Supramolecular Organization of Oligopeptides, through Complexation with Surfactants**

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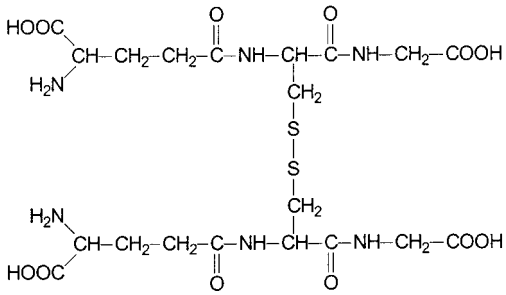
Oligopeptides are not only omnipresent in living systems but, because of their well-defined molecular structure, stereochemistry, and chemical functionality, are also ideal building blocks for a molecular tectonics or supramolecular chemistry[1] towards chemical nanostructures with a function. Organization of such building blocks can be caused by shape and complementary functional patterns,[2] but can also be induced by addition of a species that mediates additional weak and directed interactions, such as a transition-metal ion.[3] However, this chemistry is still restricted to aqueous or highly polar environments, and more extended structures and materials are only produced in this way in nature.

The approach presented here is complementary to such approaches: multiply charged, water-soluble oligopeptides are hydrophobized by complexation with appropriate organic counterions (in the present case simple oppositely charged surfactants and lipids) and the resulting hybrid structures act as building blocks for further, spontaneous supramolecular assembly. The complexation with organic counterions is expected to be very simple, because it is known that other organic compounds such as dye molecules[4] and polyelectrolytes[5] complex in a strict 1:1 stoichiometry, and the resulting product usually precipitates from water and can be isolated. Because of the amphipolar character introduced by the surfactant, the polar peptide backbone with its hydrogen-bonding system and hydrophobic tails are expected to separate into different nanophase regions. The minimization of binding and interface energy consequently results in structural complexity and a high degree of supramolecular order.

This mode of self-organization is also known from less defined polyelectrolytes, analogues with larger molecular weight and structural distributions, which form highly organized polyelectrolyte–surfactant[6–8] and polyelectrolyte–lipid complexes,[9] or from the hydrogen-bond-mediated supramolecular complexes between polar polymers and alkylphenols.[10,11] Synthetic polypeptides made of a single amino acid such as poly-L-lysine, poly-L-histidine, and poly-L-glutamic acid have also been complexed with surfactants,[12,13] mostly under preservation and sometimes stabilization of their typical α-helical structure.

Contrary to the simpler polymer–surfactant complexes, we expect the realization of new, unconventional assembly structures for the more variable and molecularily uniform oligopeptides, while their lower molecular weight should support a more rapid equilibration and higher perfection of the resulting structures.

Herein we focus on surfactant and lipid complexation of a simple model peptide, oxidized glutathion or GSSG. GSSG is an H-shaped hexapeptide composed of two γ-GluCysGly trimers which are linked by a sulfur bridge. GSSG contains



four carboxy units and two amine groups available for complexation (depending on the pH value), is cheap and easily available in larger amounts. Glutathion has a number of cellular functions: it is important as a redox buffer, maintains the sulfhydryl status of proteins, is important for a whole range of detoxification reactions, and binds easily to xenobiotic compounds.[14]

The synthesis of the complexes is rather simple, because all samples precipitate from aqueous solution. After washing, the residual amount of sodium and halogenide ions is very low (< 0.02 weight %), which indicates a close-to-perfect complexation with 1:1 stoichiometry with respect to the charges. The complexes can be redissolved in organic solvents such as THF, unlike peptides. In dilute solution, rather large aggregates are formed, as already indicated by dynamic light scattering. Drying those solutions and characterizing the dried aggregates with AFM and TEM reveals an exciting superstructure (Figures 1 and 2).

Both complexes of GSSG with lipids and surfactants form fibers with lengths of at least a few microns. A TEM of a GSSG–Lec complex (Lec = lecithin), which develops well (because of the absence of side-chain crystallinity in this complex), is shown in Figure 1. The fibers are flexible, bundled in the middle and frayed towards the ends. Each fiber is 35–45 nm wide, displaying a height of 15 nm (see AFM, Figure 2). A close-up of the aggregates shows them to be formed by left-handed spirals. The pitch of these supercoils is 40 nm with a tilt angle of 30°; the width of the coiled ribbon is about 15 nm. The diameter and height of the fibers is by far too large to be caused by a simple aggregation structure of single GSSG–surfactant units. We assume that about ten of the complexes laterally aggregate to form a nanosized ribbon which (as a wood shaving) winds up to give a left-handed spiral (Figure 3).

It is underlined that this "spirelli"-motive is a well-known superstructure for chiral surfactants and organogelators, such as those formed by modified steroids,[15] octylglucosides,[16] and dodecanoylserines,[17] which is however extended by the present systems to much larger tectonic units with higher stability. Similar superhelices made of non-natural peptide analogues were recently described by Nolte et al.,[18,19] who

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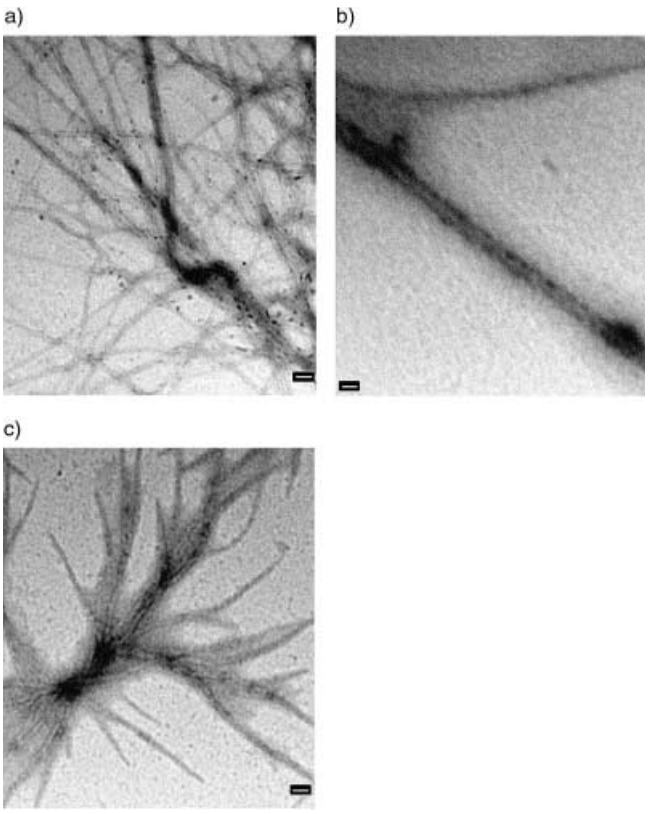

Figure 1. TEM pictures of the GSSG–lecithin aggregates in THF. Long, homogeneous, hollow tubules are formed. Scale bars: 100 (a), 40 (b), and 100 nm (c).

synthesized isocyanopeptides which also arrange via a modified β-sheet structure towards larger superhelices. The route presented here is however more simple, because we create our

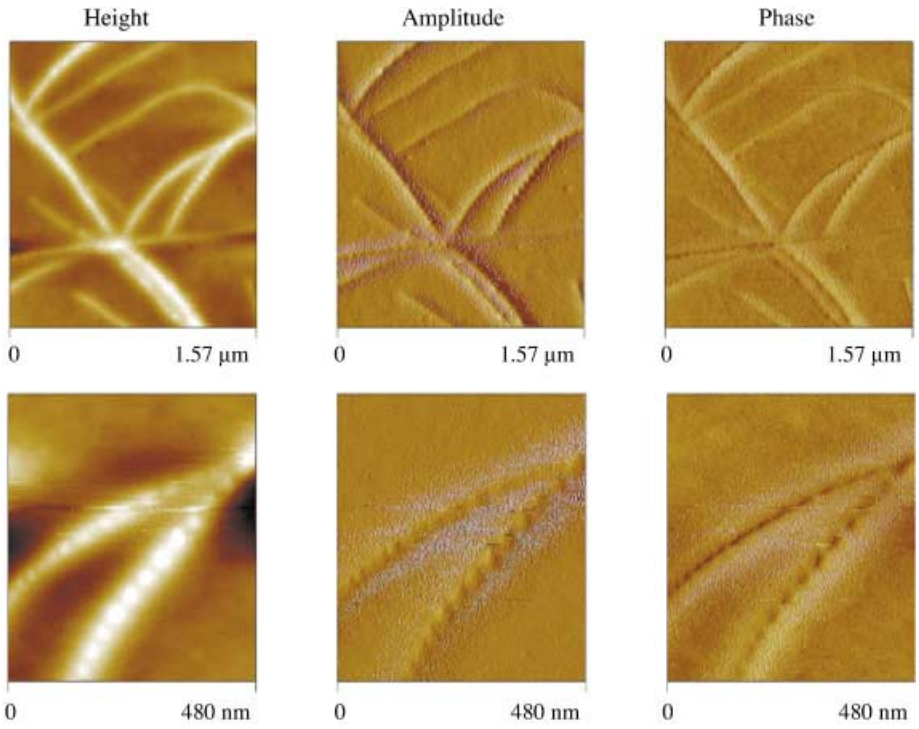

Figure 2. Tapping-AFM pictures of the GSSG–lecithin complex, different views of two selected areas; the aggregates are left-handed spirals.

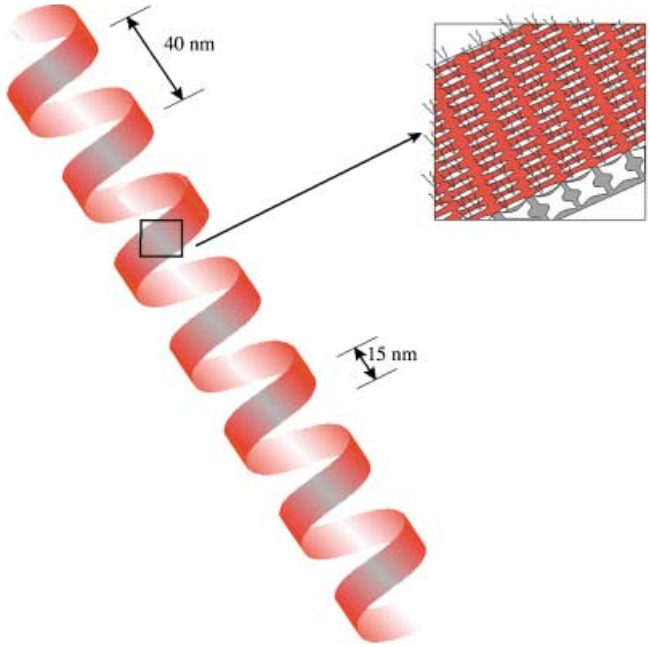

Figure 3. Schematic representation of the aggregates.

tectonic units by a primary assembly step (the "ionic self-assembly") from two simple starting products, and the method can be easily generalized towards other peptide and surfactant units.

Evaporation of the solvent leads to transparent films which are hard but flexible and show high optical birefringence, that is, the complexes possess reasonable material properties and are much harder than bare surfactant structures or films of the corresponding peptides. The nanostructure of the GSSG–lecithin complex (two lipids per oligopeptide unit) was characterized by small-angle X-ray scattering (SAXS) measurements (Figure 4).

Measurements are taken above and below a broad enthalpic phase transition found by differential scanning calorimetry (DSC) at about 60 °C, which is identified as a liquid-crystalline phase transition, since birefringence is preserved above this temperature. The formed complexes obviously exhibit high mesomorphic order on the nanometer scale. Both SAXS diffractograms are consistent with the fundamental symmetry of a lamellar, stack-of-bilayer structure, where the main scattering peak corresponds to a bilayer-to-bilayer distance of 4.76 nm. However, in the low-temperature phase there is a set of three other peaks, two of them even ahead of the main peak, which show that the unit cell is twice as large as the layer-to-layer distance and which must be interpreted as a superstructure onto the lamellae.

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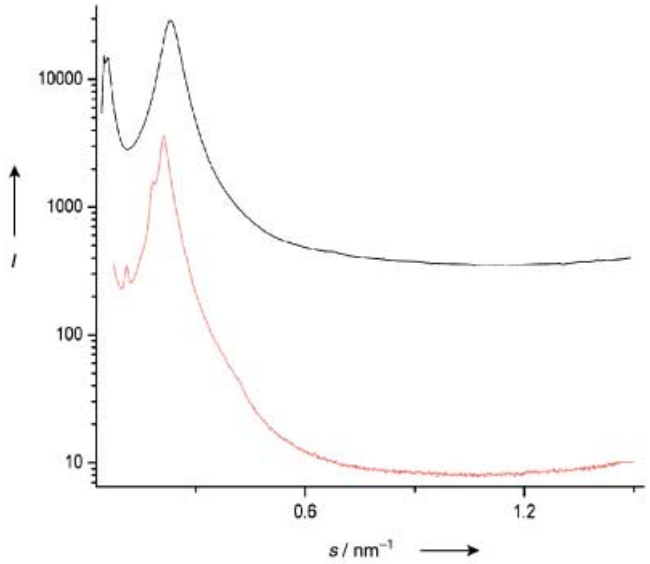

Figure 4. Small-angle X-ray scattering of solid GSSG–lecithin. Red curve: 25 °C, black curve: 60 °C. An evaluation of the structures with κ–ι analysis gave: $\kappa = 1.5$, $\iota = 1.7$.

From volume considerations and quantitative determination of the interface area per unit cell and the averaged curvature from the SAXS data (using the κ-ι approach, see ref. [20]), a so-called "tagliatelle" phase (a distorted hexagonal packing of one-dimensional channels in the lamellae with ABA symmetry, as depicted in Figure 5) is consistent with all experimental observations.

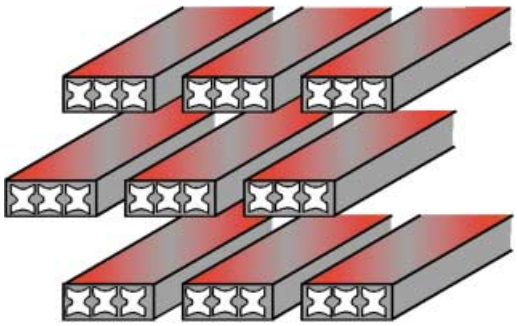

Figure 5. Schematic structural model for the solid GSSG–lipid phase. The presentaion of the separating bilayers is omitted for better visualization. The peptide subphase breaks up in stripes.

The correspondence to the solution structure is obvious: it is a similar ribbon-type secondary structure which winds up to a spiral in solution and forms a layered lyotropic phase in the solid film. At higher temperature, this hexagonal ordering of the ribbons "melts away", and statistically stacked ribbons are obtained. This structure is not specific to the lipid complex, but is also found for the double-tail surfactant. Because every GSSG unit binds four DOAC surfactants in this case (instead of two), minor differences in the superstructure arise.

The DSC trace of this complex indicates side-chain crystallinity at room temperature, which makes sample preparation more complicated; liquid crystallinity occurs only at elevated temperatures (ca. 60—80 °C). The sample clears at about 250 °C, which is attributed to the disintegration of the GSSG-subphase, but sample decomposition also occurs around the same temperature.

To summarize, the simple complexation of a model oligopeptide with appropriate surfactants leads to species soluble in organic solvents which—presumably by a combination of polarity effects, packing, and the formation of hydrogen-bonding bridges—further aggregate to rather complicated, but really well-defined superstructures and lyotropic phases. This way, it is in the hand of the chemist to encode (by the appropriate choice of the starting components) at least three levels of a hierarchical ordering process, by employing a different mode of self-assembly for each hierarchy, namely ionic self-assembly for the creation of the tectonic units, H-bond formation for the build-up of the constituent secondary ribbons, and amphipolar organization for the final nanophase structures.

Experimental Section

Synthesis of the GSSG–surfactant complexes: GSSG from Sigma, dimethyldioctadecyl ammonium chloride (DOAC, Fluka Co.) and soy lecithin "Lipopur" (Lec, Lucas Meyer Co) were used as received. Deionized water (resistance $> 18\,M\Omega$) and THF (HPLC grade, Sigma Co.) were used to prepare all solutions.

For production of the 1:1 charge ratio complex, all components were prepared as 50 mM aqueous stock solutions. For the complexation of the cationic sites of GSSG with lecithin, 2 equivalents of Lec were added to 1 equivalent of GSSG stock solution, both adjusted to a pH value of 2.0. The precipitate was collected after 24 h. For the second complex, anionic complexation was performed at a pH value of 10 with four equivalents DOAC and the precipitate was collected directly. The precipitate was centrifuged and washed three times with 30 mL of water (pH 2.0 or pH 10, respectively) to remove all unbound species. The complex was then dissolved in THF and cast to form films under vacuum.

Analysis: Small-angle X-ray-scattering (SAXS) curves were recorded by using a Nonius rotating anode ($P = 4$ kW, $Cu_{K\alpha}$) with pinhole collimation and an image-plate detector system. With the image plates placed at a distance of 40 cm from the sample, a scattering-vector range from $s = 0.05$–$1.6\ nm^{-1}$ was available ($s = 2\sin\lambda$, scattering angle 2θ, $\lambda = 0.15418$ nm). The samples were irradiated for 24 h to reduce the noise level and to obtain a sufficiently high scattering intensity. 2D diffraction patterns were transformed into a 1D radial average of the scattering intensity.

TEM was performed with a Zeiss 912 Omega electron microscope operating at 120 kV. The diluted colloidal solutions were applied to a 400-mesh carbon-coated copper grid and left to dry; no further contrasting was applied. DSC was performed on a Netzsch DSC 200 apparatus at a scanning rate of $10\ K\,min^{-1}$ by applying two heating and one cooling cycle. AFM was performed with a Nano Scope IIIa microscope (Digital Instruments, Santa Barbara, USA), operating in tapping mode. The instrument was equipped with a $10 \times 10\ \mu m$ E scanner and commercial silicon tips (model TESP, the force constant was $50\ N\,m^{-1}$, the resonance frequency was 300 kHz, and the tip radius < 20 nm). The samples were prepared by letting droplets of diluted solutions (0.01 % w/w) in THF dry on a freshly prepared gold surface at room temperature.

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Use of Enzymes Deactivated by Site-Directed Mutagenesis for the Preparation of Enantioselective Membranes**

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Many biologically active compounds, among them drugs, have chiral structures, of which only one stereoisomer has the desired activity. Chemical synthesis of such compounds often leads to racemic mixtures, whose resolution may be a tedious and expensive procedure. Enantioselective membranes can facilitate the transport of one enantiomer of a racemate, thus constituting a simple and fast resolution method. Lakshmi and Martin incorporated enzymes into porous polymeric membranes and prevented the undesired catalytic turnover of the substrates by depriving the enzymes of their cofactors.[1] Many enzymes do not use cofactors and thus a more general method would involve destroying the catalytic activity of the enzymes by site-specific mutagenesis, while maintaining their property to bind their substrates enantioselectively, that is, the enzymes are converted into receptors.

The enzymes histidine ammonia lyase (HAL) and phenylalanine ammonia lyase (PAL) seemed to us to be two excellent candidates for such an endeavor. Both enzymes have a catalytically essential electrophilic group, which for almost 30 years was believed to be dehydroalanine. The recently determined X-ray crystal structure of HAL revealed that the electrophilic prosthetic group is not dehydroalanine, but rather methylidene imidazolone (MIO).[2] Although there is currently no X-ray crystal structure of PAL, its MIO group has been identified by UV-difference spectroscopy.[3] We prepared a number of mutants of HAL and PAL that lack the MIO group or other catalytically essential amino acid residues.[3–6] Most of the mutants maintained their binding affinity for their substrates, but lost almost all catalytic activity. The two mutants PAL Y109F and HAL E414A were selected for incorporation into an artificial membrane.

To accelerate the transport through the membrane, the carrier molecule must be able to move freely in the whole pore volume. This movement is possible by physical entrapment between polymer films that serve as semipermeable barriers. Small molecules such as solvent or substrate molecules can diffuse through the polymer, but large molecules such as the carrier can not. The polymer film can be prepared, for example, by redox polymerization of pyrrole or its derivatives, or by polycondensation of dialkyl dichlorosilanes on the surface of a mesoporous membrane.

The quality of the synthetic polymer film can be best monitored by electron microscopy.[7] By using this method, we found that only poly(dimethylsiloxane) polymer films that were synthesized on the surface of an alumina membrane (Anodisc membrane, Figure 1a) could be obtained in a

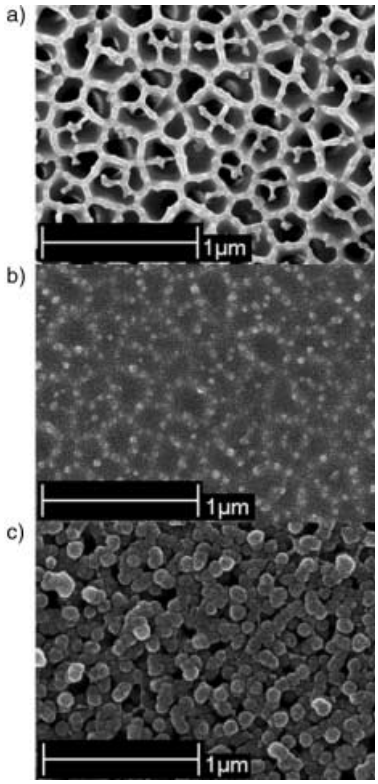

Figure 1. Electron microscope images: a) the uncoated surface of an alumina membrane (Whatman Anodisc); b) a poly(dimethylsiloxane) polymer film synthesized on the membrane surface; c) platelets of poly(pyrrole) on the surface of an alumina membrane.

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