EVIDENCE FOR STEREOSPECIFIC UPTAKE OF IRON CHELATES IN FUNGI

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1. Introduction

Iron-chelating compounds containing hydroxamate groups (sideramines) are widely distributed among various fungi [1–3]. Structures and stability constants are known for several sideramines [4–6]

Iron transport properties of these compounds were studied using the corresponding producing strains as well as some non-producing strains [7,8]. Cells without the ability to biosynthesize hydroxamates were also found to utilize iron from these chelates [9].

The transport of chelate iron into fungal cells grown under iron-deficient conditions revealed saturation kinetics and competitive inhibition by other sideramines [7] Membrane and respiratory poisons severely impaired the whole uptake process. Recent studies gave evidence that chelate-iron uptake depends on binding to the cytoplasmic membrane [10] and iron release inside the cell [11,12].

In order to confirm the earlier postulated chelate–membrane relationship, the transport behaviour of ferrichrome and enantio-ferrichrome was studied (fig.1). Enantio-ferrichrome has recently been synthesized from D-ornithine and glycine [13]. This paper supports the view that in fungi such as *Neurospora crassa* and in *Aspergillus,* sideramines are taken up by stereospecific recognition of the chelate molecule.

2. Materials and methods

2.1. *Culture and growth conditions*

Neurospora crassa (*arg*-5, *ota, aga*) is an ornithine-free mutant, and was a gift from Rowland H. Davis, Irvine, CA. Under iron-deficient conditions and without addition of ornithine the mutant can be grown sideramine-free. That means that no desferri-sideramine synthesis can occur. Cultivation medium

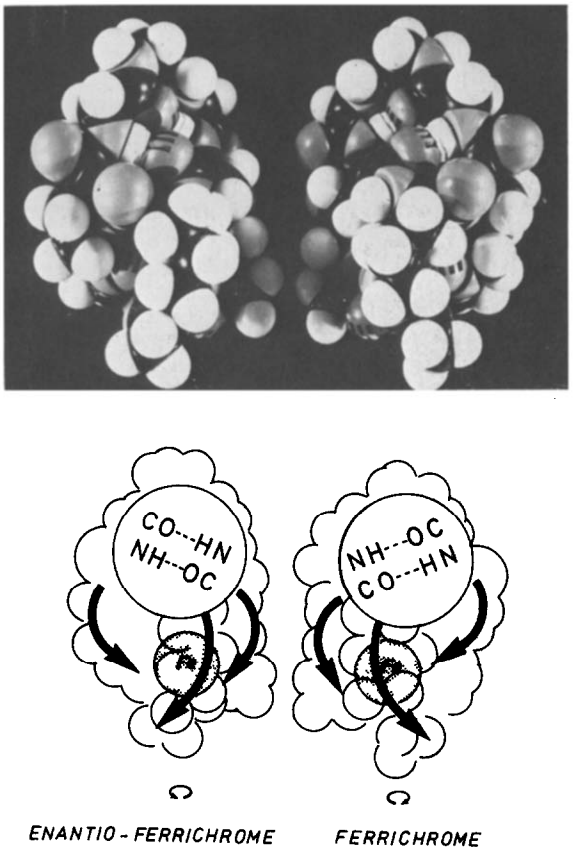

Fig.1. Models of the conformation of enantio-ferrichrome and ferrichrome showing the β-pleated sheet structure and the coordination array.

and growth conditions were essentially as in [11]. The medium was inoculated with freshly prepared conidiospores. Kinetic measurements were done with young mycelia after 16 h submerged cultivation at 27°C.

Aspergillus quadricinctus, E. Yuill, was obtained from the Centraalbureau voor schimmelcultures, Baarn. *A. quadricinctus* is a ferrichrome producer when grown under iron-deficient conditions [8]. Medium and growth conditions were as described for *N. crassa* (*arg*-5, *ota, aga*). Kinetic measurements were performed with young mycelia after 48 h incubation at 27°C.

2.2. *Iron chelates*

Enantio-ferrichrome was kindly provided by Professor Keller-Schierlein, Zurich. Ferrichrome was a gift from Professor J. B. Neilands, Berkeley. Preparation of ^{55}Fe-labelled chelates was carried out as in [8].

2.3. *Chemicals and radiochemicals*

Salts and medium constituents were from Merck, Darmstadt. $^{55}FeCl_3$ in 1 M HCl was from Amersham Buchler, Braunschweig. Unisolve I was obtained from Koch Light, Colnbrook, Bucks.

2.4. *Kinetic measurements*

For the saturation kinetics, increasing amounts of unlabelled ferrichrome and a constant amount of labelled ferrichrome were filled into glass vials on a waterbath shaker held at a constant 27°C. After adjusting to an equal volume with 0.9% NaCl solution, 1.9 ml mycelial suspension was added and vigorously shaken. After 10 min incubation the mycelia were filtered off and washed 3 times with 10 ml precooled 0.9% NaCl solution. The filters were counted after 24 h equilibration in a liquid scintillation counter (Mark I, Nuclear Chicago) using Unisolve I as a scintillation fluid.

3. Results

Using *Neurospora crassa arg*-5 *ota aga* no interference with alternative sideramines is possible, as this mutant can be grown completely sideramine-free. After 16 h submerged cultivation in a chemically-defined medium under iron-deficient conditions the mycelia were used for [^{55}Fe]ferrichrome and enantio-[^{55}Fe]ferrichrome uptake measurements (fig.2). Concentration-dependent uptake of these enantiomeric iron chelates revealed characteristic differences. Whereas the natural ferrichrome, containing L-ornithine, showed typical saturation kinetics, the enantio-ferrichrome, containing D-ornithine, showed linear uptake behaviour which indicated a diffusion-controlled uptake process.

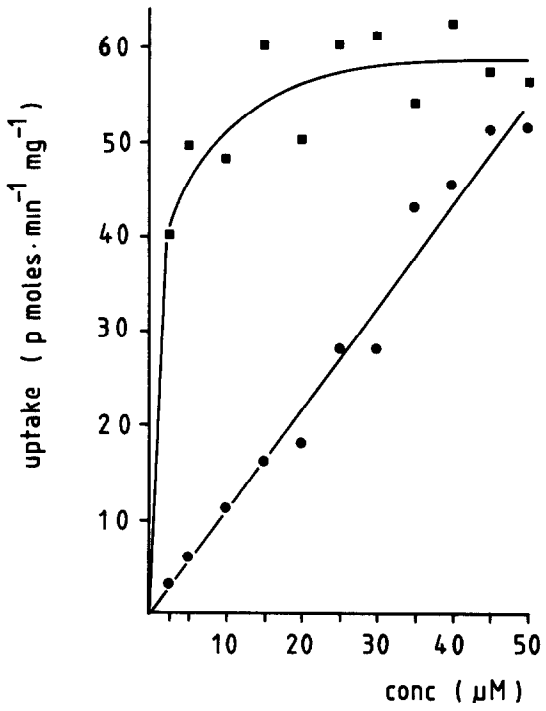

Fig.2. Concentration-dependent uptake of [^{55}Fe]ferrichrome (■) and enantio-[^{55}Fe]ferrichrome (●) by mycelia of *Neurospora crassa arg*-5 *ota aga*. The uptake assay contained increasing amounts of labelled chelate in an equal volume of 0.9% NaCl solution and was started by the addition of 1.9 ml mycelial suspension After 10 min incubation at 27°C the mycelia were filtered off and washed 3 times with ice-cold 0.9% NaCl solution. The radioactivity was counted after 24 h equilibration in a liquid scintillation counter.

Saturation during ferrichrome uptake occurred at 50 pmol min^{-1} mg^{-1}. This value is significantly lower than the corresponding values found for ferricrocin or coprogen which are normally 10-fold higher. With increasing concentration the uptake of enantio-

ferrichrome was as high as the uptake of the natural ferrichrome.

Aspergillus quadricinctus excretes desferri-ferrichrome when grown under iron-deficient conditions. Exogenously-supplied ferrichrome will always be mixed with the ferrichrome synthesized by the fungus itself. In addition, a slight iron exchange may occur when two ligands are present. This should be considered when the uptake of enantio-ferrichrome is studied on a ferrichrome-producing strain.

The uptake assay was similar to that used for *N crassa*. In *A. quadricinctus* [^{55}Fe]ferrichrome was taken up more efficiently than the corresponding enantiomeric form (fig.3). Saturation of ferrichrome

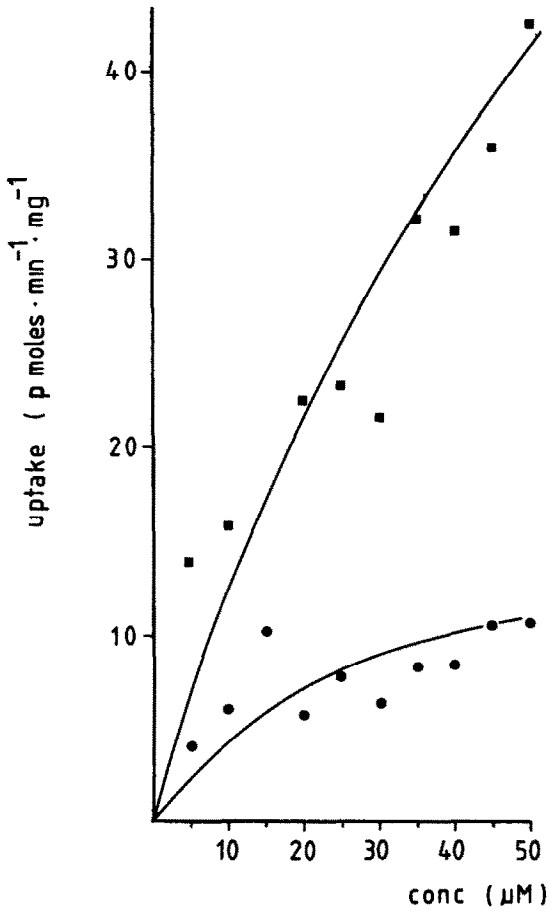

Fig.3. Concentration-dependent uptake of [^{55}Fe]ferrichrome (■) and enantio-[^{55}Fe]ferrichrome (●) by mycelia of *Aspergillus quadricinctus*. The uptake assay and conditions were as in fig.2.

uptake could not be achieved within the concentration range used in this experiment. A comparison of the uptake of both ferrichrome enantiomers clearly shows the superiority of the natural L-ornithine containing ferrichrome in a ferrichrome-producing strain. The shape of the enantio-ferrichrome uptake curve is, however, not linear. This may be the result of iron-exchange effects, which cannot be excluded using sideramine-producing organisms.

4. Discussion

This is the first report showing stereospecific uptake of iron chelates by microorganisms. Although in this investigation only the iron label was followed during uptake measurements, it can be assumed that a ligand label would lead to identical results. Earlier studies with double-labelled chelates support this assumption [11].

From spectroscopic data [13] it can be infered that ferrichrome and enantio-ferrichrome have an opposite conformation. The pronounced differences of iron chelate uptake are presumably not the result of unequal stabilities. The assumption that stereospecific reducing or hydrolyzing enzymes [12] are operative during iron-chelate uptake cannot be completely excluded. However, the K_m values of these enzymatic activities are much higher than the values reported for iron-chelate uptake [7]. The most probable explanation for the observed differences during uptake of enantiomeric ferrichromes in fungal membranes is a pronounced membrane chirality. From the work in [14,15] it is known that ion-transporting depsipeptides (e.g., the enniantins), are biologically active in both enantiomeric forms when studied for their antibiotic activity against various microorganisms. Furthermore, specific conformation properties are important for the interaction with specific complementary membrane receptors in mitochondria.

The conformation of ferrichrome has been well characterized both by X-ray [16] and PMR studies [17]. It has been shown that the peptide backbone has an anti-parallel β-pleated sheet structure as a result of two transannular hydrogen bonds (*orn*^{3}–NH - - - O = C-*gly*^{3} and *orn*^{3}–C = O - - - HN–*gly*^{3}). Thus the whole iron-containing molecule possesses a

compact globular structure allowing specific chelate–membrane interaction.

The present investigation shows that fungal membranes are able to discriminate between two enantiomeric forms of ferrichrome and probably between other sideramine conformations.

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