fluctuations and the difference in fluctuations between the two structures. Probably, the surrounding lipids have a similar effect. Even if the energies indicate that equilibrium is reached, the fluctuations may still need more time to reach their equilibrum value. Therefore, it can be concluded that the α-helix monomer is favourable as compared to the U-shaped β-sheet monomer. However, an entropy term will certainly reduce the energy difference so that it is not possible to give a quantitative estimate of the free energy difference.

4.2 Protein dimers

For the α-helix the energy per monomer is 46 kJ/mol lower for the dimer than the monomer after that the surface energy correction has been performed. This means that it is favourable for them to bind together. This seems reasonable, since electrostatic interactions and a few hydrogen bonds could favour protein/protein interactions as compared to protein/lipid interactions. The conclusion is that the M13 coat protein in the α-helices has a tendency to aggregate, but a low one.

For the β-structure the corresponding energy difference is 164 kJ/mol per monomer in favour of the parallel dimer. This means that the energy difference between α- and β-structures decreases from 266 kJ/mol to 148 kJ/mol when going from monomers to dimers. It is reasonable that this difference will continue to decrease when more protein monomers in a β-sheet conformation are allowed to aggregate. This means that for highly aggregated β-structures the energy may be similar to that of α-helical monomers and the β-sheet conformation may equally well be possible in lipid membranes as compared to the α-helix conformation. For a more accurate comparison, however, it will be necessary to properly take into account the solvent effects, as well as a quantative description of the entropy differences.

Acknowledgements

This research was supported by the Netherlands Foundation of Biophysics with financial aid of the Netherlands Organization for Scientific Research (NWO), by the commission of the European Communities, contract nr. ST2J-0088 and ST2J-0368-C, by the Swedish Natural Science Research Council under contract K-KU 3777-302 and with computer time from the Swedish National Supercomputer Center.

References

1 O. Edholm and J. Johansson, Eur. Biophys. J. 14 (1987) 203–209.
2 O. Edholm and F. Jähnig, Biophys. Chem. 30 (1988) 279–292.
3 F. Jähnig and O. Edholm, Z. Phys. B 78 (1990) 137–143.
4 R. Williams and K. Dunker, J. Biol. Chem. 252 (1977) 6253–6255.
5 S.P.A. Fodor, A.K. Dunker, Y.C. Ng, D. Carsten and R.W. Williams, in: Seventh Biennal Conference on Bacteriophage Assembly, ed. M.S. Dubow (Alan R. Liss, New York, NY, 1981) pp. 441–445.
6 R. Spruijt, C. Wolfs and M. Hemminga, Biochemistry 28 (1989) 9158–9165.
7 D. Pratt, H. Tzagaloff and J. Beaudoir, Virology 39 (1969) 42–53.
8 D.S. Ray, in: Comprehensive Virology, eds. H. Fraeckel-Conrad and R.R. Wagner, Vol. 7, (Plenum Press, New York, NY, 1977) pp. 105–178.
9 I. Rasched and E. Oberer, Microbiol. Rev. 50 (1986) 401–427.
10 J.P. Rykaert, G. Cicotti and H.J.C. Berendsen, Comput. Phys. Commun. 23 (1977) 327–341.
11 H.J.C. Berendsen, J.P.M. Postma, W.F. van Gunsteren, A.D. Nola and J.R. Haak, J. Chem. Phys. 81 (1984) 3684–3690.
12 W.F. van Gunsteren and M. Karplus, Macromolecules 15 (1982) 1528–1544.
13 L.J. Lis, et al. Biochemistry 20 (1981) 1771–1777.
14 B. Lee and F.M. Richards, J. Mol. Biol. 55 (1971) 379–400.
15 M. Levitt, Biochemistry 17 (1978) 4277–4285.
16 K.G. Valentin, D.M. Schneider, G.C. Leo, L.A. Calgano and S.J. Opella, Biophys. J., 49 (1986) 36–38.
17 R. Elber and M. Karplus, Science 235 (1987) 318–321.
18 H. Frauenfelder, G.A. Petsko and D. Tsernoglou, Nature 280 (1979) 558–563.
19 P.Y. Chou, G. Némethy and H.A. Scheraga, J. Mol. Biol. 168 (1983) 389–407.
20 J.S. Richardson, Adv. Prot. Chem. 34 (1981) 167–339.