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Synthesis, siderophore activity and iron(III) chelation chemistry of a novel mono-hydroxamate, bis-catecholate siderophore mimic: N^{α},-N^{ε}-Bis[2,3-dihydroxybenzoyl]-L-lysyl-(γ-*N*-methyl-*N*-hydroxyamido)-L-glutamic acid

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Abstract

The synthesis and characterization of a novel tripodal mono-hydroxamate, bis catecholate siderophore mimic, N^{α},-N^{ε}-bis[2,3-dihydroxybenzoyl]-L-lysyl-(γ-*N*-methyl-*N*-hydroxyamido)-L-glutamic acid (H_6L), is described. The structure of H_6L was established by 2D NMR and mass spectrometry. The chelation chemistry of H_6L with respect to iron(III) is characterized in aqueous solution through determination of ligand pK_a values and iron(III) binding constants using spectrophotometric and potentiometric titration techniques. Proton dependent iron(III)–ligand equilibrium constants were determined using a model based on the sequential protonation of the iron(III)–siderophore complex. These results were used to calculate the pH dependent speciation, the overall formation constant $\log\beta_{110}$ (31.4) and pM value (18.3) for H_6L with iron(III). The ability of H_6L to deliver the essential nutrient iron to living cells is determined through growth promotion assays using various bacterial strains.

Keywords: Iron; Siderophore; Bioavailability; Stability constant; Growth promotion

1. Introduction

Controlled iron homeostasis is essential to the survival of virtually all living organisms in that a delicate balance exists between detrimental iron deficiency and toxic iron overload [1,2]. Acute iron overload, which typically occurs in infants upon ingestion of iron supplements, is one of the most common types of accidental poisoning. Iron overload is also a chronic side-effect of transfusion therapy which is administered to treat blood disorders such as sickle cell, thalassemia and myelodysplastic syndromes. To reduce the iron burden incumbent with this type of treatment, transfusion therapy is generally accompanied by the administration of iron chelating agents with limited activity within the body which enable iron to be excreted [3–5]. An ideal therapeutic chelator should be bioavailable, have a low toxicity, be selective for iron and form an inert and thermodynamically stable complex upon binding iron [6,7].

Historically, the approach to searching for new agents for use in chelation therapy has started with the examination of naturally occurring iron chelators, such as the siderophores produced by microorganisms. These low molecular weight ligands have naturally evolved with a high affinity and specificity for ferric ion in order to scavenge iron from the microbe's host environment [2,8–12]. The first (and until recently only) FDA approved iron chelator was the naturally occurring siderophore, desferrioxamine B (Desferal®, DFB). Due to the poor bioavailability

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and short plasma half-life of DFB, chelation therapy with this chelator has required a rigorous regime of subcutaneous infusions which must be administered eight to 12 h per night, three to seven nights a week [13]. This demanding treatment regimen has resulted in a reduced quality of life and thus low patient compliance with treatment.

Recently, the FDA has approved a synthetic tridentate chelator called Exjade® (deferasirox) which shows promise as a once a day orally administered treatment with minimal side effects and an efficacy similar to that of Desferal® [14]. However, despite the dramatic gain in life expectancy that has been achieved through the use of these chelation therapies, cardiac disease caused by myocardial iron deposition is still the leading cause of death in transfusion dependent anemia patients [15]. Thus, we continue to explore other naturally occurring and synthetic siderophores as potential ligands for iron chelation therapy.

Another therapeutic use for naturally occurring and synthetic siderophore mimics is as antibiotics. Siderophores and siderophore analogues can act as antibiotic agents through several different mechanisms. First, a biologically inactive chelator (not taken up by the cell) may deprive pathogenic microbes of essential iron. This iron starvation can be achieved through a decrease in local iron concentration though competitive chelation. Alternatively, iron uptake via ferri-siderophore receptors can be inhibited by binding a nonfunctional siderophore analogue to a ferric siderophore receptor site or by introducing a siderophore bound to a metal with little or no metabolic function [16].

A second antibiotic role for siderophores involves the formation of a siderophore-drug conjugate in which a potent drug is covalently bound to a siderophore. This "Trojan-horse" approach is designed to exploit the natural active transport mechanism for ferric-siderophores [16,17]. Many of these siderophore–drug conjugates can be recognized and taken up into the cell despite the addition of the bulky drug conjugate. In addition, this mechanism of drug delivery may reduce side effects since the active drug may be delivered to the target site without creating high and potentially dangerous extracellular drug concentrations.

Several naturally occurring siderophores exhibit antibiotic activity, including albomycins, ferrimycin and salmycin [18–20]. Unfortunately, these compounds have not been successful as therapeutic agents because they are not readily available, they have a poorly understood mechanism of action, and they quickly lose their efficacy as resistance evolves against these siderophore-drugs [21]. Significant efforts have been made towards creating a synthetic siderophore–antibiotic conjugate as a therapeutic agent against infection; however microbes have quickly developed resistance against these analogues by terminating the expression of specific receptors which permit them access to the bacterial cell [16].

Heterotripodal, or mixed binding group, synthetic siderophore analogues, show promise as potential antibiotic vectors as they may have the ability to access the bacterial cell through multiple receptors. Here we examine the synthesis, characterization and biological activity of a synthetic siderophore with a unique combination of iron binding groups.

Naturally occurring siderophores utilize primarily either hydroxamate (e.g. desferrichrome, desferrioxamine B and E, aerobactin, rhodotoulic acid, alcaligin) or catecholate (e.g. enterobactin) moieties for iron binding [2,8,10]. The pyoverdins are somewhat unique among naturally occurring siderophores in that they possess a combination of binding groups including a catecholate and a hydroxamate at the chain terminus, and either an additional hydroxamate or a hydroxycarboxylic acid in the middle of the chain [22,23]. The synthesis and characterization of several heterotripodal tris-bidentate siderophore mimics containing (i) two catecholates and one 8-hydroxyquionoline, (ii) two 8-hydroxyquinolines and one catechol, (iii) two hydroxypyridinones and one catecholate, and (iv) two 8-hydroxypyridinones and one 2-hydroxyisophtalamide, have been reported [24–26]. Here we present the synthesis, iron coordination properties, and biological activity of a novel heterotripodal, mono-hydroxamate, bis-catecholate siderophore mimic, N^{α},-N^{ε}-bis[2,3-dihydroxybenzoyl]-L-lysyl-(γ-N-methyl-N-hydroxyamido)-L-glutamic acid (H_6L, Fig. 1). This bis-catecholate, mono-hydroxamate chelator is expected to bind iron through four phenolate and two hydroxamate oxygen atoms thus creating a unique hexadentate iron complex with potentially interesting iron binding characteristics.

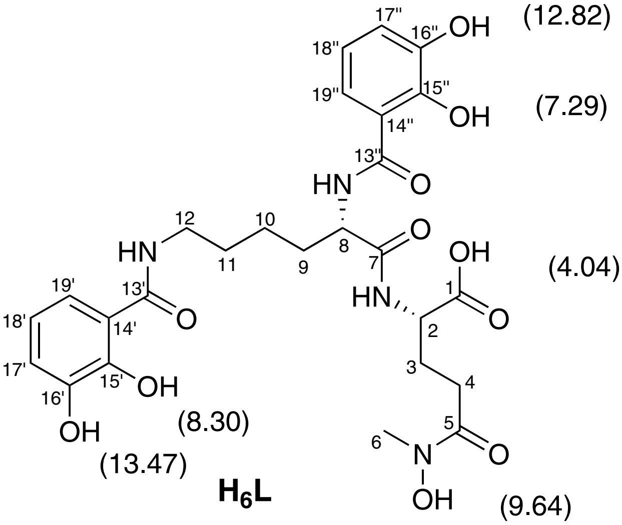

Fig. 1. Structure of the heterotripodal (bis-catecholate, mono-hydroxamate) synthetic siderophore analogue, N^{α},-N^{ε}-bis[2,3-dihydroxybenzoyl]-L-lysyl-(γ-N-methyl-N-hydroxyamido)-L-glutamic acid, H_6L. Numbers in parentheses correspond to pK_a values for the adjacent moiety (see Table 1; values assigned to –OH moieties attached to 16′/16″ C atoms and 15′/15″ C atoms are arbitrary and may be interchanged between (′) and (″) designations).