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A unique amino acid substitution in the outer membrane protein OmpA causes conjugation deficiency in *Escherichia coli* K-12

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The outer membrane protein OmpA of *E. coli* K-12 can serve as a receptor for phages and is required for stabilizing mating aggregates during F′-mediated conjugation. Selection for resistance to OmpA-specific phages yields mutants with alterations in the protein at four cell surface exposed sites. It is shown that conjugation deficiency can be caused by apparently only one type of amino acid substitution at one of these sites, the replacement of glycine-154 by aspartic acid. This suggests that, in contrast to binding of phages, a ligand of the donor cell recognizes only a very small area of the protein.

Conjugation; Outer membrane; *ompA* mutant; (*E. coli*)

1. INTRODUCTION

The 325-residue OmpA protein [1] is one of the abundant proteins of the outer membrane of *E. coli* K-12. Among other functions it plays a role in F′-mediated conjugation; it is required, in recipient cells, for stabilizing mating aggregates in liquid media [2]. It appears therefore, that a ligand (of an as yet unidentified nature) of the donor cell binds to this protein.

A model for the arrangement of the protein in the membrane has been proposed. The polypeptide is thought to cross the membrane eight times in antiparallel β-sheet conformation between residues 1 and about 175 [3,4]; the COOH-terminal moiety is located in the periplasm [1]. A large number of mutants have been analyzed which were selected for resistance to one or the other phage using the OmpA protein as a receptor. The alterations, usually amino acid substitutions or small deletions, were found exclusively around residues 25, 70, 110 and 154 and it was proposed that these areas of the protein are exposed at the surface of the cell [3,5,6]. Such an exposure has been demonstrated directly for areas 70 and 154 [5,7]. Binding of donor to recipient cells during conjugation should occur at the surface-exposed areas of the protein.

Surprisingly, of 18 different mutant alleles identified, only one, representing the substitution of glycine-154 for aspartic acid, turned out to effect a conjugation-deficient phenotype [6]. A set of *ompA* mutants (CC mutants) have been described which were selected for conjugation deficiency [8]. Because of the obvious possibility that such a selection yields mutants which cannot be recovered by selection for phage resistance and because we wished to establish which area of the protein is involved in cell-cell interaction during conjugation, we have analyzed some of the CC mutants which produce wild-type amounts of the polypeptide. We have also introduced new amino acid substitutions into area 25 which was suspected to be important in the stabilization of mating aggregates [6].

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2. MATERIALS AND METHODS

2.1. *Bacterial strains, mating and growth conditions*

The conjugation-deficient mutants (CC 227, 228, 230, 244, 246, 247, 248, 251, 253, 256, 257 and 260) are of the A2 class as described by Manoil and Rosenbusch [8]. Hosts for the M13 mp19 phages [9] and plasmid pTU500 [10], carrying the *ompA* gene, were strains JM103 [11] and UH203 ([12]; this strain does not produce a chromosomally encoded OmpA protein), respectively. The *dut ung* strain BW313 was used to obtain uracil-containing phage M13 [13]. As donor for mating strain RMT-3 [3] was employed. It carries an F′ specifying resistance to kanamycin (50 μg/ml). Donor and recipient cells (the latter are *rpsL*) were mixed at a ratio of 1:10, incubated for 30 min at 37°C and plated on selective media; streptomycin (150 μg/ml) counter-selected against the donor. Medium was always L-broth [14].

2.2. *Cloning and DNA sequencing*

The *ompA* genes of strains CC227 and CC260 were cloned (in a phage λ) and sequenced exactly as in [3,6].

2.3. *Oligonucleotide directed mutagenesis*

The oligonucleotides used are shown in fig.1. For annealing, DNA of a recombinant phage M13 mp19 which harbored part of the *ompA* gene (encoding the signal sequence and the mature protein up to residue 229) was used. To enrich for mutant clones, Kunkel's mehod [15] was used, i.e. this phage was grown on strain BW313. In each case the mismatch primer plus a universal 17-mer sequencing primer (New England Biolabs) was added [16] and the Klenow fragment of the *E. coli* DNA polymerase produced the second strand. Upon ligation with T_4 DNA ligase, the double-stranded *ompA* part of the phage DNA was excised with suitable restriction nucleases and cloned into the RF form of phage M13 mp19. Phage DNA of such clones was screened for the presence of the desired mutation by DNA sequencing [17]. A restriction fragment including the mutational alteration was then ligated into pTU500 and the presence of the base pair substitution in this plasmid was confirmed by DNA sequencing. In pTU500 the *ompA* gene is under the control of the *lac* regulatory elements. The plasmids were transformed into strain UH203 which was then grown in the presence of isopropylthiogalactoside (1 mM).

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                Asn-26
5'-ATC AAC AAC AAT GGC
3'-TAG TTG CCG TTA CCG
          Gly

                Gly-28
5'-AAC AAT GGC CCG ACC
3'-TTG TTA CTG GGC TGG
          Asp
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Fig.1. Mismatch primers. The area of the wild-type *ompA* DNA is written in the 5′-3′ direction.

3. RESULTS

Twelve independently isolated conjugation-deficient CC mutants were tested against a set of 11 different OmpA-specific phages. The resistance-sensitivity patterns were identical for all mutants and the same as that described for the glycine-154 to aspartic acid substitution [6]. This pattern is unique, no other *ompA* mutant exhibiting it. Therefore, and since most *ompA* mutants selected for phage resistance exhibit phage-resistance patterns different from each other, this result strongly suggested that the CC mutants may all have been identical. The *ompA* genes of two mutants, CC227 and CC260, were cloned and the areas which encode residues 1–275 of the protein were sequenced. In both cases the codon GGC for glycine-154 was found to have undergone a transition to GAC, specifying aspartic acid, and no other alteration could be detected. Hence, the twelve CC mutants in all probability are identical.

Several considerations led us to suspect that region 25 may also act as a receptor site for a ligand on the F′-donor cell [6]. Therefore, we introduced, using oligonucleotide-directed mutagenesis, base pair substitutions leading to the amino acid substitutions glycine-28 for aspartic acid and asparagine-26 for glycine. The first was chosen for obvious reasons and the latter because

it would disrupt the sequence isoleucine-asparagine-asparagine-asparagine-27 which is present almost identically in both regions 25 and 154. Cells expressing the mutant proteins were not resistant to any of the OmpA-specific phages and were fully competent in conjugation.

4. DISCUSSION

The data presented previously [3,6] and in this communication leave little, if any, doubt that only one amino acid substitution in the OmpA protein, glycine-154 for aspartic acid, causes conjugation deficiency. Two *ompA* mutants, isolated independently by selection for phage resistance, possessed this alteration. Eleven *ompA* mutants, isolated independently by selection for conjugation deficiency, exhibited the same unique phage resistance-sensitivity pattern which was identical to that effected by the former two *ompA* mutants. For two of the latter mutant *ompA* genes the same base pair substitution was found as that reported for the phage-selected mutants. Also, the effect of the amino acid substitution appears to be very specific. Replacement of the glycine-154 residue by serine did not affect conjugation [6]. The codon GGC for this glycine residue could easily mutate to CGC (arginine) but this apparently was not selected as conjugation-deficient phenotype. Hence, it would not be worth the effort to analyze further conjugation-deficient *ompA* mutants.

For a number of reasons discussed previously [6], it had been suspected that region 25 of the OmpA protein may also act as a receptor site for the F′-cell. Although the facts discussed so far made this quite unlikely, we introduced a glycine-28 for aspartic acid and an asparagine-26 for glycine substitution. There was no effect on conjugation proficiency. Remarkably, however, there was also no effect on the activity of the protein as a phage receptor, although the substitutions isoleucine-24 for asparagine or glycine-28 for valine effect resistance to several OmpA-specific phages [6]. These results underline the very high specificity of phage-phage receptor interactions.

It is known that for conjugation proficiency as for phage activity both the OmpA protein and lipopolysaccharide are required [8,18]. It is not known which component represents the receptor during conjugation. Our data cannot definitively resolve this question but do suggest that the protein is the primary recognition site. If lipopolysaccharide were the receptor, the OmpA protein might be necessary to bring the core oligosaccharide into the right position and the mutational alteration might interfere with this mechanism. It is difficult to visualize how this and only this amino acid substitution would have such an effect. It appears that, in sharp contrast to the function of the protein as phage receptor, only a very small area of it is recognized by the donor cell.

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