Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2309–2314

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and electrochemical studies of a new iron tetra-catecholamide complex.

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Received 1 March 1999; accepted 29 June 1999

Summary: A new tetra-catecholamide compound *N*5,*N*6-thiodipropanoyl-bis[*N*1,*N*10-bis(2,3-dihydroxybenzoyl-spermidine)] (H_8L) has been synthesised as an iron chelator of Fe (III). Cyclic voltammogram of the iron complex H_2LFe run under an argon atmosphere shows a quasi-reversible redox process with E^0 = -430 mV *vs.* SCE in CH_3OH/H_2O (60/40). This value approaches the range of biological reductants and consequently the complex may mimic the release of iron from enterobactin to the agents which are directly involved in cell metabolism.

Iron is an important element for almost all living organisms, but its ionic forms, especially Fe (III), are very insoluble under physiological conditions. To carry this metal to cells, microbes and many micro-organisms have developed an ingenious procedure; they synthesise and excrete low molecular-weight organic molecules called siderophores which sequester, solubilise and chelate Fe (III) specifically for transport into the cell. Enterobactin, originally isolated from *Escherichia coli* , is among the siderophores extensively studied. [1, 2] This type of siderophore possess catechols as iron-chelating functional groups which coordinate ferric ion octahedrally with six oxygen atoms. In *E. Coli* and under conditions of iron scarcity, enterobactin as well as other siderophores are synthesised as potent iron-chelating compounds. [3, 4] In the case of enterobactin, the machinery necessary for iron uptake is composed of an outer membrane receptor, a periplasmic protein and several inner-membrane-associated proteins.

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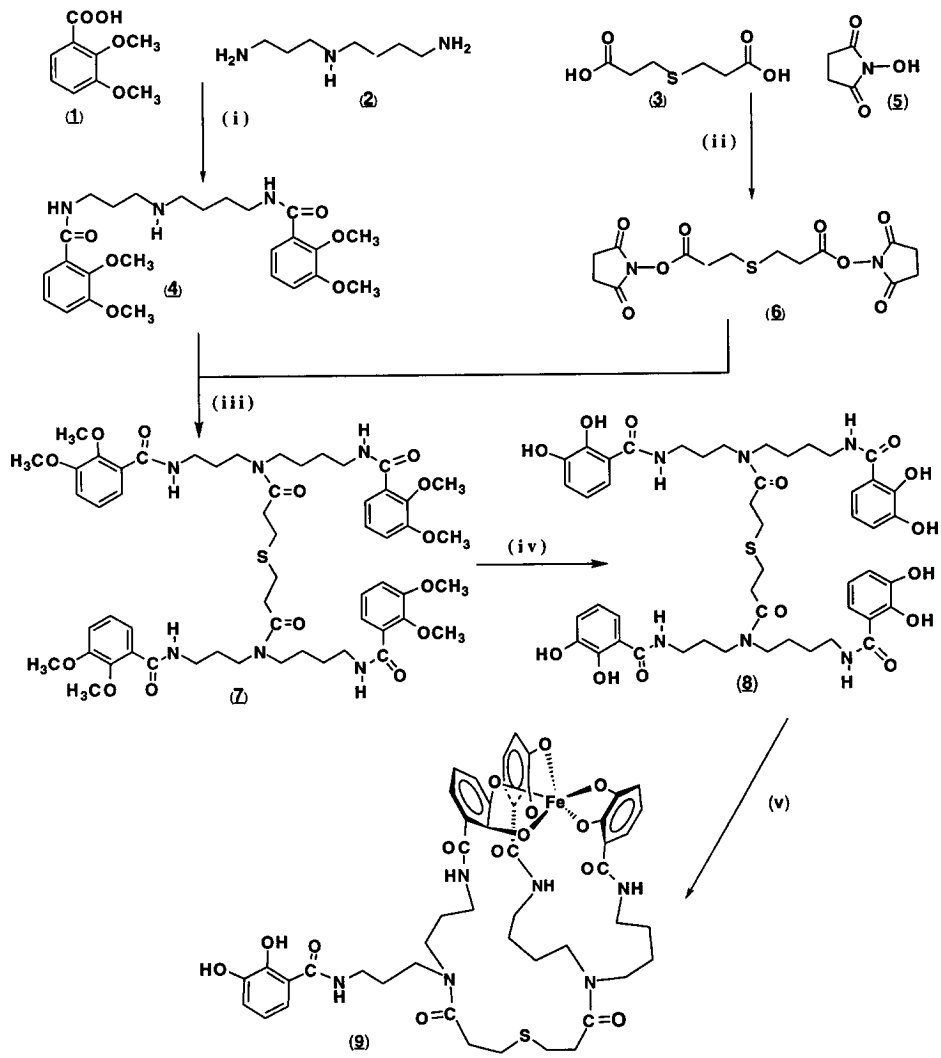
0960-894X/99/$ - see front matter
PII: S0960-894X(99)00372-8

Thus, the receptor protein in the outer membrane FepA [5] binds the ferric siderophore specifically and transports it into the periplasm. Transport of the siderophore across the cytoplasm membrane is energized by ATP hydrolysis and catalyzed by FepC proteins which are associated with the cytoplasm membrane. [6] The TonB protein [7] is required to transduce energy from the cytoplasmic membrane to the outer membrane transport proteins. However, two accessory proteins, ExbB and ExbD, are shown to play a vital role in TonB function. [8-10]. TonB interacts with ExbB and ExbD, which are both anchored in the inner membrane. The transport of iron across the inner membrane requires a periplasmic protein FepB, [11] several proteins located in the inner membrane FepG, FepD, and a polar membrane-bound protein containing two regions characteristic of nucleotide-binding proteins (FepC). [6] Once inside the cells, ferric enterobactin is hydrolysed by an esterase (the product of the fes gene) and the Fe(III) is reduced into Fe(II) by NAD(P)H-dependent ferri-reductase. [12] This enzymatic reduction to the ferrous state is one of the probable mechanisms for the release of iron from the siderophore to the agents which are directly involved in cell metabolisms. Electrochemical studies have been reported and have indicated that iron removal occurs via reduction and protonation mechanism, and this could occur in the periplasmic space of *Escherichia coli* where the environment is known to be acidic. [13-15] Therefore, comparison of siderophore complex redox potentials with those of physiological reductants can be very useful for the clarification of the iron release mechanism.

In connection with our interest for the design of siderophore models which could mimic the enterobactin iron release process and encouraged by the low potential reduction value obtained in our recent electrochemical study on tris-catecholate containing divalent sulphur, [16] it seems relevant for us to test other models of catecholamide. In this paper, we describe the synthesis of a new tetra-catecholamide siderophore analogue *N*5,*N*6-thiodipropanoyl-bis[*N*1,*N*10-bis(2,3-dihydroxybenzoyl -spermidine)] H_8L (**8**). This compound is characterised by its small size, its four catechol functions and its construction using two spermidine molecules. But the salient feature of this new ferric ion sequestering agent is the incorporation of the sulphur function into its backbone. This is expected to increase the reduction potential of the siderophore, [16] to enhance its reductive properties, and consequently to facilitate the iron release.

The synthesis of this ligand involved five steps. The methyl ether protected catechol (**1**) was treated by spermidine (**2**) in the presence of *N, N'*-carbonyldiimdazole (CDI) to give the diacylated spermidine (**4**) in quantitative yield. Next, the carboxylic acids were converted into the corresponding *N*-hydroxysuccinimide ester (**6**), in high yield, by treating the acids with *N*-hydroxysuccinimide (**5**) and DCC. These active esters (**6**) were not separated and coupled directly to the diacylated spermidine (**4**) in THF using DMAP as a catalyst, to give an octamethoxy compound (**7**). After demethylation with boron tribromide, the octahydroxy compound (**8**) was obtained in good yield. The crystalline compound (**8**) was then treated with $Fe(acac)_3$ in methanol to give ferric complex (**9**) as a new siderophore (**Scheme 1**).

The molecular structures of these compounds were characterised by ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy. [17]



Scheme: 1 i, CDI/CH_2Cl_2; ii, DCC, *N*-hydroxy succinimide; iii, THF/DMAP, iv, BBr_3/CH_2Cl_2, v, $(acac)_3$, KOH, CH_3OH.

Cyclic voltammogram were run in CH_3OH/H_2O (60/40) containing 0.1 M NaCl as the supporting electrolyte. The cyclic voltammogram of (**9**) in (Figure 1a) obtained in this

solution at pH=7.92 exhibits a chemically quasi reversible wave indicating the redox potential of Fe(III)/Fe(II) with E^0 = -430 mV *vs* SCE. The difference ΔE_p between the anodic and the cathodic potential peaks is slightly larger than 60 mV. The value of the reduction potential of this compound (**9**) is near the range of physiological reducing agents NAD(P)H and much higher than those reported at pH>10 for the natural siderophore enterobactin [18] and the synthetic siderophore TREMCAM, [19] which show one-electron oxidation-reduction wave with E_{PC} values of -1.230 V and -1.1 V, respectively, vs (SCE). However at pH=9.12, the cyclic voltammogram of ferric siderophore (**9**) exhibits a quasi reversible wave with E^0 =-440 mV vs SCE (Figure 1b).

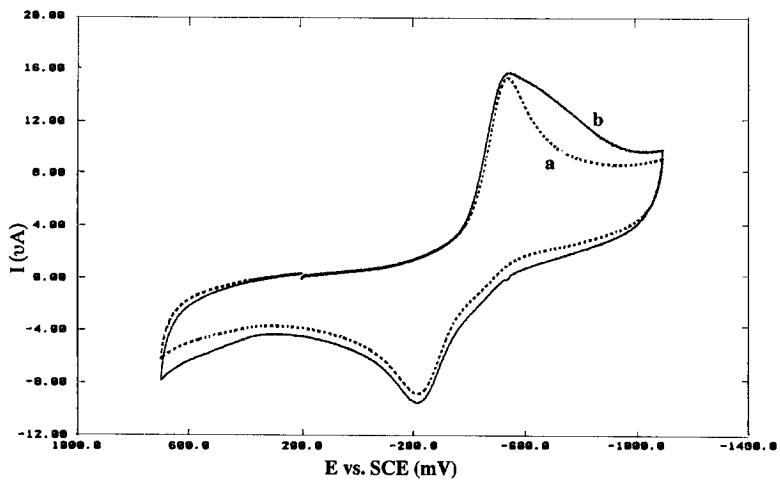


Figure 1: Cyclic voltammograms for (**9**) 2.7 10^{-4} M, NaCl 0.1 M in CH_3OH/H_2O (60/40). Working electrode was freshly polished-glassy carbon electrode, reference electrode was SCE, scan rate 200mV/s. (**a**) at pH= 7.98, (**b**) at pH= 9.12.

With this example, once again it seems that incorporation of divalent sulphur into the backbone of catecholate leads to an increase in the potential reduction of the siderophore making it approach the range of physiological reducing agents.

Materials and Methods: All reagents were of the finest quality available commercially. All solvents were distilled prior to use. TLC was conducted using Merck Silica Gel 60 F_{254} precoated plastic sheets. Column chromatography was performed using Merck Silica Gel (70-230 mesh). ^{1}H-NMR spectra were run on Bruker AM 250 and AC 200 instruments at 250 and 200 MHz, ^{13}C NMR were run on Bruker AC 200 instruments at 50 MHz. Chemical shifts are reported in parts permillion (δ) down field from internal Me_4Si. The abbreviations

used are: s= singlet, d=doublet, t= triplet, q= quadruplet. m= multiplet. J= coupling constant (Hz). IC-MS spectra determined on Nermag spectrometer using direct insertion probe a source pressure 10^{-1} torr and ammonia as the reactant gas. Thus (M^++1) and (M^++ 18) values are reported. Mass spectra were recorded at 70 eV on Nermag spectrometer, ES were recorded on Finigan spectrometer MAT 95. Electrochemical experiments were done in DMF and DMSO under anaerobic conditions. The solutions were degassed thoroughly for at least 30 min with pure argon and the gas was kept at a positive pressure during the experiments. The working electrode was 3 mm diameter vitreous carbon disk (UC, Tukai Japan) for cyclic voltammetry or a large surface area glassy carbon plate (V25, le carbon Loraine) for controlled potential electrolysis and coulometry. The reference electrode (SCE) was kept in an appropriate supporting electrolyte compartment and was separated from the working electrode compartment by a fine porosity glass frit. The counter electrode was a platinum wire with a large surface area. All electrode potentials were measured vs. (SCE). The electrochemical set-up was an EG $ 273 A driven by a PC with the 270 software.

Acknowledgements: We gratefully thank F. Perez for recording mass spectra.

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17 Dimethoxybenzoyl spermidine (**4**):δ_H (CD_3OD): 1.6 (t, 4H), 1.80 (q, 2H); 2.70 (m, 4H); 3.40-3.50 (s, 12H); 6.95 (dd, 2H); 7.00-7.10 (dd, 2H,), 7.60 (td, 2H), 8.00-8.20 (2t, 2H). IC-MS (NH_4^+): *m/z* 474 (M+1), 100).

N5,N5-thiodipropanoyl-bis [*N1,N10*-bis(3,4-dimethoxybenzoyl) spermidine]: (**7**): δ_H ($CDCl_3$): 1.4-1.8 (m, 12H, 6 x CH_2); 2.5 (m, 4H, -CH_2-CO-N tertiary amide); 2.7 (t, 4H, CH_2-S); 3.1 (m, 16 H, 4 x CH_2-NH secondary amide and 4 x CH_2-N tertiary amide); 3.8 (s, 24H, 8 OCH_3); 6.9-7.1 (m, 8H); 6.6 (dd, 4H, 4 x CH-Arom.); 7.1 (m, 4H, 4 x CH-Arom.). -7.15 (d, 4H, 4 x CH-Arom.). δ_C ($CDCl_3$) 25.72 (s, CH_2), 28.05 (s, CH_2), 33.17 (s, CH_2-C=O), 34.45 (s, CH_2-S,) 38.71 and 38.95 (s, 2 x CH_2-NCO, tertiary amide), 41.11 (s, CH_2-NHCO), 47.72 (s, CH_2-NHCO), 115.75 and 116.04 (s, 4 x CH-Arom. and 4 x CH-Arom.), 123.97 and 124.44 (s, 4 x CH-Arom.), 124.44 and 125.02 (s, 4 x C-Arom.), 146.12 (s, 4 x C-Arom.), 150.67 (s, 4 x C-Arom.), 170.81 and 171.04 (s, CON tertiary amide), 173.20 and 173.48 (s, -CONH secondary amide), IC-MS (NH_4^+): *m/z* 1089 ($(M+1)^+$, 100).

*N5,N5*thiodipropanoyl-bis[*N1,N10*bis(2,3-dihydroxybenzoyl-spermidine)] or (H_8L) (**8**): δ_H (CD_3OD): 1.1-1.5 (m, 12H, 6 x CH_2); 2.3-2.45 (m, 4H, CH_2-CO-N tertiary amide and CH_2-S); 2.95 (m, 16 H, 4 x CH_2-NH secondary amide and 4 x CH_2-N tertiary amide); 6.25 (t, J=8 Hz, 4H, 4 x CH-Arom.); 6.5 (dd, J=8 Hz and J= 1.4Hz, 4H, 4 x CH-Arom.); 6.8 (dd, J=8 Hz and J= 1.4Hz, 4H, 4 x CH-Arom.), IC-MS (NH_4^+): *m/z* = 977 ($(M + 1)^+$, 100).

K_5FeL (**9**): K_5(FeL) ESI-MS (positive): m/z 1163 (M^+-2K+ 23).

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