Iron gathering by zoopathogenic fungi

Dexter H. Howard

Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA

First published online 6 November 2003

Abstract

Iron is a metal required by most microorganisms and is prominently used in the transfer of electrons during metabolism. The gathering of iron is, then, an essential process and its fulfillment becomes a crucial pathogenetic event for zoopathogenic fungi. Iron is rather unavailable because it occurs on the earth’s surface in its insoluble ferric form in oxides and hydroxides. In the infected host iron is bound to proteins such as transferrin and ferritin. Solubilization of ferric iron is the major problem confronting microorganisms. This process is achieved by two major mechanisms: ferric reduction and siderophore utilization. Ferric reductase is frequently accompanied by a copper oxidase transport system. There is one example of direct ferric iron transport apparently without prior reduction. Ferric reduction may also be accomplished by low molecular mass compounds. Some fungi have evolved a process of iron acquisition involving the synthesis of iron-gathering compounds called siderophores. Even those fungi that do not synthesize siderophores have developed permeases for transport of such compounds formed by other organisms. Fungi can also reductively release iron from siderophores and transport the ferrous iron often by the copper oxidase transport system. There is a great diversity of iron-gathering mechanisms expressed by pathogenic fungi and such diversity may be found even in a single species.

1. Introduction

Iron is required by most microorganisms. The metal has two readily available ionization states and is often used as a cofactor in oxidation–reduction reactions. The selection of iron for this role from among other capable transition metals may be related to the fact that it is the second most abundant metal (after aluminum) in the earth’s crust and thus was abundantly available in the prebiotic world [1]. The early atmosphere of those times must have been reductive and ferrous iron was probably ‘present in the earliest Archaean environment’ [2]. With the advent of oxygen and an aerobic atmosphere iron was converted to the ferric form and combined into insoluble compounds (oxides and hydroxides) [3–5]. Iron gathering thus involves two processes: solubilization of the insoluble ferric form from environmental sources or from high affinity binding proteins in a host, and transport of the metal across the fungal membrane. This review will cover the means developed by fungi to accomplish these two goals.

Earlier I had prepared a review of the acquisition, transport, and storage of iron by pathogenic fungi [6]. Inspired by the invitation to prepare a minireview on this topic, I have refreshed the data by noting recent publications and I have reanalyzed from a different standpoint some of the work previously reviewed. The major focus will be on the diversity of iron-gathering methods among zoopathogenic fungi.

2. Ferricreduction

2.1. Cryptococcus neoformans

C. neoformans is a basidiomycetous yeast [7] that causes meningocerebralitis in immunocompromised patients. At one time it was a leading cause of death in AIDS patients. Its prevalence in developed nations has been markedly reduced by the advent of effective antiretroviral and antifungal therapy. However, in many areas where that ther-
apy is unavailable, the disease remains a serious problem [8]. C. neoformans expresses a polysaccharide capsule in a low iron environment. This structure is one of the virulence factors for the fungus. The fact that it is expressed in cryptococcosis suggests that the human host is a low iron environment in which C. neoformans must obtain iron for growth [8].

As in the yeast Saccharomyces cerevisiae [9,10], C. neoformans reduces Fe(III) with a ferric reductase that could be compared to the enzymes Fre 1p and Fre 2p characterized genetically in S. cerevisiae but not yet in C. neoformans. By analogy with the Saccharomyces system it could be assumed that the Fe(II) thus formed is bound to a transporter Ftr 1p permease which is associated with a copper ferrous oxidase, Fet 3p [9,10,13]. The oxidation step is associated with uptake in S. cerevisiae. The use of opposing enzyme functions to transport iron under iron-limited condition by opposing enzyme functions to transport iron under iron-limiting condition by S. cerevisiae was discussed in an earlier review [6]. Recently the cryptococcal permease has been cloned and genetically characterized [14]. In S. cerevisiae the ferrireductase FRE 1 and the transporter FTR 1 genes are controlled by a transcriptional regulator AFT 1 and the system is expressed at low concentrations of iron [9,10].

C. neoformans also secretes 3-hydroxyanthranilic acid which also reduces Fe(II) to Fe(II) and provides a second source of ferrous iron for transport by a copper oxidase permease system [8–12].

2.2. Candida albicans

This yeast is a commensal organism that occurs in the gastrointestinal tract and on the oral and vaginal mucosae [7]. From these locations it takes opportunistic advantage of immunocompromised individuals. In a recent study of iron transport by C. albicans two genes, CaFTR 1 and CaFTR 2, were detected [15]. The genes were identified as homologues to the iron permease gene FTR 1 of S. cerevisiae. Although screened for under conditions of iron limitation, the highest amount of the CaFTR 1 transcript was expressed under conditions of iron limitation while the greatest amount of CaFTR 2 transcript was detected under conditions of iron repletion. The Ftr 1 function requires a ferrous oxidase Fet 3. The CaFTR 1 gene was required for iron acquisition by C. albicans in iron-deficient environments in vitro and in vivo. Strains of C. albicans in which the CaFTR 1 gene was deleted were essentially avirulent [15].

A multicopper oxidase gene from C. albicans has been cloned and characterized [16]. However, a null mutant strain was not reduced in pathogenicity in a mouse model of candidiasis [16]. In S. cerevisiae the Sc Fet 3 multicopper ferroxidase requires the activity of a membrane copper permease and an intracellular copper transporting P-type ATPase, Sc Ccc 2 [17]. However, the deletion of the CaCCC 2 transporter gene in C. albicans did not result in reduced virulence of the strain and an alternative pathway involving hemin has been suggested [17].

2.3. Geotrichum candidum

This fungus is a rare pathogen of humans but a rather common phytopathogen in citrus fruits [18]. G. candidum is a yeast that reproduces by fission rather than budding [7]. Thus it resembles Schizosaccharomyces pombe in its method of growth and reproduction but appears to vary from that fungus in its iron-gathering methods [9,18]. C. neoformans does not appear to form siderophores [18]. Instead, iron uptake is mediated by two iron-regulated transport systems. One system was specific for either ferric or ferrous iron, while the other was specific for ferrioxamine B-mediated iron uptake [18]. The K_m values for ferric and ferrous ions were identical (3 µM). Experiments were designed to determine if changes in the valence form of iron occurred prior to transport. A ferric specific chelator, ethylenediamine-di(ß-hydroxyphenylacetic acid) (EDDHA), caused 60% inhibition of Fe(III) uptake whereas the ferrous trapping reagents ferrozine and dipyridyl were not effective or resulted in only slight inhibition of Fe(III) uptake. In contrast, ferrozine and dipyridyl caused 80% and 50% inhibition of Fe(II) transport, respectively, while EDDHA was only slightly inhibitory. The work with the specific chelators led the authors to state that ferric ion "may not be reduced prior to its penetration into the cells, at least during the transport period" [18]. Moreover, cells of G. candidum were unable to reduce ferric chloride; bathophenanthroline disulfonic acid was used as a chromogenic chelator of ferrous iron in these ferrireduction experiments [18].

2.4. Histoplasma capsulatum

The dimorphic fungus H. capsulatum is a facultative intracellular parasite of the mononuclear phagocytes of an infected host and must obtain nutrients necessary for growth from that immediate environment [7,19]. Perhaps because of its location in what should be an iron-restricted environment, its iron-gathering activities are diverse. Under iron-limiting conditions ferric reductase activity was expressed in both high and low molecular fractions from the fungus. The high molecular mass fraction reductase activity required reduced glutathione [20]. This reduced glutathione ferric reductase could utilize ferric chloride, ferric citrate and human holotransferrin as substrates [20]. The low molecular mass reductase activity has not been completely characterized but is a non-proteinaceous ferric reductant that resembles such activity found in C. neoformans (see above). In addition, H. capsulatum produces five hydroxamate siderophores [19]. Both dimeroxym acid and rhodotorulic acid (siderophores) act as substrates for the ferric reductase and rhodotorulic acid can remove iron from transferrin [21]. Hemin serves as a substrate for
the ferric reductase and satisfies the iron needs of *H. capsulatum* [21]. All of the iron-gathering devices are coexpressed under iron-depleted conditions [19–21]. This is the most complicated iron-gathering melange of activities detected in zoopathogenic pathogens. Though the systems seen in *C. albicans* appear to be similarly complex [6,21], not all of the activities have been as completely characterized as those in *H. capsulatum*. Recently, the hemin utilizing system in *H. capsulatum* has received additional study [22]. The results of the work suggested that *H. capsulatum* uses hemin as a source of iron and that such utilization involves a yeast cell surface receptor for hemin [22].

### 3. Siderophores

Under conditions of extreme iron stress, fungi produce low molecular mass (Mᵣ < 1500) ferric iron chelators known collectively as siderophores. These compounds solubilize ferric iron and carry it to microbial cells [3,5,23,24]. The distribution of different chemical sorts of siderophores is shown in Table 1. There is no information on siderophore synthesis by members of the phylum Chytridiomycota. Although there is one report of hydroxamate formation by *Rhizopus* spp. and *Absidia corymbifera* [25], it is the general observation that the zygomycetes form iron-regulated citric acid-containing polycarboxylates [5]. Hydroxamates are generally expressed by certain members of the Ascomycota, Basidiomycota and mitosporic fungi but there is a well-characterized phenolate siderophore in wood-rotting fungi (basidiomycetes) [26,27] and an unconfirmed report of phenolates being formed by *C. albicans* [28]. There have been reports of considerable diversity of synthesis and utilization of siderophores. For example, *H. capsulatum* synthesizes five sorts of hydroxamates and can utilize three others which it does not produce [19,22]. The synthesis of siderophores by zoopathogenic fungi has been reviewed [6].

#### 3.1. Utilization of siderophores by non-producers

In a number of instances, pathogenic fungi have been shown to use siderophores even though they cannot synthesize them. I will use the term xenosiderophores (Gr. xeno = foreign) for those iron chelates not produced by the utilizing fungus. This will prove useful in those instances where a fungus produces siderophores of its own but may also utilize others which it cannot synthesize. Examples of such utilisations are recorded in Table 2. While *S. cerevisiae* is only rarely encountered in a pathogenetic role [29], it is included here because of the diversity of the hydroxamates used.

The xenosiderophores represent iron-related growth factors. Indeed, knowledge about the fungal siderophore, coprogen, developed from early studies on growth stimulation of *Pilobolus* spp. In these studies a substance that stimulated the growth of *Pilobolus* spp. was decocted from dung. The factor was called ‘coprogen’ [30]. Thus, according to the glossary in [31]: “Coprogen (Gr. koproS = dung + gennoS = 1 give birth): a factor in dung necessary for the growth of *Pilobolus* (Zygomycota).” Hasseltine and coworkers showed that the growth factor was produced in the fermentation liquor of a number of species of bacteria and fungi [30,32]. Such diversity suggests that a number of molecularly different growth factors might have been present. They chose to work with a material from a species of *Penicillium* in order to generate enough growth factor to study chemically. A purified product was shown to contain iron and they reasoned that the growth-stimulating compound was a metalloporphyrin, certain of which were known bacterial growth factors [33]. They did show, however, that the compound was not heme [33]. The exact chemical structure was determined several years afterwards from a sample from *Neurospora crassa* [5]. The name was preserved and represents a major family of the hydroxamate siderophores. It is, of course, not certain that the growth factor(s) originally described from dung

### Table 1

<table>
<thead>
<tr>
<th>Siderophores produced by members of the phyla of the kingdom Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
</tr>
<tr>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Chytridiomycota</td>
</tr>
<tr>
<td>Zygomycota</td>
</tr>
<tr>
<td>Ascomycota</td>
</tr>
<tr>
<td>Basidiomycota</td>
</tr>
<tr>
<td>Mitosporic fungi</td>
</tr>
</tbody>
</table>

<sup>a</sup> One report of hydroxamates in *Rhizopus* spp. and *Absidia corymbifera* [25].

<sup>b</sup> One example of a well-characterized phenolate in wood-rotting fungi [26,27].

<sup>c</sup> One report on a phenolate siderophore of *C. albicans* [28].

### Table 2

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Siderophore</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Ferrioxamine B [37,38]</td>
<td></td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>Ferrioxamine B [41]</td>
<td></td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>Ferrioxamine B [13,47]</td>
<td></td>
</tr>
<tr>
<td><em>Geotrichum candidum</em></td>
<td>Ferrioxamine B [18]</td>
<td></td>
</tr>
<tr>
<td><em>Histoplasma capsulatum</em></td>
<td>Rhodotorulic acid [21]</td>
<td></td>
</tr>
<tr>
<td><em>Paracoccidioides brasiliensis</em></td>
<td>Coprogen B [48]</td>
<td></td>
</tr>
<tr>
<td><em>Pilobolus</em> spp.&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Coprogen&lt;sup&gt;b&lt;/sup&gt; [30,32,33]</td>
<td></td>
</tr>
<tr>
<td><em>Rhizopus</em> spp.</td>
<td>Ferrioxamine B [49,50]</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Not a human pathogen but added because of historical antecedents.

<sup>b</sup> The chemical structure was determined on a specimen from *Neurospora crassa* [5].
and subsequently from the fermentation liquors of a number of microorganisms [30,32] were all the iron-bearing siderophores now known as coprogen [5]. Only the name is the same.

3.1.1. S. cerevisiae

The greatest number of xenosiderophore utilizations has been reported in this species which does not synthesize its own siderophores. Not only fungal hydroxamates are used [34–37,39] but also ferrioxamine B which is formed naturally by the Actinomycetes [37,38] and enterobactin, a catecholate siderophore from a non-filamentous bacterium [40]. S. cerevisiae can use two different mechanisms, reductive and non-reductive, to take up iron. The non-reductive transport system accounts for utilization of ferrioxamine B, ferrichrome, fusarinine, rhodotorulic acid and enterobactin. Uptake may also be a reduction process at the cell membrane [41]. As the siderophore concentration rises the reduction system becomes more important as the permease system is saturated [41].

3.1.2. C. albicans

C. albicans can use xenosiderophores by reductive and non-reductive means [41]. Since the identity of hydroxamates said to be synthesized by C. albicans is unknown [25,28,42], my use of the term ‘xenosiderophore’ with reference to C. albicans may be inaccurate in detail. Although Cutler and Han [43] have reported their failure to display siderophores in C. albicans, a putative transcription factor that regulates siderophore synthesis has been identified [44]. Ferrioxamine B, ferrichrome, ferricrocin and fusarinine were utilized by C. albicans [41,45]. Exposure to serum which induces a filamentous form of C. albicans thought to be important to virulence also induced utilization of ferrichrome type siderophores [45]. The ferrichrome transporter was required for epithelial invasion [46]. Hemin is also used as an iron source by C. albicans [6,17].

3.1.3. C. neoformans

Growth of C. neoformans, which does not synthesize its own siderophores, was stimulated by ferrioxamine B (160 μM) added to an iron-depleted medium [47]. Although diffusion of the siderophore would dilute the concentration from that applied to the paper disc used, the level of siderophores close to the disc would probably be high enough to indicate a reductive mobilization of Fe(II) from the ligand assuming saturation of the permease (by analogy to S. cerevisiae [37,41]). Studies on low concentrations of the siderophore indicated ligand uptake is also possible [13].

3.1.4. G. candidum

Although G. candidum does not produce any siderophores, it has been shown to take up iron from ferrioxamine B. The uptake system had an apparent $K_m$ of 2 μM. The ferrioxamine-mediated iron transport was almost completely inhibited by ferric chloride in the medium. The ferrioxamine and ferric chloride transport systems had many similar properties but competition experiments indicated that the membrane-mediated carrier system for the siderophore was distinct [18].

3.1.5. H. capsulatum

In spite of the fact that this fungus synthesizes five hydroxamate siderophores [7], it is also able to utilize the xenosiderophores ferrichrome, rhodotorulic acid and ferrioxamine B [21]. The studies thus far have revealed that ferrioxamine B and rhodotorulic acid serve as substrates for the H. capsulatum ferric reductase while ferrichrome does not [21].

3.1.6. Paracoccidioides brasiliensis

The growth of P. brasiliensis was stimulated by coprogen B and dimerum acid that had been synthesized by B. dermatitidis [48]. P. brasiliensis may synthesize its own siderophores under conditions of iron stress, but appropriate studies have not been conducted.

3.1.7. Rhizopus spp.

The occurrence of mucormycosis in patients being treated with Desferal (a methanesulfonate salt of desferrioxamine) clearly indicates the utilization of this bacterial siderophore by Rhizopus spp. [49]. Studies on the uptake of iron from the xenosiderophore ferrioxamine B and the native siderophore rhizoferrin by Rhizopus microsporus have been reported [50]. Studies designed to clone the ferrioxamine B permease gene (FTR 1) of Rhizopus oryzae have been initiated (A.S. Ibrahim, personal communication, May 2003).

The use of Desferal in therapy may occasion other examples of secondary infections. I am not aware of the other clinical report but exacerbation of experimental cryptococcal and Aspergillus infections by Desferal has been reported [49].

4. Conclusions

Iron acquisition is crucial to most microorganisms. Among pathogenic fungi such acquisition becomes an essential aspect of pathogenesis. Thus the study of iron metabolism becomes of central importance because of the severe limitation of its availability in a host. The importance of acquisition and transport of iron by zoopathogenic fungi suggests that a thorough study of these functions may lead to new therapeutic targets that might be explored.
Acknowledgements

I am grateful to Dr. W.B. van Leeuwen for the invitation to prepare this review. It has given me the opportunity to present some ideas that have come up after my earlier review of the topic. I am grateful to the reviewers of the manuscript who made several very important corrections and suggestions. My work was in part supported by an Excess Income Account in Medical Mycology, UCLA. I thank Lois F. Howard for her careful work in typing and proofreading.

References


