THE COLICIN I RECEPTOR OF *ESCHERICHIA COLI* K-12 HAS A ROLE IN ENTEROCHELIN-MEDIATED IRON TRANSPORT

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1. Introduction

Iron can enter the *E. coli* K-12 cell in four ways. There exist three specific, high affinity systems which transport iron with the aid of either enterochelin [1], citrate [2] or ferrichrome [3]. The fourth system is a low affinity uptake system for which no such complexing agent has been described [2]. Our knowledge of the membrane components required for high affinity iron transport and for mobilization of iron from the complexes is still poor, although the isolation of specific mutants in these uptake systems should assist in defining the required components. For example, it was shown that *ton* A mutants are missing a protein [4] and are defective in ferrichrome-mediated iron transport [3]. Ferrichrome protects sensitive cells against phages T5, T1 and ϕ80 and colicin M, all of which bind to the ton A-protein [3,5,6]. Thus the ton A-protein is probably the initial binding receptor for ferrichrome. Other mutants have been described for the ferrichrome [3,4,5], citrate [2] and enterochelin (see [1] and [7] for summary) mediated transport systems, although in many cases the defects involved have not been fully characterized. Recently the isolation of *feu* mutants (defective in *f*erric *e*nterochelin *u*ptake) was described [8]. These mutants, which had essentially normal citrate- and ferrichrome-mediated iron uptake systems, mapped separately from previously described iron transport mutants (at about 65 min on the *E. coli* genetic map). They fell into two groups with respect to colicin resistance, those insensitive to colicins I and V (called here *feu* A) and those insensitive to colicins B and I (called here *feu* B). In this paper we show that the *feu* A mutant strain VR42 is resistant (as opposed to tolerant) to colicin I and is lacking a protein in its outer membrane. This protein is presumably the colicin I receptor and functions in enterochelin-mediated iron transport, possibly as the receptor for the enterochelin–iron complex. Furthermore we show that this protein is overproduced in the wild type cell as a response to low intracellular iron levels.

2. Materials and methods

The strains used were previously described mutants of *E. coli* K-12 strain AB2847 *aro* B *thi tsx* λ^r [8]. Iron deficient, extracted medium was M9 minimal medium extracted with 8-hydroxychinoline and chloroform and supplemented as described elsewhere [4]. Inoculum cultures were grown on unextracted M9 medium in the presence of 1 mM citrate, and washed twice before resuspending in the appropriate medium at an $E578$ of 0.02 – 0.04. Outer membranes were prepared using the Osborn technique [9] from cultures grown as described in fig.1 to an $E578$ of 0.6. Polyacrylamide gel electrophoresis was performed using the technique of Lugtenberg et al. [10].

Crude colicin I was obtained from K. Hantke. Adsorption of colicin I to outer membrane preparations was tested by mixing 0.2 ml of the colicin suspension with 0.2 ml of the outer membrane preparation in the presence of 1 mM $MgSO_4$ and 10 mM Tris-HCl buffer for 1 h at 37°C. Various dilutions of the reaction mixture were spotted onto plates seeded with strain AB2847.

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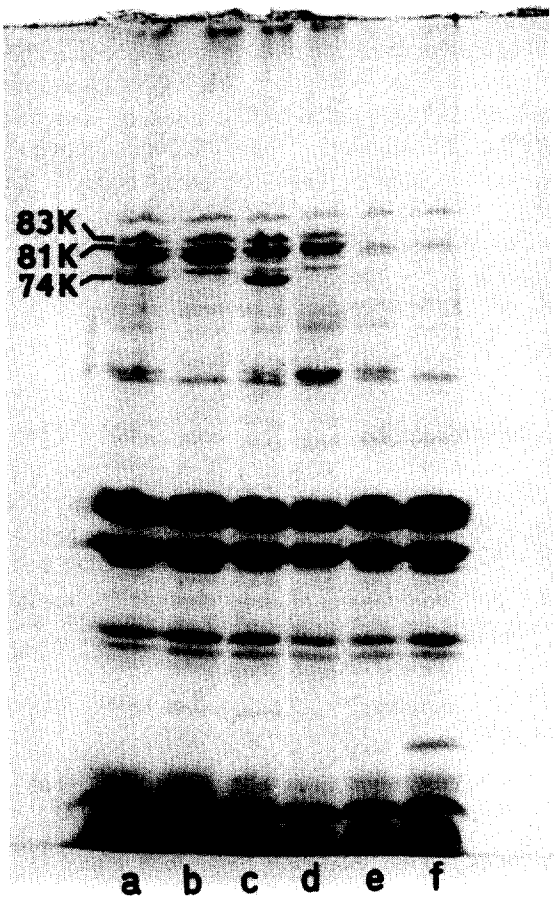

Fig. 1. Polyacrylamide gel electrophoresis of the outer membrane proteins of the wild type strain AB2847 (gels a, c and e) and its *feu* A mutant VR42 (gels b, d and f). Cells were grown in iron-deficient, extracted medium supplemented with the growth factors and with either 1 mM citrate (gels a and b), 10 μM DHB (gels c and d) or 1 mM citrate + 10 μM DHB + 10 μM $FeSO_4$ (gels e and f) to support iron uptake. The protein standards, used for localization of the bands, were phosphorylase A (94K = 94 000 mol. wt.), bovine serum albumin (67K), ovalbumin (45K), chymotrypsinogen A (25K) and cytochrome *c* (12.4K).

3. Results and discussion

Whole cells of the wild type strain *E. coli* K-12 AB2847 and its *feu* A mutant VR42 grown in tryptone–yeast broth were previously shown to adsorb colicin I very poorly [8]. Therefore we decided to use outer membrane preparations to test for colicin I binding activity. As shown in table 1, outer membranes prepared from strain AB2847 *feu*$^+$ readily adsorbed colicin I whereas those derived from strain VR42 *feu* A were unable to adsorb colicin. The *cir* mutant of *E. coli* K-12 is also unable to adsorb colicin I [11]. However since this mutant maps at a different locus (at about 41 min), we are uncertain of the relationship between *cir* and *feu* A. In contrast,

Table 1
Adsorption of colicin I by different outer membrane preparations

Outer membrane derived from strain	Amount added (mg)	Colicin I titer remaining after adsorption[a, b]
AB2847	0.25	<1
AB2847	0.75	<1
VR42 *feu* A	0.75	64
BR10 *feu* B	0.75	<1
IR20 *feu* B	0.75	<1
IR112 *feu* B	0.75	<1
BR158 *ton* B	0.75	<1
BR128 *ton* B	0.75	<1

[a] The colicin I titer is expressed as the reciprocal of the final dilution which gave a clear zone of inhibition on a plate seeded with the sensitive strain AB2847. The reciprocal colicin I titer in the control without added outer membrane protein was 1/64.

[b] The results for one set of membrane preparations, presented above, were reproducibly obtained with six other membrane preparations from each of strains AB2847 and VR42 and with one additional membrane preparation of the other strains.

the outer membranes of other colicin I insensitive mutants IR20, IR112, BR10 (all *feu* B), BR158 (*ton* B) and BR128 (mapping in the *ton* B region but sensitive to phage T1) all adsorbed colicin I.

It was recently shown that *ton* B mutants exhibit a strongly enhanced capacity for colicin I binding [8]. Furthermore, *ton* B mutants overproduce three outer membrane proteins designated the 74K, 81K and 83K proteins according to their mol. wts. (K = kilo, e.g. 74K = 74 000 mol. wt.) [4]. After partial separation of Triton X-100 + EDTA-soluble outer membrane proteins on a DEAE-cellulose column, colicin I binding activity co-fractionated with the 74K, 81K and 83K proteins [4]. This evidence suggested that one of these proteins was the colicin I receptor. When we prepared outer membranes from several strains grown in nutrient broth, we observed the following; strains AB2847 (wild type) and IR20 (*feu* B) produced low amounts of the three proteins while BR158 (*ton* B) and BR128 (which maps in the *ton* B gene region) overproduced them. Strain VR42 appeared to be lacking the 74K band, although the low level of protein involved made a positive conclusion difficult.

We have observed that high levels of the 74K, 81K and 83K proteins were produced in wild type strains grown in iron-deficient, extracted medium ([4]; see also fig.1, gels a and c) thus providing a technique for the amplification of these proteins. This production was suppressed in cells grown in iron-supplemented medium ([4]; see also fig.1, gel e). Outer membranes prepared from strain VR42 grown in iron-deficient medium supplemented with citrate (fig.1, gel b) or dihydroxybenzoate (DHB) (fig.1, gel d) were lacking the 74K protein (compare with the wild type: gels a and c respectively). We therefore conclude that the 74K protein is a constituent of the colicin I receptor.

The *feu* A mutant VR42 produced similar levels of the 81K and 83K proteins to the wild type. It was hypothesised from studies with *ton* B mutants that the 74K protein together with the 81K and 83K proteins is overproduced as a response to low intracellular iron levels [4]. We are now able to eliminate production, uptake or excretion of enterochelin as the factor leading to production of these proteins, since they are produced in the absence of enterochelin production (fig.1, gel a: strain AB2847 *aro* B cannot make enterochelin unless DHB is added to the growth medium). In this paper we have described a mutant in which only one of these proteins is missing. This suggests that while the production of these proteins might involve a common control factor (which responds to low intracellular iron levels), they are probably different polypeptides with distinct structural genes.

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