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Molecular recognition of siderophores: A study with cloned ferrioxamine receptors (FoxA) from *Erwinia herbicola* and *Yersinia enterocolitica*

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The outer membrane receptor for ferrioxamines ($FoxA_{Erw}$) of *Erwinia herbicola* (*Pantoea agglomerans*) was cloned from a cosmid gene bank and partially sequenced. A comparison of the partial amino acid sequence of $FoxA_{Erw}$ with the amino acid sequence of $FoxA_{Yer}$ from *Yersinia enterocolitica* revealed a high sequence homology. A functional analysis of $FoxA_{Erw}$ and $FoxA_{Yer}$ receptors cloned into a Fhu-negative background (HK97) revealed that ferrioxamines are recognized at very low concentrations (< 10 pmoles) in growth promotion bioassays. A collection of ferrioxamine derivatives containing varying chain lengths and ether bridges within the molecule was also accepted. However, the three ether containing ferrioxamine (Et_3) behaved differently in the two FoxA receptors. Coprogen was also recognized to a certain extent, whereas ferrichromes were completely excluded from the FoxA receptors, confirming that coprogens share some structural similarities with the ferrioxamines. *FoxA* mutants (FM13) of *Erwinia herbicola* obtained by ferrimycin selection showed no uptake of ^{55}Fe-labelled ferrioxamine E and B any more, while the transport of coprogen and ferrichrome was unaffected or even slightly increased.

Keywords: enterobacteria, ferrioxamines, FoxA receptor, iron transport, siderophores

Introduction

Members of the family Enterobacteriaceae are heterogeneous with respect to siderophore biosynthesis and utilization. While outer membrane receptors for siderophores have been well characterized in *E. coli,* other free living genera of this family like *Erwinia* and *Enterobacter* are not well understood. Seven outer membrane siderophore receptors are known in *Escherichia coli* K12, and these have been cloned and sequenced (for a review see Braun & Hantke 1997). FepA, Fiu and Cir are designed to recognize the species-own enterobactin (enterochelin) and its degradation products and derivatives. The receptors FhuA, FhuE and FecA recognize exogenous siderophores, e.g. ferrichromes, coprogens and ferric citrate, respectively, which are common siderophore products of fungi. IutA is a plasmid encoded aerobactin receptor (Braun 1981). The *Erwinia/ Enterobacter* group has much in common with *E. coli*, for example the production of enterobactin and the utilization of exogenous ferrichromes and coprogens. It differs, however, from *E. coli* by having in addition ferrioxamine biosynthethic genes and the corresponding FoxA receptor (Berner & Winkelmann 1990).

Ferrioxamines represent a group of siderophores that has previously been isolated mainly from

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Streptomyces pilosus (reviewed in Drechsel & Winkelmann 1997). Many different cyclic and linear compounds have been isolated so far, and the solution thermodynamics have been studied (Konetschny-Rapp *et al.* 1992). It has been shown earlier that some enterobacterial genera, i.e. *Erwinia, Pantoea, Enterobacter, Hafnia* and *Ewingella* also synthesize ferrioxamines E, D and G under iron limitation (Berner *et al.* 1988, Reissbrodt *et al.* 1990, Feistner & Ishimaru 1996), indicating that a great number of naturally occurring enterobacterial genera are equipped with ferrioxamine biosynthesis and uptake systems. Some of these genera also produce enterobactin either alone or together with ferrioxamines (Berner *et al.* 1991a, Feistner & Ishimaru 1996). Figure 1 shows a scheme which illustrates the transport of hydroxamate siderophores in *Erwinia herbicola* and emphasizes the analogy to *E. coli,* although TonB, ExbB,D and FhuBCD are still unknown.

Ferrioxamine transport mutants of *Erwinia herbicola* (syn. *Enterobacter agglomerans*, now transferred to *Pantoea agglomerans*) have been obtained by using a ferrimycin selection. A ferrioxamine receptor (FoxA) of 76 kDa has been identified which is expressed under iron deprivation (Berner & Winkelmann 1990). A ferrioxamine receptor has also been observed in *Erwinia amylovora*, the causative agent of fire blight in plants of the family Pomoideae (Kachadourian *et al.* 1996). *Yersinia enterocolitica*, although unable to produce ferrioxamines, can utilize iron from ferrioxamines, and the corresponding ferrioxamine receptor (FoxA) has subsequently been cloned and sequenced (Bäumler & Hantke 1992). This prompted us to clone the FoxA receptor from *E. herbicola* (*P. agglomerans*) and to compare its recognition capacity with the cloned FoxA receptor from *Y. enterocolitica.*

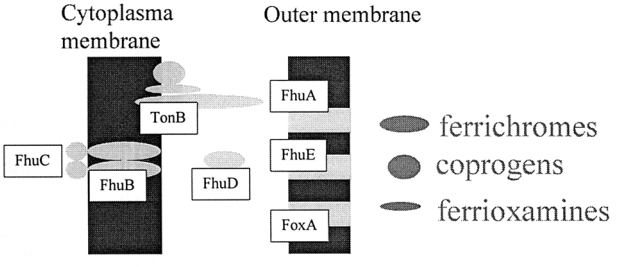

Figure 1. Scheme of hydroxamate siderphore transport in *Erwinia herbicola*. The scheme includes receptors for ferrichromes, coprogens and ferrioxamines and is otherwise analogous to the periplasmic binding protein dependent transport of hydroxamate siderophores in *E. coli.*

Materials and methods

Strains

Strains and relevant genotypes are listed in Table 1. All strains were grown aerobically in LB medium (Luria Broth Base 25 g l^{-1}) or NB medium (Nutrient Broth 8 g l^{-1}). Agar plates for growth promotion assays were prepared with tryptone medium containing per litre: tryptone 8 g, NaCl 5 g, bipyridyl 150 μM, EDDHA 150 μM, Chelex-treated glucose (0.4%) and 0.5% agar. Ferrioxamines or bipyridyl and EDDHA were sterile filtered before adding to tryptone agar. Ampicillin was added LB medium at a concentration of 100 mg l^{-1} where required. *Erwinia* strains were cultured at 30°C, while *E. coli* strains were grown at 37°C. *FoxA* mutants (FM13) were obtained by a ferrimycin selection procedure according to Berner & Winkelmann (1990).

Bioassays

Growth promotion bioassays were performed according to earlier protocols (Rabsch & Winkelmann 1991). All strains were tested on plates containing bipyridyl and EDDHA (150 μM) and 10 μl of an overnight culture of test strain. HK97 pUH60 was tested with 100 μM bipyridyl and EDDHA. Sterile aqueous solutions of siderophores were prepared in the concentration range 0.001 – 2 mM. 10 μl of the solutions were pipetted on sterile filter disks (6 mm diameter) and dried in a microwave oven. The filter disks containing 10 pmoles to 20 nmoles of siderophores were then laid on the agar surface and the plates incubated for about 12–24 hours.

Cloning of the ferrioxamine receptor ($FoxA_{Erw}$) from Erwinia herbicola K4 (wild type)

DNA manipulations, plasmid isolation and Southern blot analysis were performed according to standard procedures (Sambrook *et al.* 1977). A cosmid gene bank of *E. herbicola* in the cosmid vector pHC79 was transferred with phage lambda into a coprogen-negative background MS172 (*fhuE* $^-$). Two clones, one containing the cosmid pUH60, were obtained which showed growth in bioassays with ferrioxamine B. A 1.5 kb *Hph*I fragment from the *foxA* gene of *Y. enterocolitica* was used as a *foxA*-specific DNA probe in a Southern blot analysis of pUH60. Clear hybridization signals were obtained in *Pvu*II (4.6 and 4.8 kb fragment), *Ssp*I (2.8 and 5.0 kb) and *Pst*I (1.4 and 2.4 kb) digests. The *Pvu*II fragments were ligated in pUC19 via *Sma*I restriction site and transformed into *E. coli* strain DH5α. Positive clones (found by blue–white selection) were tested by plasmid miniprep and subsequent *Hind*III digest. Digested linearized plasmid DNA contained only a smaller insert (of about 1 kb). This DNA was sequenced and a comparison of the resulting amino acid sequence (BLAST sequence similarity searching, NCBI, Altschul *et al.* 1990) revealed a significant similarity of $FoxA_{Erw}$ with $FoxA_{Yer}$. Weaker similarities were

Table 1. Bacterial strains, plasmids and cosmids

Strains	Relevant genotype	Reference
E. coli MS172	*aroB, fhuE*	Sauer *et al.* (1990)
E. coli HK97	*aroB, ΔfhuE, fhuA*	Killmann & Braun (1992)
E. coli DH5α	*lacZΔM15*	Sambrook, *et al.* (1989)
Erwinia herbicola K4	*wild type*	Berner & Winkelmann (1990)
Erwinia herbicola FM13	Δ*foxA*	Berner & Winkelmann (1990)
Plasmids/Cosmids		
PUC19	cloning vector amp^R	Sambrook, *et al.* (1989)
pHC79	cosmid amp^R	Bäumler & Hantke (1992)
pT7-5	T7 expression vector	Bäumler & Hantke (1992)
pFU2	pT7-5, $foxA_{Yer}$	Bäumler & Hantke (1992)
pUH60	pHC97, 40 kb SauIIIA-fragment containing $foxA_{Erw}$	(this study)

observed with other siderophore receptor protein sequences (FhuA *E. coli*, ferrichrysobactin receptor *E. chrysanthemi*). The cloned FoxA receptors (pUH60, pFU2) were transformed into HK97, a FhuA/FhuE-negative background (Killmann & Braun 1992), in order to study their siderophore specificities.

Ferrioxamines and other siderophores

All natural ferrioxamines (B, E, G and ferrimycin A) were from the stock of our institute or isolated previously from strains of *Streptomyces pilosus*, *S. olivaceus* (Meiwes *et al.* 1990), *S. griseoflavus* or *Hafnia alvei* (Reissbrodt *et al.* 1990), respectively. Further ferrioxamine analogs containing shorter or longer diamine or diamino ether residues were obtained by directed or feeding fermentation (Meiwes *et al.* 1990, Konetschny-Rapp *et al.* 1992). Ferrichrome was isolated from low-iron cultures of *Ustilago sphaerogena* and coprogen was obtained from *Neurospora crassa* 74A. Ornibactin was isolated from *Burkholderia cepacia* as described earlier (Meyer *et al.* 1995). All other siderophores were from the stock of our laboratory. For structure and function of siderophores the reader is referred to a recent book on transition metals in microbial metabolism (Winkelmann & Carrano 1997).

Results

The functional activity of the FoxA receptor in *E. herbicola* was demonstrated by analyzing the transport of Fe-55-labeled ferrioxamines and other hydroxamate siderophores in the wild type *E. herbicola* K4 as well as in a *foxA* mutant (FM13)

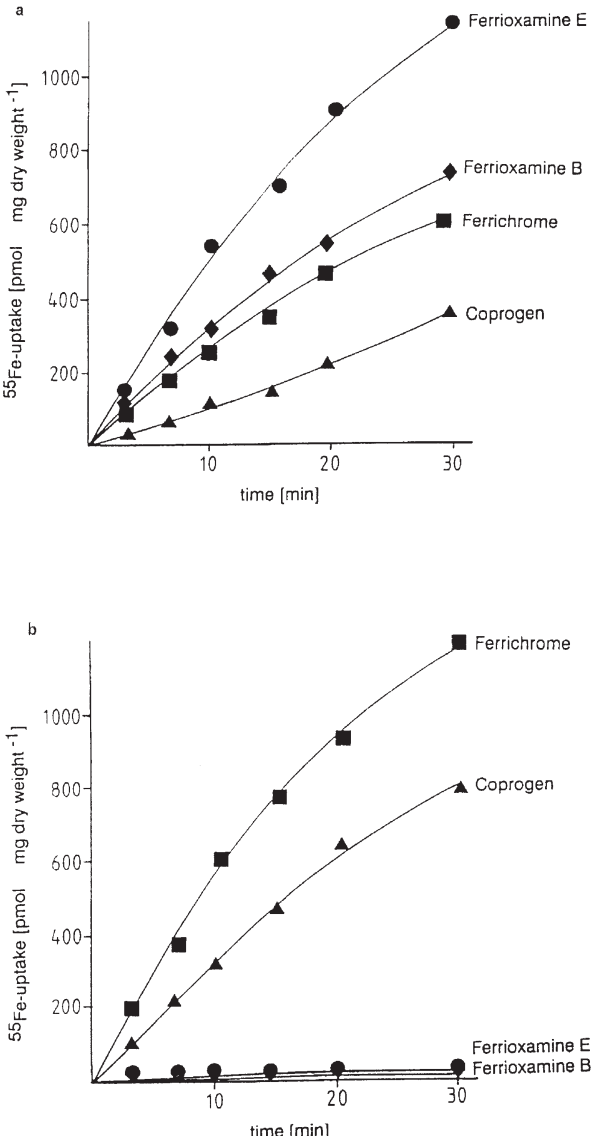

Figure 2. Transport of ^{55}-Fe-labeled ferrioxamines (B and E), ferrichrome and coprogen in *E. herbicola*: (a) wild type; and (b) *foxA* mutant (FM13).

(Figure 2). While the transport of ferrichrome and coprogen was still active and even increased, the transport of ferrioxamine B and E was completely abolished in the mutant strain. Growth promotion assays also confirmed that in this case the transport data with labeled ferrioxamines were consistent with the extent of observed growth haloes.

Due to positive hybridization signals of a *Pvu*II digest of the cosmid pUH60 with a pFU2 *Hph*I fragment ($foxA_{Yer}$) as a probe, we obtained partial DNA sequences of the outer membrane receptor for ferrioxamines ($FoxA_{Erw}$) from *E. herbicola* K4. An amino acid sequence comparison (PC/Gene, A. Bairoch/University of Geneva, Switzerland) of the obtained sequence with that of FoxA from *Yersinia* is shown in Figure 3. A high amino acid sequence homology between the two *foxA* genes was obtained, confirming that an essential part of the FoxA receptor from *Erwinia* could successfully be cloned and sequenced.

From the cosmid gene bank of the parent wild type strain of *E. herbicola* K4 the *foxA* gene was cloned into *E. coli* HK97 possessing deletions in *fhuA* and *fhuE*, so that separate testing of ferrioxamines via the cloned $FoxA_{Erw}$ receptor was possible. Using growth promotion assays we studied the utilization of various siderophores in order to determine the specificity of the different receptors, i.e. FoxA (ferrioxamines), FhuA (ferrichromes) and FhuE (coprogens). The results for FoxA from *Erwinia* and *Yersinia* are shown in Table 2. Low concentrations (< 1 mM solutions, 10 µl per filter disk = 10 nmoles) of siderophores were utilized in growth promotion assays only by those clones possessing siderophore-specific receptors. Thus, the FoxA receptors gave growth responses with receptor-specific ferrioxamines down to 10^{-3} mM (= 10 pmoles per filter disk), while unspecific utilization needed concentrations higher than 1 mM (> 10 nmoles per filter disk). Likewise, the deletion mutants containing only FhuA or FhuE receptors responded in a similar way when ferrichromes and coprogens were tested (data not shown). However, from the results shown in Table 2 it is also evident that coprogens seem to be recognized to a certain extent by the FoxA receptors, while the ferrichromes were not recognized at all within the range of the tested concentrations. Thus, the ferrioxamines and coprogens may share some common structural features which enable partial recognition by the FoxA receptor. This result confirms earlier crystallographic studies by van der Helm and coworkers showing that superimposed crystal structures of ferrioxamine D_1 and neocoprogen I possess similar topographical features (Hossain *et al.* 1987).

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FOXA ERWIN                                                  LMWQPDEDTHLILKA
FOXA YEREN  DYTDAISEH--WAFRLTGITRNSDTMYDHQREERYAIAPSLLWQPDENTSLLLRA   251
FHUE ECOLI  DLQSPLTEDGKIRARIVGGYQNNDSWLDRYNSEKTFFSGIVDADLGDLTTLSAGY   263
FHUA ECOLI  DFSDSLDDDGVYSYRLTGLARSANAQQKGSEEQRYAIAPAFTWRPDDKTNFTFLS   265
            *  ^^^^^^     *^^* ^^^^^ ^   ^^^  ^   ^ ^ ^ ^ * ^

FOXA ERWIN  YLQKDPSG-GYHGSVPG---DGSL THENGYKLSNGF-YEGNSNLDQFKRREQIY
FOXA YEREN  NLQKDPSG-GYHSAVPA---DGSI----YGQKLSRGF-FDGESNHNVFKRWQQIY   297
FHUE ECOLI  EYQRIDVNSPTWGGLPRWNTDGSSNSYDRARSTAPDWAYNDKEINKVFM------   312
FHUA ECOLI  YFQNEPET-GYYGWLPK---EGTVEPLPNGKRLPTDF-NEG-AKNNTYSRNEKMV   315
              *^ ^   ^^ ^ ^*    ^*^       ^ ^ ^ ^^  ^^ ^ ^^^^

FOXA ERWIN  SFDFAHRFNDVWSFSSTGSYSHSNVDLDQVYQIGWDSVNPD--------------
FOXA YEREN  SYEFSHKFDDVWSFRQNASYTHSNTQLEQVYQGGWNSDRT---------------   337
FHUE ECOLI  --TLKQQFADTWQATLNATHSEVEFDSKMMY--------VDAYVNKADGMLVGPY   357
FHUA ECOLI  GYSFDHEFNDTFTVRQNLRFAENKTSQNSVYGYGVCSDPANAYSKQCAALAPADK   370
              ^^^^^*^*^^^    ^   ^^ ^   ^ ^*  ^  ^  ^

FOXA ERWIN  --LLNRY-YSG----ERSSLSAWSTDNPLQAEFGTGELEHRVTLGAEYHRYKNE-
FOXA YEREN  --LMNRY-YSG----EDSSLNAFAVDNQLEADLRTAAVKHKVLLGVDFQKFRNN-   384
FHUE ECOLI  SNYGPCFDYVGGTGWNSGKRKVDALDLFADGSYELFGRQHNLMFGGSYSKQNNRY   412
FHUA ECOLI  GHYLARK-YVV----DDEKLQNFSVDTQLQSKFATGDIDHTLLTGVDFMRMRND-   419
              ^    *^^   ^ ^^ ^  ^ *  ^^^^    ^ ^*^^^ *^^^ ^ ^*

FOXA ERWIN  ITAAGG-SANQ---LNARTGEQVGDMP--DYTWAD----------TRRYYQTGLY
FOXA YEREN  LRSDSA-YATP---LNPYTGVSGGSTLYSDYLLTTPGINTSY--LSRRYEQSGVY   433
FHUE ECOLI  FSSWANIFPDEIGSFYNFNGNFPQTDWSPQSLAQDDTTH-----MKSLYAATRVT   462
FHUA ECOLI  INAWFG-YDDSVPLLNLYNPVNTDF----DFNAKDPANSGPYRILNKQ-KQTGVY   468
            ^^^      ^   ^  ^^           ^   ^ ^       ^^^   ^^^

FOXA ERWIN  LQDEMKLDRWHLDLSGRY-DRIVA---NN--SGSDRRQDDHISGRAALLYAFDNG
FOXA YEREN  LQDEMTLDNWHLNLSGRY-DRMKTENINNTANSTDERTDNNASGRASLLYSFDSG   487
FHUE ECOLI  LADPL-----HLILGARYTNW-------RVDTLTYSMEKNHTTPYAGLVFDINDN   505
FHUA ECOLI  VQDQAQWDKVLVTLGGRY-DWADQESLNRVAGTTDKRDDKQFTWRGGVNYLFDNG   522
            ^^*          ^ *^^** ^^         ^  ^ *  ^ ^^^ ^ ^^^^ ^ ^^^^

FOXA ERWIN  ISPYV
FOXA YEREN  ISPYVSYSQAITPSLFPDAQQKLLKPMTSEQYEVGIIYQPPGSTSLYSAALYDLT   542
FHUE ECOLI  WSTYASYTSIFQPQNDRDSSGKYLAPITGNNYELGLKSDWMNSRLTTTLAIFRIE   560
FHUA ECOLI  VTPYFSYSESFEPSSQVGKDGNIFAPSKGKQYEVGVKYVPEDRPIVVTGAVYNLT   577
             ^^* **^  ^ *    ^  ^ ^ * ^^^^**^*^    ^^    ^ *^^ ^^
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Figure 3. Alignment of protein sequences of $FoxA_{Erw}$, $FoxA_{Yer}$, FhuE and FhuA (* character to show that a position in the alignment is perfectly conserved, ^ character to show that a position is well conserved).

In order to compare the siderophore specificity of the cloned FoxA receptors we used a collection of ferrioxamines (Figure 4) with different chain lengths or ether links, obtained by directed fermentation of *Streptomyces olivaceus* (Tü 2718) (Meiwes *et al.* 1990, Konetschny-Rapp *et al.* 1992). Natural ferrioxamines possess 1,5-diaminopentane or 1,4-diaminobutane residues, giving either cyclic or linear ferrioxamines. Incorporation of diaminobutane residues resulted in smaller molecular sizes, while incorporation of 1,6-diaminohexane residues resulted in larger molecules. A more extended shape is obtained by incorporation of 1–3 bulky bis(2-aminomethyl)ether residues.

When natural ferrioxamines (B,G,E) and their derivatives (extended, shortened, dihydroxamic and ether containing) were analyzed in growth promotion tests there was no difference in sensitivity of the FoxA receptors observed except for the dihydroxamic derivative X_5 and the three ether bridges containing Et_3 (Figure 4). While the dihydroxamic ferrioxamine showed reduced growth promotion in both receptors, the three-ether compound Et_3 behaved differently (Table 2). The FoxA receptor from *Y. enterocolitica* responded at 0.01 mM while

Table 2. Molecular recognition of siderophores by outer membrane ferrioxamine receptors from *Y. enterocolitica* ($FoxA_{Yer}$) and *E. herbicola* ($FoxA_{Erw}$)

HK97 pFU2 ($FoxA_{Yer}$)		0.001	0.01	0.1	1	2
Ferrioxamines						
linear–NH_2	B	■	■	■	■	
linear–NH_2/–COOH	G	■	■	■	■	
cyclic normal	E	■	■	■	■	
cyclic shortened	$X_{1,2}$	■	■	■	■	
cyclic extended	X_3	■	■	■	■	
cyclic extended dihydroxamic	X_5		■	■	■	
cyclic ether (1–2)	Et	■	■	■	■	
cyclic ether (3)	Et_3		■	■	■	
Coprogens						■
Ferrichromes						
HK97 pUH60 ($FoxA_{Erw}$)						
Ferrioxamines						
linear–NH_2	B	■	■	■	■	
linear–NH_2/–COOH	G	■	■	■	■	
cyclic normal	E	■	■	■	■	
cyclic shortened	$X_{1,2}$	■	■	■	■	
cyclic extended	X_3	■	■	■	■	
cyclic extended dihydroxamic	X_5		■	■	■	
cyclic ether (1–2)	Et	■	■	■	■	
cyclic ether (3)	Et_3			■	■	
Coprogens						■
Ferrichromes						

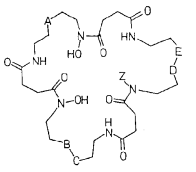
Filled bars indicate growth response at the concentrations of siderophore solutions used (0.001–2 mM). 10 µl of these solutions were pipetted on filter disks (6 mm diameter) corresponding to an actual concentration range of 10 pmoles to 20 nmoles per filter disk.

the FoxA receptor from *E. herbicola* responded only at a tenfold higher concentration (0.1 mM) indicating different recognition capacities. This might suggest that the FoxA receptor from *Erwinia* has a smaller pocket for insertion. Thus, the bulky ether containing ferrioxamine Et_3 can possibly be used to distinguish between the two FoxA receptors of different origin. Although there is a significant specificity for ferrioxamines over other siderophores, some recognition of coprogens seem to occur at higher concentrations. Furthermore, in this case a slight difference in the two FoxA receptors has been observed and the FoxA receptor from *Yersinia* seems to be more sensitive (Table 2).

A variety of other bacterial and fungal siderophores (e.g. rhodotorulic acid, fusigen, triacetylfusarinine, ornibactin, schizokinen and aerobactin) was tested with the two FoxA receptors and the receptors for ferrichrome and coprogen (data not shown). In no case was growth response seen at concentrations lower than 1 mM (= 10 nmoles per filter disk), confirming the very high specificity of the FoxA receptors for ferrioxamines.

Discussion

In the present investigation we compared the molecular recognition of siderophores by using two cloned FoxA receptors, one from *E. herbicola* and the other from *Y. enterocolitica*. The strains are members of two different genera within the family of enterobacteriaceae, both of which seem to originate from different roots in the evolutionary tree of



Desferri-oxamine	A	B	C	D	E	X	Y	Z
E	CH_2	CH_2	a	CH_2	a	OH	OH	OH
D_2	CH_2	CH_2	a	a	a	OH	OH	OH
X_1	CH_2	a	a	a	a	OH	OH	OH
X_2	a	a	a	a	a	OH	OH	OH
X_3	CH_2	CH_2	CH_2	CH_2	a	OH	OH	OH
X_5	CH_2	CH_2	CH_2	CH_2	a	OH	OH	H
Et_1	CH_2	CH_2	a	O	a	OH	OH	OH
Et_2	CH_2	O	a	O	a	OH	OH	OH
Et_3	O	O	a	O	a	OH	OH	OH

a = Structural components which do not exist in the molecule

Figure 4. Structural formula of desferrioxamines obtained by directed and feeding fermentation of *Streptomyces olivaceus* according to Konetschny-Rapp *et al.* (1992).

enterobacteria. This has made the present investigation attractive for a comparison of distantly related receptor proteins with regard to the molecular recognition of the same ferrioxamines. The FoxA receptor from *E. herbicola* was cloned, and partial sequences obtained that show high homology with the FoxA receptor of *Y. enterocolitica*. For comparison purposes we also included some *E. coli* strains (deletion mutants) possessing only a single receptor for ferrichrome (FhuA) or coprogen (FhuE) in order to confirm the specificity for ferrichromes and coprogens. These controls are important since some siderophores may enter the cells by using other receptors. Thus, we could confirm earlier observations (Sauer *et al.* 1990) that *E. coli,* while lacking a FoxA receptor, can still take up some ferrioxamine B via the coprogen receptor (FhuE). Here we have shown that coprogen is recognized by the cloned FoxA receptor(s), which also supports our previous finding with chiral linear hydroxamates as biomimetic analogus of ferrioxamine and coprogen (Berner *et al.* 1991b, Winkelmann 1997). Cloning of the FoxA receptor into a hydroxamate-negative background and partial sequencing confirmed the existence of a FoxA receptor in *E. herbicola.* A comparison of the amino acids (236–492) of the two FoxA protein sequences from *Erwinia* and *Yersinia* revealed high sequence similarities. Furthermore, a relatively high similarity was still found when the sequences of FoxA receptors were compared with those of FhuE and FhuA, suggesting that some domains may be functional equivalent in Fhu and Fox proteins. Although only a partial sequence was obtained and only one strand has been sequenced so far, this was sufficient to analyze the FoxA functions. A complete sequence is currently being prepared.

Specific and unspecific transport of siderophores can be explained by using the receptor model developed by Bäumler & Hantke (1992) and Braun (1995). Similar to the porins (Delcour 1997), outer membrane receptors for siderophores function as gated pores preventing free diffusion of siderophores. Specific recognition may be explained by interactions with inner walls of the receptor pores or loops, whereas low specificity or unspecific transport might result from weaker or no contacts at all. The actual binding sites for ferrioxamines by the FoxA receptor(s) are still unknown. Positively charged arginine residues have recently been suggested to be involved in binding of the three-negatively charged ferric enterobactin molecule in the FepA receptor (Newton *et al.* 1997). However, ferrioxamines (and other siderophores) are uncharged molecules and seem to be recognized preferentially by nonionic interactions of the overall shape of ferrioxamines. Rather, entropic effects by expelled water molecules or hydrogen bonding may be involved in siderophore recognition sites as was discussed for the specificity of periplasmic binding proteins (Quiocho & Ledvina 1996). A structure–function relationship has previously been discussed in studies with siderophores in fungi, which showed that several parts of the siderophore molecules, e.g. backbone and iron surrounding residues, are essential for molecular recognition (Huschka *et al.* 1986, Leong & Winkelmann 1998). Support also comes from studies with *enantio*-ferrichrome in fungi (Winkelmann 1979, Winkelmann & Braun 1981) and *enantio*-enterobactin in *E. coli* (Neilands *et al.* 1981) where conformation and configuration of the ferric complexes determine the interaction with the corresponding siderophore receptors.

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