Control of nano-micrometric twist and helical ribbon formation with gemini–oligoalanine *via* interpeptidic β-sheet structure formation†

Aurélie Brizard, Roni Kiagus Ahmad and Reiko Oda*

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Confinement of anionic oligo-alanine peptides at the surfaces of cationic membrane by ionic interaction can induce their secondary structure formation; such organized peptides reciprocally transfer their chirality to membranes with non-chiral amphiphiles and their supramolecular chiral structures can be tuned both by peptides and amphiphiles structures.

The confinement of molecules capable of molecular recognition at the surface of nanometric supramolecular objects can be used to reinforce inter-molecular and inter-supramolecular interactions. Such an approach allows induction of recognition mechanisms between functionalized supramolecular objects but also the nano-scale control of the molecular assemblies morphology (bottom-up architecture). In this context, designing structurally well defined assemblies[1] inspired by biological systems is particularly attractive. To this aim, the use of peptido–amphiphiles was proposed where the association of the two partners (peptides and amphiphiles) may induce structures and functionalities of assemblies which are absent if they are alone.[2] A number of examples with various induced interactions and structures of short peptides[3] confined at the surface of the lipidic assemblies are reported.[4,5] Sometimes, organized peptides can reciprocally influence the aggregates morphology, and molecular chirality of amino acids can lead to the formation of chiral supramolecular structures.[6,7] For these peptido–amphiphiles in general, peptides are covalently connected to amphiphilic molecules and the membrane organization of amphiphilic molecules assures the confinement of peptides. Is it necessary that this connection is covalent for the induction of secondary structure of peptides?

We previously showed that non-chiral gemini surfactant[8] C_2H_4-α,ω-$((CH_3)_2N^+C_mH_{2m+1})_2$ denoted as *m*-2-*m*, self-assemble to chiral fibers in the presence of chiral tartrate counterions.[9] The intimate interaction between the two lead to a cooperative effect of their organization.[10] Recently we also reported that the same gemini surfactants formed gels when they were complexed with acetyl-oligoglycine-aspartate. FTIR measurements showed the presence of weak inter-peptidic hydrogen bonds but without particular secondary structures.[11]

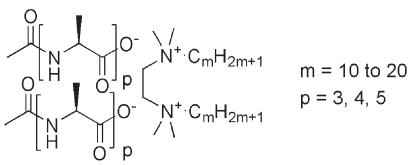
Herein we show that induced secondary structures of peptides can take place simply through ionic interaction with a new family of peptido–amphiphiles formed by a complex of cationic gemini surfactants and anionic acetylated oligoalanines and the morphology of these aggregates can be tuned both by varying interpeptidic and intergemini interaction. For the present study, we designed molecules such that two peptides, acetyl-$(Ala)_p$ ($AcAla_p$ with p = 3, 4 and 5), formed a complex with one gemini molecule (Scheme 1).

As these molecules were solubilized in water and cooled down to room temperature, precipitates were formed for some of the molecules. The microscopy images of these precipitates are summarized in Fig. 1. Interestingly, various supramolecular nano/micrometric chiral ribbons are formed although the gemini cations do not have intrinsic chiral centers! For p = 3, twisted ribbons were observed with gemini 16-2-16 and 18-2-18. For p = 4, twisted ribbons were observed with 16-2-16 whereas multilayered tubules were observed with 18-2-18. With p = 5, only helical ribbons were observed with 10-2-10 and 12-2-12. At the same concentration, the sodium salt of AcAla3 is soluble, AcAla4 and AcAla5 are precipitated without any defined structures (see ESI†).

All these ribbons have multi-bilayer structures as observed with small angle X-ray scattering (SAXS, data not shown). The time necessary for the formation of these helices can be quite long and depend largely on the hydrophobic chains and peptide length (see ESI†). For all p, only flat platelets were observed with longer chains ($\geqslant C_{20}$ for p = 3 and 4, $\geqslant C_{14}$ for p = 5) (data not shown), and with shorter chains ($\leqslant C_{14}$ for p = 3 and 4), only clear solutions or poorly defined structures were observed. It should be emphasized that such a rich polymorphism could simply be obtained by the variation of only one or two amino acids or the hydrocarbon chain length: the same gemini molecules having 3, 4 or 5 alanines as counterions can lead to very different structures!

The correlation between the emergence of such interesting chiral aggregates and the intermolecular interaction and molecular organization was investigated by FTIR and the results are summarized in Fig. 2.[13]

As expected, the wavenumbers of CH_2 stretching bands decreased with increasing m, indicating a more crystalline organization for longer hydrophobic chains (Fig. 2(B)). For p = 3, they remained in a melt state through all the investigated chain lengths and the lack of crystalline character was correlated with the value of the amide I band, all around 1640 cm^{-1}, representative of



Scheme 1 Molecular structure of cationic gemini surfactants complexed with anionic acetylated oligoalanine, *m*-2-*m*·$(AcAla_p)_2$.

Institut Européen de Chimie et Biologie, 2, Rue Robert Escarpit, Pessac, 33607, France. E-mail: r.oda@iecb.u-bordeaux.fr; Fax: (+33) 540-00-3066; Tel: (+33) 540-00-2229

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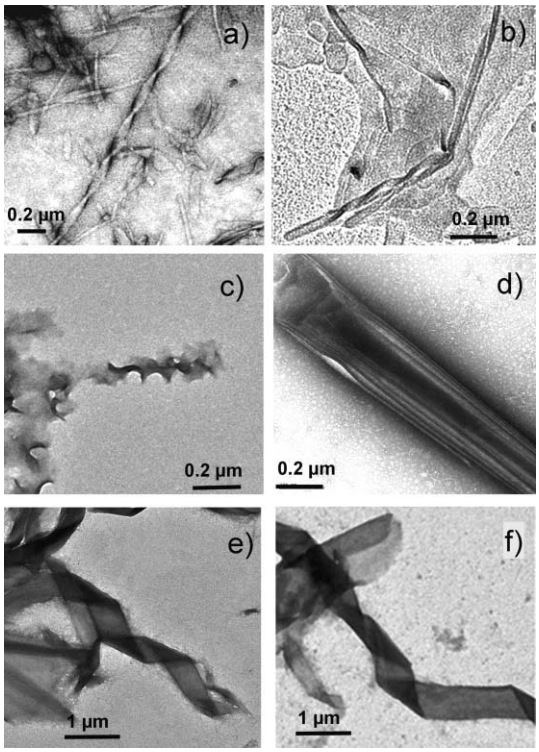

Fig. 1 TEM images of right handed chiral ribbons (negative staining) obtained at 21 °C with *m*-2-*m*·$(AcAla_p)_2$: (a) 16-2-16·$(AcAla_3)_2$, (b) 18-2-18·$(AcAla_3)_2$, (c) 16-2-16·$(AcAla_4)_2$, (d) 18-2-18·$(AcAla_4)_2$, (e) 10-2-10·$(AcAla_5)_2$ and (f) 12-2-12·$(AcAla_5)_2$. (a), (b) and (c) show twisted ribbons, (e) and (f) helical ribbons and (d) multilayered tubules.[12]

a weak hydrogen bonding network. Interestingly, when p increased to 4, the melt to gel transition of hydrophobic chains (from above to below 2920 cm^{-1} for the antisymmetric stretching band) between C_{16} and C_{20} was associated with a sharp decrease for the amide I band below C_{16} ($\sim$1640 cm^{-1}) and above C_{18} ($\sim$1630 cm^{-1}), where another peak appeared at $\sim$1685 cm^{-1}, characteristic of antiparallel β sheet organization. This particular organization was even stronger for $p = 5$, with a further decrease of amide I bands to 1620–1625 cm^{-1} for all the chain lengths whereas the chains showed melt to gel transition between C_{14} and C_{16}.[14] By comparison, the amide I band of the solution of the same molecules in methanol or sodium salt of $AcAla_5$ is found at around 1647 cm^{-1} representative of disorganized peptide structure.

Fig. 3 summarizes schematically the organization of gemini and peptides as well as the supramolecular structures. The two different morphologies of chiral ribbons observed with $p = 3$ (twisted ribbons) and $p = 5$ (helical ribbons) are worth mentioning, since FTIR revealed well defined anti-parallel β sheet organization whenever helical ribbons were observed whereas when twisted ribbons were formed, only weak interactions were present. An interesting intermediate case was observed for $p = 4$: twisted ribbons (C_{16}) were associated with weak hydrogen bonding, whereas C_{18} resulted in tubule formation in the presence of antiparallel β sheets. As discussed elsewhere,[15] helical ribbons are often precursors of tubular structures; both structures have cylindrical curvature. It is therefore likely that the formation of β sheets is indeed closely related to the induction of cylindrical curvature.

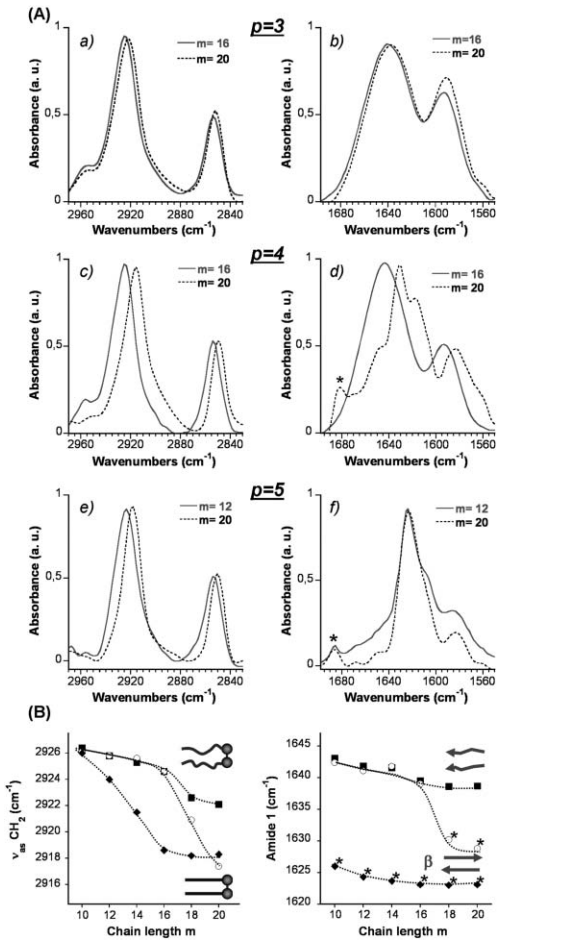

Fig. 2 (A) Typical IR spectra obtained after reaching equilibrium for the hydrophobic chains (left) and peptide (right) domains, for different gemini–oligoalanine complexes, at 100 mM in D_2O. (a) and (b) for $p = 3$, (c) and (d) for $p = 4$, and (e) and (f) for $p = 5$. (B) Values of antisymmetric CH_2 stretching bands and amide I bands as a function of hydrophobic chain length for different peptide length (■, $p = 3$; ○, $p = 4$; ◆, $p = 5$). For a given peptide length, both values decrease with chain length, indicating increasing interpeptidic and intergemini interaction. The antisymmetric CH_2 stretching bands (left) show that gemini are in a gel phase (<2922 cm^{-1}) for $p = 4$ and C_{18}, C_{20} and for $p = 5$, C_{14}–C_{20}. The amide I bands (right) show that peptides form only ill-defined secondary structure (>1635 cm^{-1}) for $p = 3$ with all studied chain lengths and for $p = 4$ with C_{10}–C_{16}, but they form antiparallel β sheets for $p = 4$, C_{18}–C_{20} and for $p = 5$ with all studied chain lengths. Data points labelled with stars indicate the presence of a second peak at around 1685 cm^{-1} (antiparallel β sheet).

In view of the data presented above, we propose that complex non-chiral gemini surfactant–oligoalanine can form chiral multilayered ribbons through close contact of oligoalanine with gemini bilayers by ionic interaction. The supramolecular chirality is observed only when the cooperative organization between the gemini molecules and the two-dimensional network between peptides matches a delicate balance: either peptides or hydrophobic chains of gemini has to be long enough to assure the formation of multilamellar structures. However, some flexibility is required for the chirality to be readily expressed, since such ribbons were only observed in a certain range either of peptides or chain lengths. The morphologies of the aggregates are thus intimately

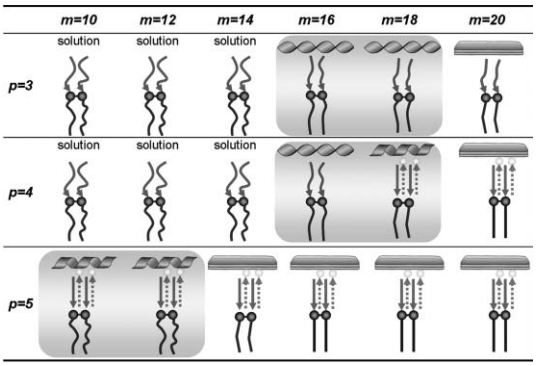

Fig. 3 Schematic representation of gemini–peptide organization at the molecular and supramolecular level as a function of peptide length and hydrophobic chain length. Solution states represent micellar solutions. At higher peptide length or hydrocarbon chain length, peptides form antiparallel β sheets with the peptides complexed with gemini in the adjacent bilayers.

related to the molecular organization. When the peptides form well defined inter-membrane antiparallel β sheets, these ribbons formed with multibilayers become rigid and form helical ribbons,[15b] whereas when the inter-membrane interaction is weaker, twisted ribbons are favored. The cooperative organization between inter-peptidic and inter-gemini interaction and their effect on the assembly morphologies can be summarized as follows: for $p = 3$, weak inter-peptidic interaction leads to weak inter-membrane interaction then to twisted membrane formation. Such gemini driven ribbon formation requires a certain hydrophobic chain length of gemini molecules (C_{16} and C_{18}). On the other hand, with $p = 5$, strong antiparallel β-sheets assures the inter-bilayer interaction, and rigidifies the ribbons, leading to helical ribbons (peptide driven ribbon formation). The cooperativity was well expressed for the case $p = 4$: with C_{16}, gemini driven ribbon (twisted ribbons) formation was observed whereas with C_{18}, stronger inter-gemini interaction cooperatively induced better inter-peptide interaction, as shown by antiparallel β sheet formation and helical ribbons. In other words, one can control the morphologies of the aggregates from platelets to helical ribbons, then to twisted ribbons both by varying chain length of gemini at fixed peptide length (C_{20}, C_{18} and C_{16} for $p = 4$) or by varying peptide length at fixed chain length ($p = 5$, 4, 3 for C_{18})

In conclusion, we have described a new membrane system with which well-defined secondary structures of short anionic peptides, acetyl oligoalanines can be induced simply by complexing them at the surface of cationic bilayers, without covalent connections between the two elements, amphiphiles and peptides. The structure formation is driven in a cooperative manner: the longer the hydrophobic chains, the better the peptide organization, and the longer the peptides, the better the gemini organization. Very small molecular structural variations have drastic effects on their assembling mechanism as well as on their supramolecular morphologies. Such a cooperative structure formation has the effect in reciprocal chirality induction to the assemblies from chiral peptides to non-chiral gemini. The ribbons formed with the gemini–peptides complex showed supramolecular chirality at nano to micrometer level and the morphology of the aggregates was easily controlled by the hydrophobic chain or peptide lengths. There is a direct relation between the secondary structure of peptides and the morphology of the chiral ribbons: in the presence of antiparallel β sheets, helical ribbons and tubules were observed whereas in the absence of such structures, twisted ribbons were observed. The access to these versatile chiral aggregates *via* cation–anion complexes of such simple biomolecules allows a new approach for the architecture of supramolecular assemblies.

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