Biol Metals (1990) 2:197-202

BIOLOGY OF METALS

Original articles

Ferrioxamine transport mutants and the identification of the ferrioxamine receptor protein (FoxA) in *Erwinia herbicola* (*Enterobacter agglomerans*)

Ingrid Berner and Günther Winkelmann

Mikrobiologie/Biotechnologie, Universität Tübingen, Auf der Morgenstelle 1, D-7400 Tübingen, Federal Republic of Germany

Received October 19, 1989

Summary. Iron deprivation of *Erwinia herbicola* (*Enterobacter agglomerans*) induces the biosynthesis of six high-M_r outer-membrane proteins and large amounts of ferrioxamine E. Mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and selection with ferrimycin A yielded mutants of *E. herbicola* K4 (wild type), defective in the expression of a 76-kDa outer-membrane protein, as determined by SDS/polyacrylamide gel electrophoresis. While in bioassays wild-type cells showed growth promotion in the presence of ferrioxamines (B, D_1, D_2, E, G), enterobactin, citrate, ferrichrome and coprogen, these mutants failed to respond to ferrioxamines. Moreover, experiments with ^{55}Fe-labelled siderophores confirmed that iron transport mediated by ferrioxamine E and B in the mutants was completely inhibited, whereas iron transport by other hydroxamate siderophores, such as ferrichrome and coprogen was unaffected. The results are evidence that the 76-kDa protein in the outer membrane represents the receptor protein (FoxA) for ferrioxamines in *E. herbicola*.

Key words: *Erwinia herbicola* - *Enterobacter agglomerans* - Ferrioxamines - Ferrioxamine receptor - Iron transport - Siderophores

Introduction

Erwinia herbicola (*Enterobacter agglomerans*) has been shown earlier to represent a group within the Enterobacteriaceae that produces siderophores of the ferrioxamine family (Berner et al. 1988). Iron-starved cultures excreted the desferri forms of ferrioxamine E as the principal product and ferrioxamine D_2 and B as minor products. It was also shown in that investigation that other siderophores, such as enterobactin, ferrichrome, coprogen and even citrate functioned as siderophores, suggesting the presence of multiple siderophore receptors in *E. herbicola*. In *Escherichia coli* six different outer-membrane receptor proteins have so far been identified (FepA, FecA, FhuA, FhuE, Fiu, Cir) which are responsible for iron transport mediated by enterobactin, citrate, ferrichrome, coprogen and for two unidentified siderophores (Hantke 1983). Strains of *E. coli* containing ColV plasmid express additionally a receptor (Iut) for aerobactin transport (Braun 1981). While *E. herbicola* resembles biosynthetically the ferrioxamine-producing Streptomycetes, the outer-membrane profile shows similarities to the Enterobacteriaceae. Ferrioxamine production has also been reported in the genus *Nocardia, Micromonospora, Chainia,* and also in *Pseudomonas, Chromobacterium* and *Arthrobacter* (Müller and Zähner 1968; Meyer and Abdallah 1980). This communication describes the isolation of mutants unable to transport ferrioxamines by use of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and a ferrimycin A selection procedure. The isolated mutants allowed the identification of the ferrioxamine receptor protein (FoxA) in the outer membrane of *E. herbicola*.

Offprint requests to: G. Winkelmann

Materials and methods

Bacterial strains and growth conditions. Bacterial strains used in this study are listed and described in Table 1. All strains were maintained on agar slants containing 0.4% yeast extract, 1% malt extract, and 0.4% glucose (YMG). Rich medium contained 0.8% nutrient broth (NB; Difco) and minimal medium contained M9 salts (Miller 1972) and 0.4% glucose. M9 medium was made iron-deficient either by the addition of ethylenediamine-*N,N'*-bis(2-hydroxyphenylacetic acid) (EDDA, Fluka), a strong nonutilizable iron chelator, or by passing it through a Chelex-100 column (Bio-Rad) as described by Matzanke et al. (1989). A stock solution of EDDA was deferrated by the procedure of Rogers (1973).

Mutagenesis. For mutagenesis of the wild-type strain *E. herbicola* K4, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG, Sigma) was used (Ankenbauer et al. 1986; Hantke 1987; Miller 1972). M9 medium + 50 μM EDDA was inoculated to a density of $A_{578} = 0.05$ with an overnight culture of *E. herbicola* K4, grown in NB medium. After growth at 27° C and 120 rpm to a density of $A_{578} = 0.2$, cells were

Table 1. Mutants obtained from the ferrimycin A selection

Strain	Sensitivity to		Growth promotion mediated by			
	Ferrimycin A	Albomycin	Ferrioxamines B, D_1, D_2, E, G	Ferrichrome	Coprogen	Enterobactin
K4 (wild type)	+	+	+	+	+	+
FM13[a]	−	+	−	+	+	+
FM1, FM 2 FM6, FM11 FM15, FM16 FM20, FM22 FM24, FM26	−	+	−	+	+	+
FM3, FM4 FM7, FM8 FM9, FM10 FM12, FM14 FM17, FM21 FM23, FM27 FM28, FM29	−	+	+	+	+	+
FM5, FM25	−	+	+	−	−	+
FM30, FM31	−	−	−	+	+	+
B 63/1[b]	−	+	−	+	+	+

[a] FM13 was isolated as a spontaneous ferrimycin-A-resistant colony from the growth inhibition zone of *E. herbicola* K4 (wild type)
[b] This mutant was obtained from a streptonigrin selection

collected by centrifugation at 6000 *g* and washed twice with 0.9% NaCl solution. Cells were resuspended in M9 medium to a density of 10^8 cells/ml. Mutagenesis was started by the addition of NG (100 µg/ml) and incubation at 27° C and 250 rpm for 30 min. Cells were sedimented at 6000 *g*, washed twice with 0.9% NaCl solution, resuspended in NB medium and incubated overnight at 27° C.

Selection of mutants. The antibiotic ferrimycin A was used to select specifically for mutants affected in the outer-membrane receptor for ferrioxamines. Ferrimycin A is a structural analogue of ferrioxamines (Bickel et al. 1966) isolated from *Streptomyces griseoflavus* (ETH9578) and is assumed to be taken up into the cells by the ferrioxamine route. Selection was carried out as follows. M9 medium + 0.1% yeast extract + 50 µM EDDA was inoculated to a density of $A_{578} = 0.05$ with NG-treated cells grown overnight in NB medium. Cells were grown to a density of $A_{578} = 0.3$ at 27° C and 120 rpm. Ferrimycin A (40 µg/ml) was added to the culture and incubation was continued for 4 h in the same conditions. Cells were collected by centrifugation at 6000 *g* and washed twice with 0.9% NaCl solution and stored at 4° C. Streptonigrin selection was carried out according to the method described by Hantke (1987).

Screening of mutants. Ferrimycin-treated or streptonigrin-treated cells were spread on NB plates containing 1.5% agar (Fluka) with a density of 50–100 colonies per plate and incubated overnight at 27° C. Colonies were replica-plated onto Chrome azurol S (CAS) plates (Schwyn and Neilands 1987) and on NB plates containing 1 mM EDDA (Ankenbauer et al. 1986; Braun et al. 1983). Colonies growing poorly on NB + 1 mM EDDA and showing large haloes on CAS were chosen for bioassays.

Bioassay. Growth promotion by different siderophores and growth inhibition by different antibiotics was studied in the following medium: 100 mM Tris (Trizma, Sigma), 0.03 g/l KH_2PO_4, 0.5 g/l NaCl, 1 g/l NH_4Cl, 1 mM $MgSO_4 \cdot 7H_2O$, 0.1 mM $CaCl_2 \cdot 2H_2O$, 0.4% glucose, 1 mM EDDA, 0.4% agar. Bioassay medium (10 ml) was inoculated with 0.3 ml of an overnight culture in NB medium and poured into sterile plates. After solidification, filter paper disks (6 mm) containing either 0.15 nmol siderophores or 0.15 µg ferrimycin A or 1.5 µg albomycin, were placed on the plates and growth or inhibition was scored after 1 or 2 days of incubation at 27° C.

Isolation of outer membranes. Isolation of outer-membrane proteins was carried out according to published procedures (Hantke 1981; Eick-Helmerich and Braun 1989) with slight variations. Cells grown in M9 medium + 50 µM EDDA (100 ml) to a density of $A_{578} = 0.5$ were collected by centrifugation and suspended in 1 ml ice-cold 0.2 M Tris/HCl pH 8.0. Then 2 ml 0.2 M Tris/HCl pH 8.0, 1 M sucrose and 0.2 ml lysozyme (2 mg/ml) were added. The suspension was frozen overnight at −70° C and thawed at 27° C, before 0.2 ml 10 mM EDTA pH 8.0 was added. The cells were incubated for 15 min at room temperature. Then, 2 ml extraction buffer (2% Triton, 50 mM Tris/HCl pH 8.0, 10 mM $MgCl_2$) and 0.2 ml DNase (1 mg/ml) were added. Incubation was continued for 20 min at room temperature. The cleared solution was centrifuged for 15 min at 6000 *g* to remove residual cells and cell debris. The supernatant was then centrifuged for 1 h at 40000 *g*. The sediment, containing the outer-membrane proteins, was washed twice with 1 ml bidistilled water and used for SDS/polyacrylamide gel electrophoresis.

Electrophoresis. SDS/polyacrylamide gel electrophoresis of membrane proteins was carried out according to Laemmli (1970). Gels (8%) were run at a constant current of 35 mA for approximately 2.5 h and stained with Coomassie blue R 250 (Serva).

Siderophores and sideromycins. Enterobactin was isolated from a *fepA* mutant of *E. coli* AN 311 (Young and Gibson 1979) by adsorption of the culture filtrate to XAD-2 (Serva, Heidelberg), desorption with methanol and purification on LH20 (Pharmacia, Freiburg). Ferrichrome was isolated from cultures of *Ustilago sphaerogena* or *Neovossia indica* (Deml et al. 1984). Coprogen was purified from culture filtrates of *Neurospora crassa* according to Wong et al. (1983). Ferrioxamine E was isolated from *Streptomyes pilosus* (Meiwes 1989). Ferrioxamine B was provided by Ciba-

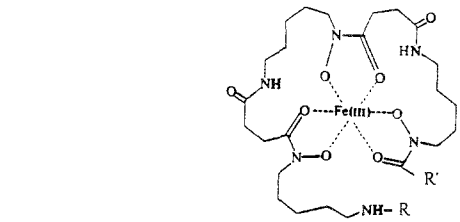
Geigy (Basel) and ferrioxamine D_1 was prepared by *N*-acetylation of ferrioxamine B. Ferrioxamine D_2 and G were gifts from W. Keller-Schierlein. Samples of ferrimycin A, danomycin and albomycin were kindly provided by H. Zähner. ^{55}Fe-labelled siderophores were prepared as described earlier (Berner et al. 1988). Streptonigrin was obtained from K. Hantke.

Transport assay. Transport of ^{55}Fe-labelled siderophores was carried out as described previously (Berner et al. 1988) with slight variations. Cells grown in M9 medium + 50 μM EDDA (100 ml) to a density of $A_{578} = 0.5$ were collected by centrifugation and washed twice with M9 + 10 μM nitrilotriacetate. The cells were finally suspended in M9 medium to a density of $A_{578} = 0.5$. Aliquots of this suspension were taken for the transport assay and preincubated for 10 min at 27° C and 120 rpm. Transport was started by the addition of ^{55}Fe-labelled siderophores to a final concentration of 0.5 μM (specific activity 2.2 kBq/nmol). Samples (1 ml, corresponding to 0.2 mg dry mass) were taken at intervals, filtered on cellulose nitrate membrane filters (0.45 μm, Sartorius) and washed twice with ice-cold 0.9% NaCl solution. The radioactivity on the filters was measured in a liquid scintillation counter.

Results

The selection procedure described for ferrimycin-A-resistant mutants of *E. herbicola* is based on the general properties of sideromycins to enter the cells via siderophore transport pathways and receptors. Sideromycins, like ferrimycin A_1, A_2 and B, danomycins A and B and albomycins (δ_1, δ_2, ε) are secondary metabolic products of *Streptomyces* (Keller-Schierlein et al. 1984). Albomycin is an antibiotically active ferrichrome analogue (ferrichrysin derivative), of which the structure has recently been revised by Benz et al. (1982). Structural formulae of ferrioxamines and ferrimycin A_1 are given in Fig. 1. Ferrimycin A_1 is the principal components of the ferrimycin mixture (A_1, A_2, B) isolated from *Streptomyces griseoflavus*. As our ferrimycin was not highly purified, we used the designation ferrimycin A in the present paper indicating that the A-type predominates in the mixture. Thus, ferrimycins represent antibiotic derivatives of ferrioxamine B both of which are dependent on the ferrioxamine receptor for entrance into the bacterial cells, as shown in the present investigation. Generally, outer-membrane receptors are not present in visible amount in SDS/PAGE gels, unless the iron content of the growth medium is significantly reduced. Therefore, the iron content of growth media was varied to different degrees in order to study the synthesis of iron-regulated outer-membrane proteins in *E. herbicola* (Fig. 2). Wild-type cells of *E. herbicola* K4 were grown in: (a) M9 medium + 200 μM $FeCl_3$, (b) M9 medium, (c) M9 medium + 50 μM EDDA, (d) M9 medium + 300 μM EDDA in order to demonstrate iron-dependent expression of outer-membrane proteins. As shown in Fig. 2, partial derepression was seen in M9 medium without further iron removal, but complete derepression of iron-regulated outer-membrane proteins could only be seen in media supplemented with EDDA.

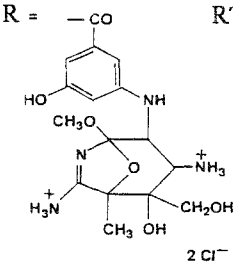
Mutagenesis with NG and subsequent ferrimycin A selection yielded 31 ferrimycin-A-resistant clones (frequency 0.2%) as shown in Table 1. Surprisingly, dano-

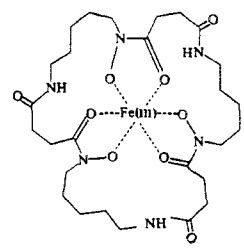


Ferrioxamine B R = H, R′ = CH_3

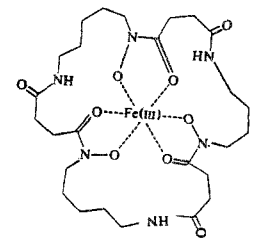
Ferrioxamine D_1 R = $COCH_3$ R′ = CH_3

Ferrioxamine G R = H, R′ = CH_2-CH_2-COOH

Ferrimycin A_1 R =  R′ = CH_3



Ferrioxamine E



Ferrioxamine D_2

Fig. 1. Structural formulae of ferrioxamines (B, D_1, D_2, E, G) and ferrimycin A_1

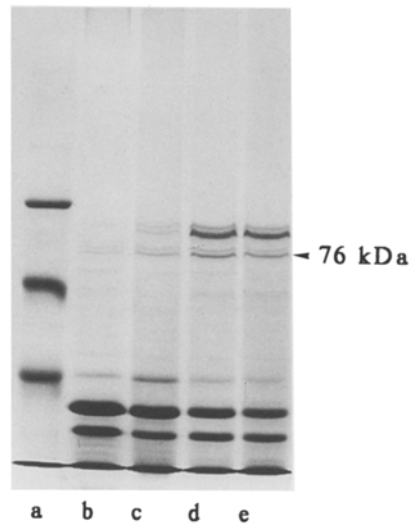


Fig. 2. SDS/polyacrylamide gel electrophoresis of outer-membrane proteins from *E. herbicola* K4 (wild type). **(a–e)** Cells were grown in M9 medium containing decreasing amounts of bioavailable iron. **(a)** Marker proteins (94 kDa, 67 kDa, 43 kDa); **(b)** M9 medium + 200 μM $FeCl_3$; **(c)** M9 medium without addition; **(d)** M9 medium + 50 μM EDDA; **(e)** M9 medium + 300 μM EDDA

mycin was not antibiotically active against *E. herbicola*, although it is a derivative of ferrioxamine G. The streptonigrin selection procedure, which has been described earlier for the selection of Fe(III)-aerobactin receptor-deficient *E. coli* strains (Braun et al. 1983), did not work well with *E. herbicola* and only one mutant (B 63/1) could be obtained which showed ferrimycin A resistance and inhibition of ferrioxamine transport.

SDS/polyacrylamide gel electrophoresis of ferrimycin-A-resistant clones revealed that several of these clones lacked a 76-kDA iron-regulated outer-membrane protein (Fig. 3). Other clones which were resistant to ferrimycin A or possessed a reduced sensitivity towards ferrimycin A_1 still contained the 76-kDa protein.

The ferrimycin-A-resistant clones which were devoid of the 76-kDa protein and those which showed a reduced sensitivity but still contained the 76-kDa protein, as well as the corresponding wild-type strain *E. herbicola* K4, were compared for their ability to utilize various siderophores in growth promotion tests. As shown in Table 1, growth promotion in wild-type *E. herbicola* K4 was positive with ferrioxamines B, D_1, D_2, E, G, as well as with enterobactin, coprogen, ferrichrome and citrate, confirming the earlier reported observation of multiple siderophore receptors in this group (Berner et al. 1988). One group of ferrimycin-A-resistant mutants (FM1–FM26) were unable to utilize ferrioxamines (E, D_1, D_2, G, B) but grew well with enterobactin, coprogen, ferrichrome and citrate. A second group of ferrimycin-A-resistant mutants (FM3–FM29) still grew with ferrioxamines and all other siderophores. Typical results of these bioassays are shown in Fig. 4. Growth zones in the presence of all siderophores and sensitivity to ferrimycin A and albomycin can be seen on plates with wild-type (Fig. 4a) and mutant FM13 (Fig. 4b). The mutant FM13 was resistant to ferrimycin A but remained sensitive to albomycin and did not grow in the presence of ferrioxamines (Table 1). Other mutants (FM5, FM25) were resistant to both ferrimycin A and albomycin (Table 1). The results clearly demonstrate that some of the ferrimycin-resistant clones were still able to utilize ferrioxamines. Growth response with other siderophores remained generally unaffected except for FM5 and FM25, where ferrichrome and coprogen utilization was absent.

A comparative transport study with cells of both wild-type *E. herbicola* K4 and ferrimycin-A-resistant mutants, using ^{55}Fe-labelled ferrichrome, coprogen, ferrioxamine E and ferrioxamine B, clearly showed that mutants lacking the 76-kDa outer-membrane protein were no longer able to transport iron complexed by ferrioxamines, while iron transport by ferrichrome and coprogen was unaffected (Table 2). It is interesting to note that in the mutant strains, where ferrioxamine up-

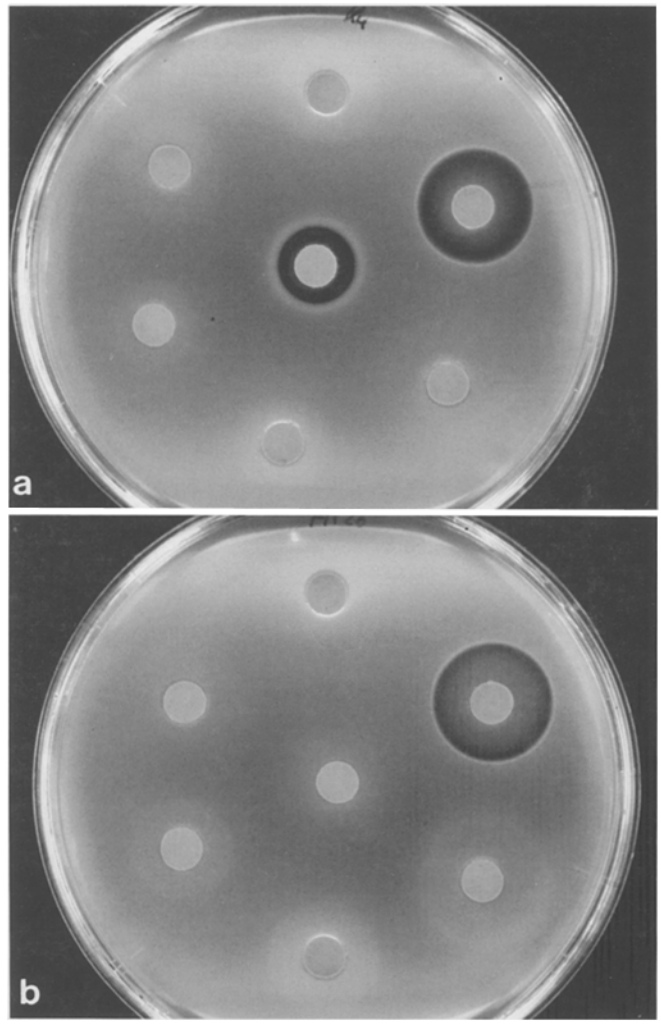


Fig. 4. Growth promotion tests with siderophores and inhibition of growth by sideromycins. **(a)** *E. herbicola* K4 (wild type), ferrimycin-A- and albomycin-sensitive; **(b)** *E. herbicola* FM13, ferrimycin-resistant. The bioassays were prepared as described in Materials and methods. Numbering is clockwise starting from the uppermost disk: (1) ferrioxamine E, (2) albomycin, (3) enterobactin, (4) coprogen, (5) ferrichrome, (6) ferrioxamine B, (7) ferrimycin A (center)

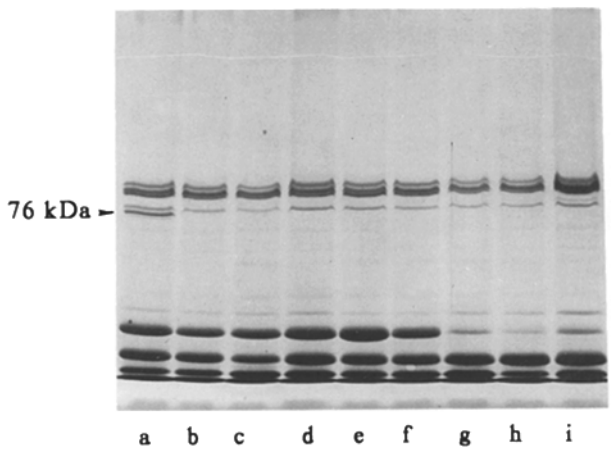


Fig. 3. SDS/polyacrylamide gel electrophoresis of outer-membrane proteins from **(a)** *E. herbicola* K4 (wild type) and **(b–i)** ferrimycin-A-resistant mutants, lacking the 76-kDa receptor protein: **(b)** FM11, **(c)** FM13, **(d)** FM15, **(e)** FM16, **(f)** FM22, **(g)** FM1, **(h)** FM2, **(i)** FM6. Cells were grown in M9 +50 μM EDDA as described in Materials and methods

Table 2. Transport of ^{55}Fe-labelled siderophores in *E. herbicola* K4 (wild type) and mutants lacking the 76-kDa receptor protein

Strain	Transport (pmol/mg dry mass) of											
	ferrioxamine E			ferrioxamine B			ferrichrome			coprogen		
	5′	10′	20′	5′	10′	20′	5′	10′	20′	5′	10′	20′
K4 (wild type)	260	510	888	165	300	560	138	277	475	45	109	294
FM2	4	5	7	2	3	5	219	426	776	168	375	680
FM13	3	5	7	2	3	6	300	569	935	165	325	632
B 63/1	4	6	12	5	5	8	291	600	1145	188	435	565

Incubation with labelled siderophores and measurement after 5 min (5′), 10 min (10′) and 20 min (20′) of uptake were as described in Materials and methods

take is blocked, transport of iron by ferrichrome and coprogen is significantly enhanced.

Discussion

The present contribution is evidence that ferrimycin A is a powerful tool for the selection of *Erwinia herbicola* mutants defective in ferrioxamine transport. Among the three ferrioxamines (B, D_2, E) produced by *E. herbicola*, ferrioxamine E is the principal siderophore (Berner et al. 1988). The ferrioxamine route is the most important iron-uptake route in these bacteria, although other siderophores like enterobactin, ferrichromes, coprogen and ferric citrate can also be used as iron donors. In this regard the herbicola group is very similar to *E. coli*, where multiple siderophore receptors have also been observed (Braun and Winkelmann 1987). The biosynthesis of catecholate siderophores has not been observed in the herbicola group but predominates in other *Erwinia* groups, e.g. in the plant pathogenic *E. chrysanthemi* which has been shown to produce chrysobactin [*N*-α-(2,3-dihydroxybenzoyl)-D-lysyl-L-serine], a novel siderophore of the catecholate family (Persmark et al. 1989).

The lack of an iron-regulated 76-kDa outer-membrane protein is very obvious in mutants obtained after ferrimycin A selection, suggesting that in these mutants the ferrioxamine receptor is absent. However, some ferrimycin-A-resistant mutants still expressed the 76-kDa protein. Subsequent growth-promotion tests revealed that these mutants still utilized ferrioxamines. Therefore, ferrimycin resistance is not only dependent on the presence or absence of a receptor protein but may also rely on other factors such as inactivation of the antibiotic residue or target insensitivity after mutation. A reduced sensitivity to ferrimycin A was also observed. Some of the ferrimycin-A-resistant mutants, which lacked the 76-kDa protein, show additional defects in the 40-kDa protein region, the function of which is still unresolved. Below the 76-kDa protein band there is another faint band to be seen in gels of the wild-type *E. herbicola* K4 which, like the 76-kDa protein, diappeared in the corresponding mutants. It remains open whether or not this protein is also involved in ferrioxamine uptake in a sense that two receptors for the same or for different ferrioxamines (E and D_2 or B) are expressed. Degradation during preparation and SDS gel electrophoresis may occur as observed for the enterobactin receptor protein (FepA) in *E. coli*.

From the results obtained with *E. herbicola*, it can be inferred that the iron-regulated 76-kDa outer-membrane protein is the ferrioxamine receptor protein which we named FoxA. Mutants of *E. coli*, defective in iron utilization from ferrioxamine B, have been reported and assigned to the *FhuF* gene locus (Braun et al. 1987). Although there is a functional similarity of the receptors in both bacterial groups, receptors and gene loci have to be defined separately unless a complete sequence identity has been demonstrated. Growth-promotion assays and iron transport measurements strongly support the assignment of a ferrioxamine-specific outer-membrane receptor. Ferrimycin A resistance alone is not sufficient to identify ferrioxamine-transport-defective strains. Only those ferrimycin-A-resistant clones which lack the 76-kDa receptor protein were unable to transport ferrioxamines. The mutants FM2, FM13, B63/1 served as typical representatives for uptake studies with ^{55}Fe-labelled siderophores. The absolute transport rates of ferrichrome and coprogen in these mutants were significantly enhanced compared to the wild-type strain, which may be attributed to a more pronounced iron deficiency in the FoxA mutants.

Acknowledgements. This research was supported in part by the *Deutsche Forschungsgemeinschaft* (Wi 628/4-1).

References

Ankenbauer R, Hanne LF, Cox CD (1986) Mapping of mutations in *Pseudomonas aeruginosa* defective in pyoverdine production. J Bacteriol 167:7–11

Benz G, Schröder T, Kurz J, Wünsche Ch, Karl W, Steffens G, Pfitzner J, Schmidt D (1982) Konstitution der Desferriform der Albomycine δ_1, δ_2 and ε. Angew Chem 94:552–553

Berner I, Konetschny-Rapp S, Jung G, Winkelmann G (1988) Characterization of ferrioxamine E as the principal siderophore of *Erwinia herbicola* (*Enterobacter agglomerans*). Biol Metals 1:51–56

Bickel H, Mertens P, Prelog V, Seibl J, Walser A (1966) Über die Konstitution von Ferrimycin A_1. Tetrahedron [Suppl 8] 1:171–179

Braun V (1981) *Escherichia coli* cells containing the plasmid ColV produce the iron ionophore aerobactin. FEMS Lett 11:225–228

Braun V, Gross R, Köster W, Zimmermann L (1983) Plasmid and chromosomal mutants in the iron(III)-aerobactin transport system of *Escherichia coli.* Use of streptonigrin selection. Mol Gen Genet 192:131–139

Braun V, Winkelmann G (1987) Microbial iron transport. Structure and function of siderophores. Progr Clin Biochem 5:67–99

Braun V, Hantke K, Eick-Helmerich K, Köster W, Preßler U, Sauer M, Schäffer S, Schöffler H, Staudenmaier H, Zimmermann L (1987) Iron transport systems in *E. coli.* In: Winkelmann G, van der Helm D, Neilands JB (eds) Iron transport in microbes, plants and animals. VCH Verlagsgesellschaft, Weinheim, pp 35–51

Deml G, Voges K, Jung G, Winkelmann G (1984) Tetraglycylferrichrome – the first heptapeptide ferrichrome. FEBS Lett 173:53–57

Eick-Helmerich K, Braun V (1989) Import of biopolymers into *Escherichia coli:* Nucleotide sequence of the *exbB* and *exbD* genes are homologous to those of the *tolQ* and *tolR* genes, respectively. J Bacteriol 171:5117–5126

Hantke K (1981) Regulation of ferric iron transport in *Escherichia coli* K12: Isolation of a constitutive mutant. Mol Gen Genet 182:288–292

Hantke (1983) Identification of an iron uptake system specific for coprogen and rhodotorulic acid in *Escherichia coli* K12. Mol Gen Genet 191:301–306

Hantke K (1987) Ferrous iron transport mutants in *Escherichia coli* K12. FEMS Microbiol Lett 44:53–57

Keller-Schierlein W, Huber P, Kawaguchi H (1984) Chemistry of danomycin, an iron-containing antibiotic. In: Krogsgaard-Larsen P, Brogger Christensen S, Kofod H (eds) Natural products and drug development. Munksgaard, Copenhagen, pp 213–227

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–686

Matzanke BF, Müller GI, Bill E, Trautwein AX (1989) Iron metabolism of *Escherichia coli* studied by Mössbauer spectroscopy and biochemical methods. Eur J Biochem 183:371–379

Meiwes J (1989) Dissertation thesis, University of Tübingen

Meyer JM, Abdallah MA (1980) The siderochromes of non-fluorescent pseudomonads: production of nocardamine by *Pseudomonas stutzeri.* J Gen Microbiol 118:125–129

Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor NY

Müller A, Zähner H (1968) Stoffwechselprodukte von Mikroorganismen. 65. Mitteilung Ferrioxamine aus Eubacteriales. Arch Microbiol 62:257–263

Persmark M, Expert D, Neilands JB (1989) Isolation, characterization and synthesis of chrysobactin, a compound with siderophore activity from *Erwinia chrysanthemi.* J Biol Chem 264:3187–3193

Rogers HJ (1973) Iron-binding catechols and virulence in *Escherichia coli.* Infect Immun 7:445–456

Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160:47–56

Wong GB, Kappel M, Raymond KN, Matzanke B, Winkelmann G (1983) Coordination chemistry of microbial iron transport compounds. 24. Characterization of coprogen and ferricrocin, two ferric hydroxamate siderophores. J Am Chem Soc 105:810–815

Young IG, Gibson F (1979) Isolation of enterochelin from *Escherichia coli.* Methods Enzymol 56:394–398