COORDINATION ISOMERS OF BIOLOGICAL IRON TRANSPORT COMPOUNDS

III. (1) TRANSPORT OF Λ-*CIS*-CHROMIC DESFERRIFERRICHROME BY *USTILAGO SPHAEROGENA*

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Received August 14,1974

Summary

Microbial iron transport studies of the structure and conformation dependent ferrichrome uptake system in *Ustilago sphaerogena* have been limited previously to kinetically labile metal ions such as the native ferrichrome complex and the aluminum(III) and gallium(III) analogs. Although two coordination isomers are possible (Λ-*cis* and Δ-*cis*), no information can be obtained concerning their biological activity using kinetically labile complexes. In this report, both the ligand and chromic ion moieties of kinetically inert Λ-*cis*-chromic [^{14}C]-desferriferrichrome are shown to be taken up in *Ustilago sphaerogena* at rates comparable to that of ferrichrome. The Λ-*cis* coordination isomer must be therefore at least one of the biologically active isomers and the transport system cannot rely on the rapid isomerization or dissociation of the labile ferric complex.

Introduction

Several microbial iron transport systems have been investigated in recent years (2). The elegant experiments of Emery (3) have outlined the ferrichrome transport system of *Ustilago sphaerogena*. Rapid cellular uptake of ferrichrome by *Ustilago* is followed by release of desferriferrichrome, which can then sequester additional extracellular iron and transport it into the cells (3). This active iron uptake system is structure and conformation dependent. Ferrichrome is a relatively specific iron transport agent for *Ustilago*; other siderochromes, including natural or synthetic hydroxamate analogs of ferrichrome, synthetic ferric chelating agents, and in particular, desferriferrichrome, are generally inactive (3, 4). However, other metal-substituted ferrichromes such as aluminum (III) and gallium(III) are transported, indicating that in this system the conformation of the metal complex is a primary factor in selective transport (3). Proton nmr data (5) and tritium exchange experiments (6) have indicated that

desferriferrichromes undergo a dramatic conformational change upon complexation of trivalent metal ions such as iron(III), aluminum(III), or gallium(III).

Two coordination isomers are possible for the ferrichromes: Λ-*cis* and Δ-*cis* (1a, 1b). No information can be obtained concerning the biological activity of coordination isomers of siderochromes with kinetically labile metal ions such as ferric, aluminum(III), or gallium(III) (7). Replacement of ferric ion by chromic ion induces kinetic inertness without introducing any structural changes. Thus, stable coordination isomers of chromic complexes can be isolated and studied.

Since microbial iron transport in such systems as *Ustilago sphaerogena* is highly conformation dependent, there is reason to postulate that specific coordination isomers are involved in iron transport. This paper will address itself to two questions: (i) Do specific isomers of chromic substituted complexes transport at rates comparable to ferrichrome? (ii) Does the cellular transport mechanism for ferrichrome rely on interconversion of isomers or rapid ligand exchange? We report here the transport of kinetically inert Λ-*cis*-chromic desferriferrichrome by *Ustilago sphaerogena*.

Experimental Procedures

Ferrichrome and chromic desferriferrichrome were obtained as described previously (1b). Ferrichrome labeled with [^{14}C] (*ca.* 10^5 cpm/μmole) was obtained by growth of *Ustilago* on medium containing [1-^{14}C]-glycine as described by Emery (8) with the following exception. [1-^{14}C]-glycine (0.1 mc, 11.5 mc/mmol) was added to 1 ℓ instead of 500 ml of a 48 hr culture of *Ustilago*. The iron was removed from [^{14}C]-ferrichrome with 8-hydroxyquinoline (9). Chromic desferriferrichrome labeled with [^{14}C] (*ca.* 5 x 10^4 cpm/μmole) was prepared as described above. A paper chromatogram of chromic [^{14}C]-desferriferrichrome, developed with n-butanol:acetic acid:water (4:1:1), was scanned with a Packard 7201 radiochromatogram strip scanner. All of the counts were found in the band of the chromic complex.

Uptake experiments were performed as described by Emery (3) using 30 ml of resuspended washed cells in 250 ml Erlenmeyer flasks. Approximately 3 ml aliquots of ferrichrome and chromic [^{14}C]-desferriferrichrome cell suspensions were removed at regular intervals, and treated as described earlier (3). The iron concentration in the ferrichrome supernatant was determined spectrophotometrically at 425 nm (ε = 2895 M^{-1} cm^{-1}) (6) with a Gilford-Beckman DU spectrophotometer, while the chromium concentration in the chromic [^{14}C]-desferriferrichrome supernatant was determined with a Perkin Elmer 303 atomic absorption spectrophotometer. The ligand concentration in the same chromic [^{14}C]-desferriferrichrome supernatant was determined by counting the radioactivity of 300 µl of supernatant with a Nuclear-Chicago Uni-Lux II counter using 3.3 ml of Bray's scintillation fluid (9). Under these conditions the counting efficiency for [^{14}C] was 64% with a background of 16 cpm. Samples were counted to a standard deviation of 5%. Uptake of ferrichrome and both the ligand and metal of chromic [^{14}C]-desferriferrichrome was linear and complete within 2.5 hr. It should be noted that some variation in the maximum rate of ferrichrome and chromic [^{14}C]-desferriferrichrome uptake was observed in different cell preparations.

Results and Discussion

Emery's transport studies of various metal substituted ferrichromes with *Ustilago sphaerogena* demonstrated that when a chromium(III) salt and [^{14}C]-desferriferrichrome are mixed in the culture medium no uptake of labeled ligand is observed (3). In contrast, he found that gallium(III) and aluminum(III) ions, which readily form complexes with a similar conformation to ferrichrome, exhibited rates of uptake comparable to that of the latter (3). In retrospect, since the chromic complex probably did not form in the culture medium, it could not have been transported. As a consequence of their large crystal field stabilization energy, chromic complexes are kinetically inert in general and their reactions are much slower than those of kinetically labile metal complexes such as gallium(III) and aluminum(III) (7). We have shown (Fig. 1) that when pre-

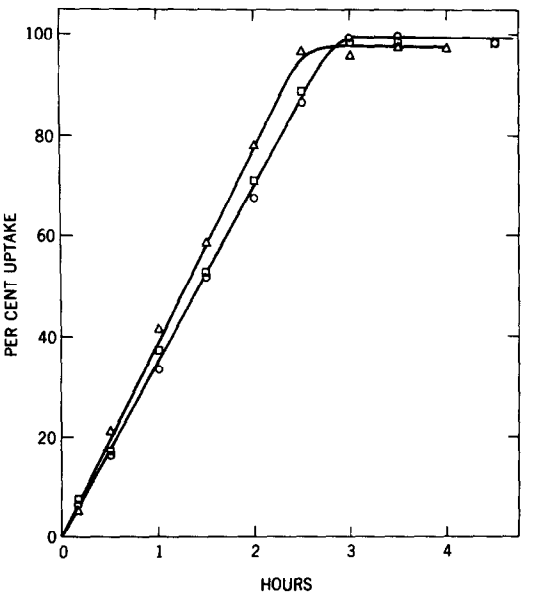

Fig. 1: Uptake of ferrichrome (△) and Λ-*cis*-chromic [^{14}C]-desferriferrichrome ([^{14}C],□ ; Cr,○) by *Ustilago sphaerogena*. The cell suspension (17 mg/ml, dry weight) was incubated at 30°C with final metal complex concentrations of 0.1 mM. Uptake was followed as described in Experimental Procedures.

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formed Λ-*cis* chromic desferriferrichrome or ferrichrome is added to cell suspensions of *Ustilago*, both ligand and metal ions are taken up at comparable rates. Since no mechanism (probably reduction in the case of the ferric complex) is available to the cells to remove the chromium from transported chromic desferriferrichrome, the complex is held within the cells and the ligand is not excreted again as in ferrichrome (3). Emery observed similar behavior with the aluminum(III) complex (3).

The conformation of the metal complex undoubtedly accounts for the specificity of the ferrichrome uptake system in *Ustilago* since desferriferrichrome is not incorporated at all, while naturally occurring and synthetic derivatives of siderochromes and ferric chelating agents are generally incorporated considerably less than ferrichrome (3, 4). An examination of molecular models of the aluminum(III), gallium(III), chromic, and ferric complexes of desferriferrichrome indicates that Λ-*cis* and Δ-*cis* optical isomers are possible, although X-ray crystallographic investigations have shown that structurally related fer-

richrome A (10) and ferrichrysin (11) both crystallize as the Λ-*cis* optical isomer. It is possible that both optical isomers of the labile complexes are in equilibrium in solution and that crystallization affords only the lesser soluble diastereoisomer.

The previous studies (3, 4) and the results reported here lead to the following conclusions: (i) Since the chromic complex of desferriferrichrome is kinetically inert and found to be transported by *Ustilago*, the Λ-*cis* optical isomer must be at least one of the biologically active isomers. (ii) Since the uptake rate of the conformationally similar ferrichrome and chromic desferriferrichrome complexes are similar, both probably share the same transport system (12). (iii) This specific iron transport system cannot rely on isomerization or partial dissociation of the complex during transport through the membrane.

Recently Zähner *et al.* (13) described uptake experiments of the iron(III), gallium(III), aluminum(III), vanadium(III), chromium(III), and cobalt(III) complexes of desferricoprogen, a trihydroxamate siderochrome, in *Neurospora crassa*, and the effect of the above inorganic metal salts on the production of desferricoprogen. The reduced rate of transport of the kinetically inert chromium(III) and cobalt(III) complexes with respect to the kinetically labile iron(III), gallium(III), and aluminum(III) complexes is not probably related to the stability of the metal-substituted complexes as the authors suggest, but may reflect the partial formation of the former kinetically inert complexes from desferricoprogen and the inorganic metal salt in the culture medium. Preformed metal substituted complexes of desferricoprogen should exhibit similar rates of transport in *Neurospora crassa* if, as with *Ustilago sphaerogena*, conformation is the primary determinant for uptake velocity.

Acknowledgment

This research is supported in part by USPHS Grants Nos. AI 04156 and AI 11744.

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