CHAPTER #

Siderocalins

Roland K. Strong*

Abstract

Siderocalin (Lipocalin 2), first identified as a neutrophil granule component, is also found in uterine secretions, in serum and synovium during bacterial infection and secreted from epithelial cells in response to inflammation or tumorigenesis. Siderocalin is a potent bacteriostatic agent in vitro and, when knocked-out in mice, confers a remarkable susceptibility to bacterial infection in the absence of any other phenotype. However, Siderocalin lacked any precise function until specific, high-affinity ligands were identified: bacterial ferric siderophores. Siderophores, small-molecule iron (III) chelators, are synthesized, secreted and reabsorbed by microorganisms in a competition to obtain iron, a scarce resource in the environment, and have been linked to virulence, though through previously undefined mechanisms. Siderocalin employs degenerate molecular recognition machinery to bind to two distinct families of siderophores: the catecholate siderophores of enteric bacteria and the mycobacterial carboxymycobactins. Siderocalin therefore functions as an anti-bacterial component of innate immune responses by sequestering iron away from invading pathogens; pathogens use siderophores that escape Siderocalin capture to help establish virulence. However, the limited pattern of Siderocalin siderophore specificity, the use of alternate or modified siderophores by bacteria and the possible existence of other siderophore-binding lipocalins ('siderocalins') clearly demonstrates that the battle for virulence is ongoing. Siderocalin may also have pleiotropic activities, having been implicated in diverse cellular processes such as apoptosis and differentiation.

Siderocalin

Lipocalin 2 (Lcn2), first identified as a lipocalin in neutrophil granules,[1-3] has been referred to by various names by various groups, reflecting the many contexts in which it has been found, including: neutrophil gelatinase-associated lipocalin (NGAL), human neutrophil lipocalin (HNL), 24p3, superinducible protein 24 kD (SIP24), uterocalin, *neu*-related lipocalin (NRL), α_2-microglobulin-related protein and, most recently, Siderocalin .[4] The last appellation ties the protein to its established ligands and a specific function, rather than some subset of its expression pattern or the chromosomal location of its gene, and is thus our preferred label. Human Siderocalin (NGAL, HNL), while also expressed by epithelial cells in response to inflammatory signals, is released from neutrophil granules as a 25 kiloDalton (kD) monomer, a 46 kD disulfide-linked homodimer and a disulfide-linked heterodimer with gelatinase-B (matrix metalloproteinase 9 (MMP-9)).[5,6] There is a single N-linked oligosaccharide site on Siderocalin and a single, conserved internal disulfide bond shared by many lipocalins. The rat ortholog (NRL) was originally identified as a protein highly overexpressed in *neu*-induced mammary

*Roland K. Strong—Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Mail Stop A3-025, 1100 Fairview Ave. North, Seattle, Washington 98109, U.S.A. Email: rstrong@fhcrc.org

Lipocalins, edited by Bo Åkerström, Niels Borregaard, Darren R. Flower and Jean-Philippe Salier.

Table 1. Sequence identities between Siderocalin and its nearest neighbor lipocalins

	Human Siderocalin	Rat Siderocalin	Murine Siderocalin	Human Lcn12	Human PDS	Murine Lcn12	Human HC	Chicken Ex-FABP	Quail Q83
Human Siderocalin	100	**62**	**60**	35	34	28	23	20	20
Rat Siderocalin		100	**81**	34	32	29	<20	20	20
Murine Siderocalin			100	31	31	28	<20	20	22
Human Lcn12				100	29	**56**	<20	28	<20
Human PDS					100	28	23	21	21
Murine Lcn12						100	<20	<20	<20
Human HC							100	<20	<20
Chicken Ex-FABP								100	**86**
Quail Q83									100

Identities between likely orthologs are in **bold**; pair-wise identities between human C8γ, human Lcn1 and all the remaining lipocalins above are all less than 20%. Only the closet PDS sequence is shown as a representative of the family. Candidate siderocalins are underlined.

cancers;[7] the murine ortholog (24p3, Sip24, uterocalin) was identified as a protein induced in response to various proliferative signals[8] and is highly expressed in uterine luminal fluids and by epithelial cells.[9] Human-mouse-rat Siderocalin pair-wise sequence identities are 60% or greater (Table 1) with marked conservation of calyx residues;[4] no other obvious orthologs have been identified so far. An equine lipocalin, also named uterocalin (on the basis of its presence in uterine secretions) or P19,[10] shows only weak sequence homology to Siderocalin (16% to 18% pair-wise identity to the three orthologs).

Siderocalin has been implicated in processes as diverse as apoptosis[11,12] and kidney cell differentiation[13,14] and, like most lipocalins, is thought to modulate these cellular processes by binding to ligand(s) and interacting with specific cell-surface receptors. Evidence for mammalian cell-surface Siderocalin receptors has been reported in two different systems,[11,13] though they remain uncharacterized, but the interaction with a third receptor, megalin (an extracellular matrix component), has been more fully characterized.[15] Megalin, a member of the low density lipoprotein family,[16] also interacts with lactoferrin[17,18] and other lipocalins: retinol binding protein, α_1-microglobulin/HC, mouse major urinary protein and odorant binding protein.[19,20] Megalin binds Siderocalin in a ligand-independent manner and can mediate its cellular uptake,[15] though the physiological role of this interaction remains speculative.

Lipocalins usually function through the ligands they bind and it is the shape of the lipocalin calyx, and the chemical character of the residues lining the calyx, that are the primary determinants of ligand specificity. [Equine uterocalin, for instance, displays a predicted calyx that is quite distinct in character from Siderocalin; therefore, it is unlikely that equine uterocalin has an analogous ligand specificity or function.] However, some of these putative lipocalin functional assignments have been made on very indirect or circumstantial evidence, without determining the actual molecular mechanisms or ligands involved. For instance, the name 'lipocalin', referring to the lipophilic character of the stereotypical calyx, is reflected in the measurable affinity many family members have for simple fatty acids or retinoids, though many studies have failed to show that such interactions are specific and not due to trivial, non-specific, hydrophobic interactions. The term 'lipocalin' itself can be misleading, as a number of family members have now been shown to have distinctly polar calyces and/or bind preferentially to non-lipid or -lipophilic ligands.

Siderocalin was initially proposed to have immunomodulatory activity by binding and clearing lipophilic inflammatory mediators,[21] such as the neutrophil chemoattractant tripeptide N-formyl-Met-Leu-Phe.[22,23] However, the initial three-dimensional structures of human Siderocalin, determined by x-ray crystallography[24] and NMR,[25] revealed that the calyx is shallower (only about 15Å deep) and broader (approximately 20Å wide at the brim) than is typical of most lipocalins and is also uncharacteristically lined with polar and positively-charged residues. The Siderocalin calyx is highly sculpted, with three prominent pockets (#1, #2 & #3) outlined by the position of the side-chains of three positively-charged calyx residues (Arg81, Lys125 and Lys134).[24] The incompatible nature of the Siderocalin calyx, together with binding data showing millimolar dissociation constants,[24,26] led to the conclusion that Siderocalin does not specifically bind N-formylated tripeptides or other proposed hydrophobic ligands,[24] leaving its physiological function in question.

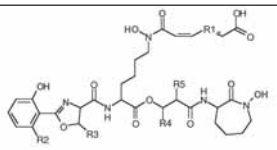
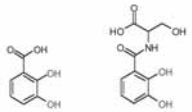
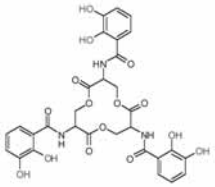
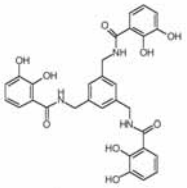
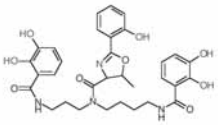
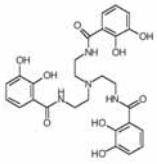
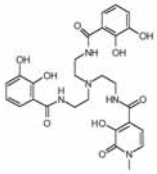
The initial crystallographic analysis of Siderocalin, using baculovirus-expressed protein, did show the presence of what was likely a fatty acid in the calyx that could be, surprisingly, displaced by sulfate.[24] This weakly-bound bound fatty acid, modeled as n-capric acid (NCA), likely represented a serendipitous ligand co-purified with Siderocalin, as has been seen in the structures of other lipocalins.[27] Distinct from the typical fatty acid-binding lipocalins, the carboxyl moiety of the fatty acid bound in the deepest pocket (#2) in the Siderocalin calyx, with the aliphatic tail trailing outwards. However, paralleling the difficulty in identifying 'true' lipocalin ligands, the low affinity, small size relative to the calyx volume and poor overall shape/chemical compatibility of the fatty acid strongly argued that this compound was also not a physiologically-relevant ligand.

Siderocalin Ligands

Building on the unexpected observation that Siderocalin co-purifies with a dark red chromophore when expressed in bacteria, our laboratory identified bacterial ferric siderophores as candidate Siderocalin ligands. A 'siderophore' is defined as a low molecular weight, virtually ferric-specific chelator involved in iron acquisition. Microorganisms secrete siderophores in a competition to scavenge scarce environmental iron in a receptor-dependent manner.[28-31] Siderophores can be divided into three broad classes depending on the chemistry of chelation: hydroxamates, phenolates/catecholates or α-hydroxycarboxylates—though other liganding chemistries can be used (Tables 2-5). Siderophore affinities for iron (III) can exceed 10^{50} M^{-1}.

Subsequent biophysical studies show that Siderocalin tightly binds (1) the catecholate-type ferric siderophore of enterobacteria, enterochelin (Ent; also known as enterobactin; K_D = 0.4 nM at 22˚C),[4] (2) a panel of related catecholate-type siderophores[32] and (3) the soluble, mixed catecholate/hydroxamate carboxymycobactin siderophores of *Mycobacterium smegmatis* and *M. tuberculosis* (CMB-S and CMB-T; Table 2).[32] Complex structures[4] show that Siderocalin binds ferric Ent (FeEnt) and related siderophores by intercalating the side-chains of the three positively-charged calyx residues (Arg81, Lys125 and Lys134; Fig. 1) between the three

Table 2. Siderophores demonstrated to bind to Siderocalin[32,44]

Compound (references)	Organism(s)	Structure	Comments (K_a for Fe(III))
Carboxymycobactins	mycobacteria		soluble form of the lipophilic mycobactins (shorter acyl chain plus a carboxylate) R1 = $(-CH_2-)_{1-9}$ R4 = $-CH_3$ or -H
2,3-Dihydroxybenzoate (DHBA) and Serine-DHB (82)	*Brucella abortus* (DHBA (83))		biosynthetic intermediates or breakdown products of Ent; three molecules together bind one iron
Enterochelin (Ent; Enterobactin) (84, 85)	enterobacteria		tris-catecholate; three DHBA groups coupled through amide linkages to a tri-serine, trilactone backbone; primary siderophore of many enterobacteria (10^{52})
MECAM (86)	synthetic Ent analog		less-hydrolyzable Ent analog, differing only in the backbone
Parabactin (87)	*Paracoccus*		similar to Ent; oxazoline group replaces one phenolate; different backbone (~10^{50})
TRENCAM (86)	synthetic Ent analog		less-hydrolyzable Ent analog, differing only in the backbone
TREN-3,2-HOPO (86)	synthetic cepabactin analog		Related to cepabactin, with one hydroxypyridinone (HOPO) ring; the complex with iron is therefore charged –2

catecholate rings of FeEnt, generating a novel hybrid of ionic (Ent is uncharged, but FeEnt carries a net -3 charge[33] delocalized over the molecule[4]) and cation-π interactions, where the interacting groups are interlaced, cation-catecholate-cation-catecholate, in a cyclically-permuted manner around the iron atom.[4]

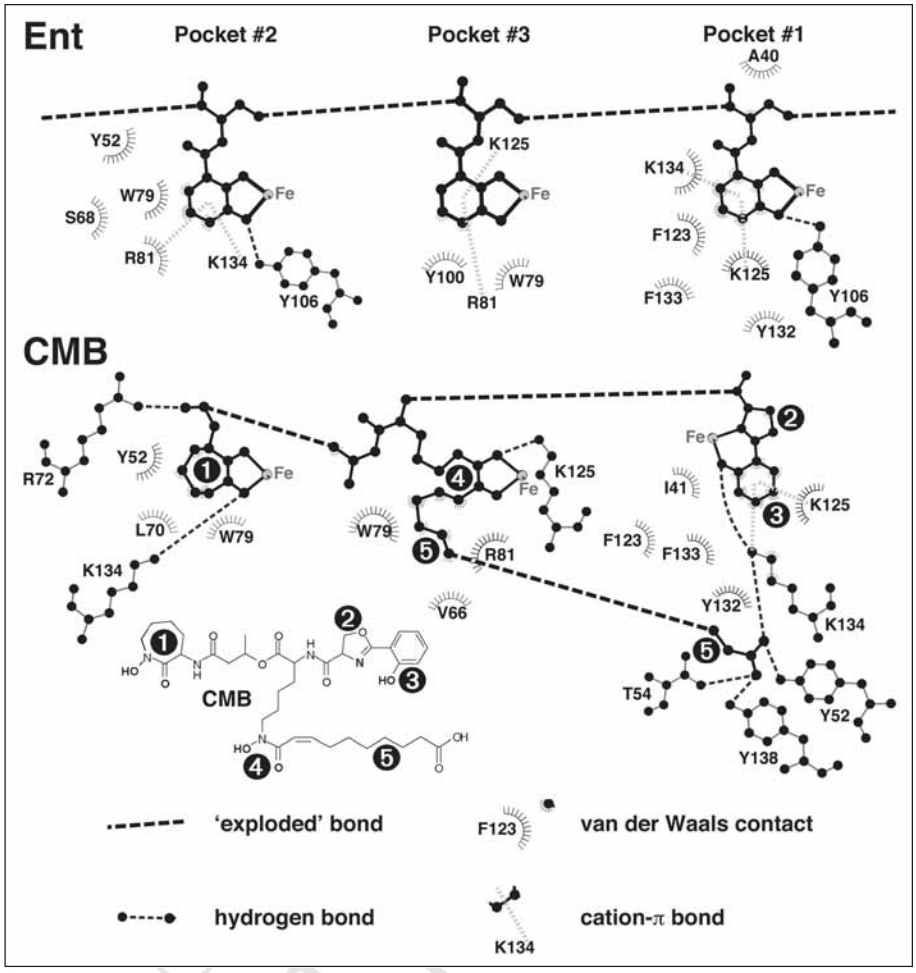
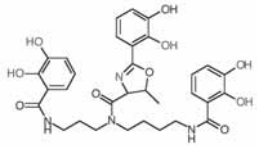
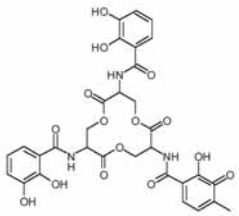
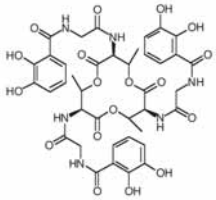
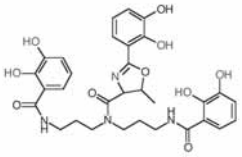
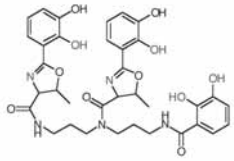
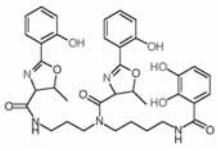


Figure 1. 'Exploded' LIGPLOT[113] representations of the interactions between Siderocalin and Ent (*top*) or CMB-S (*bottom*), with bond types shown as indicated. Representations have been exploded to isolate the protein–ligand interactions in each pocket: #1, on the right; #2, on the left; and #3, in the middle. The chemical structure of the predominant CMB variant observed in the CMB-S crystal structures is also shown; CMB substituent groups (1, the cyclic hydroxamate; 2, the oxazoline; 3, the phenolate; 4, the linear hydroxamate; 5, the fatty acid tail) are numbered on the chemical structure and the corresponding parts of the LIGPLOT diagram for clarity.

Cation-π bonds in proteins are interactions between the positive charge of lysine or arginine side-chains and the quadrupole moment associated with the delocalized π-electrons of an aromatic functional group such as tryptophan, tyrosine or phenylalanine.[34] These interactions are seen in the binding of phosphotyrosine to an SH2 domain (for example, see ref. 35, where an arginine and a lysine interact with a phosphotyrosine-containing peptide). However, in SH2 complexes the actual aromatic ring remains uncharged. The nature of Siderocalin/siderophore interactions, where the siderophore is centered in the Siderocalin calyx making multiple, direct, polar interactions, clearly demonstrates specificity and cannot be the result of serendipity. Unusually, though, the FeEnt ligand makes few other stabilizing interactions with the protein, allowing it to wiggle around in the calyx, and fails to fill all of the calyx sub-pockets

AUTHOR: PLEASE REFER TO TABLE 3 AND FIGURE 2 IN THE BODY TEXT.

Table 3. Siderophores predicted to bind to Siderocalin, based on similarities to known Siderocalin ligands and characterized elements of the recognition mechanism

Compound (references)	Organism(s)	Structure	Comments (K_a for Fe(III))
Agrobactin (88)	*Agrobacterium tumefaciens*		similar to parabactin (~10^{50})
Brucebactin (89)	*Brucella abortus*	(uncharacterized, but thought similar to enterochelin)	phenolate/catecholate-type
Cepabactin (90)	*Burkholderia cepacia*		similar to Ent; an uncharged hydroxypyridinonate (HOPO) group replaces one phenolate, reducing overall charge of iron complex to –2
Corynebactin (91)	*Corynebacterium diphtheriae*		similar to Ent, with a tri-threonine, trilactone backbone; opposite chirality to Ent (91)
Fluvibactin (92)	*Vibrio fluvialis*		similar to parabactin
Vibriobactin (93)	*Vibrio cholerae*		similar to parabactin; *vs.* Ent: oxazoline groups replace two phenolate groups; different backbone
Vulnibactin (94)	*Vibrio*		similar to vibriobactin, Ent

completely.[4,32] This behavior, suggesting that the calyx is optimized for some other ligand/s, lead to the search for other potential Siderocalin siderophore ligands, culminating in the discovery that CMBs also bind while yet many other siderophores do not.[32]

Figure 2. Unmodified CLUSTALW[114] alignments of the sequences of Siderocalin with other potential siderocalins. Dots indicate positively-charged residues with side-chains extending into the calyx, based on either direct structure determinations, homology or homology modeling; dashes indicate gaps introduced to maximize homology.

Though considerably different in structure (Table 2), CMBs bind in the same position in the Siderocalin calyx, though filling the calyx sub-pockets and crevices more completely and making more extensive interactions with the protein (Fig. 1). The CMB 2-hydroxyphenyloxazoline group is positioned in pocket #1, the cyclic hydroxamate group is positioned in the upper part of pocket #2 and the linear hydroxamate occupies pocket #3. The fatty acid tail curls under the

rest of the siderophore, crossing from pocket #3 into pocket #1, positioning the carboxylate group into the bottom of pocket #2. Interestingly, the CMB carboxylate essentially superimposes on the carboxylate group of the NCA ligand in the original crystal structure of baculovirus-expressed Siderocalin[24] and likely explains the retention of a fatty acid through purification. Lysines 125 and 134 participate in cation-π bonds to the CMB hydroxybenzoyl moiety completely analogous to those in Ent, with the hydroxybenzoyl of CMB superimposing almost identically onto the FeEnt catecholate in pocket #1. Therefore, the common element that is recognized by Siderocalin in both complexes is the highly-polarized phenyl ring of one iron chelating group sitting in what is thus revealed as likely the key binding pocket, #1, between Lys125 and Lys134.

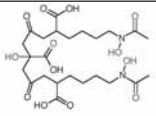
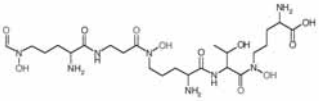
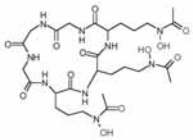
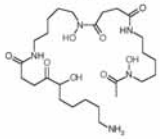
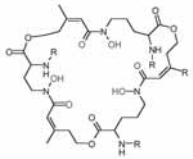
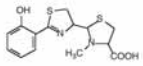
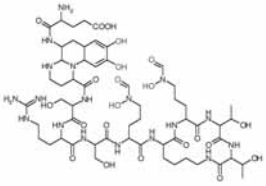
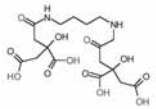
Siderocalin, Siderophores, Iron and Disease

What is the relevance of binding bacterial siderophores in a mammalian context? Iron is required by virtually all living things.[36] Within the body, the majority of iron is bound up in hemoglobin, though several proteins bind iron directly. Transferrin transports iron between cells and is normally 30 to 40% iron-saturated in the serum.[37] Iron is stored intracellularly in complex with ferritins.[38] Lactoferrin is a potent bacteriostatic agent, first discovered in milk, that is also released from neutrophil granules at sites of inflammation, directly inhibiting the growth of infecting pathogens by sequestering iron.[39,40] It has long been thought that the body generally lowers available iron in response to both infection and cancer in order to slow or stop the growth of pathogens and tumors.[40,41] The observation that giving iron supplements to patients with bacterial infections worsens their condition[40,41] demonstrates the scarcity of free iron in the body, with a serum concentration estimated to be as low as 10^{-24}M,[42] and the efficiency of iron sequestration as an antibiotic.[40] Pathogenic genera whose growth is stimulated by iron supplementation during infection in vertebrate hosts include: *Candida*, *Cryptococcus*, *Pneumocystis* and *Rhizopus* (Fungi); *Entamoeba, Leishmania, Naegleria, Plasmodium, Toxoplasma* and *Trypanosoma* (Protozoa); *Bacillus, Clostridium, Corynebacterium, Listeria, Mycobacterium, Staphylococcus* and *Streptococcus* (Gram-positive bacteria); and *Acinetobacter, Aeromonas, Alcaligenes, Campylobacter, Chlamydia, Ehrlichia, Enterobacter, Escherichia, Klebsiella, Legionella, Neisseria, Pseudomonas, Salmonella, Shigella* and *Yersinia* (Gram-negative bacteria).[41]

This ligand specificity therefore suggests a role for Siderocalin in innate immune responses: a neutrophil granule protein, secreted in response to infection or inflammation, that sequesters iron, as ferric siderophore complexes, away from microbial pathogens, thus limiting their growth and virulence. Siderocalin complements the activity of lactoferrin by binding ferric siderophore complexes rather than iron directly.[4] Siderocalin is an acute phase protein, whose serum concentration can be used clinically to differentiate between bacterial and other types of infections.[43] During inflammation, concentrations of Siderocalin can increase to levels, with concentrations approaching 20 to 30 nM in the serum, adequate to presumably bind all available iron as ferric siderophore complexes.[43] In direct support of this hypothesis, Siderocalin is a potent bacteriostatic agent in vitro against *E. coli* cultured in iron-limiting conditions,[4] functioning specifically through its affinity for FeEnt, and, when the Siderocalin gene is knocked-out, renders mice unable to fend off infections by virulent strains of *E. coli*.[44]

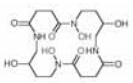
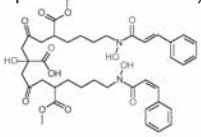
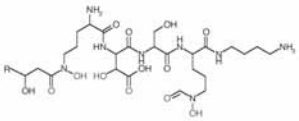
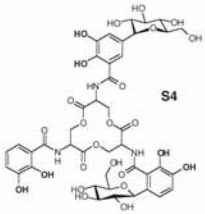
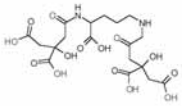
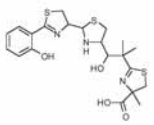
Siderocalin's binding properties may also contribute to the explanation of the association of certain siderophores with virulence. The pathogenesis islands of many bacteria (including species of *Yersinia*, *Shigella*, *Klebsiella*, *Salmonella* and *Neisseria*) encode proteins either associated with siderophore synthesis or uptake.[45-51] Biosynthesis of the siderophores aerobactin[51] (Table 4) or yersiniabactin[45] (Table 5), for instance, contributes to virulence for many bacteria. We have shown in qualitative binding assays that Siderocalin has no appreciable affinity for aerobactin[4] or pyochelin[32] (Table 4)—therefore, because of its similarity to pyochelin, Siderocalin very likely has limited affinity for yersiniabactin as well. These siderophores may thus confer virulence by allowing these bacteria to evade Siderocalin-mediated iron sequestration. This hypothesis poten-

Table 4. Siderophores demonstrated not to bind to Siderocalin[32,44]

Compound (references)	Organism(s)	Structure	Comments (K_a for Fe(III))
Aerobactin (51)	enterobacteria		citrate-based hydroxamate type; virulence factor for pathogenic enteric bacteria (ColV encoded; ~10^{23})
Exochelins (95, 96)	mycobacteria		peptide-based; linear hydroxamate-type
Ferrichromes (97)	*Microsporum, Trichophyton* and *Aspergillus*		hydroxamate-type; cyclic peptides of ornithine derivatives and Gly, Ser or Ala (~10^{30})
Ferrioxamines (98, 99)	*Actinomyces*		linear trihydroxamate-type yeast siderophores (~10^{32})
Fusarinines (Fusigens) (100, 101)	*Fusarium*, *Paecilomyces* and *Aspergillus*		linear or cyclic trihydroxamates
Protocatechuic acid (3,4-dihydroxybenzoic acid)	*Bacillus anthracis*		substituent of anthrachelin
Pyochelin (102)	*Pseudomonas aeruginosa*, *Burkholderia cepacia*		salicylate-based
Pyoverdin (also pyoverdine, pseudobactin) (103)	Pseudomonads		contains a dihydroxyquinoline chromophore, a variable peptide chain of 6 to 12 residues, and a dicarboxylic acid amide 'side-chain' (~10^{32})
Rhizoferrin (104)	Zygomycetes		citric acid-based polycarboxylate

tially explains the conundrum[30,52] of why production of a second, seemingly less efficient siderophore (aerobactin), one with a considerably lower affinity for iron (III) than Ent, would

Table 5. Siderophores predicted not to bind to Siderocalin, based on similarities to demonstrated nonbinding siderophores and characterized elements of Siderocalin recognition mechanism

Compound (references)	Organism(s)	Structure	Comments (K_a for Fe(III))
Alcaligin (105)	*Bordetella pertussis* and *bronchiseptica*		endomacrocyclic dihydroxamate; three molecules together bind two irons (M_2L_3) (~10^{24}/10^{18})
Anthrachelin (106)	*Bacillus anthracis*	(uncharacterized, but based on protocatechuic acid)	phenolate/catecholate-type
Nannochelin (107)	myxobacteria		citrate-based, cinnamoyl; variant of aerobactin; dihydroxamate siderophore
Ornibactins (108)	*Burkholderia cepacia*		linear hydroxamate-hydroxycarboxylate (R = C4, C6, C8)
Salmochelins (53)	*Salmonella enterica; uropathogenic E. coli*		Ent with glucose adducts
Staphyloferrin (109, 110)	*Staphylococcus*		citric acid-containing polycarboxylate; lysine backbone
Yersiniabactin (111, 112)	*Yersinia*		related to pyochelin

contribute to virulence. In support of this supposition, the relative susceptibility of Siderocalin-KO versus wild-type mice to *E. coli* infection is only apparent with bacterial strains unable to synthesize aerobactin; strains that can secrete aerobactin do equally well in culture in the presence or absence of added Siderocalin or in in vivo infections.[44] Infections with *Staphylococcus aureus* also show no difference in wild-type versus knock-out mice in terms of outcome, consistent with the utilization of siderophores (including staphyloferrin, Table 5) that are predicted to evade Siderocalin binding on the basis of structural homology to siderophores (rhizoferrin, Table 4) that demonstrably do not bind.[32] *Salmonella* and uropathogenic strains of *E. coli* are also able to modify Ent by glucosylation[53] yielding the salmochelins (Table 5), siderophores that would also be predicted not to bind Siderocalin because of significant steric clashes in the calyx.

However, Siderocalin-mediated anti-mycobacterial responses may be limited by the high selectivity of Siderocalin for particular CMB isoforms, in terms of fatty acid tail length, shown by the structural analysis of Siderocalin/CMB complexes.[32] While Siderocalin may be able to tolerate binding of CMB variants plus or minus one or perhaps two methylene groups in the fatty acid moieties from the optimum, though likely with significant concurrent reductions in affinity, it seems unlikely that Siderocalin could accommodate the extremes of the reported CMB-T/S spectrum (Table 2), at least while retaining the overall ligand orientation seen in the co-crystal structures. Therefore, CMB variation may reflect mycobacterial responses to Siderocalin-mediated defenses, evidenced by the obvious success of mycobacteria as human pathogens.

Siderocalin-mediated anti-bacterial responses through siderophore sequestration does not, a priori, require interactions with receptors. Therefore, the existence of Siderocalin receptors suggests that the anti-bacterial iron depletion function of Siderocalin may not be its sole physiological role.

Siderocalin and MMP-9

MMP-9 is a member of the matrix metalloproteinase family (multi-domain, zinc endopeptidases) that includes matrilysin (MMP-7), the collagenases (MMP-1, MMP-8 and MMP-13), the stromelysins (MMP 3, 10, 11 and 12), the gelatinases (-A, MMP-2 and -B, MMP-9) and a membrane associated MMP (MMP-14). MMP-9 efficiently cleaves gelatin, elastin and types V and X collagen, components of the extracellular matrix.[54] All members of the family share a homologous catalytic domain, containing the active-site zinc and are synthesized as inactive proenzymes. A cysteine in the propeptide coordinates the zinc, inactivating the proenzyme; the proenzyme can be activated in vitro through the addition of organomercurials and in vivo by plasma kallikrein.[55] MMP activity is regulated through control of the conversion of proenzyme to active enzyme and by the tight, non-covalent binding of 'tissue inhibitors of metalloproteinases' (TIMPs; ref. 56). Studies have shown that overexpression of MMPs (particularly MMP-9) can be correlated with tumor invasiveness and metastasis.[57-62] The only demonstrated consequence of a Siderocalin/MMP-9 association is a slight acceleration of the direct activation of promatrix metalloproteinases through a non-physiological pathway.[63] There is no noncovalent component to Siderocalin/MMP-9 association,[32] suggesting that the interaction may be serendipitous (murine Siderocalin lacks the corresponding cysteine and is not known to associate with MMP-9). It is also hard to imagine a functional link between Siderocalin's affinity for siderophores and the sequence-specific protease activity of MMP-9.

Siderocalin and an Alternate Iron Delivery Pathway in Mammals

Yang and coworkers[13] have shown that murine Siderocalin, in a murine tissue culture system, also acts as an iron delivery protein, acting in concert with transferrin to convert mesenchymal progenitors into tubular epithelium, forming kidney nephrons. The effect is dependent upon iron bound by Siderocalin as a complex with a chromophore—a mammalian 'siderophore'. Murine Siderocalin also apparently recycles through sub-cellular compartments distinct from the intracellular trafficking of transferrin. Passage through low-pH intracellular compartments correlates with release of iron. The murine and rat orthologs are expected to have a similar ligand specificity to human Siderocalin (experimentally confirmed for murine Siderocalin[44]) as the majority of residues lining the calyx and approaching the ferric siderophore ligands are conserved or conservatively substituted between murine, rat and human Siderocalin.[4] Therefore, the expectation is that the mammalian 'siderophore' will turn out to be something similar to one of the high-affinity bacterial siderophore ligands of Siderocalin, probably more CMB-like, as these latter compounds are clearly more calyx-complementary—thus likely better representing the 'ideal' Siderocalin ligand. If mammals do synthesize siderophores that are used to shuttle iron or act as growth factors, Siderocalin may participate in a wide variety of cellular processes by playing a role in regulating their transport, thus potentially explaining

Siderocalin's association with tumorigenesis and apoptosis. However, these effects could alternately be directly mediated by Siderocalin/receptor interactions, either ligand-dependent or -independent.

Other 'Siderocalins'?

While degenerately binding both Ent-like siderophores and CMBs, Siderocalin fails to bind to many bacterial siderophores and essentially all types of fungal siderophores.[32] Therefore, the narrow range of Siderocalin siderophore specificity leaves many holes in this potent innate immune defence, raising the question of whether there are other siderophore-binding proteins or peptides with complementary specificities. Siderocalin is the first non-bacterial siderophore-binding protein characterized and currently the only Ent-binding protein where the protein/ligand interactions have been clearly delineated. Therefore, initial attempts to identify other non-bacterial siderophore-binders have focused on lipocalins related to Siderocalin.[32] Typical of the lipocalin family, sequence identities rapidly plummet as the alignments move from the Siderocalins themselves (Table 1). A number of neighboring lipocalins are readily eliminated as having characterized functions or ligands that preclude siderophore binding: the prostaglandin D_2 synthases (PDS)[64] and human HC (also known as α_1-microglobulin),[65] a lipocalin associated with IgA that binds heme and heme-breakdown products.[66] However, candidate siderophore-binding lipocalins ('siderocalins') that display the hallmark features of the Siderocalin calyx associated with siderophore binding, a triad of positively-charged side-chains, are identifiable.[32]

Murine lipocalin 12 is found in seminal fluid,[67] but little else is known about its function; its human ortholog has only been identified through analysis of the human genome sequence.[68] Simplistic homology modelling of Lcn12 reveals a triad of positively charged side-chains in the murine Lcn12 calyx (two in human Lcn12) arranged analogously to Siderocalin. The next most-related candidate siderocalins are the highly-homologous proteins chicken Ex-FABP[69] and quail Q83.[70] Ex-FABP is expressed during chicken embryo development in hypertrophic cartilage, muscle fibers and granulocytes and is also a component of egg whites. In chondrocyte and myoblast cultures, Ex-FABP expression is induced by inflammatory agents and inhibited by anti-inflammatory agents. Q83 is a protein strongly induced in v-*myc*-transformed avian fibroblasts, though no specific candidate ligands or functions have been proposed. The NMR structure of Q83[70] again shows a triad of positively-charged amino acids in the calyx (conserved in Ex-FABP) very reminiscent, in arrangement and character, of the key siderophore-binding residues of Siderocalin. This arrangement is also echoed in the calyx of an even more distantly-related lipocalin, C8γ,[71] a well-studied member of the complement cascade and the subject of high-resolution crystallographic analyses.[72] However, preliminary binding and crystallographic analyses fail to convincingly demonstrate specific interactions between C8γ and any of the siderophores tested,[32] showing that calyx elements beyond positively-charged residues may be necessary for siderophore binding. Direct siderophore binding studies of the other candidate siderocalins, Lcn12, Ex-FABP and Q83 have yet to be reported.

Yet another lipocalin, Lcn1 (tear lipocalin, von Ebner's Gland protein), a lipocalin even more distantly removed in sequence space, is also functionally a siderocalin, broadly inhibiting the growth of bacteria and fungi through ferric siderophore sequestration, though the nature of the recognition mechanism has yet to be elucidated and where the measured dissociation constants, in the millimolar range, are surprisingly weak.[73] The structure of Lcn1,[74] though containing several disordered sections that limit the conclusions, shows no immediately recognizable structural similarity to Siderocalin in the calyx. Lcn1/siderophore complexes also have yet to be reported. It even possible that there are siderophore-binding peptides; the structure of Hepcidin,[75] an anti-bacterial and -fungal peptide hormone,[76] involved in regulating iron homeostasis through interactions with ferroportin,[77] also displays a triad of positively-charged side-chains (Arg16, Lys18 and Lys24) on its concave surface almost superimposable on the positively-charged, ligand-interacting calyx residues of Siderocalin.

Consideration of all these possibilities requires that proposals to use siderophores and siderophore analogs as therapeutics, either as antibiotics[78,79] or, through the bound iron, as oxygen radical scavengers in various clinical settings, such as the treatment of ischemia associated with congestive heart failure,[80] will need to be tempered by the possibility that endogenous siderophore-binding specificities will limit, defeat or confound such approaches.

References

1. Kjeldsen L, Johnsen AH, Sengelov H et al. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J Biol Chem 1993; 268(14):10425-32.
2. Kjeldsen L, Bainton DF, Sengelov H et al. Identification of neutrophil gelatinase-associated lipocalin as a novel matrix protein of specific granules in human neutrophils. Blood 1994; 83(3):799-807.
3. Kjeldsen L, Cowland JB, Borregaard N. Human neutrophil gelatinase-associated lipocalin and homologous proteins in rat and mouse. Biochim Biophys Acta 2000; 1482(1-2):272-83.
4. Goetz DH, Holmes MA, Borregaard N et al. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Mol Cell 2002; 10(5):1033-43.
5. Triebel S, Bläser J, Reinke H et al. A 25 kDa α_2-microglobulin-related protein is a component of the 125 kDa form of human gelatinase. FEBS Lett 1992; 3:386-388.
6. Kjeldsen L, Johnsen AH, Sengeløv H et al. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J Biol Chem 1993; 268:10425-10432.
7. Stoesz SP, Gould MN. Overexpression of neu-related lipocalin (NRL) in neu-initiated but not ras or chemically initiated rat mammary carcinomas. Oncogene 1995; 11(11):2233-41.
8. Liu Q, Nilsen-Hamilton M. Identification of a new acute phase protein. J Biol Chem 1995; 270(38):22565-70.
9. Ryon J, Bendickson L, Nilsen-Hamilton M. High expression in involuting reproductive tissues of uterocalin/24p3, a lipocalin and acute phase protein. Biochem J 2002; 367(Pt 1):271-7.
10. Suire S, Stewart F, Beauchamp J et al. Uterocalin, a lipocalin provisioning the preattachment equine conceptus: fatty acid and retinol binding properties, and structural characterization. Biochem J 2001; 356(Pt 2):369-76.
11. Devireddy LR, Teodoro JG, Richard FA et al. Induction of apoptosis by a secreted lipocalin that is transcriptionally regulated by IL-3 deprivation. Science 2001; 293(5531):829-34.
12. Kamezaki K, Shimoda K, Numata A et al. The lipocalin 24p3, which is an essential molecule in IL-3 withdrawal-induced apoptosis, is not involved in the G-CSF withdrawal-induced apoptosis. Eur J Haematol 2003; 71(6):412-7.
13. Yang J, Goetz D, Li JY et al. An iron delivery pathway mediated by a lipocalin. Mol Cell 2002; 10(5):1045-56.
14. Yang J, Mori K, Li JY et al. Iron, lipocalin, and kidney epithelia. Am J Physiol Renal Physiol 2003; 285(1):F9-18.
15. Hvidberg V, Jacobsen C, Strong RK et al. The endocytic receptor megalin binds the iron transporting neutrophil-gelatinase-associated lipocalin (NGAL) with high affinity and mediates its cellular uptake. FEBS Lett, in press.
16. Saito A, Pietromonaco S, Loo AK et al. Complete cloning and sequencing of rat gp330/"megalin," a distinctive member of the low density lipoprotein receptor gene family. Proc Natl Acad Sci USA 1994; 91(21):9725-9.
17. Willnow TE, Goldstein JL, Orth K et al. Low density lipoprotein receptor-related protein and gp330 bind similar ligands, including plasminogen activator-inhibitor complexes and lactoferrin, an inhibitor of chylomicron remnant clearance. J Biol Chem 1992; 267(36):26172-80.
18. Meilinger M, Haumer M, Szakmary KA et al. Removal of lactoferrin from plasma is mediated by binding to low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor and transport to endosomes. FEBS Lett 1995; 360(1):70-4.
19. Flower DR. Beyond the superfamily: the lipocalin receptors. Biochim Biophys Acta 2000; 1482(1-2):327-36.
20. Leheste JR, Rolinski B, Vorum H et al. Megalin knockout mice as an animal model of low molecular weight proteinuria. Am J Pathol 1999; 155(4):1361-70.
21. Bundgaard JR, Sengelov H, Borregaard N et al. Molecular cloning and expression of a cDNA encoding NGAL: a lipocalin expressed in human neutrophils. Biochem Biophys Res Commun 1994; 202(3):1468-75.
22. Sengelov H, Boulay F, Kjeldsen L et al. Subcellular localization and translocation of the receptor for N-formylmethionyl-leucyl-phenylalanine in human neutrophils. Biochem J 1994; 299(Pt 2):473-9.

23. Chu ST, Lin HJ, Chen YH. Complex formation between a formyl peptide and 24p3 protein with a blocked N-terminus of pyroglutamate. J Pept Res 1997; 49(6):582-5.
24. Goetz DH, Willie ST, Armen RS et al. Ligand preference inferred from the structure of neutrophil gelatinase associated lipocalin. Biochemistry 2000; 39(8):1935-41.
25. Coles M, Diercks T, Muehlenweg B et al. The solution structure and dynamics of human neutrophil gelatinase-associated lipocalin. J Mol Biol 1999; 289(1):139-57.
26. Bratt T, Ohlson S, Borregaard N. Interactions between neutrophil gelatinase-associated lipocalin and natural lipophilic ligands. Biochim Biophys Acta 1999; 1472(1-2):262-9.
27. Wu SY, Perez MD, Puyol P et al. beta-lactoglobulin binds palmitate within its central cavity. J Biol Chem 1999; 274(1):170-4.
28. Neilands JB. Microbial iron compounds. Ann Rev Biochem 1981; 50:715-31.
29. Neilands JB. Iron absorption and transport in microorganisms. Ann Rev Nutrit 1981; 1:27-46.
30. Ratledge C, Dover LG. Iron metabolism in pathogenic bacteria. Ann Rev Microbiol 2000; 54:881-941.
31. Winkelmann G. Microbial siderophore-mediated transport. Biochem Soc Trans 2002; 30(4):691-6.
32. Holmes MA, Paulsene W, Jide X et al. Siderocalin (Lcn 2) Also binds carboxymycobactins, potentially defending against mycobacterial infections through iron sequestration. Structure (Camb) 2005; 13(1):29-41.
33. Raymond KN, Müller G, Matzanke BF. Complexation of iron by siderophores. A review of their solution and structural chemistry and biological function. In: Boschke FL, editor. Topics in Current Chemistry. Berlin, Heidelberg: Springer-Verlag; 1984:50-102.
34. Dougherty DA. Cation-pi interactions in chemistry and biology: a new view of benzene, Phe, Tyr, and Trp. Science 1996; 271(5246):163-8.
35. Waksman G. Crystal structure of the phosphotyrosine recognition domain SH2 of the Src oncogene product complexed with tyrosine-phosphorylated peptides. Cell Mol Biol 1994; 40(5):611-8.
36. Neilands JB. Siderophores: structure and function of microbial iron transport compounds. J Biol Chem 1995; 270(45):26723-6.
37. Ponka P, Beaumont C, Richardson DR. Function and regulation of transferrin and ferritin. Semin Hematol 1998; 35(1):35-54.
38. Harrison PM, Arosio P. The ferritins: molecular properties, iron storage function and cellular regulation. Biochim Biophys Acta 1996; 1275(3):161-203.
39. Ellison RT. The effects of lactoferrin on gram-negative bacteria. Adv Exp Med Biol 1994; 357:71-90.
40. Jurado RL. Iron, infections, and anemia of inflammation. Clin Infect Dis 1997; 25(4):888-95.
41. Weinberg ED. Iron withholding: a defense against infection and neoplasia. Physiol Rev 1984; 64(1):65-102.
42. Otto BR, Verweij-van Vught AM, MacLaren DM. Transferrins and heme-compounds as iron sources for pathogenic bacteria. Crit Rev Microbiol 1992; 18(3):217-33.
43. Xu S, Venge P. Lipocalins as biochemical markers of disease. Biochim Biophys Acta 2000; 1482(1-2):298-307.
44. Flo TH, Smith KD, Sato S et al. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature 2004; 432(7019):917-21.
45. Carniel E. The Yersinia high-pathogenicity island: an iron-uptake island. Microbes Infect 2001; 3(7):561-9.
46. Carniel E. The Yersinia high-pathogenicity island. Int Microbiol 1999; 2(3):161-7.
47. Klee SR, Nassif X, Kusecek B et al. Molecular and biological analysis of eight genetic islands that distinguish Neisseria meningitidis from the closely related pathogen Neisseria gonorrhoeae. Infect Immun 2000; 68(4):2082-95.
48. Moss JE, Cardozo TJ, Zychlinsky A et al. The selC-associated SHI-2 pathogenicity island of Shigella flexneri. Mol Microbiol 1999; 33(1):74-83.
49. Vokes SA, Reeves SA, Torres AG et al. The aerobactin iron transport system genes in Shigella flexneri are present within a pathogenicity island. Mol Microbiol 1999; 33(1):63-73.
50. Zhou D, Hardt WD, Galan JE. Salmonella typhimurium encodes a putative iron transport system within the centisome 63 pathogenicity island. Infect Immun 1999; 67(4):1974-81.
51. Warner PJ, Williams PH, Bindereif A et al. ColV plasmid-specific aerobactin synthesis by invasive strains of Escherichia coli. Infect Immun 1981; 33(2):540-5.
52. Crosa JH. Genetics and molecular biology of siderophore-mediated iron transport in bacteria. Microbiol Rev 1989; 53(4):517-30.
53. Fischbach MA, Lin H, Liu DR et al. In vitro characterization of IroB, a pathogen-associated C-glycosyltransferase. Proc Natl Acad Sci USA 2004.
54. Davidson B, Reich R, Risberg B et al. The biological role and regulation of matrix metalloproteinases (MMP) in cancer. Arkh Patol 2002; 64(3):47-53.

55. Okada Y, Morodomi T, Enghild J et al. Matrix metalloproteinase 2 from human rheumatoid synovial fibroblasts. Purification and activation of the precursor and enzymatic properties. Eur J Biochem 1990; 194:721-730.
56. Murphy G, Willenbrock F. Tissue inhibitors of matrix metalloproteinases. Methods Enzymol 1995; 248:496-510.
57. Ballin M, Gomez DE, Sinha CC et al. Ras oncogene mediated induction of a 92 kDa metalloproteinase; strong correlation with the malignant phenotype. Biochem Biophys Res Commun 1988; 154:832-838.
58. Bernhard E, Muschel R, Hughes E. Mr 92,000 gelatinase release correlates with the metastatic phenotype in transformed rat embryo cells. Cancer Res 1990; 50:3872-3877.
59. Reich R, Thompson E, Iwamoto Y et al. Effects of inhibitors of plasminogen activator, serine proteinases, and collagenase IV on the invasion of basement membranes by metastatic cells. Cancer Res 1988; 48:3307-3312.
60. Nakajima M, Welch DR, Wynn DM et al. Serum and plasma M(r) 92,000 progelatinase levels correlate with spontaneous metastasis of rat 13762NF mammary adenocarcinoma. 1993.
61. Moscatelli D, Rifkin DB. Membrane and matrix localization of proteinases: A common theme in tumor cell invasion and angiogenesis. Biochem. Biophys. Acta 1988; 948:67-85.
62. Murphy G, Reynolds JJ, Hembry RM. Metalloproteinases and cancer invasion and metastasis. Int J Cancer 1989; 44:757-760.
63. Tschesche H, Zolzer V, Triebel S et al. The human neutrophil lipocalin supports the allosteric activation of matrix metalloproteinases. Eur J Biochem 2001; 268(7):1918-28.
64. Helliwell RJ, Adams LF, Mitchell MD. Prostaglandin synthases: recent developments and a novel hypothesis. Prostaglandins Leukot Essent Fatty Acids 2004; 70(2):101-13.
65. Akerstrom B, Logdberg L, Berggard T et al. alpha(1)-Microglobulin: a yellow-brown lipocalin. Biochim Biophys Acta 2000; 1482(1-2):172-84.
66. Larsson J, Allhorn M, Kerstrom B. The lipocalin alpha(1)-microglobulin binds heme in different species. Arch Biochem Biophys 2004; 432(2):196-204.
67. Suzuki K, Lareyre J-J, Sánchez D et al. Molecular evolution of a multigene subfamily encoding epididymal lipocalins localized on the [A3] locus of mouse chromosome 2. Gene, in press.
68. Strausberg RL, Feingold EA, Grouse LH et al. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. Proc Natl Acad Sci USA 2002; 99(26):16899-903.
69. Descalzi Cancedda F, Dozin B, Zerega B et al. Ex-FABP: a fatty acid binding lipocalin developmentally regulated in chicken endochondral bone formation and myogenesis. Biochim Biophys Acta 2000; 1482(1-2):127-35.
70. Hartl M, Matt T, Schuler W et al. Cell transformation by the v-myc oncogene abrogates c-Myc/Max-mediated suppression of a C/EBP beta-dependent lipocalin gene. J Mol Biol 2003; 333(1):33-46.
71. Schreck SF, Parker C, Plumb ME et al. Human complement protein C8 gamma. Biochim Biophys Acta 2000; 1482(1-2):199-208.
72. Ortlund E, Parker CL, Schreck SF et al. Crystal structure of human complement protein C8gamma at 1.2 A resolution reveals a lipocalin fold and a distinct ligand binding site. Biochemistry 2002; 41(22):7030-7.
73. Fluckinger M, Haas H, Merschak P et al. Human tear lipocalin exhibits antimicrobial activity by scavenging microbial siderophores. Antimicrob Agents Chemother 2004; 48(9):3367-72.
74. Breustedt DA, Korndorfer IP, Redl B et al. The 1.8-a crystal structure of human tear lipocalin reveals an extended branched cavity with capacity for multiple ligands. J Biol Chem 2005; 280(1):484-93.
75. Hunter HN, Fulton DB, Ganz T et al. The solution structure of human hepcidin, a peptide hormone with antimicrobial activity that is involved in iron uptake and hereditary hemochromatosis. J Biol Chem 2002; 277(40):37597-603.
76. McGrath H Jr, Rigby PG. Hepcidin: inflammation's iron curtain. Rheumatology (Oxford) 2004; 43(11):1323-5.
77. Nemeth E, Tuttle MS, Powelson J et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004; 306(5704):2090-3.
78. Roosenberg JM 2nd, Lin YM, Lu Y et al. Studies and syntheses of siderophores, microbial iron chelators, and analogs as potential drug delivery agents. Curr Med Chem 2000; 7(2):159-97.
79. Budzikiewicz H. Siderophore-antibiotic conjugates used as trojan horses against Pseudomonas aeruginosa. Curr Top Med Chem 2001; 1(1):73-82.
80. Horwitz LD, Sherman NA, Kong Y et al. Lipophilic siderophores of Mycobacterium tuberculosis prevent cardiac reperfusion injury. Proc Natl Acad Sci USA 1998; 95(9):5263-8.

81. Ratledge C, Ewing M. The occurrence of carboxymycobactin, the siderophore of pathogenic mycobacteria, as a second extracellular siderophore in Mycobacterium smegmatis. Microbiology 1996; 142(Pt 8):2207-12.
82. Cox GB, Gibson F. 2,3-Dihydroxybenzoic acid, a new growth factor for multiple aromatic auxotrophs. J Bacteriol 1967; 93(1):502-3.
83. Bellaire BH, Elzer PH, Baldwin CL et al. Production of the siderophore 2,3-dihydroxybenzoic acid is required for wild-type growth of Brucella abortus in the presence of erythritol under low-iron conditions in vitro. Infect Immun 2003; 71(5):2927-2832.
84. O'Brien IG, Gibson F. The structure of enterochelin and related 2,3-dihydroxy-N-benzoylserine conjugates from Escherichia coli. Biochim Biophys Acta 1970; 215(2):393-402.
85. Pollack JR, Neilands JB. Enterobactin, an iron transport compound from Salmonella typhimurium. Biochem Biophys Res Commun 1970; 38(5):989-92.
86. Thulasiraman P, Newton SM, Xu J et al. Selectivity of ferric enterobactin binding and cooperativity of transport in gram-negative bacteria. J Bacteriol 1998; 180(24):6689-96.
87. Bergeron RJ, Dionis JB, Elliott GT et al. Mechanism and stereospecificity of the parabactin-mediated iron-transport system in Paracoccus denitrificans. J Biol Chem 1985; 260(13):7936-44.
88. Ong SA, Peterson T, Neilands JB. Agrobactin, a siderophore from Agrobacterium tumefaciens. J Biol Chem 1979; 254(6):1860-5.
89. Gonzalez Carrero MI, Sangari FJ, Aguero J et al. Brucella abortus strain 2308 produces brucebactin, a highly efficient catecholic siderophore. Microbiology 2002; 148(Pt 2):353-60.
90. Meyer JM, Hohnadel D, Halle F. Cepabactin from Pseudomonas cepacia, a new type of siderophore. J Gen Microbiol 1989; 135(Pt 6):1479-87.
91. Bluhm ME, Kim SS, Dertz EA et al. Corynebactin and enterobactin: related siderophores of opposite chirality. J Am Chem Soc 2002; 124(11):2436-7.
92. Yamamoto S, Okujo N, Fujita Y et al. Structures of two polyamine-containing catecholate siderophores from Vibrio fluvialis. J Biochem (Tokyo) 1993; 113(5):538-44.
93. Griffiths GL, Sigel SP, Payne SM et al. Vibriobactin, a siderophore from Vibrio cholerae. J Biol Chem 1984; 259(1):383-5.
94. Okujo N, Saito M, Yamamoto S et al. Structure of vulnibactin, a new polyamine-containing siderophore from Vibrio vulnificus. Biometals 1994; 7(2):109-16.
95. Sharman GJ, Williams DH, Ewing DF et al. Determination of the structure of exochelin MN, the extracellular siderophore from Mycobacterium neoaurum. Chem Biol 1995; 2(8):553-61.
96. Sharman GJ, Williams DH, Ewing DF et al. Isolation, purification and structure of exochelin MS, the extracellular siderophore from Mycobacterium smegmatis. Biochem J 1995; 305(Pt 1):187-96.
97. Zalkin A, Forrester JD, Templeton DH. Ferrichrome-A tetrahydrate. Determination of crystal and molecular structure. J Am Chem Soc 1966; 88(8):1810-4.
98. van der Helm D, Poling M. The crystal structure of ferrioxamine E. J Am Chem Soc 1976; 98(1):82-6.
99. Dhungana S, White PS, Crumbliss AL. Crystal structure of ferrioxamine B: a comparative analysis and implications for molecular recognition. J Biol Inorg Chem 2001; 6(8):810-8.
100. Emery T. Isolation, characterization, and properties of fusarinine, a delta-hydroxamic acid derivative of ornithine. Biochemistry 1965; 4(7):1410-7.
101. Diekmann H. Metabolic products of microorganisms. 56. Fusigen—a new sideramine from fungi. Arch Mikrobiol 1967; 58(1):1-5.
102. Cox CD, Rinehart KL Jr, Moore ML et al. Pyochelin: novel structure of an iron-chelating growth promoter for Pseudomonas aeruginosa. Proc Natl Acad Sci USA 1981; 78(7):4256-60.
103. Abdallah MA, Pfestorf M, Doring G. Pseudomonas aeruginosa pyoverdin: structure and function. Antibiot Chemother 1989; 42:8-14.
104. Thieken A, Winkelmann G. Rhizoferrin: a complexone type siderophore of the Mucorales and entomophthorales (Zygomycetes). FEMS Microbiol Lett 1992; 73(1-2):37-41.
105. Hou Z, Raymond KN, O'Sullivan B et al. A preorganized siderophore: thermodynamic and structural characterization of alcaligin and bisucaberin, microbial macrocyclic dihydroxamate chelating agents(1). Inorg Chem 1998; 37(26):6630-6637.
106. Garner BL, Arceneaux JE, Byers BR. Temperature control of a 3,4-dihydroxybenzoate (protocatechuate)-based siderophore in Bacillus anthracis. Curr Microbiol 2004; 49(2):89-94.
107. Kunze B, Trowitzsch-Kienast W, Hofle G et al. Nannochelins A, B and C, new iron-chelating compounds from Nannocystis exedens (myxobacteria). Production, isolation, physico-chemical and biological properties. J Antibiot (Tokyo) 1992; 45(2):147-50.
108. Meyer JM, Van VT, Stintzi A et al. Ornibactin production and transport properties in strains of Burkholderia vietnamiensis and Burkholderia cepacia (formerly Pseudomonas cepacia). Biometals 1995; 8(4):309-17.

109. Meiwes J, Fiedler HP, Haag H et al. Isolation and characterization of staphyloferrin A, a compound with siderophore activity from Staphylococcus hyicus DSM 20459. FEMS Microbiol Lett 1990; 55(1-2):201-5.
110. Konetschny-Rapp S, Jung G, Meiwes J et al. Staphyloferrin A: a structurally new siderophore from staphylococci. Eur J Biochem 1990; 191(1):65-74.
111. Haag H, Hantke K, Drechsel H et al. Purification of yersiniabactin: a siderophore and possible virulence factor of Yersinia enterocolitica. J Gen Microbiol 1993; 139(Pt 9):2159-65.
112. Perry RD, Balbo PB, Jones HA et al. Yersiniabactin from Yersinia pestis: biochemical characterization of the siderophore and its role in iron transport and regulation. Microbiology 1999; 145(Pt 5):1181-90.
113. Wallace AC, Laskowski RA, Thornton JM. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein Engineering 1995; 8(2):127-34.
114. Higgins DG, Bleasby AJ, Fuchs R. CLUSTALW. Comput Applic Biosci 1992; 8(2):189-191.