on the contrary, against a selective replication of a master gene at each cell division (Amaldi *et al.*, 1969).

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References

Amaldi, F., and Attardi, G. (1968), *J. Mol. Biol. 33*, 737.
Amaldi, F., Zito-Bignami, R., and Giacomoni, E. (1969), *Eur. J. Biochem. 11*, 419.
Attardi, G., Aloni, Y., Attardi, B., Ojala, D., Pica-Mattoccia, L., Robberson, D., and Storrie, B. (1970), *Cold Spring Harb. Symp. Quant. Biol. 35, 599.*
Attardi, G., and Amaldi, F. (1970), *Annu. Rev. Biochem. 39*, 183.
Attardi, G., Huang, P. C., and Kabat, S. (1965a), *Proc. Nat. Acad. Sci. U. S. 53*, 1490.
Attardi, G., Huang, P. C., and Kabat, S. (1965b), *Proc. Nat. Acad. Sci. U. S. 54*, 185.
Attardi, G., Parnas, H., Hwang, M.-I. H., and Attardi, B. (1966), *J. Mol. Biol. 20*, 145.
Beaven, G. H., Holiday, E. R., and Johnson, E. A. (1955), *in* The Nucleic Acids, Vol. 1, Chargaff, E., and Davidson, J. N., Ed., New York, N. Y., Academic Press, p 493.
Birnstiel, M., Grunstein, M., Speirs, J., and Hennig, W. (1969), *Nature (London) 223*, 1265.
Birnstiel, M., Speirs, J., Purdom, I., Jones, K., Loening, M. E. (1968), *Nature (London) 219*, 454.
Brown, D. D., and Gurdon, J. B. (1964), *Proc. Nat. Acad. Sci. U. S. 51*, 139.
Callan, H. G. (1967), *J. Cell Sci. 2*, 1.
Di Girolamo, A., Busiello, E., and Di Girolamo, M. (1969), *Biochim. Biophys. Acta 182*, 169.
Fellner, P., and Sanger, F. (1968), *Nature (London) 219*, 236.
Fisher, R. A. (1950), Statistical Methods for Research Workers, Edinburgh, Oliver & Boyd.
Gould, H. J. (1967), *J. Mol. Biol. 29*, 307.
Gould, H. J., Bonanou, S., and Kanagalingham, K. (1966), *J. Mol. Biol. 22*, 369.
Grummt, F., and Bielka, H. (1968), *Biochim. Biophys. Acta 161*, 253.
Hatlen, L. E., Amaldi, F., and Attardi, G. (1969), *Biochemistry 8*, 4989.
Hatlen, L. E., and Attardi, G. (1971), *J. Mol. Biol.* (in press).
Henney, H. R., and Storck, R. (1963), *Science 142*, 1675.
Hirsch, C. A. (1966), *Biochim. Biophys. Acta 123*, 246.
Jeanteur, Ph., and Attardi, G. (1969), *J. Mol. Biol. 45*, 305.
Lerner, A. M., Bell, E., and Darnell, J. E. (1963), *Science 141*, 1187.
Mutolo, V., and Giudice, G. (1967), *Biochim. Biophys. Acta 149*, 291.
Pinder, J. C., Gould, H. J., and Smith, I. (1969), *J. Mol. Biol. 40*, 289.
Reich, E., Acs, G., Mach, B., and Tatum, E. L. (1963), *in* Informational Macromolecules, Vogel, H. J., Bryson, V., and Lampen, J. O., Ed., New York, N. Y., Academic Press, p 317.
Slater, D. W., and Spiegelman, S. (1966), *Biophys. J. 6*, 385.
Soeiro, R., Vaughan, M., Warner, J. R., and Darnell, J. E. (1968), *J. Cell Biol. 39*, 112.
Tata, J. R. (1967), *Biochem. J. 105*, 783.

Role of Ferrichrome as a Ferric Ionophore in *Ustilago sphaerogena**

Thomas Emery

ABSTRACT: The smut fungus, *Ustilago sphaerogena*, possesses a highly specific iron transport system in which the trihydroxamic acid–iron chelate, ferrichrome, serves as an iron carrier, or ferric ionophore. Intracellular removal of the metal from the chelate permits egress of the ligand, desferriferrichrome, which can then sequester extracellular iron and again shuttle the metal into the cells. In the presence of excess iron, ferrichrome is concentrated by the cells. The transport system has an optimum pH of 7 and optimum temperature of 30°, and it is inhibited by azide, *N*-ethylmaleimide, or anaerobiosis. Growth of the cells in medium containing 10 μM iron suppresses the iron transport system. Ferrichrome is a specific iron carrier for *Ustilago*. No other iron chelates tested, including natural hydroxamic acid analogs of ferrichrome, can substitute. Conversely, many other di- and trivalent metals chelated with the ligand, desferriferrichrome, are not taken up by the cells. The aluminum and gallium chelates, however, are active in the system. The specificity data indicate that conformation of the chelate, rather than charge or solubility, is the basis for recognition by the transport system.

A fundamental problem of cellular biology is the mechanism by which water-soluble extracellular nutrients enter the cell through lipophilic membranes. Specific transport systems appear to be present in most cell membranes. The transport of amino acids and sugars has been investigated, but the mechanism of the process remains unsolved. According to

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one popular hypothesis, specific carrier substances bind the metabolites and mediate their transport across the membrane. Relatively little attention has been paid to transport of inorganic ions, although a protein believed to be a carrier for sulfate has been isolated (Pardee, 1968).

Metal cations present a difficult transport problem to the cell because of their unfavorable lipid solubility. Iron is particularly difficult for cells to assimilate because of the insolubility of ferric hydroxide at pH approaching neutrality. Free-living microorganisms are faced with the problem of first solubilizing iron in an extracellular aqueous environment and then transporting the metal through the hydrophobic membrane. Neilands (1952) observed that cells of the smut fungus, *Ustilago sphaerogena*, contained an iron chelate, named ferrichrome, when the organism was grown on yeast extract medium in an iron fermentor. Subsequently it was demonstrated that a number of aerobic microbial species, including *Ustilago*, excrete the metal-free chelate, desferriferrichrome, under iron-deficient conditions. Since ferrichrome iron is available for heme synthesis by *Ustilago*, it was suggested that the substance may play a role as a specific iron sequestering agent in this organism (Neilands, 1957). Evidence presented in this paper supports this hypothesis by showing that *Ustilago* possesses an active transport system for iron in which the metal must be specifically chelated as ferrichrome. Ferrichrome is a cyclic hexapeptide containing three glycines and three δ-hydroxamic acid derivatives of δ-*N*-hydroxyornithine ideally suited to chelate ferric ion (Emery and Neilands, 1961). In the terminology of Pressman (1968), ferrichrome acts as an iron ionophore. In *B. subtilis* an iron-transport system has been reported that apparently utilizes 2,3-dihydroxybenzoylglycine as iron ionophore (Peters and Warren, 1968).

Experimental Section

Ustilago sphaerogena (ATCC 12421) was grown under iron-deficient conditions in 400 ml of culture as previously described (Emery, 1966). Twenty-four-hour cells were collected by centrifugation and washed twice in the following medium: glucose, 4 g; citric acid, 0.2 g; ammonium acetate, 0.2 g; K_2HPO_4, 8.7 g in 1 l., and the pH was adjusted to 7.0 with acetic acid. The washed cells were resuspended in this medium to give a final cell concentration of 15–20 mg per ml (dry weight). Uptake experiments were done using 10 ml of the suspension in 125-ml erlenmeyer flasks. After addition of labeled substrate, the flasks were shaken at 30°. Aliquots of about 0.5 ml were removed and quickly chilled to 0° to stop uptake. After removal of the cells by centrifugation, 200 μl of the supernatant fluid was counted as previously described (Emery, 1967). Uptake was linear with time for at least 30 min, and uptake rates were compared during the initial 10–20 min.

Ferrichrome and ferrichrome A labeled with ^{14}C (*ca.* 10^5 cpm/μmole) were obtained by growth of *Ustilago* on medium containing ^{14}C-labeled glycine or ornithine (Emery, 1966). Ferrichrome and ferrichrome A labeled with ^{59}Fe were prepared by equilibration of the compounds with isotopic ferric citrate and removal of excess iron by chromatography on DEAE-cellulose (Lovenberg and Rabinowitz, 1963). Other ^{59}Fe-labeled chelates were prepared by addition of isotopic ferric chloride to ligand present in 10% excess of the amount calculated to be necessary to satisfy the six coordination valencies of iron. The trimethyl ester of ferrichrome A was prepared by dissolution of ferrichrome A in 1 N methanolic HCl overnight at room temperature. After removal of the solvent *in vacuo*, an aqueous solution of the product was passed through a Cellex-D (Bio-Rad) column. The electrophoretically neutral triester that was eluted from the column with water was lyophilized and the ^{59}Fe-labeled chelate prepared as described above. Desferriferrichrome was prepared by previously described methods (Emery and Neilands, 1960).

Results

Ferrichrome Uptake by Ustilago sphaerogena. The rapid uptake of ferrichrome by suspended *Ustilago sphaerogena* cells was initially observed by the visual disappearance of the orange color of ferrichrome from the suspending medium and concomitant development of color in the centrifuged cell pellet. Uptake was quantitatively followed by the use of labeled ferrichrome. Using ^{59}Fe-labeled ferrichrome, we observed a rapid and quantitative removal of the iron from the medium which paralleled removal of the chelate as judged by color (Figure 1). When ^{14}C-labeled ferrichrome ligand was used, however, the uptake phenomenon proved more complex. After an initial uptake of label, counts were then released from the cells back into the medium (Figure 1). Since the metal was not released, we can conclude that the chelate has been dissociated by cellular processes; the metal is retained by the cells whereas the ligand is released. Separate experiments using the ^{14}C-labeled ligand, desferriferrichrome, confirmed the fact that the ligand is not taken up by the cells. In fact, using very heavy cell suspensions (13% by volume) we found that desferriferrichrome does not even appear to gain access into the cells by passive diffusion. The possibility that the radioactivity released from the cells is a degradation product rather than intact ligand was ruled out by reformation of the chelate by addition of iron, and identification of the chelate as ferrichrome by extraction and chromatographic comparison (Emery, 1966).

The ligand, desferriferrichrome, can act as a shuttle to bring iron into the cell. After ingress of ferrichrome and egress of the ligand, addition of iron to the medium re-forms ferrichrome, which then again enters the cells (Figure 2). The cells in these experiments make up about 3% of the total volume. Since 80% of the ferrichrome is held within the cells, the cellular and extracellular concentration difference is greater than 100-fold. As the cell's capacity to remove iron from the chelate diminishes, probably due to saturation of iron pools, more ferrichrome is held inside the cell and correspondingly less ligand is released, so that concentraion differences approaching 1000-fold can be attained.

In separate experiments it was found that the rate of [^{14}C]-ferrichrome uptake was proportional to ferrichrome concentration up to about 20 μM, after which the rate of uptake diminished rapidly. The system thus shows saturation kinetics, but accurate data could not be obtained because of the unavailability of ferrichrome of high specific activity. Nevertheless, it would appear that the half-maximal rate of uptake occurs at approximately 10 μM. It should be noted that some variation in the maximum rate of ferrichrome uptake was observed in different cell preparations.

Is the Intracellular Ferrichrome Bound? No satisfactory procedure exists to prove that a metabolite concentrated within cells has an activity coefficient approaching that in dilute solutions (Fenichel and Horowitz, 1969). That the ferrichrome accumulated by *Ustilago* is chemically unchanged was shown by its release from the cells by boiling water, or 1 N acetic acid at room temperature, and reisolation of unchanged ferrichrome. Ferrichrome can also be released from

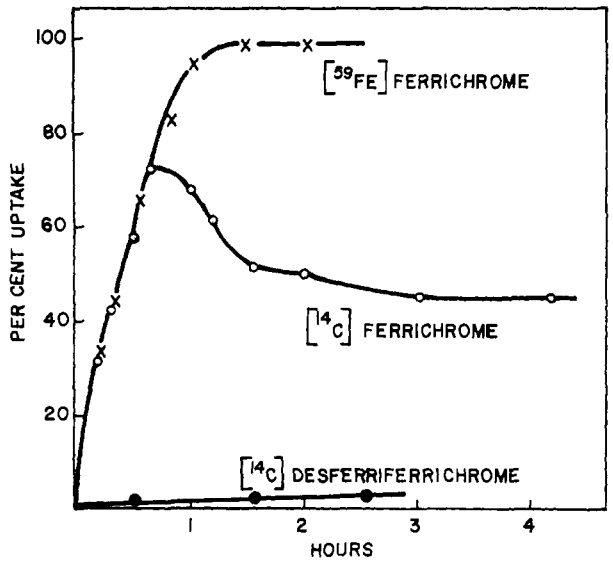

FIGURE 1: Per cent radioactivity (^{59}Fe or ^{14}C) taken up by a cell suspension of *Ustilago sphaerogena* upon addition of [^{59}Fe]ferrichrome, [^{14}C]ferrichrome, or [^{14}C]desferriferrichrome. The cell concentration was 17 mg/ml (dry weight) and the labeled substrates (0.036 mM) were added at zero time. Uptake was followed at 30° and pH 7 as described in the Experimental Section.

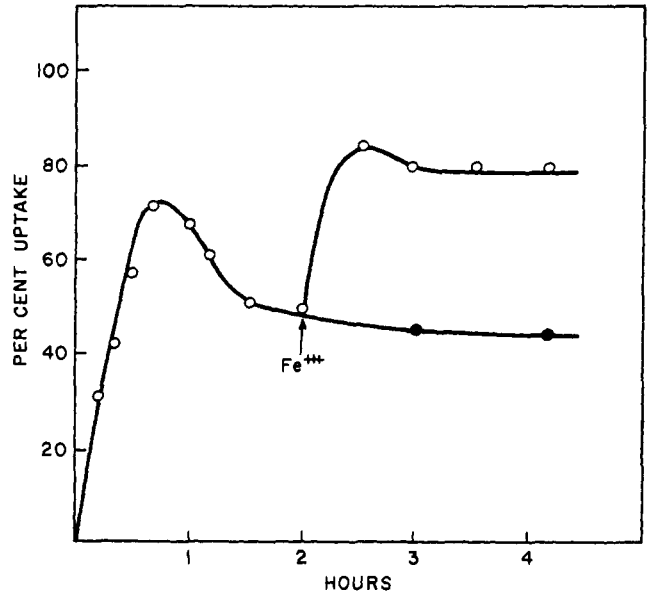

FIGURE 2: Per cent uptake of [^{14}C]ferrichrome by *Ustilago*. Experimental conditions are as for Figure 1, but at 2 hr ferrous sulfate (6.8 mM) was added. Ferrous ion is immediately oxidized to ferric in the presence of hydroxamic acids under aerobic conditions. Renewal of ferrichrome uptake is contrasted to a control flask (solid circles) to which no iron was added.

the cells by merely raising the pH of the suspension to 9.5, showing that if intracellular binding of the chelate does occur, it must be quite weak. Cellular ferrichrome does not exchange with extracellular ferrichrome, that is, it does not exhibit exchange diffusion.

Effect of pH and Temperature. The maximum rate of ferrichrome transport occurs at about pH 7 and falls to zero below pH 5 or above pH 8 (Figure 3). Although it is difficult to experimentally determine whether pH directly affects the transport system or indirectly affects transport by disturbing energy production in the cell, it should be noted that the carrier itself, ferrichrome, has no ionizable groups in the pH range studied.

Ferrichrome transport is temperature dependent, the maximal rate occurring at 30°, with complete inhibition of uptake above 50° (Figure 4).

The effects of pH and temperature are very similar to those observed for methionine transport by *Penicillium chrysogenum* (Benko *et al.*, 1969).

Effect of Inhibitors. Active transport systems show a wide range of sensitivity to various inhibitors. Table I shows the effect of various inhibitors on initial rate of ferrichrome uptake by *Ustilago*. Respiratory poisons, such as azide and cyanide, are particularly effective although the system is relatively insensitive to dinitrophenol. As in the case of magnesium uptake by *Escherichia coli* (Lusk and Kennedy, 1969), the uptake of iron in the form of ferrichrome is sensitive to *N*-ethylmaleimide. Growth of *Ustilago* requires vigorous aeration, and ferrichrome transport is also oxygen dependent.

Specificity of Transport with Respect to Ligand. Several other $^{59}Fe^{3+}$ chelates were examined for their ability to be transported by *Ustilago*. The results (Table II) show that for iron to be rapidly taken up by the cells, the metal must be in the form of the ferrichrome chelate. The process may, in fact, be absolutely specific for ferrichrome; the slow incorporation of iron from other chelates may be due to exchange of iron from the chelate to endogenous desferriferrichrome still present in the washed cells. Because of the extremely high binding constant ($K \approx 10$)[28] of ferrichrome, small amounts of this ligand would effectively remove metal from other chelates.

It is significant that other natural hydroxamate–iron chelates, such as fusarinine, rhodotorulic acid, and even ferrichrome A, which is produced by *Ustilago*, are ineffective in iron transport. [^{14}C]Ferrichrome A and its trimethyl ester were completely excluded from the cells, which further suggests that the slow uptake of ^{59}Fe from ferrichrome A is due

TABLE I: Effect of Inhibitors on Ferrichrome Uptake by *Ustilago sphaerogena*.[a]

Inhibitor	Concn (M)	Inhibition (%)
Azide	10^{-3}	100
Azide	10^{-4}	76
Nitrogen (anaerobic)		100
Cyanide	10^{-3}	100
N-Ethylmaleimide	10^{-3}	100
Iodoacetamide	10^{-3}	70
Iodoacetate	10^{-3}	0
Dinitrophenol	10^{-3}	25
EDTA	10^{-3}	61
Fluoride	10^{-3}	17
Arsenate	10^{-3}	7
Actidione	10^{-3}	0
Albomycin	10^{-4}	0
Ouabain	10^{-4}	0

[a] The cells (17 mg/ml, dry weight) were preincubated for 10 min with the inhibitor and [^{59}Fe]ferrichrome (0.1 mM) added at zero time. Uptake was followed at 30°, pH 7, and the uptake rates during the initial 20 min were compared to a control containing no inhibitor.

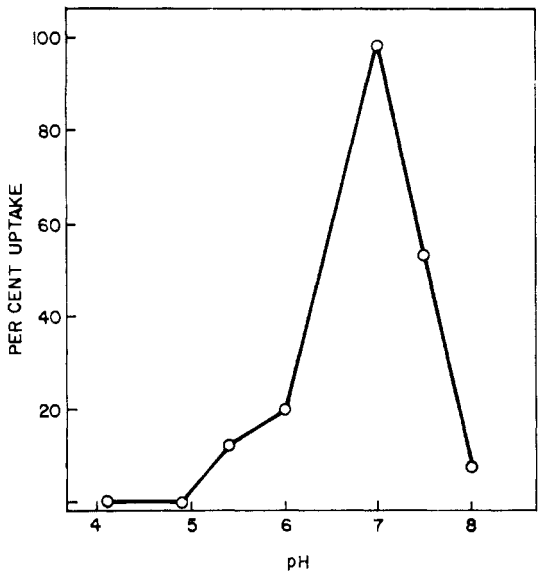

FIGURE 3: The effect of pH on the per cent uptake of [^{59}Fe]ferrichrome after 20-min incubation with *Ustilago* at 30°. Conditions are as for Figure 1.

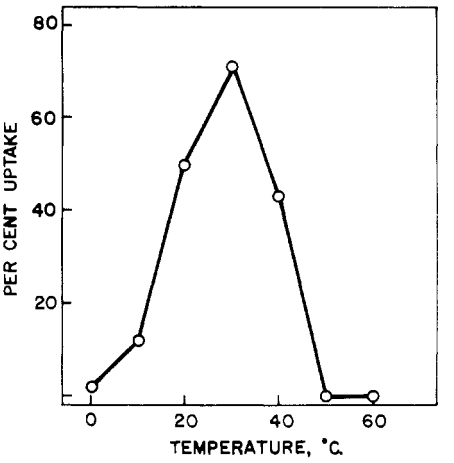

FIGURE 4: The effect of temperature on the per cent uptake of [^{59}Fe]-ferrichrome after 20-min incubation with *Ustilago* at pH 7. Conditions are as for Figure 1.

to exchange with endogenous desferriferrichrome. Ferrichrome A was not taken up at a lower pH (4.7).

Specificity of Transport with Respect to Metal. The extremely high affinity of desferriferrichrome for iron makes it impossible for other metals to compete with iron for the ligand. Thus, the presence of Cu^{2+} or Co^{2+} did not affect ferrichrome uptake. However, in the absence of iron other metals can form stable chelates with desferriferrichrome (Anderegg *et al.*, 1963). Table III, however, shows that other metal chelates of desferriferrichrome are not taken up by *Ustilago*. Notable exceptions are group III elements, Al^{3+}, Ga^{3+}, and to a lesser extent Tl^{3+}. Experiments with ^{14}C-labeled ligand showed that the uptake of the aluminum chelate differs from that of ferrichrome in that after uptake the ligand cannot be released from the aluminum chelate (Figure 5).

Aluminum chloride at concentrations as high as 10^{-4} M was found not to affect adversely the growth of *Ustilago* in iron-deficient medium.

Ferrichrome Uptake by Iron-Grown Cells. A culture of *Ustilago* was grown with 10 μM iron present in the medium. Iron at this concentration increases both the rate of growth and cell yield, but almost completely suppresses the production of extracellular desferriferrichrome. The inability of these cells to transport ferrichrome as rapidly as cells grown on iron-deficient medium is shown in Figure 6.

TABLE II: Relative Rates of Uptake of Iron Chelates by *Ustilago*.[a]

Chelate	Relative Rate of Uptake
[^{59}Fe]Ferrichrome	100
[^{14}C]Ferrichrome	100
[^{59}Fe]Ferrichrome A	30
[^{14}C]Ferrichrome A	8
[^{59}Fe]Ferrichrome A trimethyl ester	33
[^{14}C]Ferrichrome A trimethyl ester	2
[^{59}Fe]Ferrioxamine	32
[^{59}Fe]Benzohydroxamic acid	15
[^{59}Fe]Fusarinine	14
[^{59}Fe]Rhodotorulic acid	7
[^{59}Fe]Citric acid	15
[^{59}Fe]EDTA	9

[a] Conditions are the same as described for Table I. The rate of uptake of the substrates (Experimental Section) was compared during initial 20-min incubation.

TABLE III: Relate Rate of Uptake of [^{14}C]Desferriferrichrome by *Ustilago* in the Presence of Metals.[a]

Metal	log k_a[b]	Relative Rate of Uptake
None		0
Fe(III)	28.3	100
Al(III)	21.5	74
Au(III)		0
Cd(II)	7.8	0
Ce(III)	12.8	0
Cr(III)		0
Cu(II)		0
Co(II)	8.9	0
Ga(III)		70
La(III)	11.9	0
Ni(II)	9.3	0
Pb(II)	10.7	0
Ru(III)		0
Sb(III)		0
Ti(III)		0
Tl(III)		<10
Y(III)		0

[a] The cell suspension (final concentration 17 mg/ml, dry weight) was added to a solution of [^{14}C]desferriferrichrome plus the metal, each at a final concentration of 0.1 mM. The rate of uptake of radioactivity was compared during the initial 20 min. [b] Data from Anderegg *et al.* (1963).

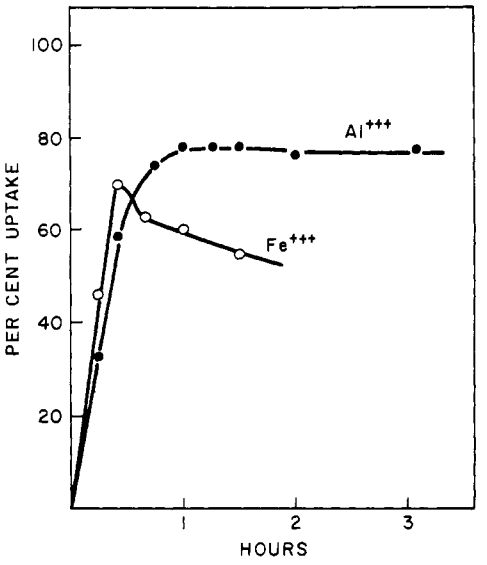

FIGURE 5: Comparison of the uptake of the ferric and aluminum chelates of [^{14}C]desferriferrichrome by *Ustilago*. The cell suspension (17 mg/ml, dry weight) was incubated at 30° with [^{14}C]desferriferrichrome, 0.10 mM. At zero time, ferric chloride or aluminum chloride was added to give a concentration of 0.10 mM in metal, and the uptake was followed as described in the Experimental Section.

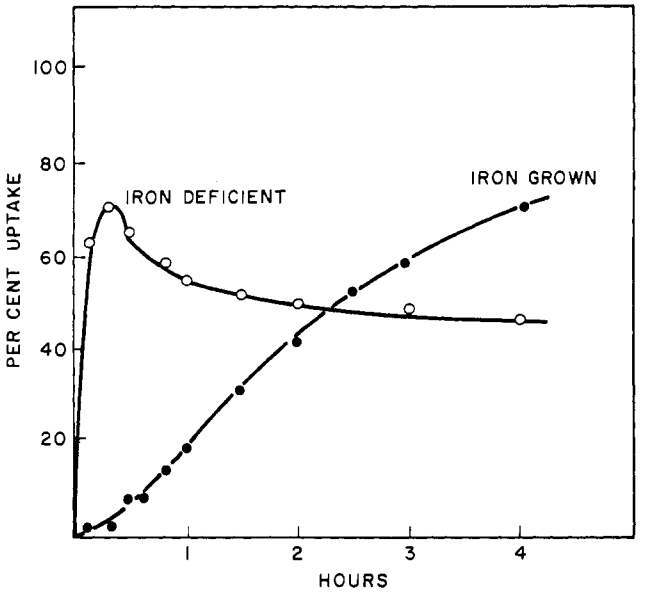

FIGURE 6: Comparison of the uptake of [^{14}C]ferrichrome by *Ustilago* grown in iron-deficient medium (Experimental Section) and in the same medium containing 10 μM iron, added as $FeSO_4$. Conditions are otherwise as for Figure 1.

Discussion

The active transport of ferrichrome by *Ustilago* possesses many features, such as pH and temperature optima, found in other transport systems (Stein, 1967). Transport is inhibited by respiratory poisons and anaerobiosis, although it is not possible in whole cell experiments to determine whether this is direct inhibition of the transport system or general inhibition of the production of energy necessary for active transport. Inhibition by *N*-ethylmaleimide, however, has often been assumed to be due to reaction with sulfhydryl groups of a specific transport protein, although this may not be the case in *Ustilago*. Although evidence has been presented that the iron hydroxamate chelate, albomycin, interferes directly with the biological function of ferrichrome in bacteria (Keller-Schierlein *et al.*, 1964), albomycin does not interfere with growth of *Ustilago*. It is, therefore, not surprising that albomycin has no effect on ferrichrome transport in this organism.

Our data indicate that the hydroxamate ligand, desferriferrichrome, can act as a ferric ionophore to shuttle ferric ion into the cell. Under iron-deficient growth conditions, desferriferrichrome is excreted by *Ustilago*. Upon chelation of extracellular iron, the ferrichrome chelate is transported into the cell against a concentration gradient. Upon cellular removal of the metal, the ligand again can pass from the cell to chelate another atom of iron. However, active transport of ferrichrome is not dependent upon the cell's ability to extract the metal from the chelate. When the cell's capacity to remove iron from ferrichrome is exceeded, the transport system continues to function and ferrichrome is concentrated in the cells. It is interesting that in spite of the fact that cellular ferrichrome appears to be unbound, ferrichrome is not released from the cells by azide or anaerobiosis, nor does it exhibit exchange diffusion with extracellular ferrichrome. The finding that transport but *not* retention is energy dependent, and that exchange diffusion is not exhibited, has also been reported for the transport of methionine by *Penicillium chrysogenum* (Benko *et al.*, 1969). Transport in these cases may be likened to a turnstyle or ratchet mechanism in which energy is required to push the turnstyle to gain entrance. Once entry has been made, however, the turnstile locks mechanically and exit is prevented without further input of energy.

The *Ustilago* iron-transport system is specific for ferrichrome. The iron of nonhydroxamate ferric chelates, such as EDTA or citrate, cannot gain entry into the cell. Although at neutral pH monohydroxamic acids form 3:1 chelates of iron with a geometry about the metal nearly identical with that of ferrichrome (Linder and Göttlicher, 1969), neither benzohydroxamic acid nor the natural monohydroxamate, fusarinine, is active. More surprising is the lack of activity of the natural trihydroxamates, ferrioxamine and ferrichrome A, the latter a product of *Ustilago*. Ferrichrome A differs from ferrichrome in that serine replaces two of the glycines in the peptide ring and β-methylglutaconate replaces acetate as the hydroxamate acyl groups. It was thought that the charged carboxylate groups of ferrichrome A might be responsible for the lack of iron-transport activity of this compound. However, the neutral trimethyl ester of ferrichrome A, which is a very close structural analog of ferrichrome, is also inactive.

The specificity of the transport system with respect to the metal is also interesting. Only the trivalent group III metals, aluminum and gallium, form chelates with desferriferrichrome that allow transport to occur, although it is known that other di- and trivalent cations form chelates with hydroxamic acids (Anderegg *et al.*, 1963). In our experiments, chelation of copper and titanium was evidenced by color formation, but these chelates were not taken up by the cells. The conformation of the chelate in the vicinity of the metal ion must be critical for recognition by the transport system (protein?). Copper forms square-planar chelates with hydroxamates, and although the geometry of other metal hydroxamate chelates is not known with certainty, it is likely that they do not give the ideal octahedral geometry known to occur with ferric ion. Since no mechanism is available to the cells to remove metal from the aluminum chelate of desferriferrichrome, the aluminum chelate is held within the cells and the ligand is not

allowed to exit. Recent work in Neilands's laboratory has also demonstrated the very similar, if not identical, structures of the aluminum and ferric chelates of desferriferrichrome (M. Llinas, M. P. Klein, and J. B. Neilands, submitted for publication). Ferrichrome A and its trimethyl ester are probably not transported because of the bulky acyl groups of the hydroxamates in the vicinity of the metal.

An increasing number of low molecular weight compounds, such as valinomycin, enniatin, nonactin, and monensin, have been implicated in the transport of monovalent cations. It has been generally accepted that the mechanism by which these substances effect transport of a metal such as potassium is chelation with the metal and replacement of the hydration shell with a nonpolar, lipid-soluble sheath (Pressman, 1968; Pinkerton and Steinrauf, 1970). Even though in some cases the resulting chelate may carry a net charge, the increased lipid solubility observed in model systems has been repeatedly stressed as the property that allows cation transport across cellular membranes *in vivo*.

Upon chelation of ferric ion by desferriferrichrome, three protons are displaced from the ligand to give a resulting chelate, ferrichrome, that is neutral and, in fact, has no ionizable groups in the pH range of 2 to 11. However, one cannot invoke lipid solubility to explain iron transport by ferrichrome. Ferrichrome is a water-soluble substance insoluble in solvents such as chloroform and hexane, which have been used to mimic the solubility properties of membranes. Ferrichrome is not even soluble in methanol, although ferrichrome A and its trimethyl ester are soluble in this solvent. On the basis of solubility, ferrichrome A trimethyl ester should be more effective than ferrichrome in iron transport, but in fact there is no observable uptake of this iron chelate by *Ustilago*. We believe that molecular conformation, not solubility, is the property that makes ferrichrome active in iron transport.

It has recently been suggested that conformation changes play an important role in the mechanism of transport of monovalent cations by depsipeptide and other macrocyclic antibiotics (Shemyakin *et al.*, 1969; Diebler *et al.*, 1969). Our results support this view. It is most significant that *both the ligand, desferriferrichrome, and the iron chelate, ferrichrome, are water-soluble, neutral compounds with no ionizable groups in the physiological pH range*. Yet ferrichrome is actively transposed by *Ustilago* and desferriferrichrome is not. The only apparent way for the cells to differentiate these two substances is the conformation change imposed by chelation of the metal. This conformational change, first observed by physical techniques (Emery, 1967; M. Llinas, M. P. Klein, and J. B. Neilands, submitted for publication), appears to be the basis of biological activity of ferrichrome in *Ustilago*.

Finally, it should be pointed out that iron is but one of the metals required by cells. In addition to the potassium transport system previously mentioned, transport systems for manganese (Silver and Kralovic, 1969) and magnesium (Lusk and Kennedy, 1969; Silver, 1969) have been reported. It may not be unreasonable to suppose that specific low molecular weight ionophores analogous to ferrichrome are used for metal transport. Should this be the case, it will be most interesting to see what organic ligands the cell has devised which can exhibit the extraordinary specificity to transport trace quantities of one particular metal to the exclusion of others that may be present in much greater concentration.

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References

Anderegg, G., L'Eplattenier, F., and Schwarzenbach, G. (1963), *Helv. Chim. Acta 46*, 1400.

Benko, P. V., Wood, T. C., and Segel, I. H. (1969), *Arch. Biochem. Biophys. 129*, 498.

Diebler, H., Eigen, M., Ilgenfritz, H., Maas, G., and Winkler, R. (1969), *Pure Appl. Chem. 20*, 93.

Emery, T. F. (1966), *Biochemistry 5*, 3694.

Emery, T. F. (1967), *Biochemistry 6*, 3858.

Emery, T. F., and Neilands, J. B. (1960), *J. Amer. Chem. Soc. 82*, 3658.

Emery, T. F., and Neilands, J. B. (1961), *J. Amer. Chem. Soc. 83*, 4903.

Fenichel, I. R., and Horowitz, S. B. (1969), *in* Biological Membranes, Robert M. Dowben, Ed., Boston, Mass., Little, Brown & Co., p 177.

Keller-Schlierlein, W., Prelog, V., and Zahner, H. (1964), *Fortschr. Chem. Org. Naturst. 22*, 279.

Linder, H. J., and Göttlicher, S. (1969), *Acta Crystallogr., Sect. B 25*, 832.

Lovenberg, W., and Rabinowitz, J. C. (1963), *J. Biol. Chem. 238*, 3899.

Lusk, J. E., and Kennedy, E. P. (1969), *J. Biol. Chem. 244*, 1653.

Neilands, J. B. (1952), *J. Amer. Chem. Soc. 74*, 4846.

Neilands, J. B. (1957), *Bacteriol. Rev. 21*, 101.

Pardee, A. B. (1968), *Science 162*, 632.

Peters, W. J., and Warren, R. A. J. (1968), *Biochim. Biophys. Acta 165*, 225.

Pinkerton, M., and Steinrauf, L. K. (1970), *J. Mol. Biol. 49*, 533.

Pressman, B. C. (1968), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 27*, 1283.

Shemyakin, M. M., Ovchinnikov, Yu. A., and Ivanov, V. T. (1969), *Angew. Chem. Int. Ed. 8*, 492.

Silver, S. (1969), *Proc. Nat. Acad. Sci. U. S. 62*, 764.

Silver, S., and Kralovic, M. L. (1969), *Biochem. Biophys. Res. Commun. 34*, 640.

Stein, W. D. (1967), The Movement of Molecules Across Cell Membranes, New York, N Y., Academic Press.