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Evidence for a Common Siderophore Transport System but Different Siderophore Receptors in *Neurospora crassa*

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Uptake and competition experiments were performed with *Neurospora crassa* and *Penicillium parvum* by using ^{14}C-labeled coprogen and ^{55}Fe-labeled ferrichrome-type siderophores. Several siderophores of the ferrichrome family, such as ferrichrome, ferricrocin, ferrichrysin, and tetraglycyl-ferrichrome as well as the semisynthetic ferricrocin derivatives *O*-(phenyl-carbamoyl)-ferricrocin and *O*-(sulfanilyl-carbamoyl)-ferricrocin were taken up by *N. crassa*. The ferrichrome-type siderophores used vary in the structure of the peptide backbone but possess a common Λ-*cis* configuration about the iron center and three identical ornithyl-δ-*N*-acetyl groups as surrounding residues. This suggests that these ferrichrome-type siderophores are recognized by a common ferrichrome receptor. We also concluded that the ferrichrome receptor is λ-*cis* specific from the inability to take up the synthetic enantiomers, *enantio*-ferrichrome and *enantio*-ferricrocin, possessing a Δ-*cis* configuration about the iron center. On the other hand, we found that coprogen, possessing a Δ-absolute configuration and two *trans*-anhydromevalonic acid residues around the metal center, was also taken up by *N. crassa* and was competitively inhibited by the ferrichrome-type siderophores. We therefore propose the existence of a common siderophore transport system but the presence of different siderophore receptors in *N. crassa*. In addition, ferrirubin, which is very slowly transported by *N. crassa*, inhibited both coprogen and ferrichrome-type siderophore transport. Contrary to the findings with *N. crassa*, transport experiments with *P. parvum* revealed the presence of a ferrichrome receptor but the absence of a coprogen receptor; coprogen was neither transported nor did it inhibit the ferrichrome transport.

It is well known that a variety of fungi synthesize more than one siderophore for iron acquisition. In *Neurospora crassa*, in addition to coprogen, which is the major siderophore of this fungus (12), several other siderophores are synthesized, among which the ferrichrome-type siderophore ferricrocin has been identified (10). *Aspergillaceae* family members are known to be a good source of various siderophores (4, 17, 23). Recently, a large number of asperchromes all belonging to the ferrichrome family were isolated from low-iron cultures of *Aspergillus ochraceus* (11). *Ustilago sphaerogena* produces ferrichrome and ferrichrome A (5). A variety of fungi and their siderophore products were listed in a recent review article (21). We have recently shown that *Neovossia indica* synthesizes ferrichrome, ferrichrome C, and the novel heptapeptide siderophore tetraglycyl-ferrichrome (2). Strains of *Fusarium* spp. are known to produce a variety of different non-ferrichrome-type siderophores such as fusigen, coprogen B, dimerum acid (3), and malonichrome (6). Although there is a great diversity among fungal siderophores, even in one strain, the production pattern within a certain genus may be highly characteristic. It has been reported that siderophore production is a useful trait in taxonomic studies of heterobasidiomycetes yeasts (1). Thus, among the smut fungi, the rhodotorulic acid-producing *Leucosporidiaceae* family members (*Leucosporidium* and *Rhodosporidium* organisms) can be differentiated from the ferrichrome-producing *Ustilaginaceae* (*Ustilago*, *Sphacelotheca*, *Cintractia*, *Farysia*, and *Sporisorium*) organisms and *Tilletiariaceae* family members (G. Deml, unpublished data). Also, the production of tetraglycyl-ferrichrome in *Tilletiales* organisms has been reported (2).

The large number of different siderophores among fungi has raised the question of whether the different siderophore structures require specific transport systems. Especially in light of recent findings that the transport of siderophores is highly stereospecific (19, 20), an analysis of the number of transport systems involved was of interest. It is now well established that siderophores adopt different optical and geometrical configurations about the iron center (16). Thus, the compounds of the ferrichrome family always possess a Λ-*cis* absolute configuration, whereas coprogen is Δ-*trans* (M. B. Hossain, M. A. F. Jalal, B. Benson, C. L. Barnes, and D. van der Helm, Abstr. Int. Conf. Coordination Chem., 29 July–3 August 1984, Boulder, Colo.). Although we have reported earlier that a competition between different siderophores may occur (18, 21), this behavior remained unexplained. Recent findings underline the importance of the coordination center and the surrounding hydroxamate acyl groups (2, 7). It is the intention of the present paper to analyze in detail the specificity of recognition and transport of siderophores in several fungi.

MATERIALS AND METHODS

Cultures and maintenance. Two fungi were used for kinetic measurements. *N. crassa* (*arg-5 ota aga*), which can be grown siderophore free if ornithine is omitted in the growth medium (19), was a gift from R. H. Davis, Irvine, Calif. The strain was maintained on YMG agar containing 4 g of yeast

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extract, 10 g of malt extract, and 4 g of glucose per liter. Conidiospores were harvested after 1 week of growth at 27°C with an NaCl solution (0.9%) containing 0.5% Tween 80. The conidiospores were washed twice with the NaCl solution by centrifugation. Packed spores (0.2 ml) were used to inoculate 100 ml of a chemically defined low-iron medium containing 5 g of L-asparagine, 1 g of $K_2HPO_4 \cdot 3\ H_2O$, 1 g of $MgSO_4 \cdot 7\ H_2O$, 0.5 g of $CaCl_2 \cdot 2\ H_2O$, 20 mg of $ZnSO_4 \cdot 7\ H_2O$, 0.01 mg of biotin, 50 mg of L-arginine, 100 mg of putrescine, and 1 liter of distilled water (pH 6). Glucose (2%) was added after separate sterilization. The spores were incubated at 27°C in a rotary shaker at 120 rpm for ca. 8 h. The length of the germination tubes at that time was about 50 to 100 μm. Larger mycelia were removed by filtration through two layers of gauze. The resulting mycelial suspension was used for kinetic measurements.

Penicillium parvum Raper and Fennell CBS 359.48 was obtained from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. The fungus was grown on YMG agar, and the spores were harvested and incubated in a chemically defined medium as described for *N. crassa*. The mycelia were used for kinetic measurements after 24 h of incubation at 27°C on a rotary shaker. The following fungi were used for siderophore production: *N. crassa* FGSC 74 A (Fungal Genetic Stock Center, Arcata, Calif.), *Fusarium dimerum* Penz var. pusillum CBS 254.50, and *Penicillium variabile* Sopp CBS 385.48. *Neovossia indica* GD 717 was from the Botanical Institute, University of Tübingen, Tübingen, Federal Republic of Germany.

Siderophores and derivatives. Ferrichrome, ferricrocin, ferrichrysin, and tetraglycyl-ferrichrome were from the stock of the institute. *Enantio*-ferrichrome, *O*-(phenyl-carbamoyl)-ferricrocin, and *O*-(sulfanilyl-carbamoyl)-ferricrocin were synthesized by H. U. Naegeli, and *enantio*-ferricrocin was synthesized by H. Leuenberger-Ryf. These compounds were kindly provided by W. Keller-Schierlein, ETH Zürich, Zürich, Switzerland.

Coprogen was isolated from low-iron cultures of *N. crassa* wild-type strain 74 A and purified as previously described (22). Coprogen B was obtained from low-iron cultures of *Fusarium dimerum* Penz var. pusillum (3). Ferric coprogen B was prepared by adsorption on Servachrome XAD-2 (250 μm) and purified on silica gel with methanol-water-acetic acid (6:2:0.1) as an eluting solvent. Ferrirubin was isolated from low-iron cultures of *Penicillium variabile* by adsorption on Servachrome XAD-2 and purification on silica gel columns with chloroform-methanol (2:1) as an eluting solvent.

Radioactive ^{14}C-labeled coprogen was prepared by *N*-acetylation of coprogen B (desacetylcoprogen) with [^{14}C]acetic anhydride (Amersham-Buchler, Braunschweig, Federal Republic of Germany). Coprogen B (10 μmol) was incubated with equimolar amounts of [1-^{14}C]acetic anhydride in water-free methanol at room temperature for ca. 48 h. After adding 10 ml of distilled water, the solution was heated to 60°C to remove unreacted anhydride, and the total siderophores were subsequently adsorbed on Servachrome XAD-2, washed with 5 volumes of distilled water, and desorbed with 1 volume of methanol. Unreacted coprogen B was removed by passage through a small column of CM-Sephadex 25 (Deutsche Pharmacia, Freiburg, Federal Republic of Germany) with 0.01 M ammonium acetate (pH 5.5) as an eluting buffer. The ^{14}C-labeled coprogen revealed a specific activity of 33.3 μCi/μmol.

^{55}Fe-labeled siderophores were prepared as described earlier (17) with $^{55}FeCl_3$ in 0.1 M HCl purchased from Radiochemical Centre, Amersham, England.

Analytical methods. The concentration of the siderophores was determined spectrophotometrically with known extinction coefficients as described previously (16, 17, 22). The purity of siderophores was analyzed by thin-layer chromatography on Silica Gel 60 (E. Merck AG, Darmstadt, Federal Republic of Germany) with chloroform-methanol-water (65:25:4). Desferri-siderophores were prepared by the hydroxyquinoline method as described earlier (17). Uptake kinetics were performed with ^{14}C-labeled coprogen and ^{55}Fe-labeled siderophores. For time-dependent uptake measurements, 20 ml of mycelial suspension was incubated with 20 μM labeled siderophores. Samples of 1 ml were taken at intervals, filtered through membrane filters (cellulose nitrate [8.0 μm]; Sartorius, Göttingen, Federal Republic of Germany) and counted in a liquid scintillation counter. Concentration-dependent uptake was carried out as previously described (19).

RESULTS

Three different types of siderophores are shown in Fig. 1, which have been used in the present investigation to analyze the specificity of siderophore recognition and transport in *N. crassa*. The first siderophore structure (Fig. 1A) shows the characteristic features of the ferrichrome-type compounds. The typical ferrichrome-type siderophore (cyclohexapeptide) contains a constant tripeptide sequence ($-Orn_1-Orn_2-Orn_3-$) and a further tripeptide sequence ($-Gly_1-Gly_2-Gly_3-$) in which Gly_1 and Gly_2 may be replaced by Ser or Ala, whereas Gly_3 seems to be a necessary constituent for the β (II) turn. Although different ornithyl-*N*-acyl groups may occur, only *N. crassa* transports those ferrichrome-type compounds which possess ornithyl-*N*-acetyl residues, such as ferrichrome, ferricrocin, ferrichrysin, or tetraglycyl-ferrichrome, which are here collectively called ferrichromes.

Figure 1B shows the mirror image of the ferrichrome-type siderophores, the enantiomeric ferrichromes, such as *enantio*-ferrichrome or *enantio*-ferricrocin, both of which have been synthesized from D-ornithine and glycine or D-serine. Consequently, the enantiomeric ferrichromes adopt a Δ-*cis* configuration about the metal center.

Figure 1D represents coprogen, which is a linear siderophore, possessing a predominantly Δ-absolute configuration about the metal center. Contrary to the ferrichromes, in which geometrical isomers are improbable, due to steric constraints, coprogen may theoretically exist as *trans*- and *cis*-geometrical isomers in solution. X-ray diffraction studies of neocoprogen, however, revealed a Δ-*trans* configuration (Hossain et al., Abstr. Int. Conf. Coordination Chem., 1984).

The two structurally very different siderophores of *N. crassa*, coprogen and ferricrocin, are transported with nearly identical uptake rates after labeling with ^{55}Fe (Fig. 2). Both siderophores are synthesized by *N. crassa* in a low-iron culture and may be regarded as species-specific siderophores. The two synthetic siderophores, *enantio*-ferrichrome and *enantio*-ferricrocin, however, were not taken up as ^{55}Fe-labeled chelates, indicating that the reversed chirality prevents recognition and transport. As has been shown earlier, uptake of other ferrichrome-type siderophores is also possible in *N. crassa*, provided the metal center possesses a Λ-*cis* configuration and three ornithyl-*N*-acetyl groups as iron-surrounding residues.

Figure 3 shows that the two ^{55}Fe-labeled ferricrocin derivatives (Fig. 1C), *O*-(phenyl-carbamoyl)-ferricrocin and *O*-(sulfanilyl-carbamoyl)-ferricrocin, produce a slightly re-

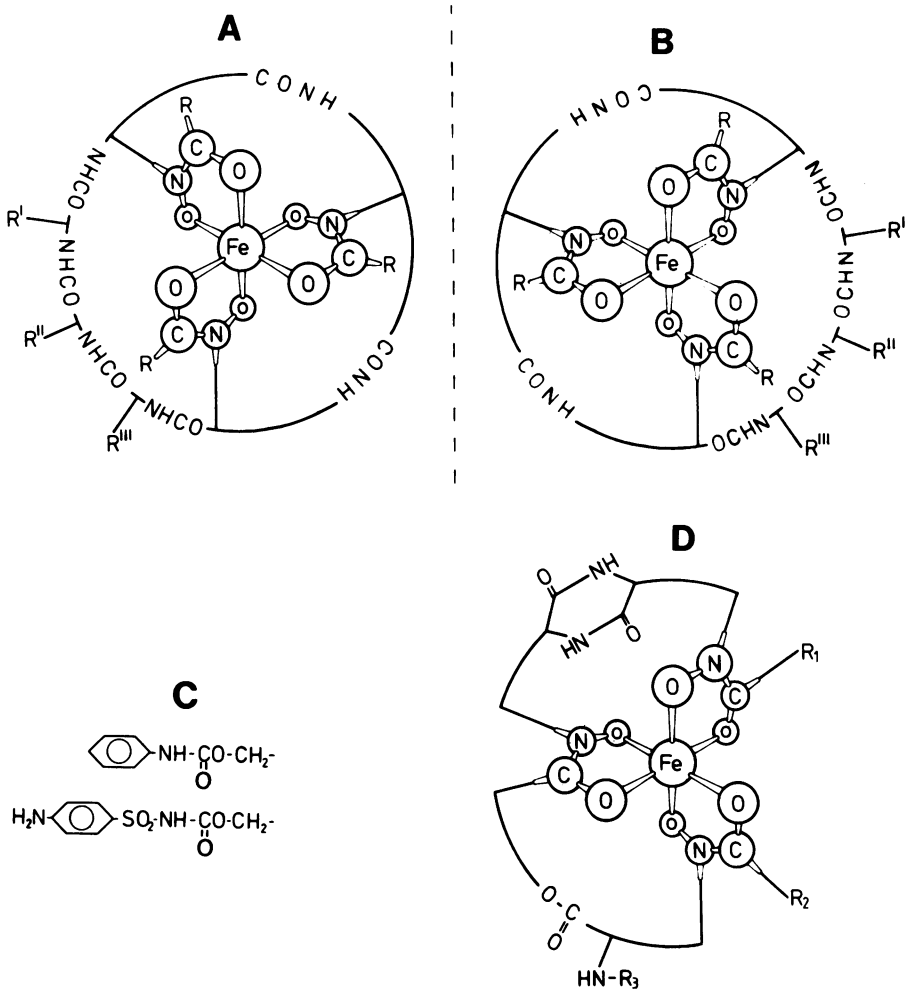

FIG. 1. (A) Schematic structure of ferrichrome-type siderophores showing the λ-*cis* configuration about the iron center and three ornithyl-*N*-acyl groups as iron-surrounding residues. Ferrichrome: R = —CH_3, R′ = R″ = R‴ = —H; ferricrocin: R = —CH_3, R′ = R‴ = —H, R″ = —CH_2OH; ferrichrysin: R = —CH_3, R′ = —H, R″ = R‴ = —CH_2OH; ferrirubin: R = —CH=$C(CH_3)$—CH_2—CH_2OH(*trans*), R′ = —H, R″ = R‴ = —CH_2OH. (B) Schematic structure of the enantiomeric ferrichromes synthesized from D-ornithine and glycine or D-serine, showing a Δ-*cis* configuration about the iron center. R, R′, R″, and R‴ are assigned as in (A). (C) Ferricrocin derivatives. *O*-(phenyl-carbamoyl)-ferricrocin: R″ = —CH_2-O—CO—NH—Ph; *O*-(sulfanilyl-carbamoyl)-ferricrocin: R″ = —CH_2-O—CO—NH—SO_2—Phe—NH_2. (D) Schematic structure of coprogen-type siderophores containing a Δ-*trans* configuration about the iron center. Coprogen B: R_1 = R_2 = —CH=$C(CH_3)$—CH_2—CH_2OH (*trans*), R_3 = H; coprogen: R_1 = R_2 = —CH=$C(CH_3)$—CH_2—CH_2OH(*trans*), R_3 = —CO—CH_3.

duced uptake compared with ^{55}Fe-labeled ferricrocin. However, ^{55}Fe-labeled ferrirubin is quite ineffective as a siderophore, indicating that alterations of the iron-surrounding residues affect the transport of ferrichrome-type siderophores more seriously than structural alterations of the peptide backbone.

Although coprogen and ferrichrome-type compounds are different in structure and configuration, we observed competition among the different types during transport in *N. crassa*. The Lineweaver-Burk plot (Fig. 4) shows that ^{14}C-labeled coprogen uptake is competitively inhibited in the presence of 5 μM and 10 μM ferricrocin. A similar competitive inhibition of coprogen uptake is also observed with ferrichrome, ferrichrysin, and tetraglycyl-ferrichrome. A determination of the inhibition constants by Dixon plots yields the following K_i values: ferrichrome K_i = 1.0 μM, ferricrocin K_i = 3.0, coprogen K_i = 3.0, and tetraglycyl-ferrichrome K_i = 8.0. The narrow range of K_i values corresponds well with the K_m values for the transport of the ^{55}Fe-labeled siderophores (5 μM). On the other hand, uptake of ^{55}Fe-labeled ferrirubin and inhibition of [^{14}C]coprogen uptake by ferrirubin yields K_m values of 0.05 μM and K_i values of 0.07 μM, which differ by a factor of 100 from the values for ferrichrome-type compounds. Furthermore, ferrirubin also inhibits the transport of the ferrichrome-type siderophores (Table 1).

However, inhibition of coprogen uptake by the ferrichromes as well as by ferrirubin is generally dependent on the transport rates of the inhibitor itself, suggesting a shared transport component for all siderophores. Uptake of [^{14}C]coprogen (20 μM) is decreased in the presence of increasing amounts of unlabeled ferrichrome, ferricrocin, and ferrichrysin (Fig. 5). The addition of *enantio*-ferricrocin, however, exhibits no effect on coprogen uptake, indicating that a Δ-*cis* configuration is recognized neither by the ferrichrome receptor nor by the coprogen receptor.

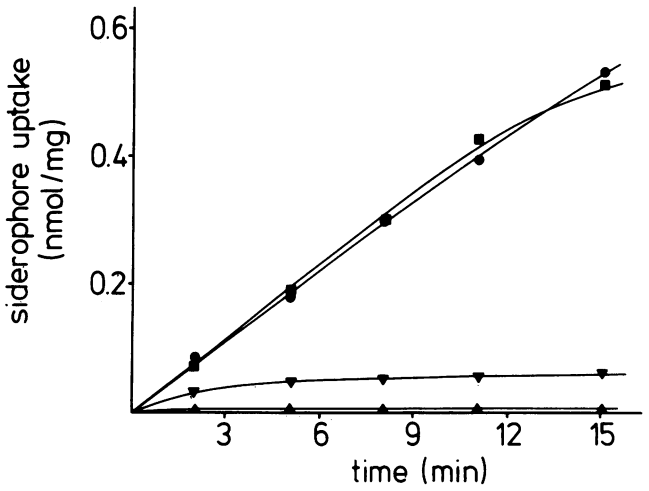

FIG. 2. Time-dependent uptake of siderophores by *N. crassa* (*arg-5 ota aga*). Ferricrocin (●), coprogen (■), *enantio*-ferricrocin (▼), and *enantio*-ferrichrome (▲) were used. ^{55}Fe-labeled siderophores (20 μM) were incubated with young mycelia and samples were taken at intervals, filtered, and counted as described in the text.

To determine whether or not the expression of different siderophore receptors is a general phenomenon among fungi, we analyzed the transport of ferrichrome as a representative of the ferrichrome-type compounds and also the transport of coprogen in the fungus *P. parvum*. The results (Fig. 6) clearly demonstrate that ^{55}Fe-labeled ferrichrome is continuously taken up by low-iron mycelia of *P. parvum*, whereas ^{55}Fe-labeled coprogen, apart from a certain degree of unspecific adsorption, is not taken up at all. These results suggest that, in *P. parvum*, a coprogen receptor is lacking. Consequently, no competition is observed between [^{55}Fe]ferrichrome and increasing amounts of coprogen. These results confirm the view that the ferrichrome receptor is structurally different from the coprogen receptor.

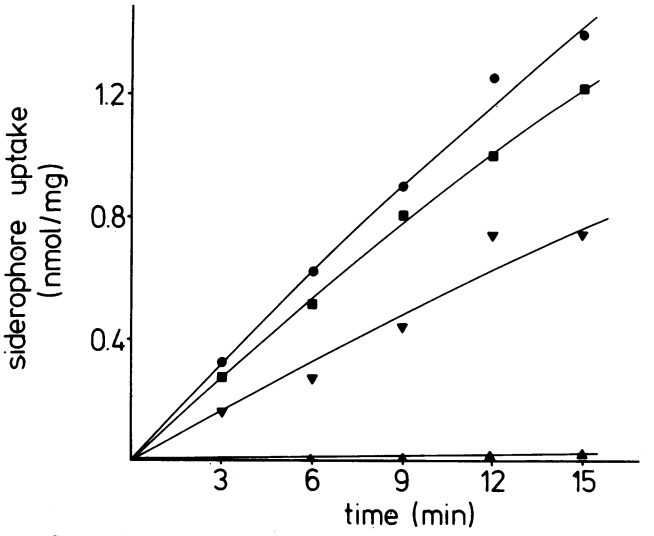

FIG. 3. Time-dependent uptake of a natural ferrichrome-type siderophore (ferricrocin), two semisynthetic ferrichrome-type siderophores possessing structural alterations of the peptide backbone [*O*-(phenyl-carbamoyl)-ferricrocin and *O*-(sulfanilyl-carbamoyl)-ferricrocin], and ferrirubin as an example of a natural ferrichrome-type siderophore possessing structural alterations at the hydroxamate acyl function. Uptake was followed after adding ^{55}Fe-labeled siderophores (20 μM) to a mycelial suspension of *N. crassa* (*arg-5 ota aga*). Ferricrocin (●), *O*-(phenyl-carbamoyl)-ferricrocin (■), *O*-(sulfanilyl-carbamoyl)-ferricrocin (▼), and ferribruin (▲) were used. Conditions were as described in the text.

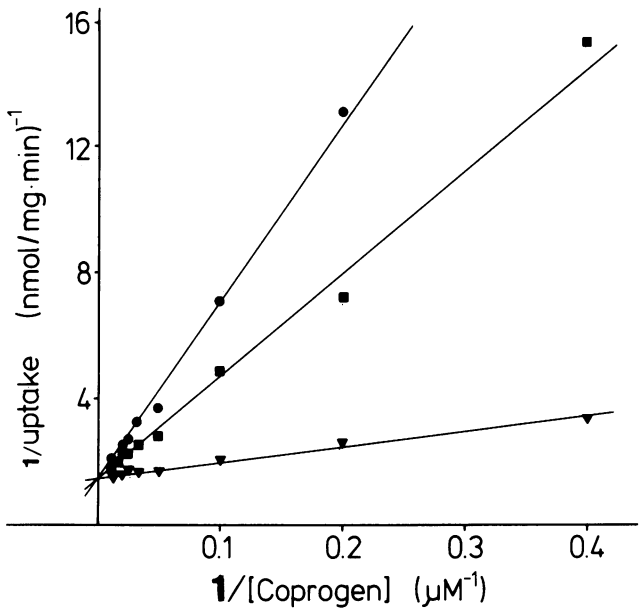

FIG. 4. Lineweaver-Burk plot of coprogen uptake by *N. crassa* (*arg-5 ota aga*) in the presence of ferricrocin as a competitive inhibitor. Concentration-dependent uptake of ^{14}C-labeled coprogen was followed without inhibitor (▼) and in the presence of 5 μM ferricrocin (■) or 10 μM ferricrocin (●).

DISCUSSION

The results of the present investigation indicate that two different siderophore receptors are required in *N. crassa*, which recognize structure and conformation of siderophores (Fig. 7). One receptor recognizes the ferrichrome-type siderophores possessing a λ-*cis* configuration about the iron center and three ornithyl-δ-*N*-acetyl groups as surrounding

TABLE 1. Transport inhibition of ferrichrome-type siderophores in the presence of ferrirubin[a]

Siderophores	Preincubation with ferrirubin	Uptake (pmol mg^{-1})
^{55}Fe-ferrichrome	−	105
^{55}Fe-ferrichrome	+	3
^{55}Fe-ferricrocin	−	95
^{55}Fe-ferricrocin	+	3
^{55}Fe-ferrichrysin	−	95
^{55}Fe-ferrichrysin	+	2
^{55}Fe-coprogen	−	82
^{55}Fe-coprogen	+	2

[a] Mycelia of *N. crassa* (*arg-5 ota aga*) grown for ca. 8 h in low-iron culture at 27°C were incubated with ^{55}Fe-labeled siderophores (10 μM) without any pretreatment (−) and with 3 min of preincubation with unlabeled ferrirubin (10 μM) (+). After 5 min of incubation, the mycelia were filtered, washed, and counted as described in the text. Uptake was calculated as picomoles per milligram (dry weight).

residues as found in ferrichrome, ferricrocin, ferrichrysin, tetraglycyl-ferrichrome, and their derivatives. We have shown earlier that the synthetic enantiomer of ferrichrome was not taken up by *N. crassa* and *P. parvum* (19, 20), indicating that ferrichrome uptake in these fungi is stereospecific. Based on the additional experiments with *enantio*-ferricrocin, it appears that the D configuration of the (-ornithyl-δ-*N*-hydroxy-δ-*N*-acetyl-)$_3$ and the resulting Δ-*cis*-absolute configuration about the iron center prevents recognition of all enantiomeric ferrichrome-type siderophores, suggesting that the receptor is a Λ-*cis*-specific receptor. As demonstrated here by the use of different ferrichrome-type siderophores and the two semisynthetic ferricrocin derivatives, alterations of the peptide backbone of the ferrichrome-type siderophores does not seriously affect the transport rates, indicating that not the peptide backbone but rather the metal center and its surrounding residues are the characteristic features for recognition by the receptors. Because of the identical recognition site of the various ferrichrome-type compounds, we conclude that a single Λ-*cis*-specific receptor exists in *N. crassa* and probably in other *Aspergillaceae* organisms.

The second receptor in *N. crassa* recognizes coprogen, which differs from the ferrichromes both in structure and absolute configuration of the metal center. Although we have shown earlier that coprogen adopts a Δ-absolute configuration about the metal center (22), the geometrical configuration has not been determined until now. Recent X-ray diffraction studies revealed that crystals of *neo*-coprogen possess a Δ-*trans* configuration (Hossain et al., Abstr. Int. Conf. Coordination Chem., 1984), indicating that in solution the Δ-*trans* isomer is one of the isomeric species. Since we cannot find any inhibition of the [^{14}C]coprogen transport in the presence of Δ-*cis*-*enantio*-ferrichrome or Δ-*cis*-*enantio*-ferricrocin, a recognition of a Δ-*cis* coprogen isomer seem to be improbable, suggesting that the coprogen receptor is Δ-*trans* specific. The additional experiments with *P. parvum* also indicate that the coprogen receptor is structurally different from the ferrichrome receptor. If the assumption of different siderophore receptors in *N. crassa* is correct, the competitive inhibition of [^{14}C]coprogen by ferrichrome-type compounds indicates the presence of a shared siderophore transport system in *N. crassa* (Fig. 7). Coprogen and ferricrocin inhibited the transport of each other. However, inhibition of [^{14}C]coprogen transport by ferricrocin was ca. 70% when equimolar concentrations were tested, suggesting a preferential transport by the ferrichrome route.

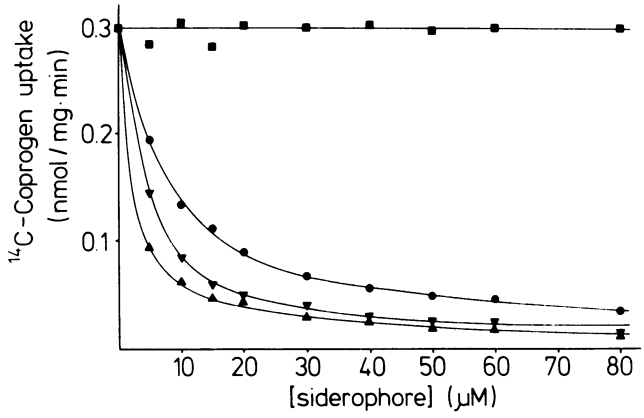

FIG. 5. Uptake inhibition of [^{14}C]coprogen (20 μM) in the presence of ferrichrome-type siderophores and *enantio*-ferricrocin. Concentration range was from 5 to 80 μM. The siderophores were added simultaneously at time zero to a mycelial suspension of *N. crassa* (*arg-5 ota aga*). After incubation for 3 min, the mycelia were filtered off, and the radioactivity was counted as described in the text. *Enantio*-ferricrocin (■), ferrichrysin (●), ferricrocin (▼), and ferrichrome (▲) were used.

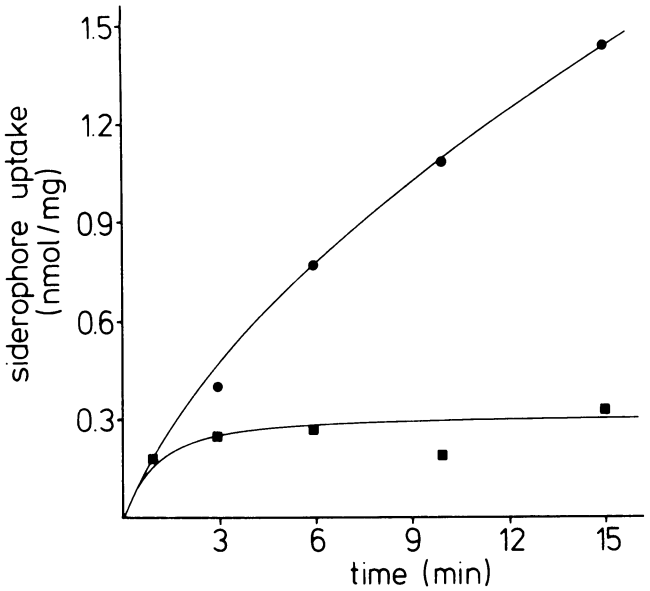

FIG. 6. Time-dependent uptake of ferrichrome and coprogen by *P. parvum*. ^{55}Fe-labeled siderophores (20 μM) were added to a suspension of young mycelia (24 h), and the radioactivity taken up was followed from samples taken at intervals. Ferrichrome (●) and coprogen (■) were used.

The fact that ferrirubin inhibits the transport of both coprogen and ferrichrome-type compounds in *Neurospora* organisms would imply that ferrirubin does not inhibit by its affinity to any of the siderophore receptors but possibly interferes at the stage of translocation by a shared transport component within the membrane. It is probable that the common component would move more slowly after binding to ferrirubin and would thereby inhibit a rapid transport of other siderophores. Why ferrirubin is apparently a transport molecule in other fungi, such as *P. variabile*, which is a ferrirubin producer, is unknown, but it may be assumed that the structural requirements for a ferrirubin receptor are different from those for the ferrichrome receptor or for the coprogen receptor, giving rise to the existence of a ferrirubin receptor. Even when a ferrirubin receptor exists in *N. crassa*, the present investigation gives evidence that the transport of all siderophores proceeds via a shared siderophore transport system. The concept of a common siderophore transport system in the presence of different siderophore receptors resembles the situation described for gram-negative bacteria, wherein various hydroxamate siderophore receptors occur which are linked to a common *fhuB* gene product responsible for subsequent iron uptake to the interior (9). However, one has to be careful to construct an analogy between the fungal and bacterial systems. The receptors and the transport system of fungi are located in the cytoplasmic membrane, as has been shown by the use of the slime mutant of *N. crassa* (13). The bacterial receptors are located in the outer membrane, and the *fhuB* gene product seems to be a periplasmic protein rather than a protein of the

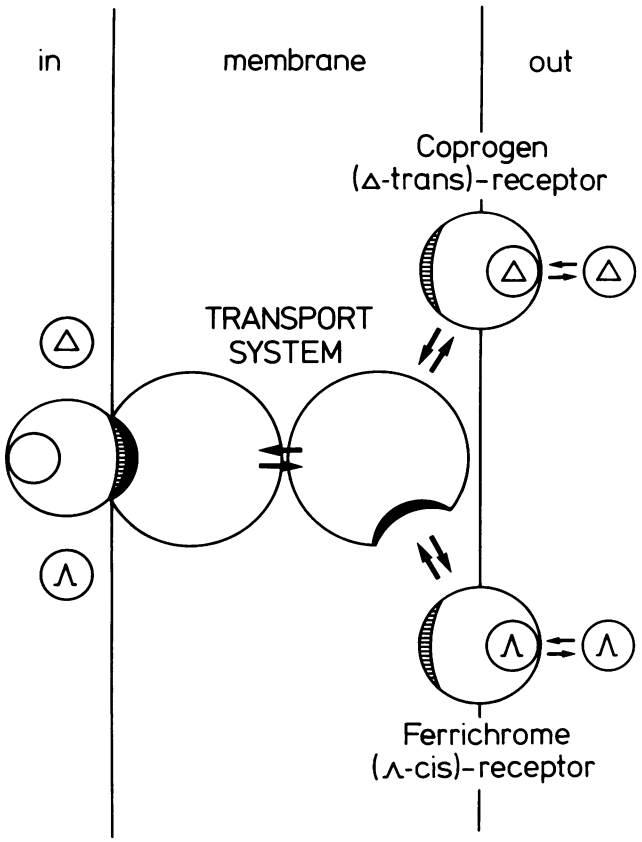

FIG. 7. Model of siderophore transport in *N. crassa* showing two different receptors and a shared transport system. It is assumed that the ferrichrome receptor recognizes ferrichrome-type compounds possessing a Λ-*cis* configuration and ornithyl-*N*-acetyl residues and that the coprogen receptor recognizes coprogen-type siderophores possessing a Δ-*trans* configuration and ornithyl-*N*-*trans*-anhydromevalonic acid residues.

inner cytoplasmic membrane, as it could not be isolated from the inner membrane (8) and was not involved in siderophore transport when inner membrane vesicles were tested (15). A further difference between fungal and bacterial systems can be seen when the specificity of the receptors is analyzed. Whereas the fungal receptors are highly specific and recognize the coordination center and its surrounding residues, the bacterial outer membrane receptors have been reported to recognize only the overall structure (8, 20).

Although the competition experiments point to a shared transport system in *N. crassa*, the postulated different receptors must not necessarily be separate entities. The kinetic results are also compatible with two receptors located on one protein. Thus, the principal statement of different receptors has to be interpreted in terms of different recognition sites, independent of their physical organization.

P. parvum was unable to transport coprogen, which may indicate a lack of a specific receptor or that subsequent transport components are lacking. As shown for ferrirubin, if translocation would be affected, inhibition or competition of coprogen transport by ferrichromes would result. Therefore, we assume that *P. parvum* does not synthesize a coprogen receptor, which makes sense as coprogen is not biosynthesized by this fungus.

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LITERATURE CITED

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