A FUNCTION COMMON TO IRON-ENTEROCHELIN TRANSPORT AND ACTION OF COLICINS B, I, V IN *ESCHERICHIA COLI*

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Received 22 August 1975

1. Introduction

Recently, we have shown that transport of ferric iron in a complex with ferrichrome is dependent on the *ton A* gene product [1] which was originally classified as the receptor for phage T5 [2], and which we have shown is a polypeptide chain of mol. wt 85 000 localized in the outer membrane of *E. coli* [3,4]. In addition, ferrichrome transport is impaired in *ton B* mutants [1]. The *ton B* function and the *ton A* protein are also both required for the productive infection of *E. coli* cells by the phages T1 and Ø80 [2] and killing of the cells by colicin M [5,6]. The action of colicin M is prevented by added ferrichrome [1]. Luckey, Wayne and Neilands independently showed that adsorption of Ø80 to cells and membrane fragments is inhibited by ferrichrome [7,8]. Therefore it was concluded that both the phage and colicin receptors evolved as components of transport systems used to overcome the permeability barrier of the outer membrane for substrates with a mol. wt of about 700 and greater.

Enterochelin is another iron complexing agent produced by *E. coli* cells [9] The questions arose whether the need for a receptor at the cell surface is restricted to ferrichrome or whether a receptor also exists for the transport of ferric iron complexed with enterochelin. Guterman, confirming earlier genetic work [12], isolated *E. coli* mutants resistant to the colicins B and I which mapped in two different regions of the chromosome, at *ton B* (27 min) and between 56 and 58 min on the genetic map of *E. coli* [10, 11]. The mutants hyperproduced and excreted enterochelin which was thought to interact directly with the colicins causing resistance. We favoured the idea that enterochelin is overproduced due to an iron shortage in the cell caused by the lack of a membrane component in the mutants involved in both iron-enterochelin uptake and killing of the cells by colicins B, I, V.

Here we show that the colicin resistance is indeed independent of the presence of enterochelin and that colicin resistant strains are unable to transport enterochelin. The described function maps outside *ton B* at about 65 min of the genetic map of *E. coli*. Tentatively we designate the function and the corresponding gene *feu* (*f*erric *e*nterochelin *u*ptake) to distinguish it from the *fep* function at 13 min which is also involved in transport of ferric enterochelin [13].

2. Materials and methods

2.1. *Strain and culture conditions*

The strains used are listed in table 1. For iron uptake studies they where grown on a CR minimal medium

Table 1
List of *E. coli* K12 strains used

Strain	Relevant characteristics	Source
AB 2847	*aro B, thi,* λ^r	[20]
CA	col Ia	P. Fredericq
P9	col Ib	D. J. McCorquodale
R 2, 1/V	col B	P. Fredericq
1, 7 a	col V	P. Fredericq
AN 311	*leu*, pro C, *try, thi, fep*-415	I. G. Young
D 22	*leu, try*, str^r, λ^r, env A	H. Boman
KL 228	Hfr, *thi, leu*	[17]
KL 14	Hfr, *thi*	[17]
PK 191	Hfr, pro B	[17]

[14] from which the iron was extracted with 8-hydroxychinolin and chloroform. The medium was supplemented with 30 mM succinate, 100 μg per ml each of tryptophane, tyrosine, phenylalanine and 40 μM *p*-aminobenzoic acid, 40 μM *p*-hydroxybenzoic acid, 1 μg per ml thiamin, 0.05% casamino acids and where indicated 1 mM citrate or 20 μM 2,3-dihydroxybenzoic acid. Conjugation procedures and selection methods were those summarized by Miller [15]. The recipient strains were made ampicillin resistant, for purposes of counter selection against donor strains while *aro* B^+ served as a selection against recipient strains not receiving donor markers.

Spontaneous colicin resistant mutants were selected by the following procedure. Agar plates were spread with the appropriate colicinogenic strain grown for 24 h at 37°C and treated for 1 hr with chloroform vapour, the cells scraped off and the colicin sensitive strain *E. coli* AB 2847 streaked on the plates. The colonies which grew were tested against the colicins B, I and V and against the phages T1 and T5 by cross-streaking. The phage titer used was about 10^8 p.f.u. per ml.

Growth on ferrichrome was tested on minimal agar plates with ferrichrome (2 μM) as the sole iron source. Filter paper discs soaked with albomycin (0.01 mg/ml) were placed on the mutant strains spread on rich medium plates. Sensitive strains gave an inhibition zone of approx. 5 mm. Iron uptake measurements were performed as described previously [1].

2.2. *Chemicals*

All chemicals used were analytical grade from Merck (Darmstadt). Highly purified δ_2 albomycin was a gift from H. Maehr (Hoffmann-La Roche, Nutley). Ferrichrome, isolated from *Aspergillus melleus*, was kindly supplied by H. Zähner of this institute. Enterochelin was prepared from *E. coli* K12 AN 311 by the method of I. G. Young (Canberra) who kindly supplied the strain and a detailed description (see also ref. 15).

Crude colicins were prepared as described by Nagel de Zwaig and Luria [16]. Adsorption of colicin was tested by incubating 0.2 ml crude colicin with 0.5 ml cell suspension (optical density of 10 at 578 nm) for 1 h at 37°C. After sedimenting the cells the various dilutions of the supernatant were spotted on streptomycin (100 μg per ml) containing plates seeded with *E. coli* D 22 as indicator strain. We thank H.-U. Schairer for providing the *E. coli* strains AB2847, KL 228, KL 14 and PK 191 and R. Hancock for the strain P 9.

3. Results

To exclude enterochelin in the medium as a cause of the resistance against colicins B, I, V we isolated spontaneously resistant mutants form *E. coli* K12 AB2847 which is an *aro B* mutant unable to synthesize enterochelin unless precursor is added. Depending on the colicins used for selection different resistance patterns were obtained as shown in table 2. This type of cross-resistance to these colicins and phages when only one agent was used for selection has been described previously [10–12] except for the antibiotic albomycin. To distinguish between mutations near *ton B* or near 58 min cotransfer of colicin sensitivity with *aro B* at 65 min was tested with three Hfr strains and the colicin resistant *aro B* mutants as recipients. The B, I and I, V resistant strains (table 2 columns 3,4) showed 80–100% cotransfer of the colicin sensitivity marker with *aro B*. The B, I, V, albomycin and the T1, B, I, V, albomycin resistant strains (table 2, columns 2,3) in contrast showed no cotransfer with *aro B*, indicating a similarity to the known mutation in the *ton B* region.

The physiology of some selected strains from table 2 with respect to transport of ferric iron complexed with enterochelin, citrate and ferrichrome and with regard to colicin resistance is listed in table 3. All mutants which are converted to wild type by cotrans-

Table 2
Resistance pattern of colicin B, I, V resistant mutant from *E. coli* K12 AB2847

Colicin used for selection	Resistant mutants obtained against			
	B, I, V, T1 Albomycin	B, I, V Albomycin	B, I	I, V
B	24	1	145	0
I	26	3	143	0
V	25	0	0	122

Table 3
Comparison of the different mutant types with respect to their resistance pattern and transport

Strain	Resistance	Transport of			Phenotype
		Fe^{3+}-enterochelin	Fe^{3+}-citrate	Ferrichrome	
AB 2847	no	+	+	+	wild type
IR 20	BI	–	+	+	*feu*
IR 35	BI	–	+	+	*feu*
VR 27	IV	–	+	+	*feu*
VR 42	IV	–	+	+	*feu*
BR 10	BI	–	+	+	*feu*
BR 128	BIV al.	–	–	–	*ton B*[a]
IR 107	BIV al.	–	–	–	*ton B*[a]
BR 158	BIV T1 al.	–	–	–	*ton B*
BR 185	BIV T1 al.	–	–	–	*ton B*
IR 112	BIV T1 al.	–	–	–	*ton B*
IR 114	BIV T1 al.	–	–	–	*ton B*

+, Transport as in the wild type; –, transport at least reduced by 70%,[a] *ton B* gene region despite T1, Ø80 sensitivity, in analogy with the literature [2,10,12], not cotransferred with *aro B*; al. means albomycin.

fer of the colicin sensitivity marker with the *aro B* marker are unable to transport Fe^{3+}-enterochelin but they transport Fe^{3+}-citrate and ferrichrome. As an example transport of strain VR 42 is shown in fig.1. The mutants which map in the *ton B* region were all impaired in the uptake of all three iron complexes which supports our previous results with strains of different origin (*E. coli* W) [1].

Enterochelin protects sensitive cells against colicins B, I, V [10] which we have confirmed using our wild type strain. However, competition between enterochelin and the colicins for a common binding site could not be demonstrated because adsorption of the colicins to the wild type strain in the absence or presence of enterochelin or to the mutant strains was uniformly low. Thus the reduction expected in the latter two cases could not be measured and the function of the *feu* gene product remains unknown. Unexpectedly all of the *ton B* mutants showed a strongly enhanced adsorption (factor 32 to 256 stronger than that of the wild type) pointing to major changes in the cell envelope which will be investigated further.

4. Discussion

The new function involved in Fe^{3+}-enterochelin uptake described in this paper is different from the hitherto known *fep* function [9,13] since both functions map at different loci on the genetic map of *E. coli*. With all three different Hfr strains, we obtained 80–100% cotransfer with the marker *aro B* at 65 min. Especially noteworthy is the cotransfer with the Hfr strain KL14 since the first known gene transferred is *arg G* at 61 min and the last one is *met C* at 58 min [17]. Our mapping data are at variance with those of Guterman [11] who localized her colicin B, I resistant, enterochelin excreting mutants within 1 min of *ser A* at 56 min. Also none of our mutants in contrast to those of Guterman was a methionine auxotroph. Since our mutants most likely map between 60 and 75 min we call them tentatively *feu* and not *exb B*. This distinction will have to be proven by a genetic fine structure analysis. The B, I and I, V linkages of the resistant strains, which may suggest deletions comprising different genes, require also a more

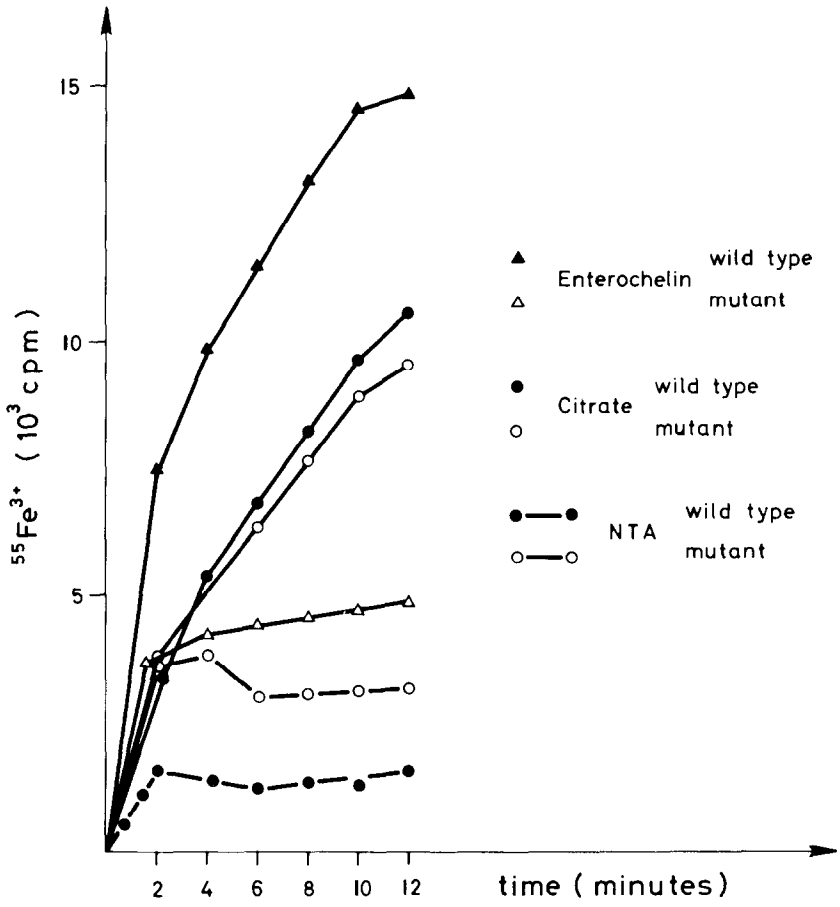

Fig.1. Fe^{3+}-uptake by the wild type strain *E. coli* AB2847 and the *feu* mutant VR 42 (see table 3). Ferrichrome uptake (not shown) of the wild type and the mutant follows the uptake curve of enterochelin for the wild type. 100 μM nitrilotriacetate (NTA) was added in all uptake measurements to supress the low affinity iron uptake system [19]. 0.3 μM Fe^{3+} and 0.3 μM enterochelin, 1 mM citrate and 0.3 μM desferri-ferrichrome respectively were added to the uptake systems. Details of the procedures used have been published [1].

accurate genetic analysis. The wild type and the *feu* mutants are colicin E1 sensitive so that the *tol C* locus at 59 min [2], conferring tolerance to E1, is not involved. Since, as shown in this paper, enterochelin hyperproduction most likely is the consequence but not the cause of the colicin B, I, V resistance the designation *feu* (ferric enterochelin uptake) is more appropriate than *exb B* (enterochelin excretion). The *feu* mutants are colicin resistant without enterochelin present. The *feu* function is both involved in enterochelin transport and killing of cells by the colicins B, I, V. In *feu* mutants uptake of Fe^{3+}-enterochelin is reduced by 70%. Iron shortage leads to enterochelin hyperexcretion since the intracellular iron concentration regulates enterochelin production [9]. Enterochelin is only an 'inhibitor' of the colicins B, I, V in the sense that it probably competes for the same component in the membrane. At present we do not know, but it is possible that the *feu* function is localized in the outer membrane and can be considered as an analogue to the *ton A* protein for the ferrichrome transport. The *feu* and the *ton A* function together with the *ton B* function may channel the rather large iron complexes (mol. wt 740) through the outer mem-

brane which could otherwise act as a permeation barrier. However the location of the common component in the cytoplasmic membrane is not excluded since the colicins B and I and perhaps also V (our unpublished results) interfere with energy metabolism and the *feu* function could be a part of the active transport system of Fe^{3+}-enterochelin, similar for example to the binding proteins of amino acids and sugars [18].

Acknowledgements

We thank Mrs Ursula Holzwarth for expert technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 76).

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