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Hydroxamate Recognition During Iron Transport from Hydroxamate-Iron Chelates

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Kinetics of radioactive iron transport from three structurally different secondary hydroxamate-iron chelates (schizokinen-iron, produced by *Bacillus megaterium* ATCC 19213; Desferal-iron, produced by an actinomycete; and aerobactin-iron, produced by *Aerobacter aerogenes* 62-1) revealed that *B. megaterium* SK11 (a mutant which cannot synthesize schizokinen) has a specific transport system for utilization of ferric hydroxamates with a recognition capacity based on the chemical structure of the hydroxamate. Both Desferal and schizokinen enhanced iron uptake in this organism; however, Desferal-iron delivered only one-sixth the level of iron incorporated from the schizokinen-iron chelate. Desferal-iron did not generate the rapid rates of iron transport noted with schizokinen-iron at elevated iron concentrations. Assays containing large excesses of either iron-free Desferal or iron-free schizokinen suggested that the iron-free hydroxamate may compete with the ferric hydroxamate for acceptance by the transport system although the system has greater affinity for the iron chelate. Aerobactin-iron did not stimulate iron uptake in *B. megaterium* SK11 and aerobactin inhibited growth of this organism, indicating that *B. megaterium* SK11 cannot efficiently process the aerobactin-iron chelate.

Microbial iron uptake is a complex and multistep process. In some microorganisms one of the early events is binding of ferric iron to a low molecular weight secondary hydroxamic acid produced by the cells. The hydroxamates have been found in a variety of microbial types; cultivation in low-iron medium usually causes increased secretion of the hydroxamates (13). These compounds have a high and specific binding capacity for ferric iron (13). The structures of three natural hydroxamates are shown in Fig. 1.

Lack of hydroxamate (schizokinen) production in *Bacillus megaterium* strain SK11 confers some interesting characteristics on the organism. In a simple sucrose-mineral salts medium containing no schizokinen supplement, this strain can reach maximal population densities only from high inocula, and it is capable of incorporating relatively low amounts of radioactive iron from this medium (5, 6). However, strain SK11 cannot effectively compete for iron with certain other systems which bind iron, such as low concentrations of aluminum in the medium which inhibit growth and block uptake of iron from an iron salt, probably by co-precipitation with iron. Kinetic examinations of $^{59}Fe^{3+}$ transport from $^{59}FeCl_3$ and the two hydroxamate chelates schizokinen-$^{59}Fe^{3+}$ and Desferal-$^{59}Fe^{3+}$ revealed that these hydroxamates increased total $^{59}Fe^{3+}$ incorporation and overcame aluminum inhibition of $^{59}Fe^{3+}$ transport in *B. megaterium* SK11 (5). It was concluded that uptake of iron from the schizokinen-iron chelate represents the primary mode of iron transport in *B. megaterium*, and that it is accomplished by a special ferric hydroxamate transport system independent from an alternate process which can incorporate iron from an iron salt (5).

Iron-free schizokinen is secreted by the cells and forms its iron complex either at the cell surface or in the medium. Thereafter, the iron complex may cross the lipid boundary of the cell as an intact unit, or the schizokinen-iron chelate may be bound at the membrane with subsequent removal of the iron and release of iron-free schizokinen. Regardless of the precise events which occur when the schizokinen-iron chelate approaches the cell membrane, either of the above possibilities suggest that operation of the transport process requires an association between the ferric hydroxamate and specific

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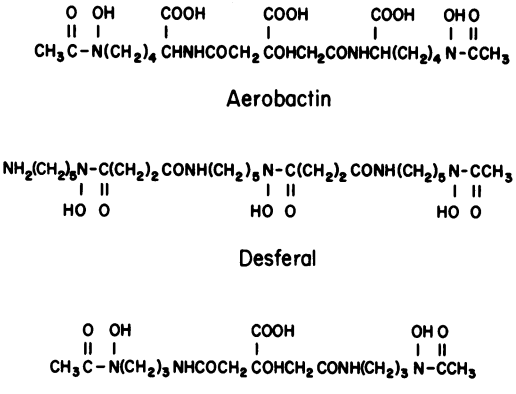

FIG. 1. *Structures of schizokinen (11), Desferal (4), and aerobactin (9), from Haydon, et al. (10).*

protein molecules. If this is true, then the system should show varying affinities for hydroxamates of different chemical structure.

The studies reported here with the hydroxamates schizokinen (produced by *B. megaterium* ATCC 19213) (3), Desferal (produced by a species of *Streptomyces*) (4), and aerobactin (produced by *Aerobacter aerogenes* 62-1) (9) were undertaken to determine if iron transport kinetics could be related to hydroxamate structure. Studies of the fate of tritium-labeled hydroxamates during iron transport are reported in a subsequent paper (1).

MATERIALS AND METHODS

Bacterial strain and cultivation methods. *B. megaterium* strain SK11, which is unable to produce the secondary hydroxamate schizokinen (2, 6) was the test organism. It was cultivated in a sucrose-mineral salts medium, treated with the metal chelating resin Chelex 100 (Bio-Rad Laboratories, Richmond, Calif.) to reduce trace metal contamination. The medium was supplemented with high purity magnesium (20 μg/ml), manganese (2 μg/ml), and iron (varying concentrations). Methods for preparation of the medium and for determination of growth kinetics of this strain have been described (6).

Assay for iron transport kinetics. Uptake of radioactive iron by *B. megaterium* SK11 was estimated by the method of Davis and Byers (5). Cells used in the assay were cultivated in low-iron medium (0.003 μg/ml) which contained no schizokinen supplement (5). Radioactive iron was purchased as $^{59}FeCl_3$ from either New England Nuclear Corp., Boston, Mass., (usual specific activity 7–12 Ci/g) or from Amersham-Searle, Arlington Heights, Ill., (usual specific activity 5–10 Ci/g). Hydroxamate-$^{59}Fe^{3+}$ chelates were prepared by mixing the iron-free hydroxamate with $^{59}FeCl_3$ at varying molar ratios of hydroxamate: $^{59}Fe^{3+}$, and allowing at least 30 min for chelation.

Preparation of hydroxamates. Desferal was a gift of Ciba Pharmaceutical Co., Summit, N. J. Aerobactin was prepared from culture filtrates of *A. aerogenes* strain 62-1 by the methods of Gibson and Magrath (9). Schizokinen was purified from culture filtrates of *B. megaterium* ATCC 19213 by previously used methods (3).

To obtain preparations of schizokinen which were essentially iron-free, solutions of schizokinen were passed slowly through a column (1 by 4 cm) of the cation exchange resin Dowex-50 (J. T. Baker, Phillipsburg, N.J.). Iron-free schizokinen is not retained by the resin. The column was washed with glass-distilled water until all schizokinen had been eluted as determined by tests of the eluate with 2% $FeCl_3$ in 0.005 N HCl (wt/vol). The eluate was then concentrated by either flash evaporation or by lyophilization.

Concentrations of iron-free schizokinen and aerobactin were determined by measuring the color produced by reaction of the hydroxamates with $FeCl_3$ in acidic solution. Ten to 50 μliters of the hydroxamate solution was mixed with sufficient 2% $FeCl_3$ in 0.005 N HCl (wt/vol) to give a final volume of 0.3 ml and the absorbance at either 500 or 510 nm was determined for aerobactin and schizokinen, respectively. Concentrations were estimated from the molar extinction coefficient for aerobactin in this test, 2×10^3 (9), and by comparison of the absorbance of the unknown schizokinen-Fe^{3+} solutions with the absorbance of known concentrations of schizokinen-Fe^{3+} prepared form crystalline schizokinen-Fe^{3+} in the same $FeCl_3$ solution.

For some experiments the Desferal was subjected to additional purification. One-milligram amounts were chromatographed ascendingly on paper by using butanol-water-acetic acid (5:4:1, vol per vol per vol) as the solvent system. After drying, the Desferal was located by spraying a strip cut from the edge of the paper with 2% $FeCl_3$ in 0.005 N HCl (wt/vol). Desferal contained in the center of the $FeCl_3$-reactive spot was eluted with glass-distilled water and the eluate brought to dryness by flash evaporation. The residue was dissolved in 0.5 ml of glass-distilled water, and the concentration of Desferal was determined from the absorbance at 500 nm of mixtures of the purified Desferal and 2% $FeCl_3$ in 0.005 N HCl as described above.

Assay for residual iron in "iron-free" preparations of schizokinen. One-tenth milliliter of 6 N HCl was added to 0.5 ml of a schizokinen solution containing 16 to 20 mg of schizokinen per ml, and the solution was autoclaved at 121 C for 15 min. After cooling, the hydrolysate was assayed for iron by using bathophenanthroline (G. F. Smith Chemical Co., Columbus, Ohio) as the chromogenic reagent (3). The iron concentration was determined by comparison with a standard curve prepared from known concentrations of high purity iron (Johnson, Matthey Chemicals, London) which had been similarly treated. Iron contamination in schizokinen preparations varied slightly, but was usually only about 30 ng of iron per mg of schizokinen. Assays with Desferal indicated iron contamination of this hydroxamate as below detectable limits of the test as employed (less than 5 ng of iron per mg of Desferal).

RESULTS

Transport of $^{59}Fe^{3+}$ by B. megaterium SK11 from schizokinen-$^{59}Fe^{3+}$ and Desferal-$^{59}Fe^{3+}$. Previous studies (5) with *B. megaterium* strain SK11, which cannot secrete the secondary hydroxamate schizokinen, revealed that both schizokinen and Desferal stimulated total uptake of $^{59}Fe^{3+}$. These examinations were done at a single $^{59}Fe^{3+}$ concentration (42 pmol/ml). In the present work, $^{59}Fe^{3+}$ transport kinetics from $^{59}FeCl_3$, schizokinen-$^{59}Fe^{3+}$ and Desferal-$^{59}Fe^{3+}$ were determined at iron concentrations from 4.2 to 8,475 pmol/ml. The hydroxamates were added at concentrations sufficient to produce a 10- to 15-fold excess of iron-free hydroxamate at all iron levels tested, assuming that 1 mol of hydroxamate binds 1 mol of iron. Residual iron contamination in the schizokinen preparations was taken into account (see above). At all $^{59}Fe^{3+}$ concentrations, the cells reached apparent saturation between 2 and 6 min after addition of the $^{59}Fe^{3+}$ sources. Apparent rates of $^{59}Fe^{3+}$ uptake were calculated from samples collected 2 min after the initiation of the uptake assay; total uptake was estimated from samples collected 15 min after initiation.

Apparent rates of $^{59}Fe^{3+}$ uptake from the schizokinen-$^{59}Fe^{3+}$ complex increased sharply as the substrate was elevated; highest rates (about 140 pmol of $^{59}Fe^{3+}$ per min per ml) were observed at a schizokinen-$^{59}Fe^{3+}$ concentration of about 1,700 pmol of $^{59}Fe^{3+}$ ml, although at greater concentrations the rate dropped to 90 to 102 pmol of $^{59}Fe^{3+}$ per min per ml (Fig. 2A). There also was an increase in total uptake from schizokinen-$^{59}Fe^{3+}$ as the $^{59}Fe^{3+}$ concentration was increased. The system reached maximal $^{59}Fe^{3+}$ incorporation (about 410 pmol of $^{59}Fe^{3+}$/ml) at schizokinen-$^{59}Fe^{3+}$ concentrations of 1,700 pmol of $^{59}Fe^{3+}$ or greater (Fig. 2B). The marked stimulation of $^{59}Fe^{3+}$ transport by schizokinen was evident again in these experiments (Fig. 2A, B).

Similar plots of $^{59}Fe^{3+}$ uptake from the Desferal-$^{59}Fe^{3+}$ chelate demonstrated a clear difference in the response of *B. megaterium* SK11 to this iron source (Fig. 2A, B). At $^{59}Fe^{3+}$ concentrations of 85 pmol per ml, or less, both rates of iron uptake and total iron incorporated were very similar for the schizokinen-$^{59}Fe^{3+}$ and Desferal-$^{59}Fe^{3+}$ chelates. Elevation of the Desferal-$^{59}Fe^{3+}$ concentration above 85 pmol of $^{59}Fe^{3+}$ per ml did not produce a corresponding increase in either of the parameters, as was observed with schizokinen-$^{59}Fe^{3+}$. Therefore, the most rapid rate of uptake and greatest level of $^{59}Fe^{3+}$ incorporation obtained with Desferal-

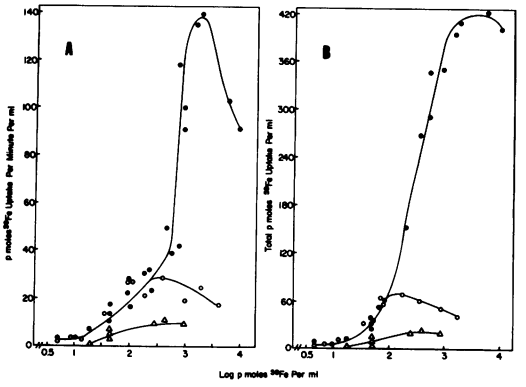

FIG. 2. *$^{59}Fe^{3+}$ uptake in B. megaterium SK11 at various $^{59}Fe^{3+}$ concentrations from $^{59}FeCl_3$ (open triangle), and the two ferric hydroxamates schizokinen-$^{59}Fe^{3+}$ (closed circle), and Desferal-$^{59}Fe^{3+}$ (open circle). The hydroxamates were added at 10 to 15 times the concentration required to bind the $^{59}Fe^{3+}$ added. A, Apparent rates of uptake calculated from samples collected 2 min after addition of the $^{59}Fe^{3+}$ sources. B, Total $^{59}Fe^{3+}$ uptake calculated from samples collected 15 min after addition of the $^{59}Fe^{3+}$ sources.*

$^{59}Fe^{3+}$ represented only about one-sixth of the values achieved with schizokinen-$^{59}Fe^{3+}$.

It is possible that the Desferal preparation was contaminated with a substance which inhibited iron uptake, and at the higher Desferal concentrations required as the $^{59}Fe^{3+}$ addition was increased this inhibitor reached an effective concentration. In an effort to eliminate this possibility, a sample of Desferal was subjected to additional purification (see above). This additionally purified Desferal did not stimulate $^{59}Fe^{3+}$ above that noted with the usual Desferal preparations. Although these procedures do not eliminate the possible presence of a contaminating inhibitor of iron transport in the Desferal, they do suggest that the differences noted in $^{59}Fe^{3+}$ transport between Desferal-$^{59}Fe^{3+}$ and schizokinen-$^{59}Fe^{3+}$ were due to inherent differences in the capacity of *B. megaterium* SK11 to utilize the two chelates. It should be remembered that schizokinen is the secondary hydroxamate synthesized by *B. megaterium* 19213.

Effect of high concentrations of iron-free hydroxamates on iron transport. Incorporation of iron from the ferric hydroxamate chelate presents some interesting questions regarding possible discrimination between the iron-free form of the hydroxamate and its ferric chelate. Emery (8) has indicated that the secondary hydroxamate ferrichrome, produced by *Ustilago*, undergoes a conformational shift after

chelation of iron. Other hydroxamates probably show similar changes. If the system which incorporates iron from ferric hydroxamates has some affinity for the iron-free form, then it might be predicted that assays which contained an excess of the iron-free hydroxamate would show alterations in iron uptake kinetics due to competition between the two forms of the hydroxamate. Apparent rates of $^{59}Fe^{3+}$ uptake estimated at 2 min of assay and total $^{59}Fe^{3+}$ uptake at 15 min for *B. megaterium* SK11 were determined by using assay mixtures which contained 85 pmol of $^{59}Fe^{3+}$ per ml and increasing concentrations of Desferal (Fig. 3). Molar ratios of Desferal: $^{59}Fe^{3+}$ of 1:1 or 10:1 produced an initial $^{59}Fe^{3+}$ uptake rate of about 27 pmol per min per ml; mixtures containing a 500-fold, or greater, excess of iron-free Desferal demonstrated a decline in the rate of $^{59}Fe^{3+}$ uptake, and assays containing about 10,000 molecules of iron-free Desferal per molecule of the Desferal-$^{59}Fe^{3+}$ chelate had an apparent uptake rate of only 1.7 pmol per min per ml. Total $^{59}Fe^{3+}$ uptake during the testing period also was reduced by excesses of iron-free Desferal (Fig. 3).

Iron uptake assays containing high concentrations of iron-free schizokinen produced a more complex pattern. Marked reduction was observed in total $^{59}Fe^{3+}$ incorporated (15 min of assay) at schizokinen: $^{59}Fe^{3+}$ molar ratios of 3,000 to 4,000:1 (Fig. 4B). However, this effect of iron-free schizokinen was noted only at $^{59}Fe^{3+}$ concentrations of 340 pmol per ml, or greater. Excessive iron-free schizokinen had little effect on the apparent rate of $^{59}Fe^{3+}$ uptake during the first 2 min of assay. For example, at an $^{59}Fe^{3+}$ concentration of 340 pmol per ml, various molar ratios of schizokinen: $^{59}Fe^{3+}$ from 1:1 to 4,000:1 did not cause a significant change in the apparent rate of $^{59}Fe^{3+}$ transport (Fig. 4A). Cells confronted with a 4,000-fold excess of iron-free schizokinen accumulated less $^{59}Fe^{3+}$ (Fig. 4A). The lowered uptake of $^{59}Fe^{3+}$ in the presence of this concentration of iron-free schizokinen was due to an abrupt halt in $^{59}Fe^{3+}$ transport which usually occurred at about two minutes of assay (data not shown).

It was considered possible that the effect of excess iron-free schizokinen might have been due to the presence of a chemically unrelated inhibitor in the preparation since reduction in total iron incorporated appeared only at the higher concentrations of iron. These assays received correspondingly higher amounts of iron-free schizokinen to maintain the 3,000- to 4,000-fold excess of iron-free schizokinen. This

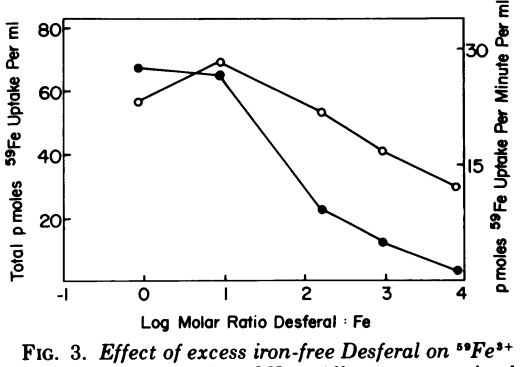

FIG. 3. *Effect of excess iron-free Desferal on $^{59}Fe^{3+}$ uptake in B. megaterium SK11. All assays contained 85 pmol of $^{59}Fe^{3+}$ per ml. Apparent rates of uptake (closed circle) calculated from samples collected 2 min after addition of the $^{59}Fe^{3+}$ source; total uptake (open circle) calculated from samples collected after 15 min.*

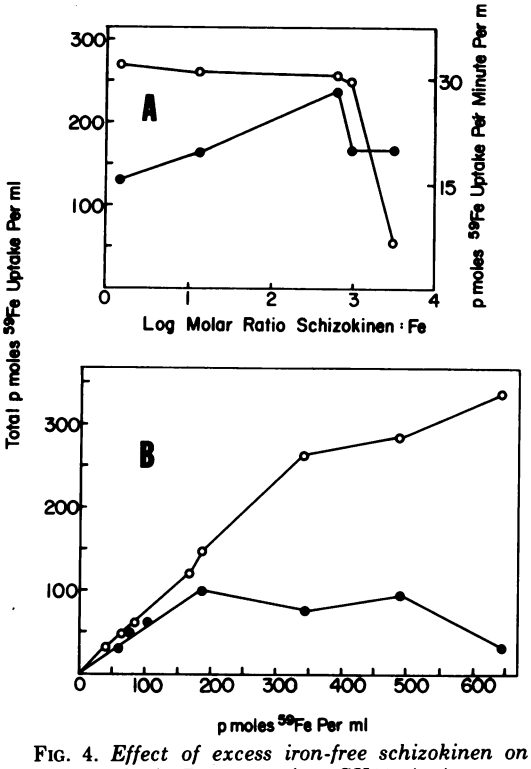

FIG. 4. *Effect of excess iron-free schizokinen on $^{59}Fe^{3+}$ uptake in B. megaterium SK11. A, Apparent rates of uptake (closed circle) calculated from samples collected at 2 min, and total uptake at 15 min (open circle); these assays contained 340 pmol of $^{59}Fe^{3+}$ per ml. B, Effect of excess iron-free schizokinen on total $^{59}Fe^{3+}$ uptake in B. megaterium SK11 at various $^{59}Fe^{3+}$ concentrations. Schizokinen: $^{59}Fe^{3+}$ molar ratios were 10 to 15:1 (open circle) and 3,000 to 4,000:1 (closed circle). Total uptake calculated from samples collected at 15 min.*

consideration was discounted partly by the results of assays in which sufficient $^{59}Fe^{3+}$ was added with the elevated schizokinen to achieve only a 10- to 15-fold excess of iron-free schizokinen. Cells in these assays rapidly incorporated $^{59}Fe^{3+}$ to the predicted saturation level.

Effects of aerobactin on $^{59}Fe^{3+}$ transport and growth in B. megaterium SK11. The results above suggest that the system transporting iron from ferric hydroxamates has a recognition capacity which distinguishes between hydroxamates of different structure. Additional evidence supporting this conclusion was obtained with aerobactin, the hydroxamate produced by *A. aerogenes* 62-1. When tested at 42 pmol $^{59}Fe^{3+}$ per ml, molar ratios of aerobactin: $^{59}Fe^{3+}$ of 1:1 to 100:1 did not stimulate uptake of $^{59}Fe^{3+}$ above the level observed with $^{59}FeCl_3$ as the iron source (Fig. 5). In the same assay, transport of $^{59}Fe^{3+}$ from the schizokinen-$^{59}Fe^{3+}$ was typically rapid reaching saturation in about 2 min.

Since *B. megaterium* SK11 appears to have low affinity for the aerobactin-iron chelate it follows that this chelate should be a poor source of iron for growth of this organism. To test this possibility, growth kinetics in the presence of aerobactin was determined by previously described methods (6). Cultures of *B. megaterium* SK11 containing 4.4 nmol of aerobactin per ml received varying iron supplements to yield aerobactin: iron molar ratios from 0.25:1 to 100:1. Control cultures containing no aerobactin were also prepared at each iron concentration. Total growth in the aerobactin-containing cultures was determined when the controls without aerobactin reached maximal population densities. Cultures containing about a 10-fold excess of iron-free aerobactin were unable to reach full growth (Fig. 6), and the population densities reached in these cultures were equivalent to the total growth achieved by control cultures which received no iron supplement. This indicates that little of the iron supplied as aerobactin-iron became metabolically active.

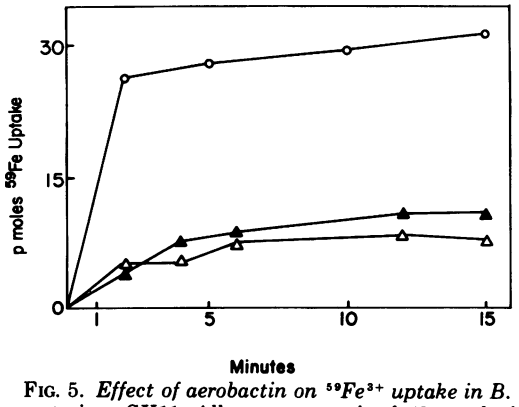

FIG. 5. *Effect of aerobactin on $^{59}Fe^{3+}$ uptake in B. megaterium SK11. All assays contained 42 pmol of $^{59}Fe^{3+}$ per ml. $^{59}Fe^{3+}$ sources were $^{59}FeCl_3$ (closed triangle), aerobactin-$^{59}Fe^{3+}$ at a 10-fold excess of iron-free aerobactin (open triangle), and schizokinen-$^{59}Fe^{3+}$ at a 10-fold excess of iron-free schizokinen (open circle).*

DISCUSSION

The most attractive hypothesis explaining the role of schizokinen as a cofactor in iron transport by *B. megaterium* suggests secretion of iron-free schizokinen into the environment where it binds iron and returns it to the cell as the ferric hydroxamate chelate. The next step in the transport process must involve an association between the ferric hydroxamate and the cell surface for either transport of the chelate or removal of the iron with release of the iron-free chelate. Recent experiments with radioactively labeled hydroxamates indicate that the chelate probably penetrates the cell as an intact unit (1, 8). Since most hydroxamates have low solubility in lipid solvents, movement of the ferric hydroxamate into or across the lipid boundary of the cell must require participation of another component(s), perhaps a membrane associated protein which binds the hydroxamate. Moreover, removal of iron from the chelate must require special processes. In *Mycobacterium smegmatis* this is accomplished by an apparent enzyme-catalyzed reduction of iron which releases it from the ferric hydroxamate, ferricmycobactin (15). Other microbial species may follow the same pattern. If there are specific

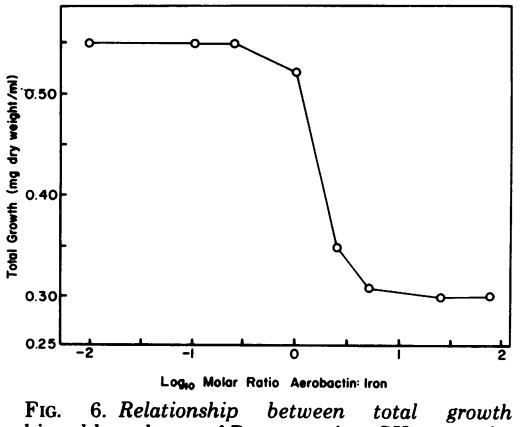

FIG. 6. *Relationship between total growth achieved by cultures of B. megaterium SK11 containing 4.4 nmol of aerobactin per ml and a variable iron supplement.*

receptor sites for hydroxamates, and if enzymic reduction of iron is required to release it for metabolic use, then the transport system should show varying affinities for hydroxamates of different chemical structures. This contention is supported by the present work in which kinetics of iron transport from the two chelates, Desferal-iron and schizokinen-iron, were different.

The ferric chelate of aerobactin inhibited growth of *B. megaterium* SK11 possibly by withholding iron from iron-utilizing systems. Iron uptake assays showed some response to aerobactin-iron; however, the amount of iron taken up from this chelate did not exceed that obtained from $FeCl_3$. This is interesting since *B. megaterium* SK11 is able to grow with only iron salts as the iron source, provided the inoculum is high (2, 6). It may be that *B. megaterium* cannot remove iron from the aerobactin-iron chelate, even though it can transport low levels of this chelate.

Other evidence supports a special ferric hydroxamate transport system with recognition capacity for certain hydroxamates. Spontaneous mutants derived from *B. megaterium* SK11, which are resistant to the ferric hydroxamate antibiotic A22765, also have lost capacity to transport iron from the structurally similar hydroxamate, Desferal (6). Similar mutants have been described in *Staphylococcus aureus* (16). Emery (8) concluded that of the two hydroxamates produced by *Ustilago* (ferrichrome and ferrichrome A), only ferrichrome is a cofactor for iron transport. Ferrichrome A also was inactive in *B. subtilis*, although ferrichrome, mycobactin, and Desferal stimulated iron uptake in this organism (7, 14). Recently Luckey et al. (11) have defined a hydroxamate-utilizing system in *Salmonella typhimurium* which also was subject to mutational alteration of its specificity.

Iron-free hydroxamates may compete with the iron chelate for acceptance by the transport system. This was suggested by the assays with excess iron-free Desferal in which both rates of uptake and total iron incorporated were reduced by high concentrations of iron-free Desferal. These results might be explained by assuming the existence of a hydroxamate recognition site which can bind with either form of Desferal. An excess of iron-free Desferal would impede binding of the iron chelate, causing a reduction in the observed rate of iron uptake and in the total iron incorporated during a short time interval. However, the large excess of iron-free Desferal required to produce significant alterations in $^{59}Fe^{3+}$ uptake kinetics may indicate greater affinity for the Desferal-$^{59}Fe^{3+}$ chelate. Excess iron-free Desferal also reduced total iron uptake in *B. subtilis* and caused apparent iron-starvation in *A. aerogenes* (W. B. Davis, Ph.D. Dissertation, University of Mississippi School of Medicine, Jackson; D. N. Downer and B. R. Byers, Bacteriol. Proc. p. 130, 1970).

Excess iron-free schizokinen caused a reduction in total iron removed by *B. megaterium* SK11 only at elevated iron concentrations. The lack of interference with apparent rates of uptake by iron-free schizokinen at all iron levels tested may indicate that the cells are able to select the iron chelate from a mixed population of the two forms of schizokinen. This does not explain early termination of iron transport which was noted at elevated iron levels with excess iron-free schizokinen. It may be that removal of the schizokinen-iron complex during transport produced a critical overload of the iron-free form. These results also might be compatible with the assumption that the schizokinen-iron chelate and the iron-free hydroxamate are transported equally well. Such a system might become saturated at high levels of schizokinen, leading to the observed halt in incorporation of iron. This does not seem a likely explanation since Arceneaux et al. (1), who demonstrated binding and possible transport of ^{3}H-labeled iron-free schizokinen in *B. megaterium* SK11, have shown that the picomoles of $^{59}Fe^{3+}$ transported by this organism from equimolar concentrations of ^{3}H-schizokinen and $^{59}Fe^{3+}$ exceeds the picomoles of ^{3}H-schizokinen retained by the cells at high concentrations of the ^{3}H-schizokinen-$^{59}Fe^{3+}$ chelate. At certain lower concentrations of the chelate there was nearly equivalent uptake of schizokinen and iron (1). The data do indicate an interaction between *B. megaterium* SK11 and the iron-free schizokinen molecule.

It should be noted that the levels of iron-free schizokinen tested here probably would not be encountered during growth of schizokinen-producing strains. Although *B. megaterium* ATCC 19213 can secrete as much as 280 nmol schizokinen per ml during iron starvation (3), very high concentrations of schizokinen are not reached until the maximal stationary phase of the culture.

The studies reported here, as well as those from other laboratories, support the existence of a special ferric hydroxamate transport system with a recognition capacity based on the chemical structure of the ferric hydroxamate. This recognition may reside in the factors responsible for binding or transport of the ferric hydroxamate, or both, or in the enzyme(s) releasing iron from the chelate.

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