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Author's Review

From Biomimetic Ion Carriers to Helical Structures

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Biomimetic chemistry aims at reproducing the functions of natural compounds with the simplest possible synthetic molecules. Our strategy in this endeavor involved: (i) first reproduction of elementary processes such as molecular recognition, mass- transport, electron-transport, and signaling, and (ii) subsequently integration of several of these properties into single molecules.

We approached the problems of molecular recognition and mass-transport by concentrating on the design and synthesis of all-artificial iron(III)-carriers that mimic the properties of microbial siderophores (iron(III) carriers): (i) the capability to effectively bind iron(III), (ii) to interact with specific membrane receptors as their iron(III)-complexes, and (iii) to transport iron(III) into the cells' interior. Conjugation of the synthetic carriers with fluorescent markers enabled us to couple molecular recognition with signaling and to thereby provide diagnostic tools for the identification of specific microorganisms. The knowledge gained in the course of this work was then applied to the synthesis of (i) triple-stranded binders that form helical, dinuclear complexes, and of (ii) helical structures where four elementary process are integrated into a single molecule to provide molecular »redox-switches«.

INTRODUCTION

Biomimetic chemistry deals with chemical modeling.[1] It aims at the synthesis of task-oriented molecules by using natural compounds as guides. Its methodology relies on identifying the minimal essential features of complex biological systems and on incorporating these features into the simplest possible synthetic molecules.

The targets of biomimetic chemistry are reproduction of basic, elementary phenomena. We consider as elementary phenomena those processes that can not be further resolved. Examples are: molecular recognition,[2–6] mass-[7–10] or electron transport,[11,12] and signal generation.[13,14] Scientists in this field believe, that once these elementary phenomena are mastered, their integration will provide artificial solutions to enzyme catalysis,[3,15–19] light harvesting[20–22] and even replication.[23,24] This approach is envisioned to ultimately yield all-artificial molecular devices of technological relevance, either by incorporating the desired properties into single molecules, or by assembling them into supramolecular architectures.[5,6,14,20,25–28]

In an attempt to obtain insight into the rules that govern recognition and transport, we concentrated on iron(III)-carriers (termed siderophores), and on their microbial iron(III) uptake,[9,29–33] and aimed at the synthesis of all-artificial analogs that would mimic the properties of the natural compounds. Siderophore-mediated microbial iron uptake appeared to us an attractive system as it combines several processes: (i) recognition of iron(III) by the siderophore, and (ii) recognition and transport of the resulting siderophore-iron(III)-complex by microbial membrane components.

Once we had advanced along this line, and had traced some of the rules that govern metal binding and transport, we applied our findings to the solution of more intricate problems: the synthesis of multinuclear metal centers[34–36], and particularly helical metal complexes, and the integration of several elementary processes into a single molecule.

In the following we outline our efforts in this area during the last few years, emphasize the guiding rational, highlight the most crucial experimental results and draw the emerging conclusions.

MOLECULAR RECOGNITION AND MASS TRANSPORT

Introduction

The central role of molecular recognition in biological systems has stimulated increasing interest in the study of non-covalent, inter-molecular interactions[37,38] and in the design of artificial binders.[2–5,26,39] These investigations led to remarkably selective and efficient binders for a large variety of metal ions.[2,3,5,26,39] Artificial receptors were also developed for charged molecules,[2,3] and in rare cases even for heterocyclic ring systems.[4,40]. Yet, the development of a general approach towards binders that would recognize organic substrates lagged behind. Both because of the weakness of most non-covalent bonds, and the large conformational space of most molecular substrates. [38,41–43]

The success in the synthesis of artificial »receptors« for metal ions led us to reason, that further advances in this areas might be possible by concentrating on molecular substrates that would be conformationally restricted. Metal complexes of organic ligating molecules, specifically microbial iron(III)-carriers (siderophores), appeared to be promising candidates. Siderophores are low-molecular weight iron(III) binders of microbial origin that are excreted into the environment where they effectively scavenge iron(III).[9,30–33,44] While the free siderophores fail to penetrate biological membranes, the structurally well-defined siderophore-iron(III) complexes are taken up by microbial cells *via* highly specific membrane receptors. They transverse the biological membranes in energy dependent processes, and release the bound iron(III) to the cytoplasma (Figure 1). Release of the iron(III) may involve reduction, ligand exchange, or decomposition of the siderophore to its constituents. Whatever the detailed mechanism, the dependence of most living systems on iron for growth and development, the large variety and wide distribution of siderophores, and of their matching membrane receptors, offer a superb play-ground for the study of recognition phenomena.[9,29–32,33 ,44,45]

In our search for biomimetic siderophores that would mimic some of the properties of the natural siderophores both *in vitro* and *in vivo*, we were confronted with a formidable task: namely the requirement to match a »key«

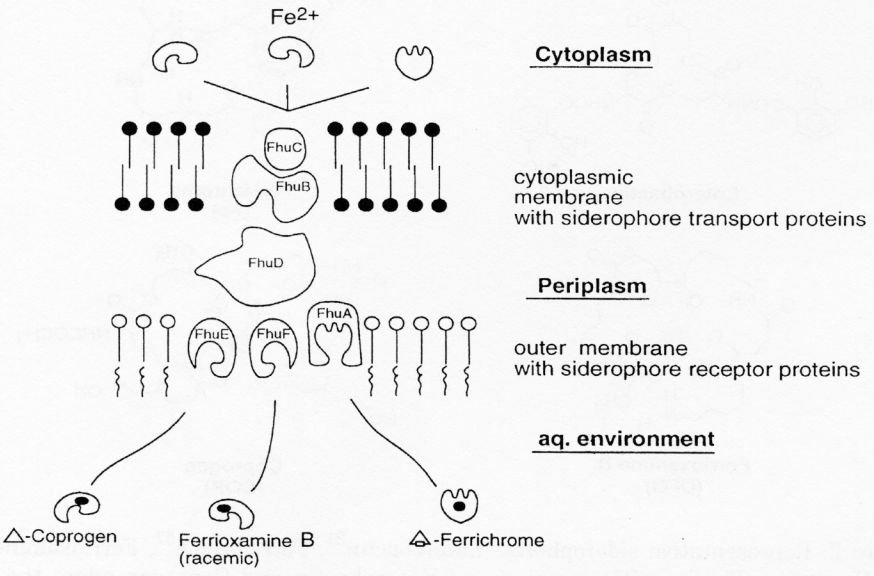


Figure 1. Schematic representation of siderophore mediated iron(III) uptake across microbial membranes.

to a »lock« of still unknown structure. In order to succeed in this endeavor, it is necessary to hit the »bull's eye« already with the first generation of compounds. As achieving any biological response, even if very modest, is essential to provide a point of departure for future optimizations. In an attempt to meet these stringent demands, we developed a strategy that combines three elements in an iterative fashion:[46–50]

(i) a modular, »lego-type« design and synthesis of the target molecules which enables systematic modifications till optimal performance is achieved,

(ii) the use of theoretical calculations for the detailed design of the molecules, and

(iii) fast and reliable assays to probe the performance of the compounds prepared.

The modular assembly facilitated systematic modifications such that each generation of compounds profited from progress made with earlier ones. Theoretical modeling helped to select from the many possible structures the most promising for synthesis, to interpret the experimental findings, and to suggest improved structures for further reiteration processes.

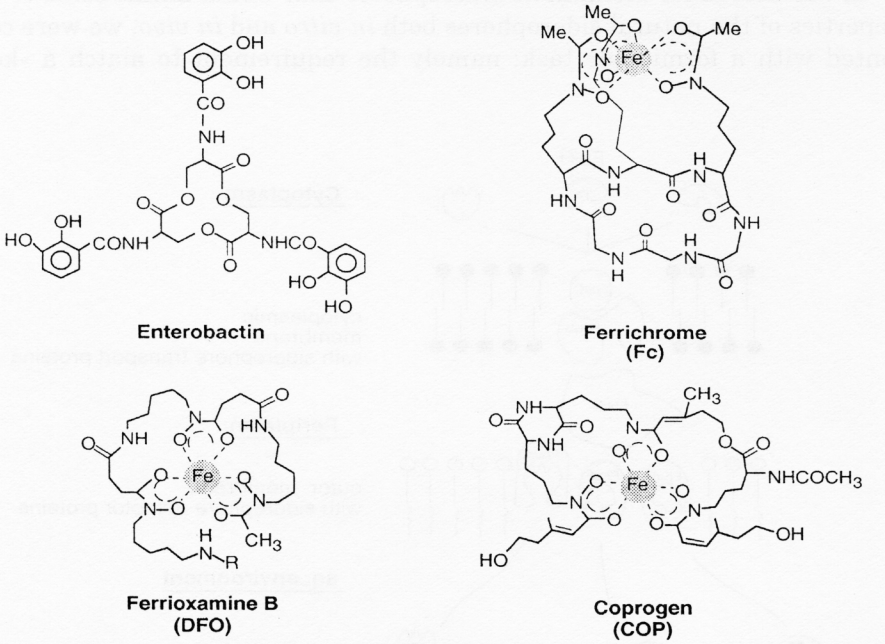


Figure 2. Representative siderophores; Enterobactin[31], Ferrichrome[57], Ferrioxamine[44] and Coprogen. The iron(III) complexes of Enterobactin and Coprogen adopt the Δ-configuration, and the Ferrichrome complex the Λ-configuration. Ferrioxamine B lacks chiral centers and forms five isomeric complexes, each as a racemic mixture.[58]

As test systems we employed whole microorganisms (actually mutants that do not produce siderophores), and compared the activity of the synthetic siderophores with that of the natural counterparts. In order to identify the nature of the receptors involved in the carrier mediated iron(III) uptake, we applied a combination of methods that relied on either of two principles[51,52]: (i) growth promotion and cellular $^{55}Fe^{3+}$- uptake in competition with the natural siderophores, and/or (ii) comparison of the response of wild type organisms with that of mutants that lack specific receptors. [30,53]

According to these principles four families of biomimetic siderophore analogs, analogs of Enterobactin,[54]Ferrichrome,[55] Ferrioxamine B [56] and Coprogen [56] (Figure 2) were prepared and their properties examined.

We selected the above four siderophores as target structures since they may be regarded as prototypes of the more than 200 siderophores known so far, many of which had been characterized by X-ray diffraction studies.[9,30–33,44] They encompass representatives of the two major classes; those that are based on catecholates as ion binding sites, and those that make use of hydroxamates. Moreover, they include the two major topologies: the tripodal one and the linear one, both of which embed iron(III) into an octahedral ion binding cavity, and they include derivatives that possess chiral centers which determine the absolute configuration of the iron(III)-complexes. The absolute configuration of siderophore-iron(III) complexes may often be crucial in determining microbial activity, since many siderophore receptors exhibit high enantioselectivity.[45,59,60]

Enterobactin Analogs

Our first target molecules were Enterobactin analogs[54,61] We chose Enterobactin as first system because of its high symmetry and exceptionally high iron(III)-binding efficiency.[31] Enterobactin is a tripode-like molecule consisting of a 12-membered trilactone ring which serves as an anchor, and three symmetrically projecting side chains that bear the ion binding catecholates.[31] When binding iron(III), the catecholates generate an octahedral cavity of Δ-*cis* configuration. Pioneering work of Raymond and coworkers demonstrated, that the proximity of the amide NH group to the catecholate group is essential for efficient binding to occur, and synthesized the first triscatecholates with Enterobactin-like activity.[31,62–64] Yet, the still large difference between the binding efficiency of the natural and the synthetic compounds led us to speculate, that there must be additional structural elements that are responsible for Enterobactin's superiority.

In an attempt to identify the stereochemical peculiarities of Enterobactin, we proceeded in a stepwise fashion. We first established the very nature of Enterobactin's trilactone anchor by synthesizing the parent trilactone and establishing its structure by X-ray diffraction. [65] The trilactone

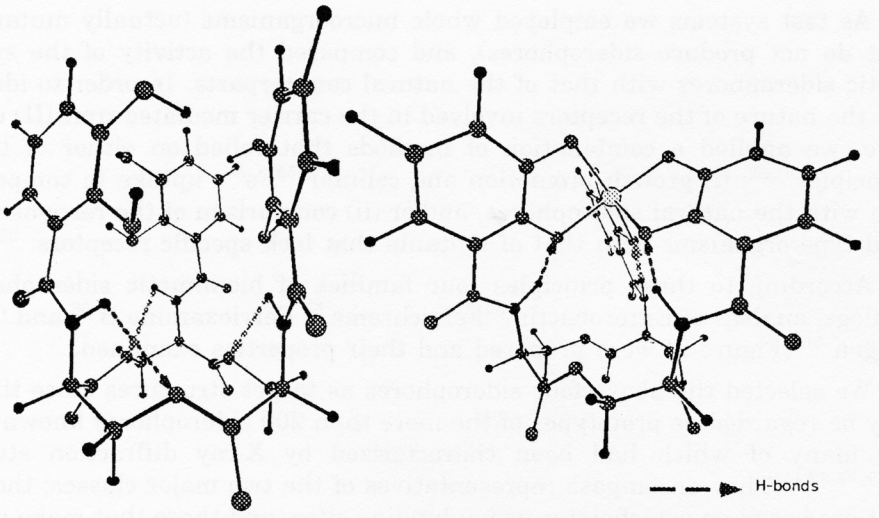


Figure 3. Calculated lowest energy conformation of Enterobactin (left) and of its iron(III)-complex (right).[61] The H-bonds between the amide NH-protons and the lactone oxygens in the free ligand (left) shift upon complexation to form H-bonds between the amide NH-protons and the catecholate oxygens.

ring was found to adopt a C_3-symmetric conformation and to have its lactone-carbonyl and lactone-ether groups pointing to opposite faces of the molecule's ring plane. We then extended the parent trilactone symmetrically with three benzamide groups, thereby obtaining a structural analog of Enterobactin, trisbenzamide TBA, with the trilactone functioning as anchor (Figure 4).[61] TBA, in contrast to Enterobactin, did lend itself to full spectroscopic examination and structural characterization. It thus served us as sounding board to test the reliability of EFF (empirical force field) calculations and to link experiment and theory. Once, the validity of such calculations had been established for TBA, they were expanded to the natural Enterobactin in order to provide insight into its structural characteristics and their effect on the molecule's binding properties.

The above studies[61] revealed that TBA, as well as free Enterobactin, adopt a propeller-type conformation with the side chains axially positioned and the amide-NH groups H-bonded to the lactone oxygen of the ring. Upon ion binding, a rearrangement of the H bonding network occurs: the H-bonds between the amide-NH groups and the ring oxygens become weakened and replaced by H-bonds between the amide-NH groups and the catecholate anions (Figure 3). Enterobactins H-bonding network thus shapes the molecule in the free state to adopt a conformation prone to ion binding, and rearranges to stabilize the complex once formed. (We like to term this H-bond-

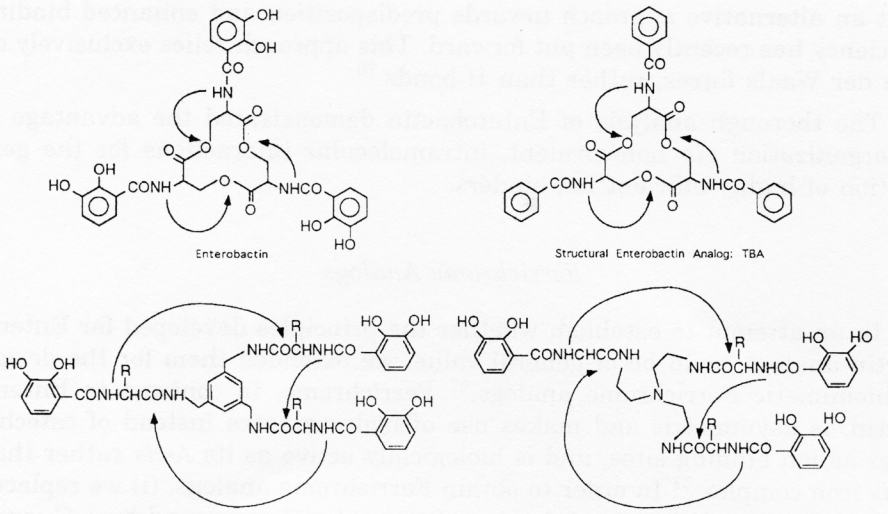


Figure 4. H-bond networks that impose propeller-like conformations in Enterobactin and in its structural analog TBA[61] (top), and in biomimetic Enterobactin analogs[54] (bottom).

shift »wiper-shift«, as it is reminiscent of the movement of windshield wipers.) To our big satisfaction, recent X-ray diffraction studies of crystalline V(IV)-Enterobactin complex[66] confirmed to the very details the structure of the Enterobactin-iron(III) complex that had been derived from calculations[61]

In our search for biomimetic Enterobactin analogs we aimed to reproduce the conformational characteristics of the natural compound with synthetically versatile, and easily accessible molecules. The idea was to reproduce Enterobactin's H-bond network with simple, tripodal structures by replacing Enterobactin's H-bonds between the side chains and the ring with belts of H-bonds between the chains (Figure 4). Guided by this rational, we synthesized chiral molecules that make use of trisamines as »anchors« and extending amino acid residues as structural units (H-bond formers).[67] These trispeptides, indeed, proved to form inter-strand H-bonds between different kinds of amide linkages, and to thereby adopt propeller-like arrangements of preferred chiral sense, as established by a combination of experimental and theoretical tools.[67,68] Extension of these structures with catechol groups provided binders that form iron(III) complexes of preferential Δ-*cis* configuration, as does the Enterobactin-iron(III) complex.[69] Moreover, the iron(III)-binding efficiency of these compounds exceeded that of the non-chiral precursor, and proved to be related to the strength of their H-bond networks, where some of the derivatives approached the binding efficiency of the natural siderophore within three orders of magnitude.[54] It is interesting to note,

that an alternative approach towards predisposition and enhanced binding efficiency has recently been put forward. This approach relies exclusively on van der Waals forces, rather than H-bonds.[70]

The thorough analysis of Enterobactin demonstrated the advantage of preorganization *via* non-covalent, intramolecular interactions for the generation of highly efficient ion binders.

Ferrichrome Analogs

In an attempt to establish whether the principles developed for Enterobactin analogs could be of general value, we extended them for the design of biomimetic Ferrichrome analogs.[57] Ferrichrome, in contrast to Enterobactin, is asymmetric and makes use of hydroxamates instead of catecholates as ion binding sites, and is biologically active as its Λ-*cis* rather than Δ-*cis* iron complex.[57] In order to obtain Ferrichrome analogs, (i) we replaced the non-symmetric hexapeptide ring of the natural compound by a C_3-symmetric triscarboxylate as anchor, (ii) compensated for the concurrent loss of chirality by extending the anchor with amino acids as variable chiral elements, and (iii) terminated the chains with hydroxamate groups. [55] Inspite of their greatly simplified structures, some of these binders (type 2, m = 2, Figure 5) proved to fully mimic Ferrichrome in its iron binding properties and microbial activity.[51,71,72]

Taking advantage of the favorable solubility properties of the Ferrichrome analogs and of their metal complexes, we examined their structures in further detail.[55] We demonstrated that networks of inter- and in-

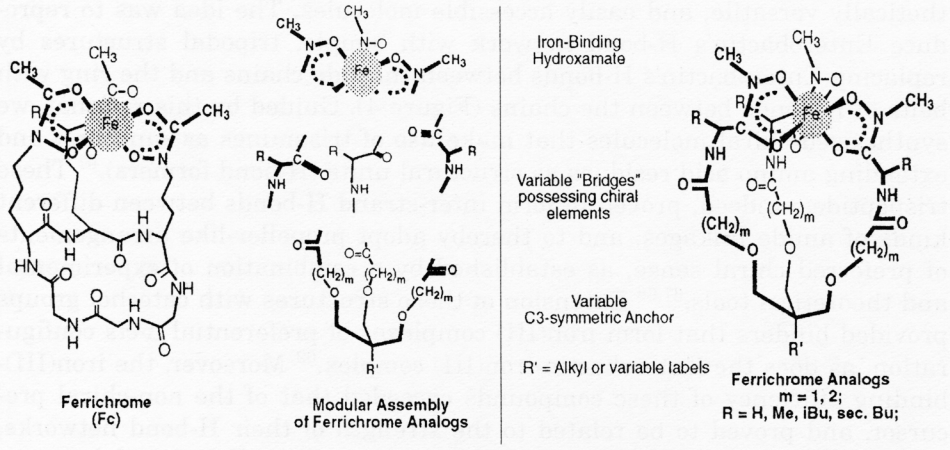


Figure 5. Design of Ferrichrome analogs.

tra-strand H-bonds shape the conformations of the free ligands towards propeller-like arrangements in both families of analogs prepared (type 1, m = 1 and type 2, m = 2, Figure 5). When loaded with metal ions, van der Waals forces, H-bonds, or a combination of both stabilize the complexes formed, and thereby promote the formation of well-defined 1:1 complexes of high isomeric and optical purity.[55],[50] In the absence of such intramolecular forces mixtures of polynuclear and polymeric complexes are obtained.

Some of the hydrophilic type 2 analogs, but none of the type 1 analogs, exerted quite remarkable microbial activity.[50,51] Within the bioactive family of compounds some fully mimicked Ferrichrome as iron carrier, while others acted as inhibitors.[51] Moreover, the binders' activity differed from organism to organism. Thus the L-ala derivative (m = 2, R = Me, Figure 5) acted as iron(III) carrier and growth promoter in *Arthrobacter flavescens,*[71] but as inhibitor in *Pseudomonas putida.*[72] The gly derivative (m = 2, R = H, Figure 5), on the other hand, acted as iron(III) carrier even in *Pseudomonas putida.*[72,73] In close analogy to the natural siderophore,[74] the enantiomeric (D-ala) derivative (type 2, m = 2, Figure 5) did not show Ferrichrome-like activity in any of the organisms examined.

The scale of activities of the different Ferrichrome analogs enabled us to identify those domains, that are critical for attaining biological activity.[51] They showed that the domain around the iron(III) center and its chiral sense have to be preserved, while the anchor may be modified. The pronouncedly different behavior of type 1 and type 2 analogs are believed to derive from long range conformational effects: While in type 2 analogs the iron(III)-binding domain is well exposed, in type 1 analogs the side chains shield the iron(III)-binding domain prohibiting recognition (Figure 6).[50]

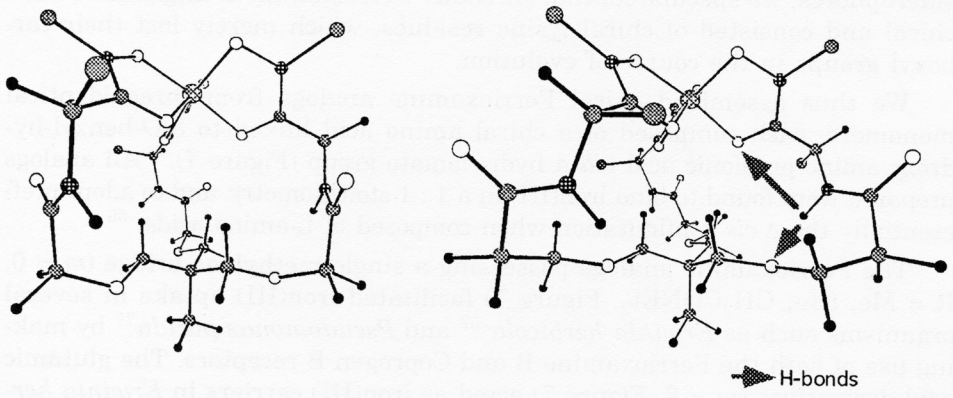


Figure 6. Calculated lowest energy conformation of Ferrichrome analogs (left, biologically inactive type 1 analogs; right, bioactive, type 2 analogs, with intramolecular H-bonds).[50]

Ferrioxamine Analogs

The success with the Ferrichrome analogs encouraged us to approach a more complex problem: the synthesis of Ferrioxamine analogs (Figure 7).[75] The Ferrioxamines, similarly to Ferrichrome, make use of hydroxamate groups as ion binding sites.[44,75,76] Yet, at variance with the Ferrichromes, the Ferrioxamines are linear structures that often lack chiral centers. They all possess three hydroxamate groups on a string which are bridged by amide or ester containing methylene chains. The most studied representative is the linear Ferrioxamine B, which became the most used drug for the treatment of iron overload. Ferrioxamine B forms a total of five isomers when binding three-valent metal ions, as established by examination of its kinetically inert Cr(III) complexes.[58]

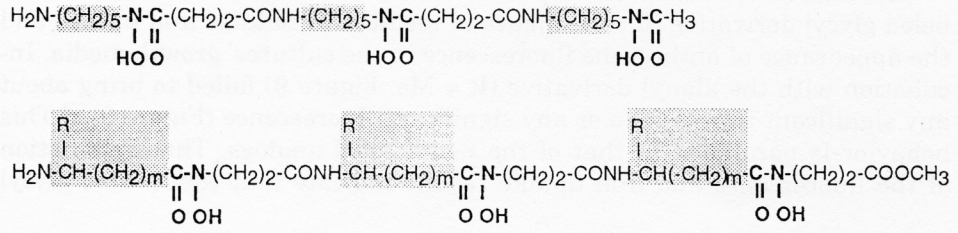
In attempt to examine the possibility of geometric discrimination by the Ferrioxamine receptor, we aimed at Ferrioxamine B analogs that would form a smaller number of configurational isomers when binding iron(III).[56] Considering the exclusive formation of cisoid complexes from both Enterobactin and Ferrichrome, we settled for Ferrioxamine analogs of cisoid configuration. Towards this goal we shortened the bridges between the binding groups, and reversed the directionality of the hydroxamate linkages relative to that in the natural compound (Figure 7). This reversal was conceived not to affect the compounds' biological activity (as earlier shown with Ferrichrome analogs), [71,72,74] but to greatly facilitate synthesis by relying on natural amino acids as building blocks. The use of natural amino acids as synthons also enabled us to introduce chiral centers that direct the configuration of the complexes to either the left- or right-handed chiral sense (Figure 7).[56], and to examine whether the Ferrioxamine B receptor could differentiate between enantiomers. Considering the preponderance of chiral siderophores, we speculated, that »archaic« Ferrioxamine B might have been chiral and consisted of chiral lysine residues, which merely lost their carboxyl groups in the course of evolution.

We thus assembled chiral Ferrioxamine analogs from three identical monomers, each composed of a chiral amino acid linked to 3-*O*-benzyl-hydroxy-amino-propionic acid *via* a hydroxamate group (Figure 7).[56] All analogs prepared were found to bind iron(III) in a 1 : 1 stoichiometry, and to adopt preferentially the Δ-*cis* configuration when composed of L-amino acids. [56]

The Ferrioxamine analogs possessing a single methylene bridge (m = 0, R = Me, *i*Bu, CH_2CONEt_2, Figure 7) facilitated iron(III) uptake in several organisms such as *Erwinia herbicola* [52] and *Pseudomonas putida*[77] by making use of both the Ferrioxamine B and Coprogen B receptors. The glutamic acid derivatives (m = 2, Figure 7) acted as iron(III) carriers in *Erwinia herbicola,* but as selective inhibitor of the Ferrioxamine receptor in *Pseudomonas putida*. The inhibition of the D-glu derivative of Λ-*cis* configuration was found to be 2–3-fold more effective than that of its enantiomer. The lat-

ter observation demonstrates that the Ferrioxamine receptor indeed exerts some chiral discrimination, although far from that reported for the receptors of Enterobactin[59] or Ferrichrome. [51,60] This result might indicate conformational tolerance by the receptor, poor match between the synthetic analogs and the receptor, or a combination of both.

The activity of the lower homologues (m = 0, Figure 7) as Ferrioxamine and Coprogen analogs, independent on the nature of the amino acid component, is indicative of their fit to both siderophore receptors. This may be attributed to the small molecular diameters of these iron(III) complexes that can easily adopt themselves to the respective recognition sites. The differential action of the higher homologues (m = 2, Figure 7) is attributed to these compounds' more stringent stereochemical requirements. The latter findings indicate that related receptors of different organisms may differ in their stereochemical demands.[51]



m = 0; R = Me (ala), iBu (leu), CH_2CONEt_2 (asp)
m = 2:; R = $CONEt_2$ (glu)

Figure 7. Natural Ferrioxamine B (top),[75] and biomimetic Ferrioxamine analogs (bottom).[56]

The above results illustrate the advantage of the modular strategy of design and synthesis and of theoretical calculations in the search for bioactive molecules. This approach provided molecular probes that differentiate between the structural requirements for receptor recognition and for transport, and led to both (i) artificial substrates that act as »master keys« and recognize more than one type of receptor, and (ii) substrates that act as »custom-made keys« and differentiate between the same kind of receptor in different organisms. The latter observations indicate that there are structural differences between analogous receptors of different organisms. Yet, the very nature of receptor-siderophore interactions is still a matter of speculation, and structural information on representative receptors will be needed to help unravel this problem.

Fluorescent Siderophore Analogs

The pronounced species-specificity of some of the synthetic siderophore analogs led us to contemplate, whether it would be possible to use such compounds as diagnostic tools for the identification of specific organisms. Realization of this idea would require the development of derivatives that would signal the microbial iron uptake processes. The presence of a tetrahedral carbon as anchor in the Ferrichrome analogs proved here of advantage, as it provided us with a site for labeling, which would neither interfere with iron binding, nor with receptor recognition. The bioactive Ferrichrome analogs (glycyl derivative, m = 2, R = H and alanyl derivative, m = 2, R = Me, Figure 5), were thus labeled with a fluorescent label at the carbon anchor (Figure 8). [78,79] To our satisfaction, iron(III) binding by the labeled derivatives expressed itself by quenching of the label's fluorescence. This quenching process was reversed upon addition of a competing chelator, indicating, that the quenching process occurs intramolecularly.

Incubation of *Pseudomonas putida* JM218 with the iron(III) loaded, labeled glycyl derivative (R = H, Figure 9) resulted in cellular iron uptake and the appearance of anthracene fluorescence in the cultures' growth media. Incubation with the alanyl derivative (R = Me, Figure 9) failed to bring about any significant iron uptake or any significant fluorescence (Figure 9).[79] This behavior is parallel with that of the non-labeled analogs. The combination of the fluorescence and iron uptake results indicate that the labeled glycyl

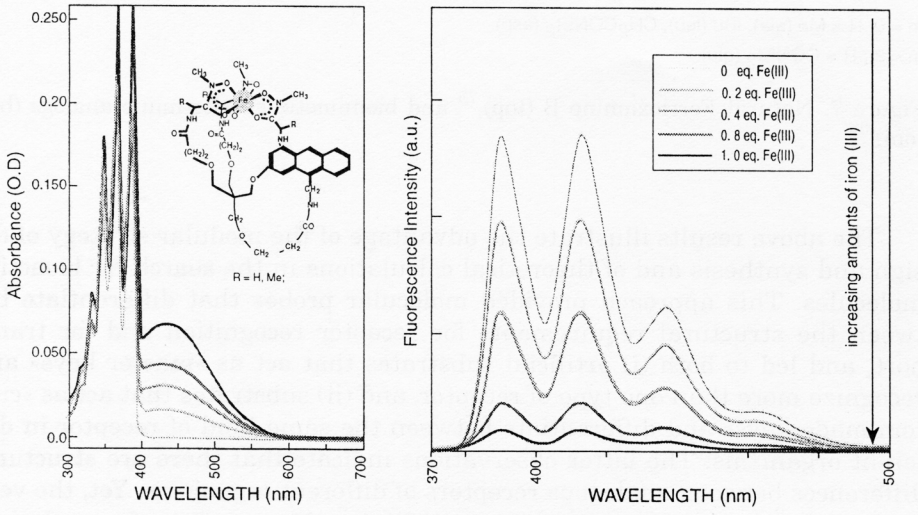


Figure 8. UV/VIS (left) and fluorescence titration (right) of fluorescent ferrichrome analogs with iron(III).[79]

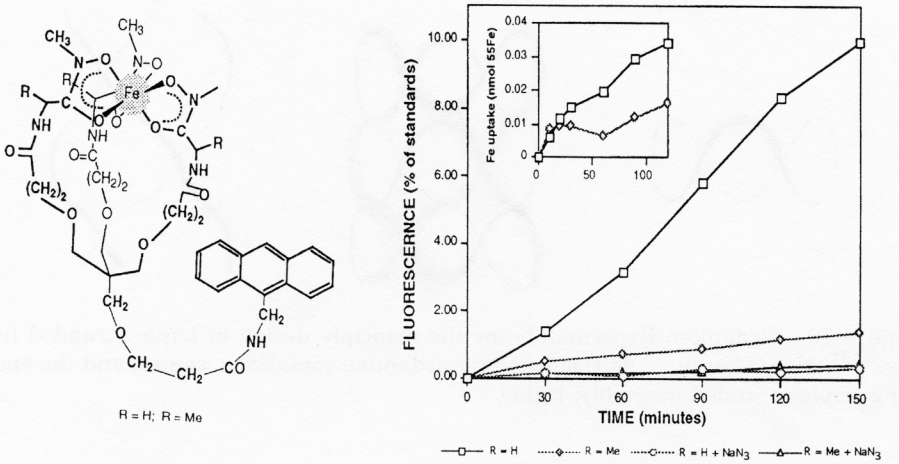


Figure 9. Appearance of anthracene fluorescence in the culture media of *Pseudomonas putida* JM218 upon treatment with labeled Ferrichrome analogs. Insert: ^{55}Fe-uptake with the Ferrichrome analogs. [79]

derivative acts as Ferrichrome analog, delivers iron(III) to the microorganism and is then released to the medium, while the alanyl derivative fails to deliver iron(III), acts as inhibitor of the Ferrichrome receptor and remains bound to the membrane receptor as an intact complex. Quite remarkably, other *Pseudomonas* strains such as *Pseudomonas fluorescens S680* or *Pseudomonas fluorescens WCS3742* failed to respond to the labeled Ferrichrome analogs, highlighting their pronounced specificity. [79]

The above data demonstrate the potential of labeled iron carriers as species-specific diagnostic tools. The energy-dependence of siderophore-mediated iron uptake renders these agents specific to live organisms. The latter point is believed to be of particular relevance for future applications.

The modular design and synthesis of the biomimetic analogs greatly facilitates labeling with spectroscopic markers, thereby providing derivatives where recognition and transport are coupled with optical signaling.

HELICAL STRUCTURES

Having established the structural parameters that promote iron(III) binding, the road had been paved towards the generation of multinuclear metal centers.[34–36] Towards this end we extended the tripodal binders along their main axis by adding a second ion binding cavity. For the detailed design of such binders we resorted to a conceptual »Gedanken-Experiment«.[80,81]

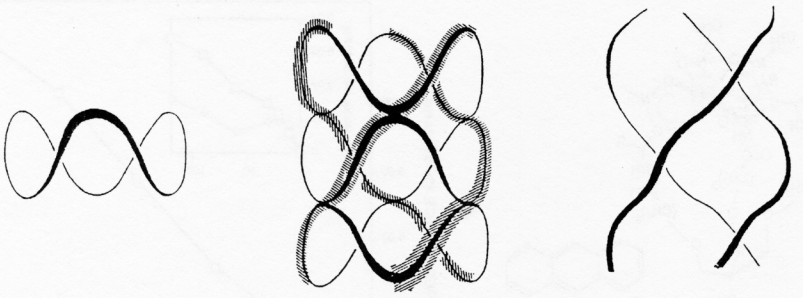


Figure 10. »Gedanken-Experiment« for the principle design of triple stranded helices.[80,81] (Nocardamine[76], left; stacked nocardamine complexes, center, and the emerging triple-stranded assembly, right).

This »Gedanken-Experiment« relied on stacking macrocyclic, Nocardamine iron complexes[76] (cyclic tris-hydroxamate based siderophore complexes) in such a way, that their ion binding backbones merge into chains of tripodal structures. Simultaneously, the chains consisting merely of methylene bridges are deleted, as schematically represented in Figure 10. The resulting structure is a triple stranded helix, where the sequence of its chains can be read off directly as consisting of alternating hydroxamate and

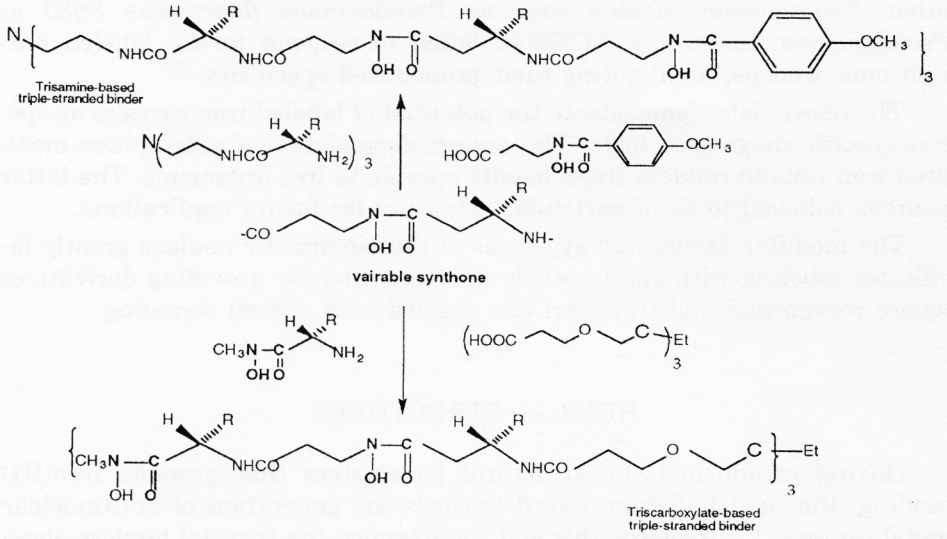


Figure 11. Modular assembly of triple-stranded, ditopic iron binders by the use of »Synthons«.[80,81 82](The trisamine-based binder (top), and triscarboxylate-based binder (bottom) are assembled from identical synthons (middle)).

amide groups. Here the hydroxamates serve again as binding sites, the amides as structural units.

Following this approach triple-stranded, ditopic ion binders were assembled as illustrated in Figure 11.[82] Symmetric trisamines or triscarboxylate anchors were extended with amino-acid like synthons, H_2N-CH*R-$(CH_2)_m$-CONOH-CH_2CH_2COOH, and terminated with single-chain hydroxamates possessing either free amino- or free carboxyl groups. The intermittent synthons, H_2N-CH*R-$(CH_2)_m$-CONOH-CH_2CH_2COOH, possess several functional and structural elements, that impart to the target molecules the desired properties and simultaneously facilitate synthesis. The functional elements include hydroxamates as ion binding sites, asymmetric centers as chiral probes and methylene chains of variable length for size adjustment. The structural elements are created by amidation of the terminal amino- and carboxyl groups *via* facile condensation processes. The use of N- and C-terminals in these synthons also enables their attachment to either C_3-symmetric, triscarboxylate anchors (similar to those used in the Ferrichrome analogs, Figure 5) or trisamino anchors (similar to those used in the Enterobactin Analogs, Figure 4, right). The latter options provide binders and complexes that merely differ in the directionality of their functional groups.

Adopting this strategy we prepared several families of triple-stranded ditopic ion binders and their dinuclear iron(III) complexes. Particular emphasis was placed on establishing the complexes' helical nature. Towards this goal we applied two criteria: identical absolute configuration around each metal center, and amplification of the chiral preference in the dinuclear complexes when compared with the mononuclear ones.[80,82] The usefulness of these criteria is their general applicability and the ease by which they can be traced, using CD-spectroscopy. Examination of the binders and their complexes revealed that the presence of amide linkages is essential for amplifying the chiral preference of the dinuclear complex relative to the mononuclear one, indicating the importance of structural elements. Independent support for the »in series« alignment of the metal ions was obtained by monitoring iron(III) exchange with EDTA. Iron(III) exchange was found to occur in a stepwise fashion *via* two bimolecular steps and an intermittent monomolecular step. We attribute the monomolecular step to iron(III) translocation from the internal to the external cavity (Figure 12).[81,82]

The successful synthesis of triple-stranded, ditopic ion binders encouraged us to also consider heterotopic ion binders possessing a »hard«, internal hydroxamate binding cavity and a »soft«, external bipyridine binding cavity. Such binders were anticipated to preferentially accommodate iron(III) in the internal cavity, and iron(II) in the external one. Should iron translocation from one cavity to its neighbor be possible upon application of reducing- and/or oxidizing -agents, molecular redox-switches were conceived to become available. [83–86]

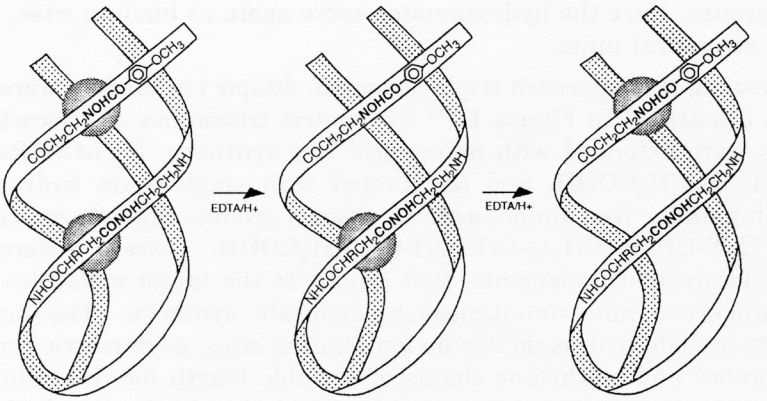


Figure 12. Stepwise iron(III)-release from helical, triple-stranded, dinuclear metal complexes.[81,82]

This idea was realized with several triple-stranded, heterotopic ion binders (Figure 13).[87] When exposed to iron(III), the internal hydroxamate cavity became occupied as evident from the appearance of the tan color at around 430 nm. Upon addition of ascorbic acid or dithionite, reduction of iron(III) to iron(II) took place with subsequent translocation of the metal ion to the external bipyridyl cavity and concurrent color change to deep purple. Addition of persulfate caused reversal of this process.

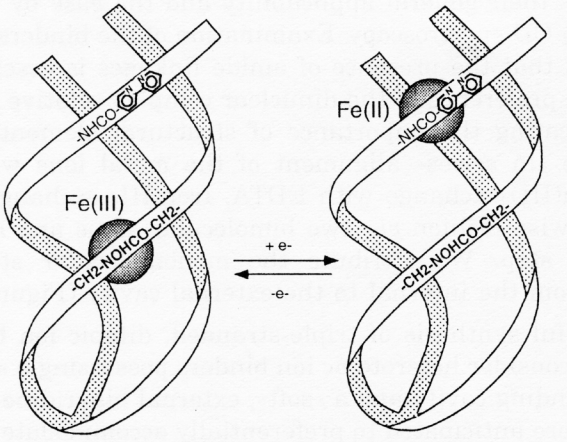


Figure 13. Schematic representation of triple-stranded metal complexes that function as molecular switches by inter converting between the light-brown Fe(III)-complex and the purple Fe(II)-complex upon exposure to reducing and oxidizing agents respectively.[87]

The orientation of the bipyridyl chromophores in these switches deserves particular attention. In some of these binders[87] the chromophores' orientation is random in the free ligand, but becomes increasingly aligned when transformed to the internal iron(III)-hydroxamate and subsequently to the external iron(II) complex. These observations demonstrate that the internal metal center can direct the helicity of the external one, and that the helical sense of some of these complexes is conserved during the reductive switching process.

The modular, »lego-type« assembly of the molecular redox-switches described above enables optimization of their performance, while their helical topology promises ready incorporation into monolayers for future »wiring« to the external world. Current investigations aim at triggering these switches by electrochemical and photochemical means, at improving their performance in terms of addressing, reading, and response rate, and at immobilizing them on conducting surfaces.

Modular extension of the triple-stranded ion binders led to helical derivatives that act as redox switches by intergrating four elementary processes: (i) molecular recognition of specific metal ions, (ii) electron transfer from an external reducing agent to an iron(III)-complex, (iii) translocation of a reduced iron-ion from the internal to the external cavity (mass transport), and (iv) optical signaling of these processes by the accompanying color changes.

CONCLUSION

Aiming at chemical models for molecular recognition, mass transport and signaling, we concentrated on analogs of microbial siderophores and on their helical extensions. The characteristics of these arrangements are the presence of an inner space and an outer envelope. The inner space can be modified to fit a given guest ion. The envelope can be adjusted for specific intermolecular interactions to occur such as to promote molecular recognition by biological receptors. The basic theme in the design of these molecules is the separation of functional elements from structural elements, and the use of chiral elements as structural probes. We define here as functional elements those parts that carry the compound's desired ion binding properties, and as structural elements those that shape the molecules three dimensional arrangement in support of their function. Although nature does not necessarily separate between functional and structural elements, this separation was conceived advantageous in allowing systematic modifications of each, and evaluation of their interdependence.

Adopting the above outlined strategy we succeeded to prepare all-synthetic iron carriers that simulate some of the properties of the natural

siderophores.[47,50,51,54–56] Some of these compounds provide tools for growth promotion of desired organisms, others for growth inhibition of unwanted pathogens,[47,88,89] and still others diagnostic agents for the identification of specific organisms.[79] We then extended this work to the synthesis of homonuclear[80,82] and heteronuclear,[87] triple-stranded, helical structures, and finally to molecular redox-switches that integrate four elementary processes.

On the basis of these results future efforts are directed towards the creation of functional assemblies that can be wired. First successes in this direction have been achieved by assembling biomimetic ion binding molecules on gold to provide highly selective sensors.[90,91] Current efforts aim at the generation of photoconducting assemblies, and possibly the creation of memory units from molecular redox-switches.

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SAŽETAK

Od biomimetskih prenosilaca iona do helikalnih struktura

Abraham Shanzer i Jacqueline Libman

Biomimetska kemija nastoji reproducirati funkcije prirodnih spojeva najjednostavnijim mogućim sintetskim molekulama. Naša strategija u tom poduhvatu uključuje: (i) prvo reprodukciju elementarnih procesa kao što su prepoznavanje molekula, transport mase, transport elektrona i signalizacija, zatim (ii) integracija tih različitih svojstava u jednu molekulu.

Pristupili smo problemu molekularnog prepoznavanja i transporta mase usmjerivši se na nacrtovanje i sintezu potpuno artificijalnih nosača za željezo(III) koji oponašaju svojstva mikrobnih siderofora (prenosilaca željeza(III)): (i) sposobnost efektivnog vezanja željeza(III), (ii) sposobnost interakcije nastalih kompleksa sa specifičnim membranama receptora, (iii) sposobnost transporta željeza u unutrašnjost stanice. Konjugacija sintetskih prenosilaca s fluorescentnim biljegom omogućila nam je da povežemo prepoznavanje molekula sa signalizacijom i tako dobijemo dijagnostičko sredstvo za identifikaciju specifičnih mikroorganizama. Ta su znanja zatim primijenjena u sintezi (i) trovlaknastih povezivača koji formiraju helikalne, dinuklearne komplekse, i (ii) helikalnih struktura u kojima su četiri elementarna procesa integrirana u jednoj molekuli dajući molekulski »redoks-prekidač«.