An Enterobactin Analogue Prepared by Cross-Linking a Preassembled Iron(III) Complex of a Catechol Derivative with Poly(ethylene imine)

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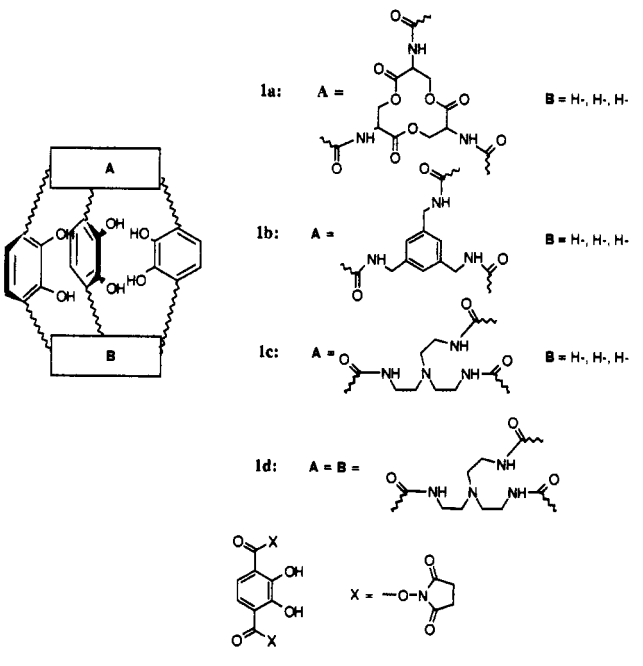
Design of host molecules capable of highly selective complexation of metal ions is an important area of molecular recognition. Metal-binding ability of the host can be adjusted by varying the shapes of spacers connecting ligating sites. If the ligands of a preassembled complex are cross-linked with a spacer that allows conservation of the geometry of the coordination site, little strain in the molecular geometry and, consequently, stable and inert binding are expected for the resulting complex.

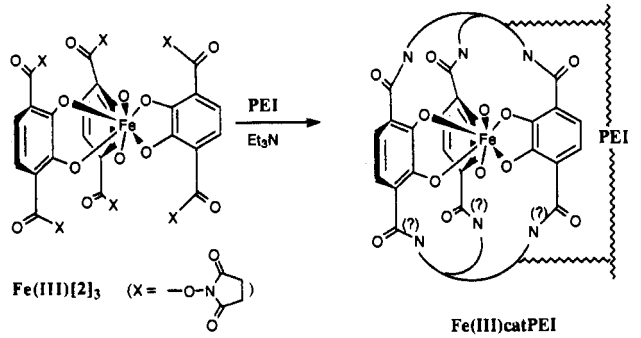
When spacers of small sizes are used, fine adjustment of the molecular geometry is difficult to achieve. In this regard, poly(ethylene imine)[1-4] (PEI; MW ~60 000) may be employed as a spacer to cross-link a preassembled metal complex in view of the availability of many amine nitrogens as well as its structure containing many branches. PEI contains ethylamine as the repeating unit. Among about 1400 nitrogen atoms of PEI, 350 are tertiary amines representing the branching sites on the polymer backbone, 350 are primary amines, and 700 are secondary amines.

In the present study, the idea of cross-linkage of a preassembled metal complex with PEI was tested by aiming at design of an enterobactin analogue. Enterobactin (**1a**),the acyclic siderophore



with the highest binding constant among the microbial iron(III)-sequestering agents, was chosen as the target since several analogues have been synthesized by cross-linking three molecules of catechol derivatives with various spacers (e.g., **1b–d**) and their physicochemical behavior has been well characterized.[5-22] In addition, PEI may be regarded as a polymer of the spacer of **1c,d**.

The Fe(III) complex ($Fe^{III}[cat]_3PEI$) of the enterobactin analogue built on PEI was prepared by cross-linking the preassembled Fe(III) complex (5.6×10^{-5} mol, generated *in situ*) of **2**[21] with PEI (3.3×10^{-2} mol of monomer residue mol, i.e. 2.4×10^{-5} mol of polymer) in dimethyl sulfoxide (150 mL) in the presence of triethylamine[21](1 mmol) at room temperature and



purified by dialysis against aqueous ethanol and water. Preassembly of the Fe(III) complex of **2** before cross-linking with PEI was indicated by the characteristic visible spectra[21] (450–700 nm). Attack at the activated ester linkages of $Fe^{III}[2]_3$ by excess amines of PEI is expected to occur readily. The formation of amide linkages was reflected in the IR spectrum (1641 cm^{-1}) of the lyophilized $Fe^{III}[cat]_3PEI$, although whether bicapped structures were actually obtained with $Fe^{III}[cat]_3PEI$ in this investigation is not certain.

Some of the Fe(III)-binding sites of $Fe^{III}[cat]_3PEI$ may have free carboxylate groups unattached to the polymer backbone. To mimic enterobactin, however, it is not necessary to cross-link all of the six carboxyl groups of the preassembled Fe(III) complex of **2** as long as the three catechol moieties are contained in a molecular entity. If at least one catechol moiety of the preassembled complex were not linked to the polymer, the Fe(III) ion would have dissociated easily under the conditions of competition experiments with *trans*-1,2-cyclohexylenedinitrilotetraacetate (CDTA) mentioned below.[9]

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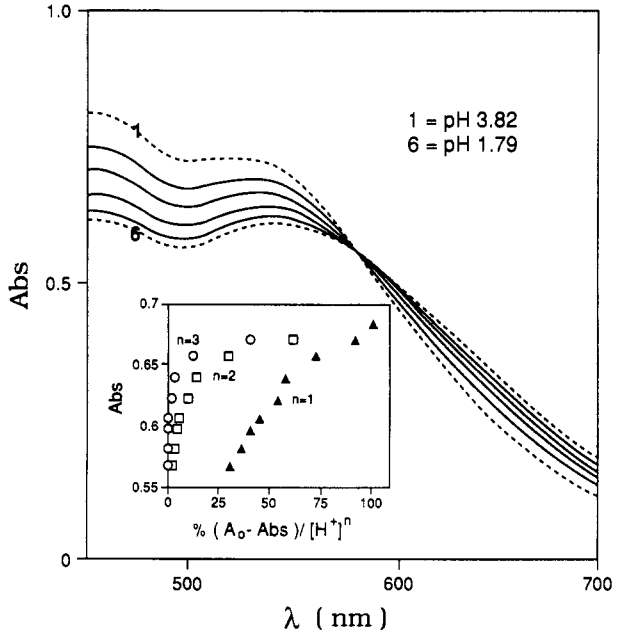

Figure 1. Isosbestic region for the titration of $Fe^{III}[cat]_3PEI$ (concentration of Fe(III) site = 1.42×10^{-4} M; no additional salt added) at pH 3.82–1.79. Inset: Schwarzenbach plot,[21] for which the best straight line is obtained when $n = 1$ (log (equilibrium constant) = 2.31). Curvature of the line may be related to the presence of several species with different proton affinities in $Fe^{III}[cat]_3PEI$.

Table 1. Thermodynamic Data for Fe(III)-Sequestering Agents[a]

complex	pH at midpoint of catecholate protonation	pM
Fe^{III}[**1a**]	4.89	33.5[b] (pH 7.4)
Fe^{III}[**1b**]	7.08	29.1 (pH 7.4)
Fe^{III}[**1c**]	5.59	27.8 (pH 7.4)
Fe^{III}[**1d**]	5.33	30.7[b] (pH 7.4) 24.3[c] (pH 5)
$Fe^{III}[cat]_3PEI$	2.31	>31.2[d] (>30.6)[e] (pH 7.4) >27.7[d] (27.0)[e] (pH 5)

[a] Data were taken from literature for the Fe(III) complexes of **1a–d**.[8,9,15,21] Measured at 25 °C. [b] Estimated in the original study from the results of competition experiments performed at lower pHs. [c] Calculated in this study by using parameters[21] reported in the literature. [d] Calculated by assuming the detection limit of Fe(III) transfer as 5% of $[Fe^{III}[cat]_3PEI]_0$. [e] Calculated by assuming the detection limit of Fe(III) transfer as 10% of $[Fe^{III}[cat]_3PEI]_0$.

Assuming that the molar extinction coefficient at λ_{max} in the visible spectrum of the $Fe^{III}[cat]_3$ moiety built on PEI is identical with that[21] of Fe^{III}[**1c**], the chemical yield for attachment of the $Fe^{III}[cat]_3$ moiety to PEI is estimated as 80%. Thus, each molecule of $Fe^{III}[cat]_3PEI$ contains 1.9 $Fe^{III}[cat]_3$ moieties on the average, and the molecular weight of the polymer increases by about 2% upon attachment of the $Fe^{III}[cat]_3$ moiety.

Visible spectra of $Fe^{III}[cat]_3PEI$ recorded at various pHs are illustrated in Figure 1. Protonation of the catecholate oxygens of Fe(III) complexes of enterobactin analogues is known to destroy the tris(catecholate)–Fe(III) structures.[23] $Fe^{III}[cat]_3PEI$ manifests much greater resistivity to acid compared with the Fe(III) complexes of enterobactin or other enterobactin analogues (Table 1; pH at midpoint of catecholate protonation). This is attributable to cationic microenvironments provided by the ammonium ions of $Fe^{III}[cat]_3PEI$. Stability at acidic pHs generally improves the utility of metal-sequestering agents.

The chelation stability of $Fe^{III}[cat]_3PEI$ was measured through a competition experiment[21] using CDTA. At pH 5 and 7.4, transfer of Fe(III) from $Fe^{III}[cat]_3PEI$ (concentration of the Fe(III) site: 9.3×10^{-5} M) to CDTA (0.001–0.05 M) was not positively detected over a period of up to 6 weeks. When Fe(III) is transferred from $Fe^{III}[cat]_3PEI$ to CDTA, the absorbance of $Fe^{III}[cat]_3PEI$ at >500 nm (Figure 1) becomes undetectable.[21] Binding of CDTA to $Fe^{III}[cat]_3PEI$ was found to be insignificant by the dialysis method.[24] With the assumption that the detection limit of the Fe(III) transfer is 5–10% of the initially added concentration of $Fe^{III}[cat]_3PEI$, the lower limits of pM (–log [Fe(III)] where [Fe(III)] stands for concentration of uncomplexed Fe(III) in equilibrium with enterobactin analogues)[25] are estimated;[21] these are summarized in Table 1. Metal-sequestering ability under particular conditions is best represented by pM. The pM of $Fe^{III}[cat]_3PEI$ is greater than those of several other synthetic entrobactin analogues, although the exact value is not available.

Failure to observe Fe(III) transfer from $Fe^{III}[cat]_3PEI$ to CDTA for up to 6 weeks might be ascribed to kinetic inertness instead of thermodynamic stability. In this case, the kinetic inertness is extraordinary since the Fe(III) transfer to CDTA reaches equilibrium within several days for enterobactin and analogues studied previously.[21]

Although the structures of the individual metal-binding sites are unknown, $Fe^{III}[cat]_3PEI$ is more stable in acidic pHs and manifests pM values greater than those of several other enterobactin analogues.[26,27] This suggests that cross-linkage of preassembled metal complexes with PEI could be extended to the design of effective host molecules for other metal ions.

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(23) Acidification and the subsequent reneutralization of $Fe^{III}[cat]_3PEI$ restore the visible spectra of the $Fe^{III}[cat]_3$ moiety. The Fe(III) ion may be removed by acidification of $Fe^{III}[cat]_3PEI$. Attempts were not made, however, to isolate the metal-free form of the catechol attached to PEI, since catechol derivatives are easily oxidized by air and it is practically impossible to purify the partially oxidized form of a polymeric catechol derivative.

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(25) pM is defined as –log [Fe(III)] at $[Fe(III)]_{tot} = 10^{6}$ M and $[ligand]_{tot} = 10^{-5}$ M. Although the conventional definition of pM requires pH 7.4, we extend the definition to variable pHs.

(26) Selectivity of the metal-binding site for Fe(III) and Fe(II) can be calculated from the reduction potential for the Fe(III) center.[21] Reversible redox patterns, however, were not observed for $Fe^{III}[cat]_3PEI$ by cyclic voltammetry.

(27) The greater pM observed at pH 7.4 is more attributable to the stable binding for $Fe^{III}[cat]_3PEI$ instead of the cationic microenvironment of PEI, since all the catechol phenolates of Fe^{III}[**1a–d**] as well as $Fe^{III}[cat]_3PEI$ are deprotonated at this pH.