OUTER MEMBRANE OF ESCHERICHIA COLI K-12: DEMONSTRATION OF THE TEMPERATURE SENSITIVITY OF A MUTANT IN ONE OF THE MAJOR OUTER MEMBRANE PROTEINS.

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Summary

A con mutant of E. coli K-12 previously described as having an altered protein 3A present at a level not detectable on polyacrylomide gel electrophoresis, is shown to have a temperature dependent phenotype. It has no detectable protein 3A at 30°C, and is defective as a recipient in conjugation and is resistant to bacteriophage K3 and colicins K and L. At 42°C the protein is detectable and the strain is sensitive to phage K3 and shows a marked increase in recipient-ability. However, full sensitivity to the colicins is not regained.

Introduction

The outer membrane of Escherichia coli K-12 consists of a number of major outer membrane proteins (1,2), and of these protein 3A (using the nomenclature of Schnaitman, ref. 1) is the second most abundant. This protein has been shown to be missing in con mutants (2), which are resistant to bacteriophage K3 (3) and tolerant to colicins K and L (5,6). The loss of protein 3A also results in the loss of receptor activity for bacteriophage K3 (2,3) and protein 3A has now been shown to be the receptor (Manning and Reeves, manuscript in preparation). Con mutants are also defective as recipients for most F-like plasmids (2,3).

We have recently isolated a series of con mutants unable to plaque wild type

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bacteriophage K3, but on which we were able to isolate host range mutants (Manning, Puspurs and Reeves, submitted to J. Bacteriol.). In this communication we demonstrate that amongst these bacterial mutants are a type which is cold sensitive, in that no detectable protein 3A is observed at 30°C but in which the protein can be identified at 42°C. The temperature dependence of a number of properties of these mutants is also described.

Materials and Methods

The bacterial strains used are all derivatives of Escherichia coli K-12 and are listed in Table 1. Bacteriophage and colicinogenic strains are those we have used previously (2). Nutrient media and minimal media supplemented with the appropriate growth factors and carbon source were as previously described (7,8).

Recipient-ability in conjugation was measured as previously described (9), with the exception that the bacteria were grown and mated at the test temperature and after plating out for recombinants the plates were incubated at 37°C.

Bacteriophage and colicin sensitivity was measured as previously described (2,4,5), using the conventional cross-streak plate test.

Table 1.

Bacterial strains

Strain	Characteristics	Source
P400	F^-/thi, argE, proA, thr, leu, mtl, xyl, ara, galK, lacY, supE, non, λ^-	3
P460	con-1 mutant of P400	3
P1675	con-19 mutant of P400	a.
CSH23 (E5014)	F'lac^+ $proA^+$, B^+ / Δ(lac pro)	Cold Spring Harbor

a. This is one of a series of mutants selected as resistant to bacteriophage K3 and on which host range mutants could be isolated. They will be described elsewhere (Manning, Puspurs and Reeves, submitted to J. Bacteriol.)

Outer membrane preparations were obtained and prepared for electrophoresis on SDS-polyacrylamide gels with the pH7.2 buffer system of Maizel (11) and the pH11.4 buffer system of Bragg and Hou (12) using the methods of Schnaitman (1,10).

Results and Discussion

We have previously shown that under our conditions protein 3A always runs

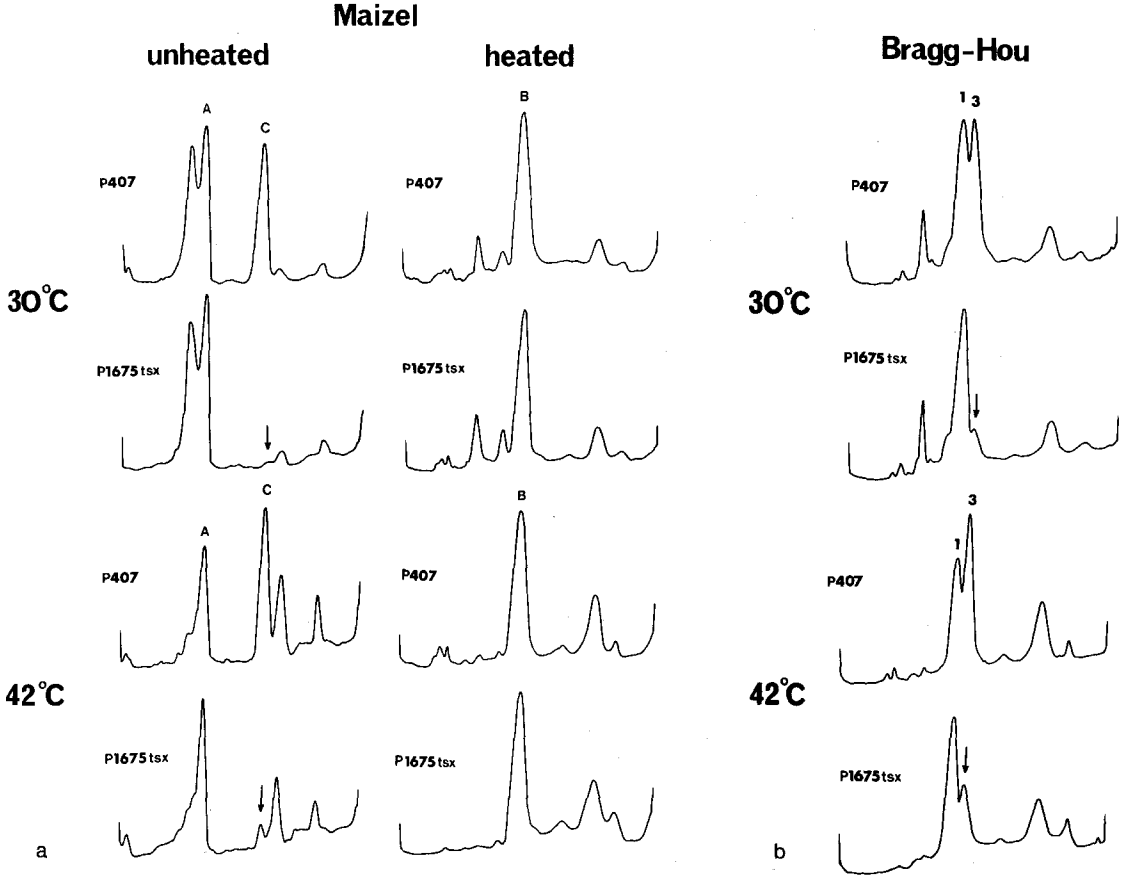

Figure 1a : Densitometer traces of SDS-polyacrylamide gels run using the pH7.2 buffer system of Maizel (11), comparing a tsx mutant of P1675 with P407 (a tsx mutant of P400, the parent strain of P1675).

Figure 1b : Densitometer traces of SDS-polyacrylamide gels run using the pH11.4 buffer system of Bragg and Hou (12), comparing a tsx mutant of P1675 with P407 (a tsx mutant of P400, the parent strain of P1675).

on SDS-polyacrylamide gel electrophoresis with other major outer membrane proteins : with the tsx-protein in unheated samples on Maizel gels, with 1 and 3B in heated samples on Maizel gels and with 3B on Bragg-Hou gels (2). We therefore made tsx mutants from our mutant and the parent strain by selecting for resistance to bacteriophage T6, and it is the outer membranes from these strains which we present in figure 1. These strains have no tsx-protein as shown by the absence of any residual peak C using heated samples on Maizel gels and also the tsx-protein peak is absent in the Bragg-Hou gels. We can therefore say that the peak C observed in the strains represents only

Table 2.

Sensitivity to bacteriophage K3

Strain	E.O.P. of wild type K3(h^+)	
	30°C	42°C
P400	1	1
P460	nd*	nd
P1675	nd	0.43

Strain	E.O.P. of K3 host range mutants											
	h1		h3		h30		h40		h44		h47	
	30°	42°	30°	42°	30°	42°	30°	42°	30°	42°	30°	42°
P400	1	1	1	1	1	1	1	1	1	1	1	1
P460	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
P1675	0.6	0.2	0.5	0.5	0.6	0.6	10^{-4}	0.4	0.2	0.3	0.4	0.3

* nd = not detectable; eop of less than 10^{-6}.

protein 3A. Thus, from the Maizel gels with the unheated samples, it can be seen that strain P1675 has no protein 3A at 30°C but that significant amounts are present at 42°C, although not the full normal amounts. The con mutant P460 shows no protein 3A at either temperature (Manning and Reeves, unpublished results), whereas its parent strain P400 has the protein at both temperatures. From figure 1. it can also be seen that protein 3B, which constitutes the majority of the protein in the peak running immediately faster than peak C, increases significantly in proportion to the other proteins at 42°C, as shown by comparing this peak using unheated and heated samples on the Maizel gels (fig. 1), since protein 3B is known to move into peak B upon heating (1,2).

From table 2 it can be seen that P1675 becomes sensitive to wild type bacteriophage K3 at 42°C, and that a similar effect is observed with one of the host range phages tested, namely K3h40.

Table 3.

Colicin Resistance Patterns

Strain	Colicins			
	K-235		L-JF246	
	30°	42°	30°	42°
P400	S	S	S	S
P460	P	P	R	R
P1675	P	Sl	R	P

Resistance was determined using the convential cross-streak plate test (5). S = sensitive, Sl = slight resistance, P = partial resistance and R = full resistance (S<Sl<P<R)

The fact that full colicin sensitivity is not regained at 42°C (table 3) suggests that the protein may be altered so as to affect its role in sensitivity to colicins K and L, as some mutants with undetectable amounts of protein 3A have previously been shown to be sensitive to colicins K and L (Manning, Puspurs and Reeves, submitted to J. Bacteriol.)

The increase in recipient ability in conjugation at 42°C (table 4) correlating to the return of protein 3A, is further evidence supporting our hypothesis (2, 3) that protein 3A is required for efficient conjugation with most F-like plasmid bearing donors.

Table 4.

Recipient-ability with F' lac pro

Recipient	% Transfer with respect to donor*	
	30°C	42°C
P400	22.5	29.0
P460	0.006	0.009
P1675	0.14	8.8

* Each is the mean of at least three experiments.

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