Amphiphiles containing aromatic groups in the hydrophobic part

Visscher, Inge

Summary

Aggregation processes are essential for life on this planet. For example, the membranes of all living cells are bilayered aggregates, consisting of lipid molecules, proteins and steroids. In many biological processes, aggregates play a role. The main driving force for aggregation of amphiphiles is hydrophobic interaction. Both micellar and bilayer aggregates have the polar or hydrophilic groups of the amphiphiles situated on the outside of the aggregate, whereas the apolar or hydrophobic tails avoid contact with water. Amphiphiles can form a wide variety of aggregate morphologies. The morphology of the aggregate is determined by the molecular structure and is the result of the interplay between the attractive hydrophobic interactions between the tails and the repulsive electrostatic and hydration shell overlap interactions between the headgroups. A micelle is a highly dynamic aggregate of surfactant molecules, in which the headgroups and the two methylene groups nearest to the headgroups have considerable contact with the aqueous phase. The apolar part of the surfactant molecules forms the hydrophobic core of the micelle. Vesicles consist of an aqueous compartment enclosed by a bilayer composed of amphiphiles. Again, the hydrophobic part of the amphiphiles forms the inner part of the bilayer, whereas the hydrophilic headgroups are located at the bilayer–water interface.

Noncovalent interactions involving aromatic moieties are an important class of interactions. They are present in DNA and proteins, and play an important role in protein–ligand interaction.

This led to the idea to incorporate aromatic units in the hydrophobic part of amphiphiles. This thesis deals with the design and synthesis of single–tailed and double–tailed amphiphiles containing an aromatic moiety in the hydrophobic part and the consequences of incorporation of an aromatic moiety for the aggregation behaviour and aggregate properties.

In Chapter 2 describes the synthesis of a number of single-tailed surfactants with different aromatic moieties in the hydrophobic part combined with a phosphate headgroup, *n*–alkyl phosphates. The *n*–alkyl phosphates containing the larger aromatic moieties, *viz.* a naphthoxy or biphenoxy group, did not dissolve in water. Replacing three methylene groups at the end of the alkyl chain by a phenoxy group (C_{11}OphenPO_4) reduced the Krafft temperature and increased the cmc. It was also found that the headgroup size was increased considerably compared to that of *n*–tetradecyl phosphate ($C_{14}P O_4$). The phenoxy group was found to fold towards the micellar interphase, causing the increase in headgroup size both by its presence and by changing the pKa of the phosphate in the micelle. This has as a consequence that in micelles of C_{11}OphenPO_4 more phosphate headgroups are

dianionic than in $C_{14}PO_4$ micelles in bidistilled water, leading to the increased cmc and the increased headgroup size.

Also di–*n*–alkyl phosphates containing two naphthyloxy or biphenyloxy moieties in the hydrophobic part did not dissolve in water. By contrast, the amphiphiles containing a phenyl or phenoxy group in the hydrophobic part did form aggregates in water.

Incorporating a phenyl group at the end of the two alkyl chains decreased the main phase transition temperature (T_m) compared to that of the reference compound di–*n*–tetradecyl phosphate. The decrease in T_m was even larger for phosphates containing the phenyl or phenoxy group in the middle of the chain, the main phase transition temperatures of these compounds were even reduced below 0 °C. A small increase of T_m was observed in case of a phenoxy at the end of the chains of a di–*n*–alkyl phosphate. The permeability for carboxyfluorescein was increased for vesicles composed of amphiphiles containing a phenyl group at the end or a phenyl or a phenoxy group in the middle of the hydrophobic tail. The di–*n*–alkyl phosphate containing a phenoxy at the end of the hydrophobic chain could not even entrap carboxyfluorescein. All these observations lead to the conclusion that the packing of the bilayer is significantly disturbed upon incorporating an aromatic moiety in the alkyl chain.

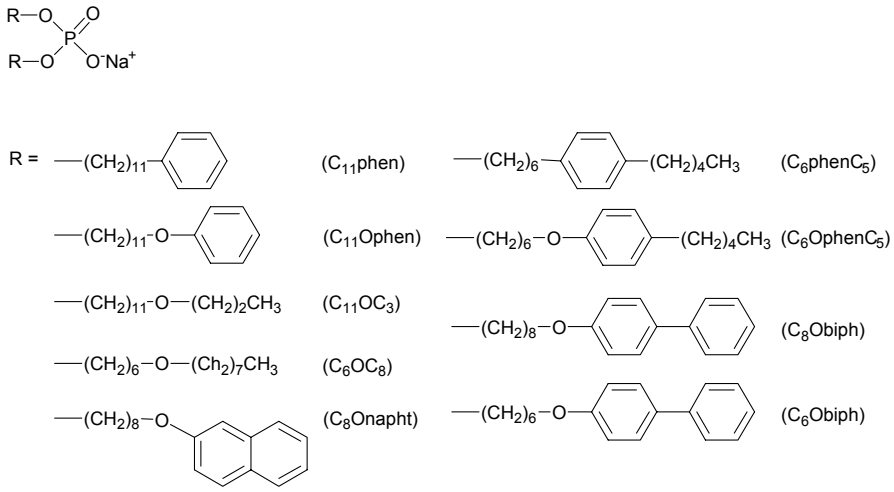

Figure 1 Molecular structures of the di–*n*–alkyl phosphates studied in Chapters 3 and 4.

In Chapter 4 the aggregate morphology of seven di–*n*–alkyl phosphates was investigated with cryo–electron microscopy. In all aggregate solutions that displayed a T_m below 0 °C, spherical vesicles were observed. If freeze thawing was used in the preparation, the observed vesicles contained small inclusions. No spherical vesicles were observed below T_m for the di–*n*–alkyl phosphate containing a phenyl at the end of the tails (C_{11}phen), although vesicles formed from C_{11}phen could entrap carboxyfluorescein above T_m. Curved bilayer fragments and bicelles were observed for C_{11}Ophen and $C_{11}OC_3$. Investigations using the membrane probe Laurdan showed that the interface of bilayers composed of C_{11}phen in the gel state contains more water than bilayers composed of C_{14}P in the gel state. Bilayers formed from amphiphiles containing a phenyl or a phenoxy in the middle of the alkyl chain contained more water at the interface between 15 and 70 °C than bilayers composed of C_{14}P and C_{11}phen. In monolayers formed from di–*n*–alkyl phosphate amphiphiles, the cross sectional surface area of an amphiphile containing phenyl or phenoxy moieties in the tails was increased compared to that of C_{14}P. FTIR studies showed that upon incorporating a phenyl group, either in the middle or at the end of the hydrophobic chain, the conformational order decreased.

A strategy to study the mechanism of membrane fusion using phospholipids with an oligomerisable headgroup and aromatic groups in the hydrophobic part is described in Chapter 5. The oligomerisable group, a nitrostyrene attached to the headgroup, can also be hydrolysed to a benzaldehyde at high pH. This enables the formation of vesicles of which solely the inner leaflet is oligomerised. Oligomerisation of the inner leaflet decreases the fusion rate. In an attempt to decrease this rate even more, aromatic units were incorporated into the hydrophobic part. It was expected that aromatic interaction between the tails would increase the attractive interaction between the tails and that this would decrease the fusion rate. Possibly this would allow direct observation of a hemifusion intermediate. The synthesis of a phospholipids derivative containing a biphenyl unit was completed, but purification problems prevented the isolation of pure lipid derivatives. Preliminary measurements on aggregates formed from impure lipid derivatives showed that these aggregates form H–aggregates and have a high T_m (79.5 °C). The oligomerisable headgroup is temperature labile, so heating above T_m resulted in decomposition of the headgroup. Since formation of vesicles and studies of vesicle fusion require heating above T_m, it can be concluded that this system is unsuitable for the study of the fusion mechanism.

Chapter 6 provides a critical review of the conclusions drawn from the research described in this thesis. To find answers on questions resulting from the research described in this thesis and to further investigate interesting findings in this study, possibilities for further research are suggested.