Suppression by the ColV, I-K94 Plasmid of a Growth Lesion in *ompA* Mutants of *Escherichia coli*

By CAROLINE M. M. DEENEY, MARGARET GOODSON,
FREDA T. ROSSOUW AND ROBIN J. ROWBURY*

Department of Botany and Microbiology, University College London, Gower Street,
London WC1E 6BT, UK

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Organisms of three independently isolated *ompA* mutants of *Escherichia coli* failed to form colonies on glucose minimal agar (glucose MA) at 44 °C after growth in glucose minimal salts medium at 37 °C, although all three strains formed colonies on nutrient agar at 44 °C. Supplementation of the glucose MA with individual amino acids including L-methionine and/or L-cysteine did not allow colony formation at 44 °C, although addition of 0.1% Casamino acids was effective; replacement of glucose with other energy sources or ammonium ions with glutamate also did not allow growth at 44 °C. The failure to form colonies at 44 °C was not due to killing of the organisms, because colonies were formed if plates of the *ompA* mutant initially incubated at 44 °C were shifted to 30 °C after 16 h. Introduction of the ColV, I-K94 plasmid into P678-54 *ompA*, 1131 *ompA* or an *ompC ompA* mutant suppressed the 44 °C growth lesion, but other plasmids (F *lac*, R483ColIa, RI, ColB-K98, R124) tested in P678-54 *ompA* did not. Growth of the ColV, I-K94⁺ derivative at 44 °C was due to a suppressing effect of the plasmid rather than to introduction of the plasmid into a variant with normal or altered OmpA protein. An attempt was made to ascertain which component(s) encoded by ColV, I-K94 was (were) responsible for allowing growth at 44 °C. Transfer components appeared unlikely to be involved and plasmids which conferred individual colicins (plus the corresponding immunity component) did not suppress. The findings that the ColV, I-K94-encoded VmpA protein resembles the OmpA protein (a) immunologically and (b) in being a transmembrane component of the outer membrane suggested that it might be the presence of the VmpA protein which allowed growth at 44 °C. Several experiments were in accord with this possibility.

INTRODUCTION

The outer membrane of strains of *Escherichia coli* contains a few so-called major proteins in very large amounts. In *E. coli* K12 derivatives there are four such proteins under most cultural conditions, the lipoprotein and the OmpA, OmpC and OmpF proteins (Lugtenberg *et al.*, 1975). Aside from their functioning as phage receptors and in colicin attachment or conduction, important functions have been established for the OmpC and OmpF proteins as porins for small hydrophilic molecules (Luktenhaus, 1977), whilst the lipoprotein has been implicated in attachment of the outer membrane to the murein layer (Braun, 1978). In contrast, although the OmpA protein acts as a receptor (e.g. for phages K3 and TuII*), as a component in colicin L conduction and in stabilization of conjugal aggregates (Skurray *et al.*, 1974; Manning *et al.*, 1977), its specific role in normal growth has not been precisely defined. On the basis that glutamine and proline are taken up less well by *ompA* strains than by *ompA*⁺ ones, there is the possibility of a porin function for the protein (Manning *et al.*, 1977), although the properties of the OmpA protein differ markedly from those of the other porins (Nikaido, 1979). The finding that *ompA* mutants lose viability in stationary phase might, however, indicate a more general role for OmpA protein in morphogenesis or in membrane or envelope stability or integrity

(Manning *et al.*, 1977), a role also suggested by studies of morphological mutants (Sonntag *et al.*, 1978).

The OmpA protein is a transmembrane protein of 325 amino acids (Chen *et al.*, 1980). Of these, about 148, at the C terminal region, protrude inwards into the periplasmic space (Chen *et al.*, 1980). A loop (at residues about 60–70) appears to protrude to the outside (Cole *et al.*, 1983); it is possible that about 70 N terminal residues protrude, since this segment becomes sensitive to proteolytic enzymes in one *ompA* mutant (Cole *et al.*, 1983). Other loops of the OmpA protein chain may protrude to the outside (e.g. at residues about 110 and 154; Morona *et al.*, 1984), but substantial numbers of amino acids appear to be buried in the membrane. It is this part of the protein which might play a role in permeation or in membrane integrity under certain conditions.

The plasmid ColV, I-K94 is a colicinogenic plasmid which resembles F in transfer properties and incompatibility (Hardy, 1975). Like F, ColV, I-K94 confers derepressed transfer properties on strains which carry it. Additionally, it encodes the production of the major VmpA protein (Rowbury *et al.*, 1985) and the secretion of colicin V and colicin Ia as well as the corresponding immunity components (Hardy, 1975).

As part of a study of ColV-encoded proteins and of the role of ColV plasmids in pathogenicity, ColV⁺ derivatives of the minicell producer P678-54 and its *ompA* mutant have been made (Moores & Rowbury, 1982; Rowbury *et al.*, 1985). It has previously been shown (Manning *et al.*, 1977) that *ompA* mutants grow poorly in minimal medium at 42 °C. During the present work it was noticed that an *ompA* mutant of strain P678-54 grew poorly in a number of minimal media and failed to form colonies at 44 °C on glucose minimal media; the same was true for two other *ompA* mutants tested. This communication reports that the presence of ColV, I-K94 in any of these *ompA* mutants reverses this 44 °C growth lesion.

METHODS

Bacterial strains. All strains used here except the colicin L producer (*Serratia marcescens* JF 246) were of *E. coli*. Most of these were derived from *E. coli* K12, but two strains used in ColV plasmid transfer (*E. coli* dig 41 and raw 8) were isolates from sewage (ColV-41 and ColV-8 derive from these). The plasmid ColV-M40(5) is a mutant form of ColV, I-K94 which forms virtually no VmpA protein in P678-54 *ompA*. The origin and characteristics of strains P678-54, PCO 479 and 1131 *ompA* were described by Rossouw & Rowbury (1984). From P678-54, *ompA* mutants were prepared by plating organisms with excess phage K3 and isolating resistant colonies. All non-mucoid strains resistant to phage K3 map at the *ompA* locus (Manning *et al.*, 1976; Henning *et al.*, 1976) but the *ompA* strains used here were also resistant to phage TuII* and to colicin L and were devoid of OmpA protein. One *ompA* mutant of strain P678-54 was used to prepare plasmid-bearing strains. After construction (see below), all plasmid-bearing derivatives of *ompA* mutants were examined for the presence of the OmpA protein in their membranes, by SDS-PAGE of membranes prepared as described below, and were tested for sensitivity to phage K3 and colicin L. All the plasmid-bearing *ompA* derivatives were devoid of OmpA protein; except for R483 ColIa⁺ derivatives, they were all resistant to phage K3 and colicin L. All four tested R483 ColIa⁺ derivatives from P678-54 *ompA* proved sensitive to phage K3 and colicin L although their membranes were devoid of OmpA protein. It is assumed that this resistance plasmid encodes an envelope component which allows attachment/conduction of these inhibitory agents.

Derivatives of strain 1829 carrying ColV, I-K94 and other F-like plasmids were used as donors in conjugation. These strains have been described by Rossouw & Rowbury (1984) and Rowbury *et al.* (1985).

Growth media. The minimal medium used for growth was that of Davis & Mingioli (1950) with glucose (0.2%, w/v) as energy source and with required DL-amino acids added at 20 µg ml⁻¹ and other growth factors at 5 µg ml⁻¹. This medium is referred to in the text as supplemented glucose MM. In some experiments, glucose was replaced with other energy sources and ammonium sulphate with other nitrogen sources. The enriched medium was Oxoid no. 2 broth (broth). Where required, these media were solidified with 2% (w/v) Difco Bacto agar to give supplemented glucose MA and NA.

Plasmid transfer by conjugation. Derivatives of strain 1131 and strain P678-54 *ompA* carrying F-like plasmids were made either by plasmid transfer in nutrient broth at 37 °C with gentle shaking (Rossouw & Rowbury, 1984) using derivatives of *E. coli* 1829 (Rowbury *et al.*, 1985) as donors, or by filter conjugation. For this purpose, unless otherwise stated, donor strains were derivatives of strain 1829; 1 ml samples of donor and recipient strains grown to exponential phase (about 2 × 10⁸ organisms ml⁻¹) in nutrient broth at 37 °C were filtered onto 0.22 µm pore size Millipore filters (sterilized by UV irradiation) and filters incubated on nutrient agar plates for 2–3 h at 37 °C. After

incubation, filters were transferred to 2 ml supplemented glucose MM and, after dilution, cells were plated on appropriate selective media.

Growth and tests for colony formation on minimal agar. Organisms were grown overnight with shaking at 37 °C in supplemented glucose MM, then diluted (time zero) 40-fold into the same medium and growth continued with shaking at 37 °C. At time zero and after 24 h, samples of suitable dilutions were plated on supplemented glucose MA and plates incubated at 30, 37 and 44 °C. Colonies were counted after 48–72 h.

Membrane preparation, separation and analysis. For analysis of membranes for presence or absence of the OmpA protein, the organisms were grown in broth and membranes prepared and analysed by SDS-PAGE as described by Rossouw & Rowbury (1984). For separation of membrane fractions, organisms were lysed and membranes prepared as described by Ito *et al.* (1977) and Burnell *et al.* (1980). Fractions were analysed as described by Rossouw & Rowbury (1984). For preparation and use of activated dextran, the procedure of Kamio & Nikaido (1977) was used, membrane fractions being prepared and analysed as above.

Where appropriate, gels were scanned with a Joyce-Loebl Chromoscan 3.

Antibody preparation. The VmpA protein and OmpA protein were partially purified from strains P678-54 *ompA* ColV, I-K94⁺ and P678-54 *ompA*⁺, respectively, as follows. Outer membrane peptidoglycan complexes were prepared as described by Burnell *et al.* (1980), washed in 50 mM-Tris/HCl pH 8.0 and then incubated in the extraction buffer of Rosenbusch (1974) for 30 min at 60 °C. The mixture was then diluted with an equal volume of the same buffer and centrifuged at 110000 g for 45 min at 20 °C. The proteins in the soluble fraction were precipitated with 95% (v/v) ethanol at –70 °C and collected by centrifugation (35000 g for 30 min at 4 °C). The pellet was suspended in SDS sample buffer (Rossouw & Rowbury, 1984) heated at about 100 °C for 5 min and samples were run on a polyacrylamide gel. After electrophoresis, the required bands were sliced out, crushed and the crushed fragments wetted with a small volume of 0.1 M-Tris/HCl buffer pH 7.0 containing 0.1% (w/v) SDS. The proteins were eluted from the wetted gel by 2 vols 0.1 M-Tris/HCl pH 7.0 containing 0.1% (w/v) SDS, concentrated by precipitation with 90% (v/v) acetone overnight at –20 °C and the protein recovered from several gels pooled. Samples were stored at –15 °C in Tris buffered saline (TBS: 10 mM-Tris/HCl pH 7.3, 0.15 M-NaCl, 1 mM-EDTA) or phosphate buffered saline (0.14 M-NaCl, 2.7 mM-KCl, 1.5 mM-KH₂PO₄, 8.1 mM-Na₂HPO₄ pH 7.3) in 0.5 ml samples with protein at about 10 mg ml⁻¹.

The partially purified proteins (0.5 mg) were mixed with Freund's complete adjuvant (0.2 ml) and injected intramuscularly into separate rabbits. Booster injections (about 0.2 mg protein) were given at 2 week intervals for 6 weeks; 3 weeks after the third booster, large blood samples were taken by heart puncture. Serum was collected and stored frozen at –20 °C in 0.5 ml samples.

Antibody adsorption. Cell envelopes [prepared by the method of Ito *et al.* (1977)] from strain P678-54 *ompA* were washed in TBS by centrifugation and then heated at 100 °C for 15 min in TBS containing 0.1% (w/v) SDS and 5% (v/v) glycerol. After cooling, samples were mixed with equal quantities of each of the sera at 1 mg or 10 mg protein ml⁻¹ and incubated for 2 h at 37 °C followed by 17 h at 4 °C. The envelope–antiserum mixture was centrifuged at 11000 g for 15 min at 4 °C and the supernatant (representing adsorbed antiserum) collected and used as described below.

Ouchterlony tests. All antisera and agars were prepared in or diluted with TBS, whereas dilutions of antigen were made in TBS containing 1% (v/v) Triton X-100. Ouchterlony plates [1% (w/v) agar containing 0.02% sodium azide] were prepared in 25 ml Petri dishes with 3 mm holes around a central hole. Antigen, i.e. the appropriate partially purified protein or cell envelope preparation [prepared by the method of Ito *et al.* (1977)] at a protein level of about 10 mg ml⁻¹, was heated at 100 °C for 15 min in TBS containing 1% SDS and 5% glycerol and, after dilution, 10 µl of each concentration (undiluted and 2-, 4-, 8-, 16- and 32-fold diluted) placed into the outer wells while 10 µl of undiluted or two- or fourfold diluted antiserum was placed in the centre well. Control experiments were done in parallel using pre-immune serum. Plates were kept in a humid atmosphere at room temperature for 24–48 h and then stained with Coomassie Blue to enhance the precipitin lines.

RESULTS AND DISCUSSION

Growth of ompA mutants at 44 °C

Organisms of strain P678-54, after growth in supplemented glucose MM at 37 °C, formed colonies when plated on supplemented glucose MA at 30, 37 or 44 °C (Table 1), although sometimes the number of colonies formed at 44 °C was somewhat less than at 30 or 37 °C (Table 1). In contrast, organisms of P678-54 *ompA* formed colonies on supplemented glucose MA at 30 and 37 °C but not at 44 °C (Table 1). Organisms of the *ompA* mutant formed colonies on NA or MA + Casamino acids at 44 °C but not on supplemented glucose MA or on supplemented glucose MA plus individual amino acids. In the latter studies, each of the 18 common protein amino acids (other than threonine and leucine, which were present in all cases) was tested individually

Table 1. *Effect of ColV, I-K94 on growth at 44 °C*

Bacteria were grown in supplemented glucose MM shaken at 37 °C and plated after 24 h. Colony no. is given as a percentage of that at 30 °C.

<i>E. coli</i> strain	Relative no. of colonies formed on supplemented glucose MA at:	
	37 °C	44 °C
P678-54 <i>ompA</i> ⁺	87	37
P678-54 <i>ompA</i>	72	0
P678-54 <i>ompA</i> ColV, I-K94	187	75
P678-54 <i>ompA</i> , cured*	116	0
P678-54 <i>ompC ompA</i>	98	0
P678-54 <i>ompC ompA</i> ColV, I-K94	85	17
PCO479 <i>ompA</i> ⁺	103	100
1131 <i>ompA</i> †	105	0
1131 <i>ompA</i> ColV, I-K94	104	91

* This is a Col⁻ strain derived from P678-54 *ompA* ColV, I-K94 by a temperature regime (Rowbury *et al.*, 1985).

† Derived from PCO479.

(in the DL form except for glycine) and some were also tested in pairs (methionine plus cysteine, isoleucine plus valine, glutamate plus glutamine and aspartate plus asparagine); none was able to allow growth of P678-54 *ompA* on supplemented glucose MA at 44 °C.

Strain 1131, an *ompA* derivative of PCO 479 (Lugtenberg *et al.*, 1975), also failed to form colonies on supplemented glucose MA at 44 °C, unlike the *ompA*⁺ parent (Table 1). The same was true for a double *ompC ompA* mutant derived from P678-54.

Even at 37 °C, the growth lesion was apparent in the *ompA* mutants. Although colonies were formed on supplemented glucose MA at 37 °C, growth was slower, so that whereas colonies of the *ompA*⁺ parents on this medium were easily visible after 16 h at 37 °C, the colonies of the *ompA* mutants could not be seen with the naked eye at this time.

Effect of ColV, I-K94 on growth at 44 °C

Although P678-54 *ompA* grew poorly in liquid minimal medium at 37 °C, on introduction of ColV, I-K94, the derivative behaved like the parental P678-54. The ColV, I-K94⁺ derivative isolated from P678-54 *ompA* formed colonies normally on supplemented glucose MA at 44 °C (Table 1). The same was true for ColV, I-K94⁺ derivatives of strains 1131 and P678-54 *ompC ompA*. The introduction of ColV, I-K94 also led to better growth of *ompA* mutants on supplemented glucose MA at 37 °C. Colonies were, as for the *ompA*⁺ strains, visible to the naked eye after 16 h incubation, whereas this was not so for the Col⁻ *ompA* strains.

It seemed likely that the presence of ColV, I-K94 in the *ompA* strains suppressed the growth lesion. Another possibility, however, was that the plasmid had been introduced into mutants carrying normal or altered OmpA protein, these mutants being already present in the *ompA* cultures. Since *ompA* mutants are Con⁻ with respect to F-like plasmids such as ColV, I-K94, any such variants might be more efficient recipients for the plasmid. Several findings rule out this possibility. Firstly, the ColV, I-K94⁺ derivatives arising from the *ompA* mutants showed no OmpA protein in their outer membranes (Fig. 1), nor any new protein of similar *M_r* (i.e. a possible OmpA protein variant), except for the VmpA protein, which is known to be plasmid-encoded (Moores & Rowbury, 1982; Rowbury *et al.*, 1985). Secondly, none of the ColV, I-K94⁺ derivatives tested showed sensitivity to phage K3 or colicin L (sensitivity depends on the presence of OmpA protein). Thirdly, a cured derivative isolated from the *ompA* ColV⁺ strain by a 42 °C growth regime (Rowbury *et al.*, 1985) had lost both the plasmid and the ability to grow at 44 °C on supplemented glucose MA (Table 1).

Other F-like plasmids tested did not suppress the 44 °C growth lesion; derivatives of strain P678-54 *ompA* carrying F *lac*, RI, ColB-K98 or R124 failed to form colonies on supplemented glucose MA at 44 °C (Table 2).

Fig. 1. VmpA protein in ColV, I-K94⁺ strains of *E. coli*. P678-54 *ompA* and derivatives were grown in broth at 37 °C and whole membrane preparations isolated, analysed and run on SDS-PAGE gels. (a) P678-54 *ompA*; (b) P678-54 *ompA* ColV, I-K94; (c) P678-54 *ompA*, cured; (d) P678-54 *ompA* R124; (e) P678-54 *ompA* ColV, I-K94, R124; (f) P678-54 *ompA* ColB-K98; (g) P678-54 *ompA* ColV, I-K94, ColB-K98; (h) P678-54 *ompA* ColV-41; (i) P678-54 *ompA* ColV-M40(5); (j) P678-54 *ompA* ColV-8; (k) P678-54 *ompA*⁺ ColV, I-K94; (l) P678-54 *ompA* F *lac*; (m) P678-54 *ompA* R483 ColIa; (n) P678-54 *ompA* R1. Positions of OmpF protein (F), OmpA protein (A) and VmpA protein (V) are arrowed.

Table 2. *Plasmids and growth on supplemented glucose MA at 44 °C*

Bacteria were grown and plated as described in Table 1.

Plasmid(s) present in P678-54 <i>ompA</i>	Plasmid properties				Relative colony no.† on supplemented glucose MA at:	
	Incompatibility group	Transfer properties	Colicins	Resistances*	37 °C	44 °C
None	NA	None	None	None	75	0
ColV, I-K94	FI	Derepressed	V, Ia	None	105	61
F <i>lac</i>	FI	Derepressed	None	None	115	0
R1	FII	Repressed	None	Ap, Sm, Cm, Su, Km	107	0‡
ColB-K98	FIII	Repressed	B, M	None	163	0
R124	FIV	Repressed	None	Tc	48	0
R483 ColIa	I	Repressed	Ia	Sm, Tp	92	0
ColV, I-K94, ColB-K98	FI, FIII	Repressed	V, Ia, B, M	None	68	86
ColV, I-K94, R124	FI, FIV	Repressed	V, Ia	Tc	82	30

NA, Not applicable.

* Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin; Sm, streptomycin; Su, sulphonamides; Tc, tetracycline; Tp, trimethoprim.

† Colony no. is given as a percentage of that at 30 °C.

‡ Slight background growth.

Plasmid components and growth at 44 °C

Presumably, it is a ColV, I-K94-encoded outer membrane protein which suppresses the growth lesion. A number of ColV, I-K94 transfer components, including the TraT gene product, are located in the outer membrane. It is, however, unlikely that any of them are responsible for the suppression, since the introduction of a *fi*⁺ plasmid, such as R124 or ColB-K98, into P678-54 *ompA* ColV, I-K94 did not abolish growth on supplemented glucose MA at 44 °C (Table 2), although the presence of the *fi*⁺ plasmids abolished transfer properties, MS2 phage sensitivity and the formation of transfer components such as the TraT product. Additionally, the transfer properties of F *lac* are very similar to those of ColV, I-K94 (Hardy, 1975), but F *lac* did not suppress the 44 °C growth lesion in the *ompA* mutant (Table 2).

Fig. 2. Location of the VmpA protein. Membranes from P678-54 *ompA* ColV, I-K94 were prepared and separated by the method of Burnell *et al.* (1980) and analysed, run by PAGE and scanned as described in Methods. (a) Cytoplasmic membrane fraction; (b) outer membrane fraction. The positions of the porins (1) and VmpA protein (2) are arrowed.

It is unlikely that the presence of colicin V and its immunity component in the outer membrane is, alone, able to suppress the *ompA* growth lesion, because introduction of ColV-41 or ColV-8 (ColV plasmids from *E. coli* isolates from sewage), which encode colicin V and its immunity component, failed to allow growth of the *ompA* mutant on supplemented glucose MA at 44 °C.

A plasmid which encodes colicin Ia and its immunity component (R483 ColIa) also failed to suppress the *ompA* growth lesion (Table 2). Strikingly, this plasmid appears to encode sensitivity to phage K3 and colicin L. In spite of this sensitivity, all tested R483 ColIa⁺ derivatives of P678-54 (see Methods) were devoid of the OmpA protein (Fig. 1). It is possible that these strains contain a trace of this protein, not detected on PAGE, or alternatively, this plasmid may confer a new protein present in low amounts or masked, perhaps because of its size, by other outer membrane proteins. For example, a smaller protein, related to the OmpA protein but missing part of the C terminus, might confer sensitivity to phage K3 and colicin L, but be masked by other proteins or be present at low level; a larger protein with a similar N-terminus might be masked by the porins.

Some ColV plasmids encode an iron chelation system which uses plasmid-encoded outer membrane proteins for transport. It is very unlikely that a component of such a system is involved in suppression of the *ompA* growth lesion because ColV, I-K94 apparently does not encode this system (Williams, 1979).

The ColV, I-K94 plasmid, like most ColV plasmids, encodes the VmpA protein. This is, like the OmpA protein, located exclusively in the outer membrane. This was established by analysing cytoplasmic membrane and outer membrane fractions from P678-54 *ompA* and P678-54 *ompA* ColV, I-K94. The porins (in both strains) and the VmpA protein (in P678-54 *ompA* ColV, I-K94) were almost exclusively in the outer membrane fractions (Fig. 2). Moreover, it appears that, like the OmpA protein, the VmpA protein spans the outer membrane, with segments protruding on both sides. The trypsin sensitivity of the VmpA protein in membrane preparations (giving rise to a fragment with an M_r of about 31 000) but not in whole cells suggests that a segment (probably about 10–20 amino acid residues in length) extends inwards into the periplasmic space (Moores & Rowbury, 1982; Rowbury *et al.*, 1985). That a part of the VmpA protein protrudes to the outside is suggested by the finding that when cyanogen bromide-activated dextran is added to whole cells of P678-54 *ompA* ColV, I-K94 and membranes prepared from them, then the level of VmpA protein is reduced about eightfold compared to that in the control membranes from cells not treated with activated dextran (Fig. 3); a similar reduction occurs for the porins (Fig. 3). This indicates that most of the VmpA protein has reacted with the non-penetrating activated dextran and must therefore protrude to the outside.

Fig. 3. Reaction of outer membrane proteins with cyanogen bromide-activated dextran. Whole cells of P678-54 *ompA* ColV, I-K94 were treated with cyanogen bromide-activated dextran. Outer membrane fractions from (a) untreated and (b) treated organisms were then analysed, run by SDS-PAGE and scanned as described in Methods. The positions of the porins (1) and VmpA protein (2) are arrowed. Equal amounts of protein were loaded for each sample but 3.07 times as much protein from the untreated sample entered the gel as for the treated sample.

Table 3. *Immunological relatedness of OmpA protein and VmpA protein*

Sera were used undiluted; essentially identical results were obtained with two- or fourfold diluted sera.

Sera	Membranes used for adsorption	Extent of reaction with antigen:							OmpC protein undiluted	OmpF protein undiluted
		VmpA protein at dilution:			OmpA protein at dilution:					
		None	× 8	× 32	None	× 8	× 32			
Pre-immune serum	None	—	—	—	—	—	—	—	—	
Adsorbed serum raised against VmpA protein	P678-54 <i>ompA</i>	++	++	+	++	++	+	—	—	
Adsorbed serum raised against OmpA protein	P678-54 <i>ompA</i>	++	++	+	+++	++	+	—	—	

Additionally, the VmpA protein resembles the OmpA protein in certain properties such as solubility in 2% SDS at 60 °C (Moore & Rowbury, 1982) and its derivation from a slightly larger pro-protein precursor (Rowbury *et al.*, 1985).

The OmpA and VmpA proteins appear to be immunologically related. To demonstrate this, partially purified OmpA and VmpA proteins (see Methods) were tested for reaction with antibodies raised against the corresponding purified proteins. The methods used to prepare the antigens (see Methods) ensured that the partially purified VmpA protein contained no OmpA protein and similarly that the partially purified OmpA protein contained no VmpA protein (accordingly, the serum raised against each protein was free from specific antibodies against the other). Pre-immune sera gave no reaction when tested against any of the dilutions (1/1 to 1/32) of partially purified porins, OmpA protein or VmpA protein. Partially purified porins gave no reaction at any of the tested dilutions (1/1 to 1/32) with adsorbed sera raised against VmpA protein or OmpA protein. In contrast, all tested dilutions of VmpA protein gave marked precipitin reactions with adsorbed serum raised against OmpA protein and vice versa; the patterns of interaction were essentially identical for each antibody with its own antigen and with the other antigen (Table 3).

These similarities between the two proteins suggested that the VmpA protein might be a plausible replacement for certain OmpA protein functions. Three findings are in accord with the VmpA protein being responsible for suppressing the 44 °C growth lesion in *ompA* mutants. Firstly, three ColV plasmids, which either do not encode the VmpA protein (ColV-41 and ColV-8), or fail to confer VmpA protein synthesis on strain P678-54 *ompA* [ColV-M40(5)], did not allow growth of P678-54 *ompA* on supplemented glucose MA at 44 °C; in contrast, ColV-K30, which like ColV, I-K94 encodes the VmpA protein (Williams, 1979), allowed growth of P678-54 *ompA* on supplemented glucose MA at 44 °C. The failure of the other tested plasmids to suppress the growth lesion (Table 2) accords with none of them leading to the synthesis of appreciable amounts of VmpA protein (Fig. 1). Secondly, prior growth with magnesium or calcium ions (0.005–0.05 M) prevented colony formation by P678-54 *ompA* ColV, I-K94 on supplemented glucose MA at 44 °C. Cells grown under these conditions failed to form VmpA protein. Thirdly, organisms of P678-54 *ompA* ColV, I-K94 also failed to form colonies on supplemented MA at 44 °C if grown static rather than shaken at 37 °C prior to plating. Organisms of this (and other ColV⁺ strains) failed to form the VmpA protein in static culture.

Survival of ompA strains on supplemented glucose MA at 44 °C

Low power microscopy of supplemented glucose MA plates which had been seeded with P678-54 *ompA* and then incubated at 44 °C for 40 h showed the presence of microcolonies; i.e. some growth and division took place at this temperature. It seemed possible, therefore, that the elevated temperature had led to starvation for some component rather than having a more profound effect, e.g. on membrane integrity. Accordingly, survival of the *ompA* mutants on supplemented glucose MA at 44 °C was examined by shifting plates to 30 °C after 16 h at 44 °C. Most of the organisms formed colonies under these conditions; i.e. they had not been killed by incubation at 44 °C.

One possible cause of starvation might be the failure of *ompA* organisms to synthesize a specific metabolite at 44 °C. A mixture of amino acids allowed growth on supplemented glucose MA at 44 °C and, accordingly, the synthesis of one or more of these might be aberrant. No individual amino acid allowed growth at 44 °C; even L-methionine (the synthesis of which is switched off at elevated temperature; Ron, 1975) on its own or together with L-cysteine was ineffective.

Alternatively, starvation might result from a failure of essential components of the growth medium to permeate at a sufficient rate at 44 °C into *ompA* organisms; such organisms are claimed to have permeability defects (Manning *et al.*, 1977). Growing on minimal medium, the organism has to make all its sulphur compounds from sulphate, all its nitrogen-containing ones from NH₄⁺, and has to provide energy and carbon skeletons from glucose. However, an uptake defect for sulphate is unlikely to be the sole cause of growth failure at 44 °C, because supplementation of minimal medium with L-methionine plus L-cysteine did not allow growth at 44 °C, but adding the other 18 (non-sulphur-containing) amino acids allowed growth. If uptake of ammonium ions were aberrant, this would lead to a reduction in synthesis of most or all nitrogenous cellular components. Such a defect would be in accord with the observations because a mixture of amino acids would have a 'sparing' effect and, therefore, might allow growth. If there were a specific defect in uptake of ammonium ions then P678-54 *ompA* should grow on supplemented glucose MA at 44 °C if other nitrogen sources were provided. This is not so. Replacement of ammonium sulphate with glutamic acid did not allow growth of P678-54 *ompA* on supplemented MA at 44 °C although glutamic acid (250 µg ml⁻¹; L-form) is an effective nitrogen source for P678-54 *ompA* on supplemented glucose MA at 30 or 37 °C and for P678-54 *ompA*⁺ and P678-54 *ompA* ColV, I-K94 at 30, 37 or 44 °C on the same medium. Accordingly, if the growth lesion involves a defect in uptake of ammonium ions then this defect applies to glutamate as well.

An alternative possibility is that there is a specific glucose uptake defect at 44 °C. This might also be in accord with reversal of the defect by a mixture of amino acids because this could have a sparing effect. If such a specific defect were responsible for failure of the P678-54 *ompA* strain to grow on supplemented glucose MA at 44 °C, then growth ought to occur if glucose were

replaced with other energy sources. In fact, no growth of strain P678-54 *ompA* occurred if glucose was replaced by maltose, glycerol, succinate or acetate (0.2%), although all of these are effective as energy sources for growth of this strain on supplemented MA at 30 or 37 °C and for growth of P678-54 *ompA*⁺ and P678-54 *ompA* ColV, I-K94 at 30, 37 or 44 °C. Accordingly, if failure of the *ompA* mutant to grow at 44 °C results from failure of energy sources to permeate, then the failure is general and not specific to glucose.

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