Chelating Ligands

Self-Assembly of an Amphiphilic Iron(III) Chelator: Mimicking Iron Acquisition in Marine Bacteria

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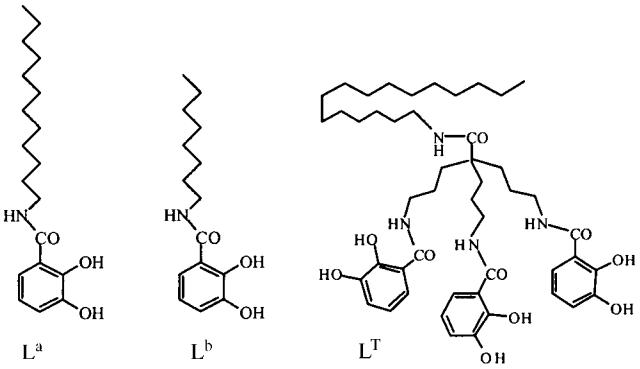
Iron is one of the most common elements on Earth, but its availability to living organisms is limited because of its extremely low solubility. Siderophores are low-molecular-weight iron(III) chelators that were evolved by microorganisms for the uptake of physiologically essential iron.[1] Among the mechanisms proposed for iron solubilization and transport into cells,[2] the self-assembly of amphiphilic siderophores from marine bacteria observed by Butler et al.[3] is a noteworthy process. Hydrophilic siderophores (marinobactins and aquachelins) with polar peptidic head groups and hydrophobic fatty acid tails are surface-active amphiphiles that form self-assembled structures. We thought that the biologically inspired use of this strategy might allow the resolution of the crucial problem of the hydrophilic/lipophilic balance encountered with abiotic siderophores designed for iron nutrition.[4] Amphiphilic iron chelators may also offer a new approach in iron chelation therapy.

Herein, we present a preliminary report on the self-assembly properties of amphiphilic chelators and their iron complexes, and discuss the first results concerning iron nutrition of *Erwinia chrysanthemi* and some mutants defective in the production of one or both of its siderophores. Three synthetic catechol-based chelators were investigated (Scheme 1).

The two monopodal ligands L^a and L^b were synthesized by reaction of 2,3-dimethoxybenzoyl chloride with dodecylamine and octylamine, respectively, followed by a deprotection step with BBr_3. The tripodal ligand L^T was obtained from our previously described[5] tripodal precursor 2,2,2-tris[3-(2,3-dimethoxybenzamido)propyl]acetic acid (by reaction of the acid chloride with hexadecylamine and then deprotection of the catechol groups). The ligands were characterized by mass spectrometry and ^1H and ^{13}C NMR spectroscopy (see Supporting Information). The pH-dependent equilibria of the ligand species and their ferric complexes were determined by potentiometric and spectrophotometric titrations to obtain their net charge at physiological pH, which tunes the amphiphilic properties.[6–8] The deprotonation constants of the ferric complexes $pK_{a,[Fe(LH)]}$, $pK_{a,[Fe(LH_2)]}$, and $pK_{a,[Fe(LH_3)]}$ are 7.9, 7.4, and 6.4, respectively (the fully deprotonated ligand is denoted L^{6-}), which indicates that $[Fe(L^TH)]^{2-}$ and $[Fe(L^TH_2)]^-$ are the major species at pH 7.4. The UV/Vis spectrum of the $[Fe^{III}(L^T)]$ system at pH 7.4 ($\lambda_{max} = 540$ nm, $\varepsilon = 4000\,M^{-1}cm^{-1}$) is similar to that for the $[Fe^{III}(CacCAM)]$ complex previously described,[5] which indicates a mixed salicylate (coordination with carbonyl and *o*-hydroxy oxygen atoms) and catecholate coordination. The pFe value of CacCAM is 27.5 at pH 7.4 ([L] = 10 μM, [Fe] = 1 μM). A similar value could be expected for ligand L^T, since we have shown that a change of backbone does not significantly alter the iron chelating efficiency of the ligand.[5] Notably, at pH 9 the spectral parameters ($\lambda_{max} = 490$ nm, $\varepsilon = 4300\,M^{-1}cm^{-1}$) indicate a tris-catecholate coordination. For ligands L^a or L^b, the spectrum of the ferric complexes at pH 7.4 ($\lambda_{max} = 540$ nm, $\varepsilon = 3300\,M^{-1}cm^{-1}$) is characteristic of a bis-salicylate coordination, which corresponds to the $[Fe(LH)_2]^+$ species. Despite an expected lower pFe value for ligands L^a or L^b compared with L^T (for example, pFe = 15 at pH 7.4, [L] = 10 μM, [Fe] = 1 μM for the dimethylamido derivative of catechol[9]), solutions of the ferric complexes of ligands L^a or L^b are very stable and no ferric hydroxide appears after several days.



L^a L^b L^T

Scheme 1. Chemical formulae of the synthetic amphiphilic ligands.

In water/methanol (95/5, v/v) solution containing 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer at pH 7.4, the free ligands and their iron complexes exhibit tensioactive properties with a critical micelle concentration (CMC) of $< 10^{-5}$ M.[10] The low CMC values of the iron-free ligands are in the same range as that for marinobactin.[3a] Dynamic light-scattering studies[11] on solutions (10^{-4} M) of the Fe^{III} complexes of ligands L^a, L^b, and L^T in the same medium revealed the presence of spherical particles of diameter 200–250, 100–110, and 130 nm, respectively. In all cases the distribution is rather broad (polydispersity index of 0.2–0.3). The free ligands, which do not scatter light, are probably limited to micellar assembly. Similar observations have been made for the siderophores from marine bacteria.[3a] Cryo-transmission

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electron microscopy (cryo-TEM) images of 1.4×10^{-3} M solutions of the Fe^{III} complexes showed polydisperse spherical particles with diameters ranging from 100 to 250 nm.[12] An example is given in Figure 1b for the $[Fe^{III}(L^{T})]$ system. The

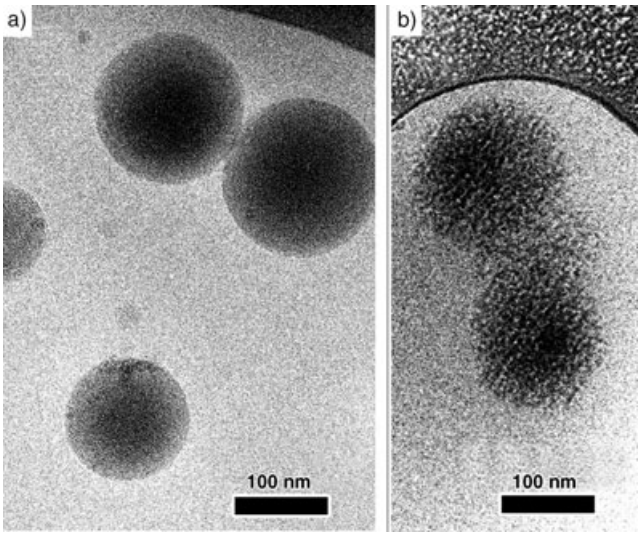

Figure 1. Cryo-TEM images of $[Fe^{III}(L^{n})]$ complexes (10^{-3} M) in water/methanol (95/5, v/v) solution at pH 7.4 (MOPS buffer) embedded in vitreous ice;[10] a) ligand L^{a} 3 h after addition of 1% octanol and b) ligand L^{T}.

micrographs depicted in Figure 1 compare well with those of Fe^{III}-marinobactin vesicles.[3a] In the experiment depicted in Figure 1a, octanol (1 vol%) was added to the $[Fe^{III}(L^{a})]$ solution to favor structuration of the particles. Successive cryoelectron micrographs allowed visualization of the evolution of the aggregates with time: vesicles of octanol first appeared at the surface of the aggregates (see Supporting Information) and after several hours perfectly spherical particles were observed. The contrast in the images of the particles (clear external surface and dark interior) suggests that they are filled. Furthermore, this observation indicates an aggregation of the molecules of the complexes, in agreement with an external hydrophilic surface as schematically depicted in reference [3a].

The ligands studied herein are good models for the physicochemical properties of the siderophores from marine bacteria. For a better understanding of the biological relevance of the self-assembly process (in correlation with the interesting discussion in reference [3a]), studies of bacterial iron nutrition were performed with *E. chrysanthemi*.[13–15] Preliminary results show that: 1) the iron aggregates allow nutrition; 2) the best results are obtained with the iron complex of ligand L^{a}; 3) the natural siderophore way is used when available (wild-type bacteria); and 4) nutrition is allowed in mutants without the siderophore way. Moreover, we showed that 70–75% of iron transport involves the TonB-dependent receptors Fct and/or 88 Da with Cbu for the inner membrane (see ref. [14] for a description of the iron transport system in *E. chrysanthemi*). For the remaining 25–30% of iron transport, it can be suggested that the aggregate merges with the outer membrane, which releases iron in the periplasm and then Yfe internalizes iron (Yfe is not TonB-dependent).

Further experiments are needed to reach more definite conclusions about the precise role of the self-assembly process in iron acquisition. This process, which is evidenced in marine bacteria, may allow new perspectives for applications in iron nutrition and for iron chelation therapy.

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[6] The ligands and their ferric complexes have low solubilities in aqueous solution, therefore the potentiometric titrations were carried out in water/methanol (50/50, v/v; ligand concentration 0.5 mM) and the spectrophotometric titrations in water/methanol (95/5, v/v; ligand concentration 0.1 mM). The methods have been previously described.[4a] Data analysis of the potentiometric and spectrophotometric titrations was performed using the Hyperquad[7] and Letagrop[8] programs. The spectrophotometric titration of a mixture of Fe^{III} and ligands L^{a} or L^{b} in a 1:3 molar ratio was carried out over the pH range 5–12.
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[10] Surface tension measurements were carried out with a drop tensiometer (Tracker; I.T. Concept, Longessagne, France) at 25 °C, which allowed the determination of the interfacial tension by analyzing the axial symmetric shape (Laplacian profile) of the rising air drop in solution. The CMC values were estimated from the intersection of the extrapolated linear portions of the plot of surface tension versus ligand or complex concentration.
[11] The size of the particles was measured by quasielastic light scattering. The movement of particles in suspension is characterized by a diffusion coefficient, and the particles undergo Brownian motion. A Malvern zetasizer 300HsA instrument was used at a scattering angle of 90°. This apparatus includes a 5-mW He–Ne laser that operates at a wavelength of 633 nm. The detector is an avalanche photodiode and the numerical correlator (type 7132CN with 256 channels) is controlled by the AutoSizer software version XY. The sample is contained in a flow-through cell kept at 23 °C. The data were analyzed by the CONTIN method. The translational diffusion coefficient is

related to particle diameter using Stoke's diffusion law and the Einstein equation for Brownian motion.
[12] Thin liquid films of the suspensions were fast-frozen in liquid ethane at −171 °C with a method described by C. Poncet-Legrand, D. Cartalade, J.-L. Putaux, V. Cheynier, A. Vernhet, *Langmuir* **2003**, *19*, 10563. The samples were mounted in a Gatan 626 cryoholder at −180 °C, transferred into a Philips CM200 "cryo" transmission electron microscope, and observed under low-dose conditions at low temperature. Images were recorded on Kodak SO163 films.
[13] We used the Gram-negative *Erwinia chrysanthemi*, which produces two siderophores upon iron deficiency: chrysobactin (a monocatecholate) and achromobactin, which is produced only when chrysobactin synthesis is repressed.[14] The wild-type *E. chrysanthemi*, a chrysobactin-negative mutant, and a chrysobactin–achromobactin–tonB mutant were used. Iron complex solutions (0.5–50 mM) in culture medium were employed (iron complexes were added at the beginning of iron carency). The experimental protocol for iron nutrition is described in ref. [15].
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