ENVELOPE PROTEIN CHANGES IN MUTANTS OF *ESCHERICHIA COLI* REFRACTORY TO COLICIN E2

A.C.R.SAMSON and I.B.HOLLAND
Department of Genetics, University of Leicester, England

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1. Introduction

Colicins are antibacterial proteins which induce specific changes in membrane-associated functions of sensitive bacteria. The fixation of colicin E2 is followed by a complex series of surface changes apparently culminating in the formation of a specific conformational state of the membrane which promotes rapid degradation of the bacterial chromosome [1].

Previous studies have indicated that colicin action may be blocked at several post-adsorption steps [2–4] and that at least some of these steps involve specific proteins [5]. In this study we have attempted to confirm that mutants which still adsorb colicin E2 but remain insensitive to its presence, produce altered envelope proteins. The mutants examined were of two types: CetB mutants are specifically refractory to colicin E2 at low temperatures (25°) but are largely sensitive at high temperature (37°) [6]; CetC mutants are also refractory to colicin E2 at 25° but show varying degrees of refractivity at 37°. CetB and CetC loci are closely linked [7] but CetC mutants, as distinct from CetB mutants, as distinct from CetB mutants, are also UV-sensitive [8, 9]. Genetic studies have also shown that mutations to CetC refractivity are pleiotropic [7] and that in addition to UV-sensitivity, these strains also show detergent sensitivity, recombination deficiency, and in some cases filament formation or abortive growth of bacteriophage λ [9].

In this report we show that envelope preparations of both types of Cet mutant, when analysed on sodium dodecyl sulphate (SDS) acrylamide gels, have an almost identical large increase in one polypeptide peak when compared to the wild type parent. This enlarged peak is not observed in revertants of CetC mutants which have a wild type response to colicin E2. In contrast, UV-resistant revertants which are still refractory to E2 still produce enhanced amounts of the polypeptide.

2. Materials and methods

Litre cultures of each bacterial strain were grown in nutrient broth plus 80 μg/ml thymine at 25°, harvested in the exponential phase at 5×10^8 cells/ml and washed with 10 mM sodium phosphate buffer pH 7.1. Cell envelopes were prepared by sonication of whole cells followed by low and high speed centrifugation [10]. The pellets obtained by centrifugation at 100,000 *g* for 30 min were rigorously washed by resuspension in wash buffer with the aid of brief (0.2 min) sonication followed by centrifugation at 100,000 *g* for 30 min. This procedure was then repeated twice more. Typically, the final pellet contained approximately 9% by weight of the sonicate protein and less than 0.03% of the sonicate RNA. The washed pellet was resuspended at 5 mg protein/ml in 10 mM sodium phosphate buffer pH 7.1, containing 1% w/v SDS and 1% v/v 2-mercaptoethanol and dialysed 14 hr at 37° against the same buffer containing 0.2% w/v SDS, 0.1% v/v 2-mercaptoethanol. Acrylamide gels were prepared as described by Weber and Osborn [11] and had the final composition: 10% w/v acrylamide, 0.26% w/v *N,N'*-methylenebisacrylamide 0.1% w/v SDS, 0.06% v/v *N,N,N',N'*-tetramethylenediamine, 0.05 w/v ammonium persulphate, 100 mM sodium phosphate buffer pH 7.0.

Envelope preparations (0.02 ml, 100 μg protein) were mixed with an equal volume of 20% w/v sucrose containing 0.001% w/v bromphenol blue as anti-con-

vectant and tracking dye respectively, and applied to 6 mm X 90 mm gels. Both buffer reservoirs contained 0.1% w/v SDS in 40 mM sodium phosphate buffer pH 7.0. Samples were electrophoresed at 3 mA per gel for 3 hr followed by 6 mA per gel for 5 hr. Gels were fixed in 50% w/v aqueous trichloroacetic acid (TCA) overnight at 37°, stained with 0.1% w/v coomassie brilliant blue in fresh 50% w/v TCA for one hr at 37°, and destained by several changes of fresh 7% v/v aqueous acetic acid. Gels were scanned on a Joyce-Loebl microdensitometer using a red filter.

3. Results and discussion

The CetB, CetC and the revertant strains used in this study are shown in table 1. Envelope fractions of bacterial strains were prepared as described above and microdensitometer tracings of typical gel profiles are shown in figs. 1 and 2. The appearance of the gels is shown in fig. 3. Eleven major polypeptide peaks (or groups) can be discerned (*a–k*) which occur in all six strains. On the basis of repeated gel analysis of these strains, however, we conclude that peak *b* at least is probably complex. Furthermore, the variation in this region observed in different runs is in part due to its proximity to the top of the gel where adverse heating effects are maximal, and protein concentrations are initially highest. Examination of the gel profiles clearly demonstrates that both CetB (ASH101) and CetC mutants (ASH114 and ASH111) show an increased peak *e* compared with the wild type ASH1. The enlarged peak *e* was in all cases obtained whether the Cet mutants were originally grown at 25° or 37°, and this

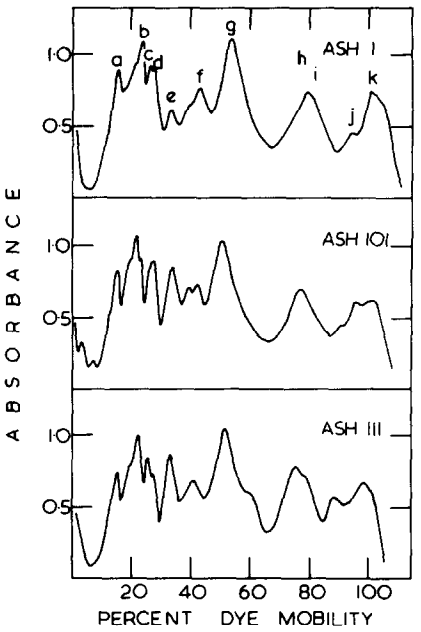

Fig. 1. Joyce-Loebl microdensitometer tracings of coomassie blue stained 10% acrylamide, 0.1% SDS gels of envelope proteins from strains ASH1, ASH101 and ASH111.

feature is not therefore conditional upon the growth temperature of the cells. Mutants resistant to colicin E2 may also be obtained which have lost the capacity to adsorb E2 (and related colicins). These receptor-negative strains do not show pleiotropic effects and are genetically quite distinct from Cet mutants [2]. Analysis of envelope preparations of these mutants has also been carried out, but no deviations from the wild type pattern have been revealed.

Table 1

Strain number	Immediate parental derivation	Cet status	Response to colicin E2* 37°	25°	Response to UV* 25°	Area ratio**
ASH1	–	+	S	S	R	41.86
ASH101	ASH1	CetB	S	R	R	90.61
ASH111	ASH1	CetB	S	R	S	69.5
ASH114	ASH1	CetC	S	R	S	110.49
ASH706	ASH114	–	S	R	R	100.79
ASH711	ASH114	–	S	S	S	45.25

* R = resistant; S = sensitive to colicin E2 and UV [8].

** Area *e* expressed as a percentage of the sum of areas *c* and *d* measured on naphthalene black stained gels.

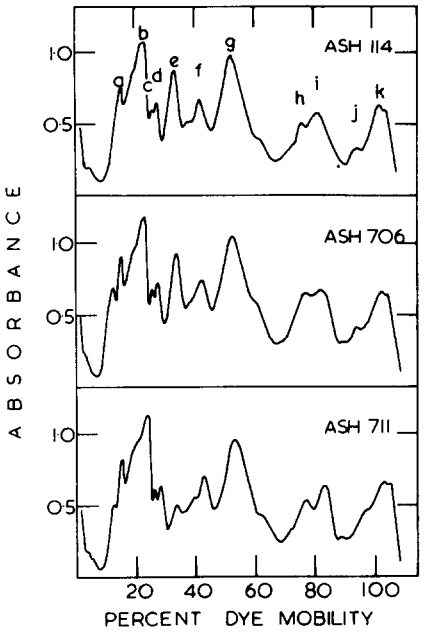

Fig. 2. Joyce-Loebl microdensitometer tracings of coomassie blue stained 10% acrylamide, 0.1% SDS gels of envelope proteins from strains ASH114, ASH706 and ASH711.

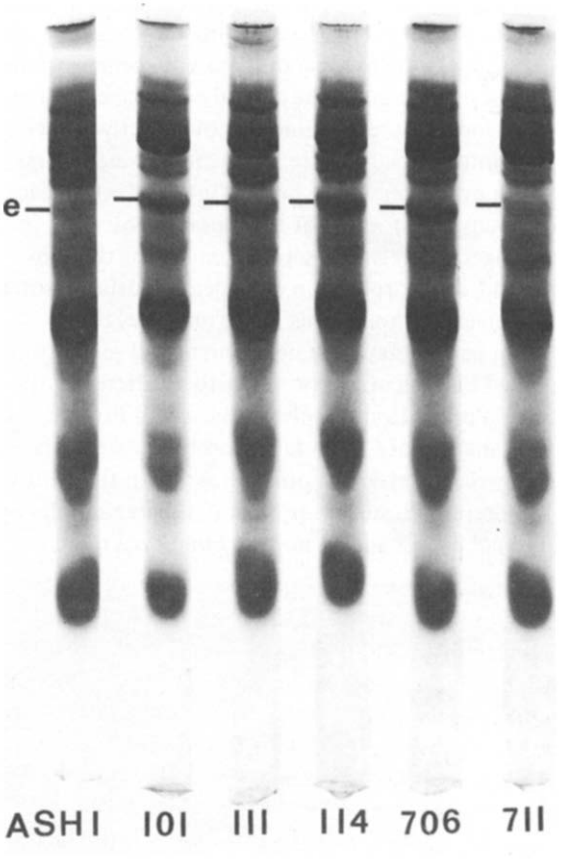

Fig. 3. Appearance of coomassie blue stained 10% acrylamide, 0.1% SDS gels of envelope proteins from strains ASH1, ASH101, ASH111, ASH114, ASH706 and ASH711. The peak *e* is shown as a bar line adjacent to the peak position.

Confirmation of the specificity of the observed changes in gel profiles was obtained when revertants of ASH114 were examined. As shown in fig. 2, envelope preparations of ASH711, a colicin E2-sensitive revertant of CetC mutant ASH114, shows peak *e* to be reduced to the original parental level. It is important to note that this revertant strain is however still UV-sensitive. Moreover, all UV-resistant revertants of ASH111 and ASH114 (e.g. ASH706) which are still refractory to E2 retain the enlarged peak *e* characteristic of their mutant parents. The enlargement of peak *e* therefore appears to be specific to changes in E2 refractivity of CetC mutants. Since previous genetic analysis of CetC mutants has nevertheless indicated that both UV-sensitivity and E2 refractivity arise through a single point mutation, the precise relationship between these two characters remains obscure.

Analysis of the gel profiles shown in figs. 1, 2 and 3 also provides some information with regard to the properties of the altered polypeptide(s) in mutant strains. The mean relative dye mobility of peak *e* is 33.7% which according to the data of Weber [11] indicates a molecular weight of about 4.3×10^4 daltons. Similar results have also been obtained using napthalene black as protein stain and this has facilitated a quantitative estimate of the protein content of peak *e* [12]. Thus in wild type strains peak *e* constitutes about 3% of the total envelope protein and increases to about 6% in most mutant strains.

The results described above do not yet establish the precise location within the cell surface of the altered polypeptide(s) present in the mutants. Although the properties of these strains strongly suggest that they have altered cytoplasmic membranes, modification of other protein containing layers in the cell surface has not been excluded. Further studies will now attempt to clarify this question and also how increased amounts

of polypeptide *e* in the cell envelope can arise. The appearance of an enlarged peak *e* may for example result from (1) the binding of a protein normally present in the cytoplasm to the altered cell surface; (2) incorporation of increased amounts of defective envelope protein into the cell surface; (3) increased incorporation of a normal envelope protein into the cell surface in compensation for loss of the mutant protein; (4) changes in a regulatory gene controlling the synthesis and/or incorporation of a specific surface protein. Alternatively, although this seems unlikely, the increase in peak *e* may only be apparent and result from increased binding of the two dyes to an altered polypeptide. Purification of polypeptide *e* and further genetic analysis of Cet mutants and their revertants may now be necessary in order to establish the nature of the altered cell surface protein *e*, its precise relationship to colicin E2 and its normal functional role in the cell.

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