

## Ferrous Iron Uptake in *Cryptococcus neoformans*

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Previous studies have implicated ferric reduction in the iron uptake pathway of the opportunistic pathogen *Cryptococcus neoformans*. Here we studied iron uptake directly, using  $^{55}\text{Fe}$  in the presence of reductants. Uptake was linear with respect to time and number of yeast cells. The plot of uptake versus concentration exhibited a steep rise up to about  $1\ \mu\text{M}$ , a plateau between  $1$  and  $25\ \mu\text{M}$ , and a second steep rise above  $25\ \mu\text{M}$ , consistent with high- and low-affinity uptake systems. A  $K_m$  for high-affinity uptake was estimated to be  $0.6\ \mu\text{M Fe(II)}$ ;  $1\ \mu\text{M}$  was used for standardized uptake assays. At this concentration, the uptake rate was  $110 \pm 3\ \text{pmol}/10^6\ \text{cells/h}$ . Iron repletion ( $15\ \mu\text{M}$ ) and copper starvation drastically decreased high-affinity iron uptake. Incubation at  $0^\circ\text{C}$  or in the presence of  $2\ \text{mM KCN}$  abolished high-affinity iron uptake, suggesting that uptake requires metabolic energy. When exogenous reducing agents were not supplied and the culture was washed free of secreted reductants, uptake was reduced by  $46\%$ ; the remaining uptake activity presumably was dependent upon the cell membrane ferric reductase. Further decreases in free  $\text{Fe(II)}$  levels achieved by trapping with bathophenanthroline disulfonate or reoxidizing with potassium nitrosodisulfonate reduced iron uptake very drastically, suggesting that it is the  $\text{Fe(II)}$  species which is transported by the high-affinity transporter. The uptake of  $\text{Fe}$  was stimulated two- to threefold by deferoxamine, but this increment could be abolished by copper starvation or inhibition of the ferric reductase by  $\text{Pt}$ , indicating that  $\text{Fe}$  solubilized by this molecule also entered the reductive iron uptake pathway.

Iron is required for oxidoreductase enzymes in almost all living organisms. Although iron is widely distributed in the environment, under neutral pH and aerobic conditions oxidation of iron to poorly soluble  $\text{Fe(III)}$  causes ambient concentrations to be very low. Microbes mobilize environmental iron for uptake according to one of three basic strategies: by secreting iron-chelating chemicals, by reducing insoluble environmental  $\text{Fe(III)}$  to soluble  $\text{Fe(II)}$ , or by expressing receptors and deferration mechanisms for iron-containing proteins. The most widely studied microbial iron uptake systems use secreted hydroxamate or catecholate  $\text{Fe(III)}$  chelators, termed siderophores, and cell surface receptors for the ferrated complex (21). One such siderophore, deferoxamine (Desferal; Ciba-Geigy), is manufactured by fermentation and is used clinically to mobilize trivalent metals. *Saccharomyces cerevisiae* (16), *Listeria monocytogenes* (5), *Legionella pneumophila* (14), and higher plants (18, 23) use the second strategy, reducing  $\text{Fe(III)}$  to  $\text{Fe(II)}$  prior to uptake of the iron. The obligate pathogens *Neisseria gonorrhoeae* and *Neisseria meningitidis* use the third strategy, binding and deferrating human transferrin and lactoferrin (19); *Hemophilus influenzae* scavenges iron directly from heme and hemoglobin (24).

Iron uptake mechanisms in pathogenic fungi are incompletely understood. Hydroxamate siderophores have been detected in culture supernatants of *Histoplasma capsulatum* (2). *Rhizopus* species express surface receptors for ferri-deferoxamine but do not secrete hydroxamate siderophores (1); thus, deferoxamine represents an iron uptake-related vitamin in *Rhizopus*, as it does in the pathogenic bacterium *Yersinia enterocolitica* (25). In *Cryptococcus neoformans*, deferoxamine stimu-

lates growth in the presence of iron starvation, but this fungus does not secrete  $\text{Fe(III)}$  chelators, nor does it express a specific transferrin receptor (12, 13). Rather, *C. neoformans* and *Candida albicans* reduce extracellular  $\text{Fe(III)}$  to the much more soluble form,  $\text{Fe(II)}$ , like *S. cerevisiae*, and are presumed to transport nascent  $\text{Fe(II)}$  into their cells (20, 22).

In what has been termed nutritional immunity, vertebrates use unsaturated transferrins to withhold their iron from microbes and respond to infections by further sequestering their stores of iron, even to the point of anemia (3). This inference is supported by the observation that saturation with physiologic iron chelators leads to opportunistic infections (28). It has been hypothesized that the administration of therapeutic iron-sequestering agents may further protect host iron stores and thereby alleviate infections. Such compounds are being actively sought, but currently the only chelator which can be safely administered is deferoxamine, a microbial hydroxamate siderophore. One might expect that this compound would sequester iron and block iron uptake by microbes which lack receptors for the iron chelate; however, the compound also might be predicted to stimulate uptake in microbes which express such receptors. Indeed, it has been found that the administration of deferoxamine alleviates malaria (9) but promotes opportunistic infections by *Rhizopus* species (1). Thus, microbial iron uptake mechanisms can powerfully affect the host-parasite balance, and understanding them has the potential to lead to better management of infections.

*C. neoformans* is the etiologic agent of a meningitis which complicates many immunodeficiency diseases, including  $10$  to  $20\%$  of those resulting from the human immunodeficiency virus (17). Although the symptoms of cryptococcosis can often be suppressed by treatment with antifungal agents, in most cases the infection is incurable and lifelong treatment is required (29). Because control of iron uptake has decided the outcome of infections in the model cases cited above, we investigated iron uptake in *C. neoformans* with the hope of being

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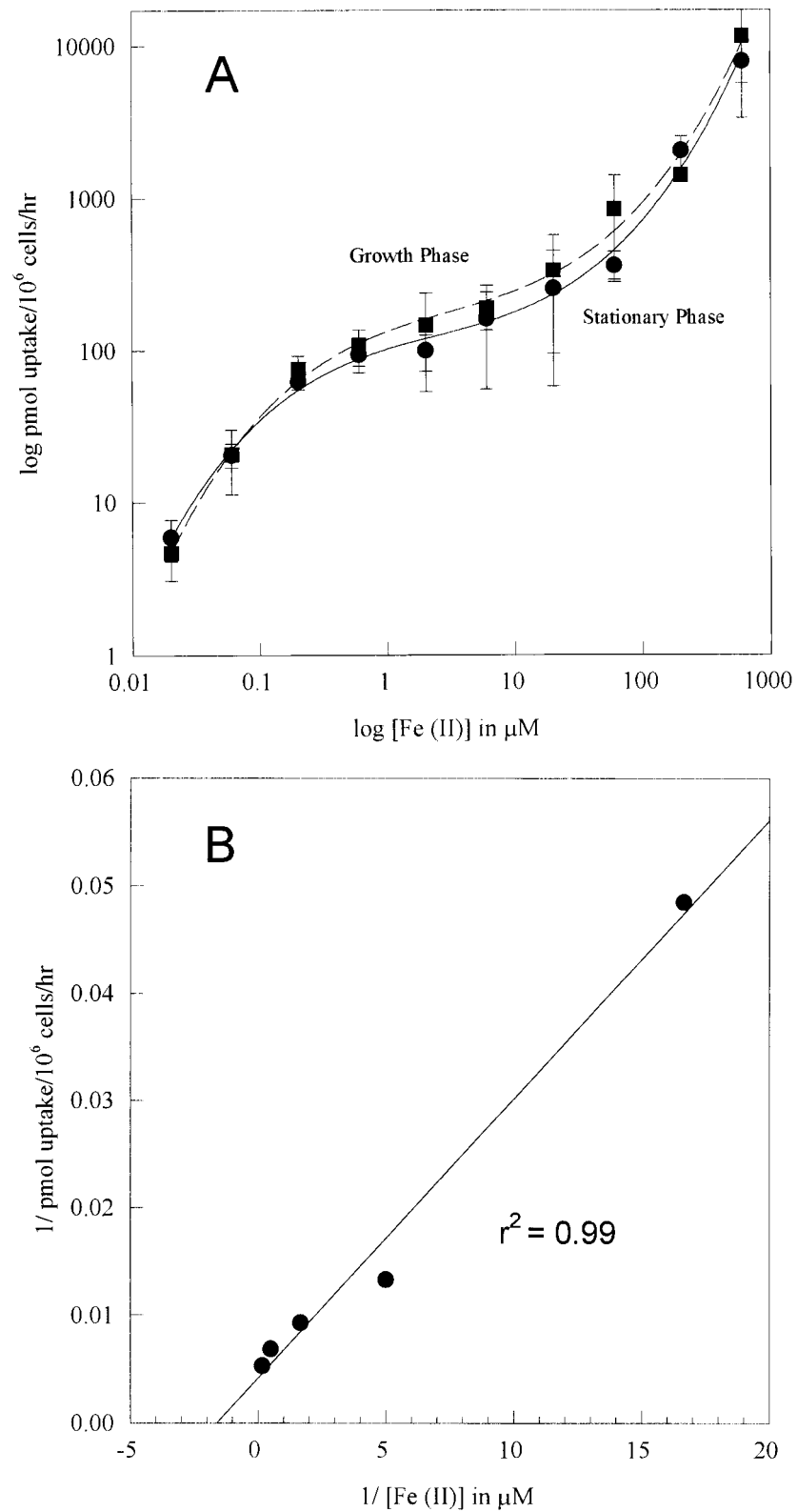


FIG. 1. Fe(II) uptake by growth-phase and stationary-phase cells. (A) Velocity depicted as a function of concentration. A logarithmic plot was used in order to encompass a 5-log-unit variation in Fe(II) concentrations. Error bars indicate standard deviations. (B) Double-reciprocal plot of low-concentration data from an exponentially growing culture. The experiment was performed three times, and representative results are shown.

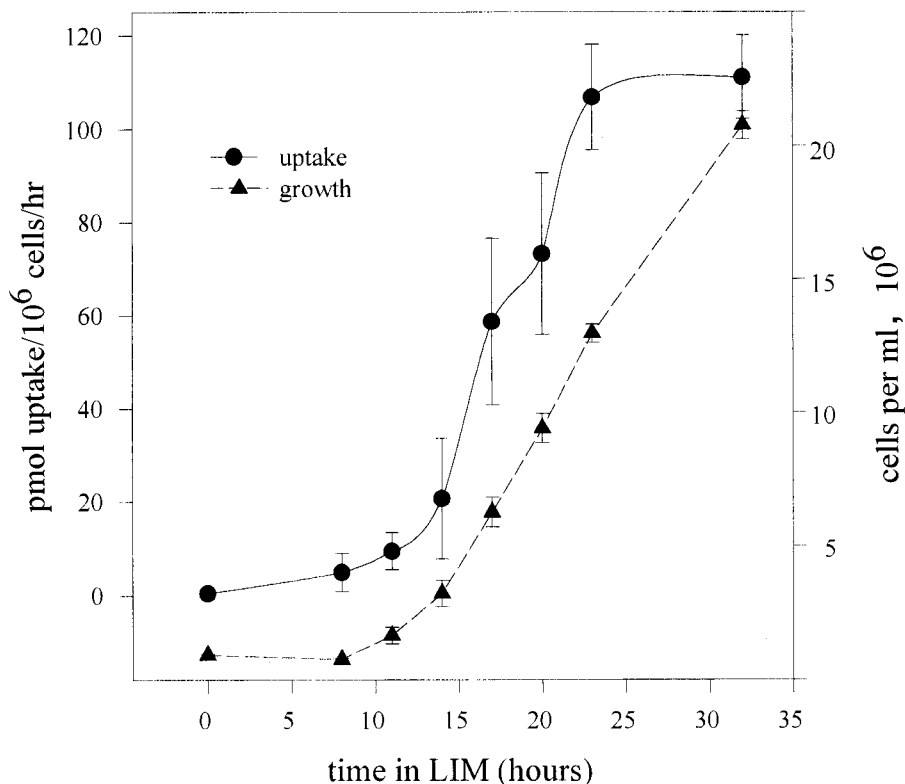


FIG. 2. Expression of high-affinity uptake system following transfer from GYE medium to LIM medium. Cells from a stationary-phase culture in GYE were inoculated at  $10^6$ /ml and agitated. Growth was monitored optically at 700 nm, and high-affinity Fe(II) uptake was measured periodically. Results represent averages of three separate timed cultures assayed in duplicate. Error bars indicate standard deviations.

able to bolster nutritional immunity. We previously described two mechanisms by which this organism reduces extracellular iron: expression of a cell membrane ferric reductase and secretion of the nonspecific reductant 3-hydroxyanthranilic acid (22). In the present report, we describe iron uptake physiologically. We chose to use a serotype D strain because of the availability of both meiotic and molecular analyses in the serotype D cryptococcal system.

#### MATERIALS AND METHODS

**Yeast strains and culture conditions.** We used strain B3501, *MAT $\alpha$* , serotype D, obtained from the National Institutes of Health culture collection. Cultures were stored on brain heart agar slants, from which liquid cultures were started in 2% glucose–2% yeast extract (GYE; Becton Dickinson and Co.) broth. Limited-iron medium (LIM) contained, per liter, 20 g of glucose, 5 g of asparagine, 400 mg of  $K_2HPO_4$ , 100 mg of  $MgSO_4 \cdot 7H_2O$ , 50 mg of  $CaCl_2 \cdot 2H_2O$ , 1 mg of thiamine, 57  $\mu$ g of boric acid, 396  $\mu$ g of  $CuSO_4 \cdot 5H_2O$ , 72  $\mu$ g of  $MnCl_2 \cdot 4H_2O$ , 4.2 mg of  $ZnCl_2$ , and 37  $\mu$ g of  $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ . It was buffered with 50 mM 2-(*N*-morpholino)ethanesulfonic acid adjusted to pH 6.0 with NaOH. Salts of polyvalent metals were dissolved in water treated with Chelex-100 (Bio-Rad) and filter sterilized. Other components were purified over a Chelex-100 column and filter sterilized. Cultures in defined medium were started from stationary-phase cultures in GYE broth, washed twice with LIM, and inoculated at a density of  $10^6$  cells/ml. All glassware was soaked in Citranox acid detergent overnight and rinsed with distilled, deionized water before use with LIM. Iron repletion was accomplished by adding the desired concentration of Fe(III)-hydroxyethyl-enediaminetriacetic acid (FeHEDTA) to LIM. All cultures were grown at room temperature with agitation at 200 rpm. Platinum inhibition was achieved by use of LIM saturated with platinum(II) chloride; the concentration was determined by atomic absorption spectroscopy (Galbraith Laboratories, Knoxville, Tenn.).

**Iron uptake assays.** Cells were grown in LIM with or without various concentrations of FeHEDTA to the late growth phase ( $<2 \times 10^7$  cells/ml) or to the stationary phase. The cells were washed twice and diluted in LIM to a concentration of  $4 \times 10^6$  cells/ml. To assay ferrous iron uptake, an equal volume of LIM containing 100 mM ascorbate and 1 mM dithiothreitol (DTT) at pH 6.0 (LAD) was added to 5 ml of cells. To assay Fe(III) uptake, an equal volume of LIM

(without reductants) was added to 5 ml of cells. Iron was added to the cultures at the desired concentration as FeHEDTA diluted in LAD [for Fe(II)] or LIM [for Fe(III)] containing  $^{55}FeCl_3$  (0.2  $\mu$ Ci; New England Nuclear Corp.). Immediately upon the addition of iron, a 3-ml initial-time sample was filtered through a GF/A glass microfiber filter (Whatman) and washed twice with 10 ml of 0.1 M disodium EDTA (pH 6.0). Cultures were incubated with agitation at 150 rpm and room temperature for 20 min, after which a second 3-ml sample was obtained. Counts on filters were determined with 10 ml of scintillation fluid (Biosafe II; Research Products International) by use of a model 1410 liquid scintillation counter (Wallac). Uptake was calculated by subtracting the counts per minute of the initial sample from that of the 20-min sample and comparing the result to an internal standard. Deferoxamine mesylate was a gift from the Ciba Corporation. Statistical comparisons were done with Student's *t* test.

#### RESULTS AND DISCUSSION

**Kinetics of Fe(II) uptake.** The uptake of Fe(II) was studied with  $^{55}Fe$  in the presence of the reductants DTT and ascorbate. Uptake was linear with respect to time for 40 min and linear with respect to number of cells assayed for up to  $10^7$  cells/ml (data not shown). Suspensions of *C. neoformans* grown in LIM were assayed for uptake in various concentrations of Fe(II). Each concentration was studied in triplicate, several experiments were performed, and a typical experiment is shown in Fig. 1. A log-log plot of uptake versus concentration shows a steeply sloping portion well below 1  $\mu$ M, a shallowly sloping, relative plateau between 1 and 25  $\mu$ M, and a second steeply sloping portion above 25  $\mu$ M (Fig. 1A). This pattern is consistent with a combination of high- and low-affinity uptake systems, as described for *S. cerevisiae* (6, 7), and indicates saturation of the high-affinity uptake system in the range of 1 to 5  $\mu$ M. A double-reciprocal plot indicated a  $K_m$  of approximately 0.6  $\mu$ M for the high-affinity system (Fig. 1B; data for a growth-phase culture are shown). Clearly, the high-affinity uptake sys-

tem is tuned to concentrate ambient iron in the range of 0.1 to 1  $\mu\text{M}$  very efficiently. Because it is very difficult to reduce the iron content of growth media below 100 nM, these data seem to explain why *C. neoformans* grows readily, even in iron-depleted media; in order to observe the failure of growth due to iron limitation, we found it necessary to use an avid synthetic chelator of Fe(II) or Fe(III) (12, 13).

We chose 1  $\mu\text{M}$  Fe(II) as the standard concentration for assaying the high-affinity system in order to minimize the effect of changes in substrate concentration. At 1  $\mu\text{M}$ , the uptake of Fe(II) was  $110 \pm 3$  pmol/ $10^6$  cells/h, close to the  $V_{\text{max}}$  for the high-affinity system. When the temperature of incubation was reduced to 4°C, high-affinity uptake was 96% inhibited ( $27 \pm 5$  versus  $1 \pm 0$  pmol/ $10^6$  cells/h; the experiment was performed in triplicate), indicating that active respiratory metabolism is required for transport. That inference was supported by the finding that 2 mM KCN inhibited uptake by 79% ( $38 \pm 8$  versus  $8 \pm 1$  pmol/ $10^6$  cells/h; the experiment was performed in triplicate). These results are very similar to those obtained for *S. cerevisiae*, except that for the latter, the  $K_m$  for high-affinity iron uptake is somewhat lower (0.15  $\mu\text{M}$ ) (7).

**Regulation of ferrous transport.** Cultures in the late growth phase (24-h incubation time) and in the stationary phase (72-h incubation time) in LIM exhibited comparable uptake activities at all concentrations of Fe(II) studied (Fig. 1). Cultures transferred from GYE (approximately 25  $\mu\text{M}$  Fe) to LIM (approximately 0.2  $\mu\text{M}$  Fe) did not immediately express the high-affinity (low-substrate-concentration) uptake system; such cultures began to express that uptake system only after 10 h of growth in LIM, reaching maximum activity at about 24 h (Fig. 2; an experiment with duplicate time points is shown). This result suggests that the high-affinity uptake system was regulated by iron in the culture and that the culture had outgrown the iron stores left over from the complex medium. Indeed, we observed that uptake activity was down regulated 50% after 24 h of growth in LIM containing 1  $\mu\text{M}$  Fe(III) (data not shown) and that both high-affinity and low-affinity (high-concentration) uptake systems were down regulated 90% by growth in 15  $\mu\text{M}$  Fe(III), as shown in Fig. 3 [uptake was studied once at a range of  $^{55}\text{Fe}$ (II) concentrations; the result was confirmed by four uptake studies at 1  $\mu\text{M}$   $^{55}\text{Fe}$ (II), each performed in triplicate]. Thus, the high-affinity uptake system is potentially resistant to iron overloading at up to 25  $\mu\text{M}$ , partly because of the saturation phenomenon, whereby increases in iron concentrations from 1 to 25  $\mu\text{M}$  result in very little incremental uptake, and partly because the regulation of uptake activity (presumably genetic regulation of expression of the ferrous transporter) begins at about 1  $\mu\text{M}$  ambient iron.

Given the sensitivity of the high-affinity iron uptake system to regulation by only 15  $\mu\text{M}$  Fe, the utility of high-velocity iron uptake at high concentrations (25 to 200  $\mu\text{M}$ ) is not at all clear, yet above 25  $\mu\text{M}$  an upward curve in the concentration-velocity plot suggests a contribution by a low-affinity, high-velocity uptake mechanism (Fig. 1, 3, and 4). The low-affinity system concentrated approximately 1 nmol/ $10^6$  cells/h at 200  $\mu\text{M}$  Fe (II), and the lack of a second plateau in the plot suggests that the low-affinity mechanism is not saturable. This second system appeared to be down regulated during growth by the same relatively low concentration of iron (15  $\mu\text{M}$ ) as that which regulated the high-affinity system (Fig. 3A). Thus, if low-affinity uptake were not also regulated, the iron-replete plot should have converged upon the iron-depleted plot at high iron concentrations in the log-log plot, since the constant equal to the saturated high-affinity uptake system should represent a diminishing fraction of the increasing logarithms. Accordingly, both high- and low-affinity systems appeared to have been down

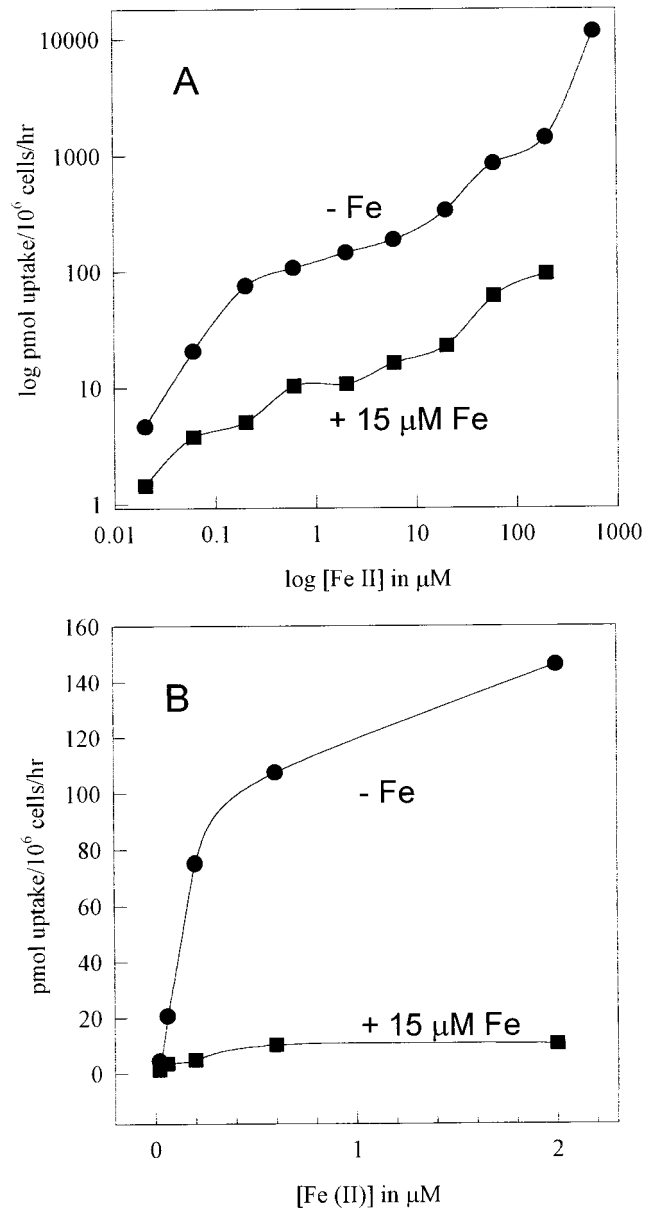


FIG. 3. Effect of Fe(III) in growth medium upon Fe(II) uptake activity. Cells were grown in LIM with or without (-Fe) 15  $\mu\text{M}$  Fe, washed in LIM, and assayed for Fe(II) uptake at various concentrations. (A) Double-logarithm plot. (B) Arithmetic plot of low-concentration data.

regulated 1 log unit by 15  $\mu\text{M}$  Fe(III). Curiously, at 200  $\mu\text{M}$  Fe(II), the low-affinity system of the high-iron culture transported about as much iron (100 pmol/ $10^6$  cells/h) as the  $V_{\text{max}}$  for the maximally induced high-affinity system of the low-iron culture, making regulation of the high-affinity system seem futile. One can assert that an Fe(II) concentration of 100  $\mu\text{M}$  may not be physiologic, but even with aerobic cultures we have observed such a concentration for a constitutive ferric reductase regulatory mutant (11).

**Dependence upon exogenous copper.** Perhaps the most striking finding for the *S. cerevisiae* system has been the dependence of iron uptake upon a copper-containing protein thought to function as an oxidase (4). Indeed, there is evidence for the direct association of a copper-containing protein with the ferrous transporter in *S. cerevisiae*, and it is inferred that copper-

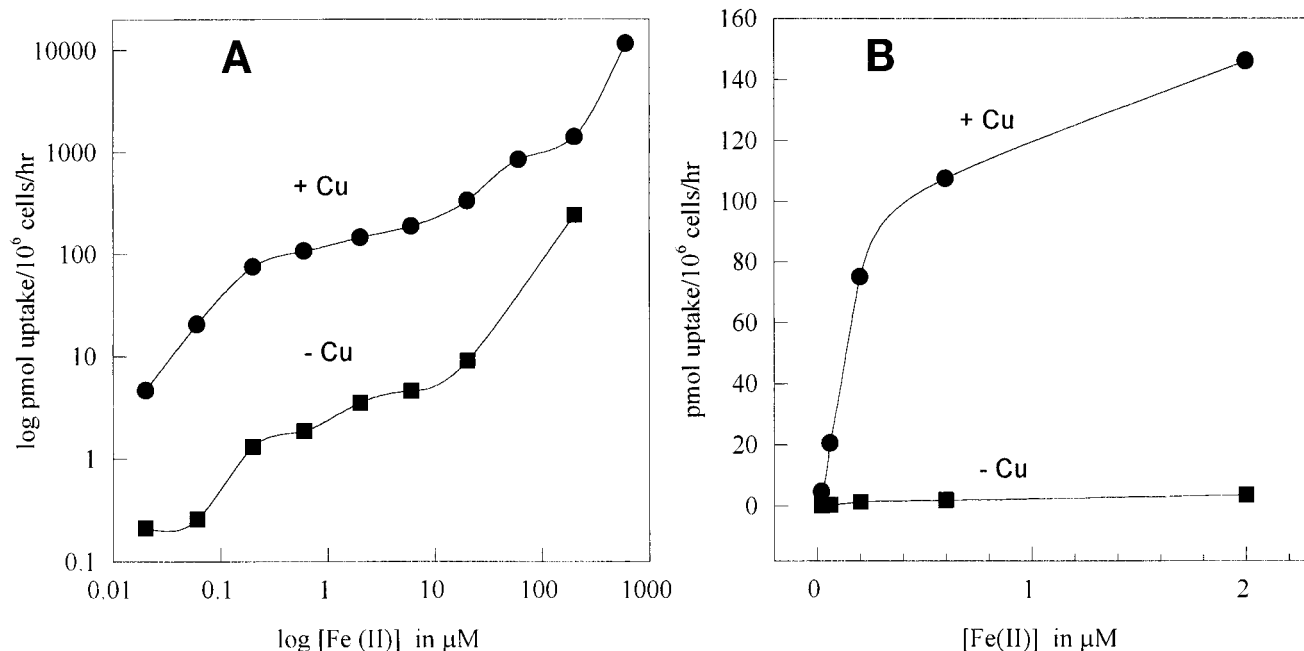


FIG. 4. Effect of culture copper deprivation upon Fe(II) uptake. Cells were grown in LIM containing 1.6  $\mu\text{M}$   $\text{CuSO}_4$  (+Cu) or in LIM formulated without  $\text{CuSO}_4$  (-Cu). (A) Double-logarithm plot. (B) Arithmetic plot of low-concentration data.

containing oxidases may be widely required for eukaryotic iron transport (15, 26). Nascent Fe(II) released from the cell membrane ferric reductase is presumed to be reoxidized by the copper-containing protein at the time of transport into the cell; thus, the main uptake pathway may perhaps be called "reductive/oxidative." We assayed Fe(II) uptake in copper-starved *C. neoformans* cultures. Cryptococcal LIM normally contains 1.6  $\mu\text{M}$  Cu(II). When this copper was not supplied, 24-h cultures reached optical densities 10% lower than those of cultures containing 1.6  $\mu\text{M}$  Cu(II). Under these conditions, the high-affinity system was 90% suppressed, as shown in Fig. 4 (uptake was studied once at a range of concentrations; the result was confirmed by an uptake study performed in triplicate at 1 mM  $^{55}\text{Fe}$ ). Thus, it seems likely that a copper-containing factor is also required for high-affinity iron uptake in *C. neoformans*.

**Specificity for Fe(II) of the iron uptake step.** Iron assimilation (iron-dependent growth) in *C. neoformans* appears to require the reduction of Fe(III) to Fe(II) as a first step (13, 22, 27). We defined two main mechanisms for the reduction of extracellular Fe(III) prior to uptake. The first is a plasma membrane ferric reductase, and the second consists of secreted ferric reductants, one of which has been identified as 3-hydroxyanthranilate. Expression of the enzyme and secretion of the reductant are both down regulated by environmental iron, a finding which supports the above hypothesis (22). In our uptake assays, we usually fixed iron in the Fe(II) state with the exogenous reducing agents ascorbic acid and DTT. We tested whether reduction is required for physiologic iron uptake. When a 24-h growth-phase culture was assayed for the uptake of 1  $\mu\text{M}$  Fe(III) without the exogenous reductants DTT and ascorbic acid but still in the presence of its culture supernatants, the uptake rate was only slightly lower than that in the presence of the exogenous reductants (no reductants,  $118 \pm 26$  pmol/10<sup>6</sup> cells/h; reductants,  $144 \pm 38$ ; three experiments were performed in triplicate;  $P = 0.3$ , not significant). However, washing the cells twice in fresh LIM resulted in a 46% decrease

in iron uptake, presumably by the removal of secreted 3-hydroxyanthranilate (washed, no exogenous reductants,  $60 \pm 16$  pmol/10<sup>6</sup> cells/h; exogenous reductants,  $111 \pm 8$ ; three experiments were performed in triplicate;  $P = 0.05$ ). The substantial remaining uptake activity presumably depended upon the reduction of extracellular Fe(III) by the cell membrane ferric reductase (22). This hypothesis was tested in further experiments with washed cells without exogenous reductants (Table 1, experiment A). Bathophenanthroline disulfonate (BPDS) (1 mM), an avid chelator of Fe(II), was found to abolish iron uptake quantitatively; the mild oxidant potassium nitrosodisulfonate (1 mM) inhibited the uptake of iron by 94%, although at this concentration it did not inhibit growth. Thus, reduction of Fe(III) to Fe(II) appeared to be essential for high-affinity uptake. Interestingly, the velocities at which physiologic reduction and uptake occur are very different, for the reduction of Fe(III) can occur in a burst at 60 nmol/10<sup>6</sup> cells/h (23) while the maximum velocity for Fe(II) uptake is 120 pmol/10<sup>6</sup> cells/h, about 500-fold lower. Thus, it is possible for Fe(II) to accumulate in the extracellular fluid, where it may represent a pool of reducing

TABLE 1. Iron uptake in the presence of various reagents<sup>a</sup>

Expt	Reagent	Uptake, pmol/10 <sup>6</sup> cells/h (mean $\pm$ SD)	<i>P</i> (vs no reagent)
A	None	128 $\pm$ 32	
	BPDS (1 mM)	0 $\pm$ 0	<0.01
	(KSO <sub>3</sub> ) <sub>2</sub> NO (1 mM) <sup>b</sup>	8 $\pm$ 1	<0.01
B	None	92 $\pm$ 15	
	DFO <sup>c</sup> (1 $\mu\text{M}$ )	188 $\pm$ 14	<0.01
	DFO (1 $\mu\text{M}$ ) and Pt (5.6 $\mu\text{M}$ )	104 $\pm$ 15	0.23

<sup>a</sup> Uptake was studied with LIM containing 1  $\mu\text{M}$  Fe(II). Data represent two experiments performed in triplicate.

<sup>b</sup> Potassium nitrosodisulfonate (Fremy's salt).

<sup>c</sup> DFO, deferoxamine.

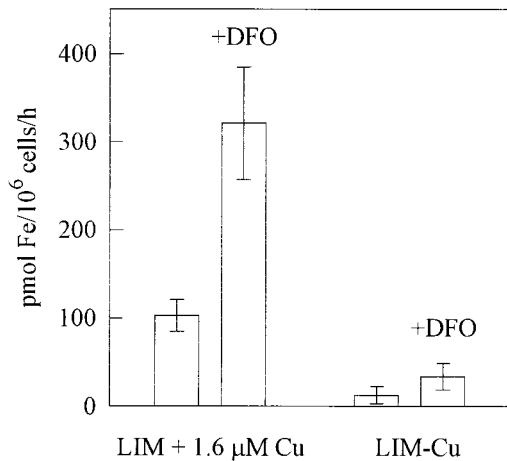


FIG. 5. Stimulation of iron uptake by 1  $\mu$ M deferoxamine (DFO). Cultures grown in LIM with and without (Lim-Cu) 1.6  $\mu$ M CuSO<sub>4</sub> were washed and assayed for iron uptake without exogenous reductants. The results are averages of two experiments performed in triplicate. Error bars show standard deviations.

equivalents available for electrochemical work, such as the reduction of melanin (11).

**Stimulation of iron uptake by deferoxamine.** Although *C. neoformans* does not secrete siderophores, its response to them is of considerable interest. We have observed that deferoxamine relieves iron starvation caused by the avid synthetic chelator ethylenediaminedi(*o*-hydroxyphenylacetic acid) (12), while others have found deferoxamine to exacerbate experimental cryptococcosis in the guinea pig (1). Thus, *C. neoformans* behaves as though it expresses a receptor for Fe(III)-deferoxamine. We tested the effect of 1  $\mu$ M deferoxamine mesylate (added 10 min prior to the experiment) upon the uptake of Fe(III) by 24-h growth-phase cultures of *C. neoformans*. The experiment was performed without exogenous reductants because deferoxamine is specific for Fe(III). The threefold stimulation observed (Fig. 5) did not seem attributable to the nonspecific solubilization of Fe(III) by deferoxamine, because iron was always used with equimolar HEDTA, a synthetic iron chelator and solubilizer. We tested whether deferoxamine-stimulated uptake was independent of the copper-dependent uptake pathway but found that deferoxamine-stimulated uptake was 90% abolished by growth without copper (Fig. 5). Thus, deferoxamine-mediated uptake apparently proceeds via the copper-dependent uptake pathway.

We also considered the relationship of deferoxamine to the iron reduction step. The fate of Fe(III) in the *S. cerevisiae* uptake model is believed to be reduction to Fe(II) by the cell membrane ferric reductase, followed by reoxidation by the copper-containing uptake-linked oxidase during transport (4, 15, 26). Assuming an equivalent pathway in *C. neoformans*, Fe(III) complexed by deferoxamine may enter the uptake pathway either prior to the reductive step or following reoxidation. Since the ferric reductase is subject to inhibition by Pt(II)Cl<sub>2</sub> (6), inhibition of deferoxamine-mediated iron uptake by Pt(II) would suggest that deferoxamine does not allow Fe(III) to bypass the reductive step, while the maintenance of deferoxamine-stimulated uptake in the presence of Pt inhibition of the reductase would suggest the donation of Fe(III) by deferoxamine after the copper-mediated oxidase step. We found approximately 80% inhibition of the cryptococcal ferric reductase by 4  $\mu$ M Pt(II); when we performed uptake experiments with 5.6  $\mu$ M Pt(II), deferoxamine-stimulated uptake was inhibited by Pt (Table 1, experiment B). This result suggests that the

input of Fe(III) by deferoxamine precedes the enzyme-mediated ferric reduction step. Moreover, because deferoxamine-stimulated iron uptake is abolished by copper starvation (see previous paragraph), it is doubly unlikely that the Fe(III)-deferoxamine complex bypasses the copper-dependent oxidase step. Thus, a deferoxamine receptor protein may be associated with the plasma membrane ferric reductase. Alternatively, deferoxamine may simply be more efficient than FeHEDTA at presenting Fe(III) to the ferric reductase. The deferoxamine stimulation of iron uptake that we observed with *C. neoformans* is similar to observations made with *Rhizopus* species, which cause opportunistic infections in deferoxamine-treated patients (1). It is not clear why cases of deferoxamine-associated cryptococcosis have not likewise been reported.

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