

## Polytopic chiral binders

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**Abstract** - The design, synthesis, and ion binding properties of two families of polytopic ion binders are described: single chain binders that form single stranded helical metal complexes and triple chain binders that form triple stranded helical complexes.

In an attempt to generate models for transmembranal ion transport through ion channels (ref. 1), we synthesized ion binders that accommodate a string of metal ions in their interior space. Two families of binders were synthesized and examined: single chain binders and triple chain binders that create single stranded and triple-stranded helical structures when binding metal ions (see Fig. 1)

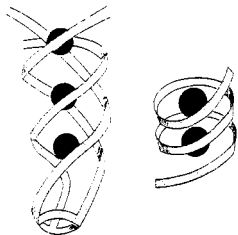
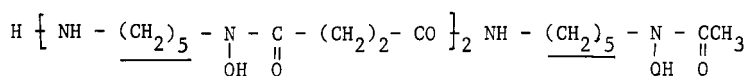


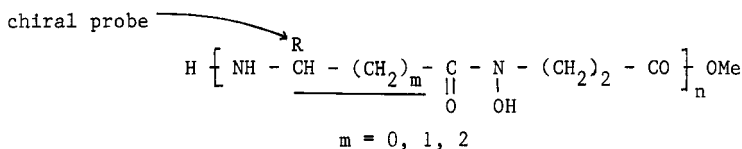
Fig. 1. Schematic representation of triple- and single-stranded helical complexes.

We chose to line these binders with hydroxamate groups that serve as binding sites for iron and other three valent metal ions (ref. 2) and allow analysis by a large range of spectroscopic tools.

The design of the single-stranded helical complexes was based on desferal, a natural iron carrier or siderophore (ref. 3), as guiding model (see Fig. 2).



Desferal



$n = 3$

monotopic binder

$n = 6$

ditopic binder

Fig. 2. Principle design of single stranded helical complexes

Desferal is a linear trishydroxamate that embeds ferric ions in an octahedral ion binding cavity by folding around in a loop. However, desferal is not suitable for the generation of single stranded helical complexes: (i) it forms ill defined complexes of both cis and trans configuration due to its long methylene bridges, (ii) it is not extendable since one of its termini is blocked by an acetyl group, and (iii) it does not allow probing of the helicity of the complexes formed as it lacks chiral centers. In an attempt to obtain well defined and

extendable, chiral complexes we chose to assemble such ligands from synthetic monomers that possess hydroxamate groups as ion binding sites, shortened methylene bridges ( $m = 0, 1, \text{ or } 2$ ), extendable amino and carboxyl groups at their termini and asymmetric carbons (CHR) in their backbone.

Such trimeric monotopic binders can bind octahedrally by forming either a right or left handed loop. In the presence of chiral centers the two types of structures become diastereomeric, and one of them preferentially populated. Distinction between the right and left handed structures becomes then possible by measuring the Cotton effects of the complexes, and specifically the Cotton effects around 230 nm brought about by exciton coupling between the ligating chromophores. On extension to hexameric, ditopic binders either right or left handed helical complexes are *a priori* possible, or non-helical S-shaped structures. The formation of a helix implies that both metal centers adopt the same absolute configuration and exhibit enhancement of the chiral preference relative to the monotopic complexes due to chiral induction. In non-helical, S-shaped dinucleating complexes the Cotton effects should be twice those of the monotopic binders when both metal centers adopt the same absolute configuration, or less than twice when the metal centers preferentially adopt opposite configurations.

The first compounds synthesized were the monotopic ( $n = 3$ ) and ditopic ( $n = 6$ ) binders derived from glu ( $m = 2, R = \text{CONEt}_2$ ). CD-analysis of each of these binders established small chiral preference for the monotopic complexes ( $n = 3$ ), but significant chiral preference in the ditopic complexes ( $n = 6$ ) when binding trivalent ions of medium size such as iron, gallium, or indium. Moreover, the Cotton effects almost doubled upon addition of the second metal ion.

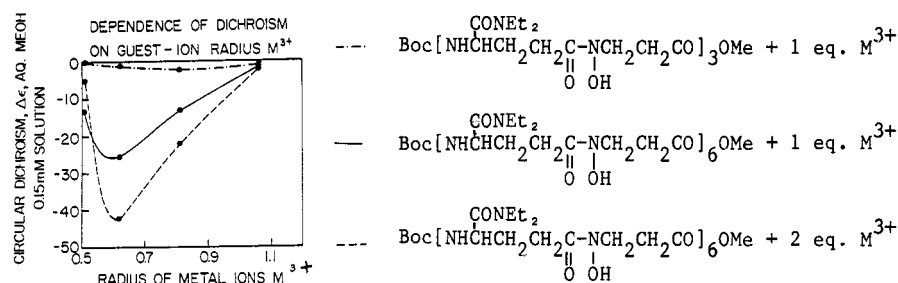


Fig. 3. Dependence of helical preference (dichroism) on guest ion radius.

This indicates identical configurations around each metal ion and therefore the presence of a helical structure. The smaller ions, such as aluminium, and the larger ions, such as lanthanum, did not conform to this pattern, but gave rise to rather small Cotton effects and small or "negative" enhancement upon addition of the second equivalent.

It therefore appears, that the configuration of these complexes is highly adaptable and that a perfect match between the guest ion and the host diameter is a prerequisite for helix formation. And, indeed, shortening of the methylene bridges in these systems by replacing glu with leu ( $m = 0$ , and  $R = \text{iBu}$ ), provided ditopic binders ( $n = 6$ ) where only the bis-aluminium complex showed significant chiral preference, implying helix formation.

The adaptability of the single stranded systems to the size of the guest ion where helical complexes are being formed either with aluminium or with gallium, might imply, that selective ion channels could be obtained from these structures when incorporated into bilayer membranes. An alternative solution for transmembranal ion flow might be provided by triple stranded helices.

In an attempt to generate such triple-stranded, polytopic ion binders we resorted to a topological "Gedanken-Experiment" (see Fig. 4)

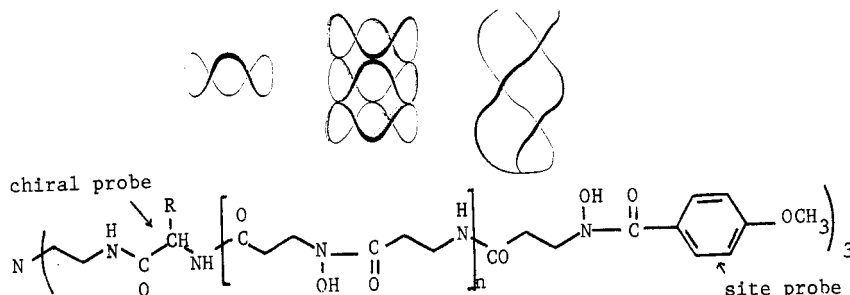


Fig. 4. Principle design of triple stranded helices.

This "experiment" relied on stacking macrocyclic, nocardamine iron complexes (ref. 3) in such a way, that their ion binding backbones interlink and collapse into chains of tripodal structures. Simultaneously, the chains consisting merely of methylene bridges are deleted, as schematically represented in Fig. 4. The emerging structure is a triple stranded helix, where the sequence of its chains can be read off directly as consisting of alternating hydroxamate and amide groups (Fig. 4). In order to facilitate the structural examination of the complexes formed, we introduced as probes chiral centers ( $R = iBu$ ) and terminal benzoate groups.

CD-spectra of the first ditopic complexes revealed identical configuration around each metal ion in the case of  $Fe^{3+}$ -ions (ref. 4), as well as  $Ga^{3+}$ - and the smaller  $Al^{3+}$ -ions, namely delta-cis, and enhancement of the chiral preference with the second guest ion.

*A priori*, ion uptake could occur (i) randomly, (ii) in a cooperative manner, or (iii) in an anti-cooperative manner. Chromatographic analysis of the Fe-complexes obtained after addition of 1 equivalent of  $FeCl_3$  demonstrated formation of a single product which was different from that obtained after addition of 2 equivalents of  $FeCl_3$ . This result eliminates the first two possibilities and indicates formation of the mono-complexes with the metal either at the outer or the inner position. UV-titration indicated preferential binding to the outer position by showing higher extinction values for the mono-ferric complexes than the increments obtained after addition of the second metal ion.

At this stage the possible occurrence of  $Fe^{3+}$ -translocation from one to the second cavity was examined. Towards this end the iron release of the bis-ferric complexes upon addition of EDTA as competing complexing agent was followed by monitoring the decrease of the ferric-hydroxamate absorption in the UV/VIS region (Fig. 5). These experiments revealed a delay between the release of the first and the second guest ion. Computational simulation of the observed kinetic behavior is in agreement with three successive processes, a bimolecular process, a monomolecular process, and another bimolecular process, according to the scheme shown in Fig. 5.

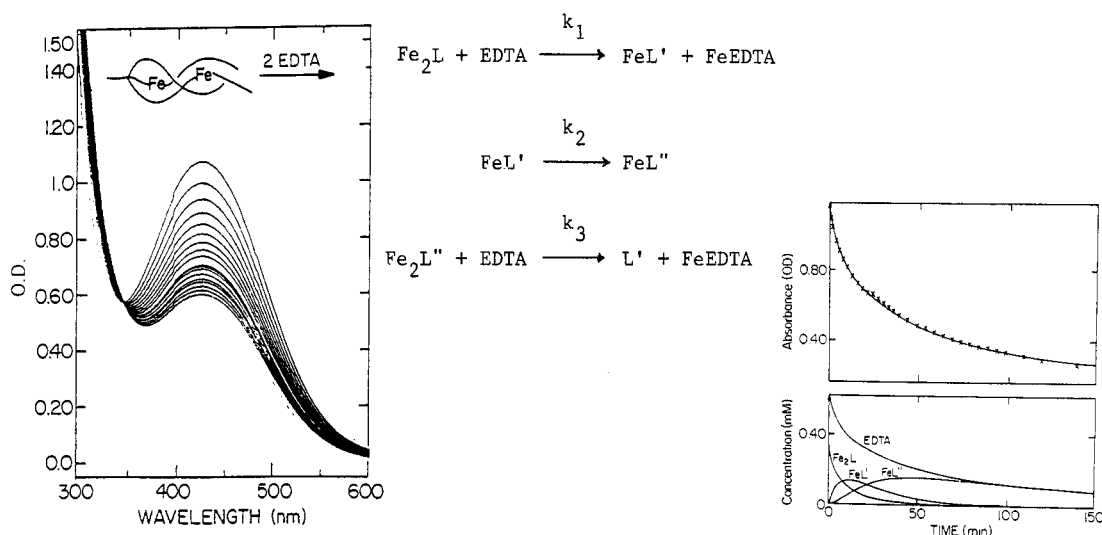


Fig. 5. Rate of iron removal from bis-ferric complex (Left: UV/VIS spectral changes during iron removal. Right Upper: Optical density curves, x = experimental, — = simulated. Right Lower: Concentration curves, simulated.)

Attempts to fit the experimental data with models based on two rate constants only consistently failed.

Our model is as follows: the ditopic binders provide two sites for the iron, an "internal" and an "external" one. Release to EDTA can occur only from the "external" site; release from the "internal" one necessitates prior site-to-site ion translocation with concurrent conformational change.

The same kinetic pattern was also found for other dinucleating, helical complexes, although with different rate constants. The differences in the rate constants are attributed to differences in the H-bonding patterns, which may stabilize (or destabilize) the transition states relative to the ground states and thereby accelerate (or reduce) the iron(III) exchange processes. The ion translocation step in these systems is likely to occur via a transition state where the  $Fe^{3+}$  is bound to the C=O groups of the "internal" cavity and the N=O groups of the "external" one.

These results demonstrate that helical complexes may be obtained from both single and triple stranded systems. These systems differ in their adaptability to the guest ion and are expected to also differ in their ion translocation modes. Whether either of them, or both, will generate hydrophilic pores when incorporated into membranes and therefore allow transmembranal ion flow, is still an open question.

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#### REFERENCES

1. G. Eisenman and J.A. Dani, Ann. Rev. Biochem. Biophys., 16, 205-226 (1987); C.L. Schauf, Sci. Prog. (Oxford), 71, (284), 459-478 (1987).
2. G. Schwarzenbach and K. Schwarzenbach, Helv. Chim. Acta, 46, 1391-1400 (1963).
3. J.B. Neilands, Structure and Bonding, 58, 1-24 (1984); K.N. Raymond, G. Mueller, and B.F. Matzanke, Top. Current Chem., 123, 49-102 (1984); R.C. Hider, Structure and Bonding, 58, 25-87 (1984).
4. J. Libman, Y. Tor, and A. Shanzer, J. Am. Chem. Soc., 109, 5880-5881 (1987).