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Host Range Mutants of Bacteriophage Ox2 Can Use Two Different Outer Membrane Proteins of *Escherichia coli* K-12 as Receptors

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The *Escherichia coli* K-12 outer membrane protein OmpA functions as the receptor for bacteriophage Ox2. We isolated a host range mutant of this phage which was able to grow on an Ox2-resistant *ompA* mutant producing an altered OmpA protein. From this mutant, Ox2h5, a second-step host range mutant was recovered which formed turbid plaques on a strain completely lacking the OmpA protein. From one of these mutants, Ox2h10, a third-step host range mutant, Ox2h12, was isolated which formed clear plaques on a strain missing the OmpA protein. Ox2h10 and Ox2h12 apparently were able to use both outer membrane proteins OmpA and OmpC as receptors. Whereas there two proteins are very different with respect to primary structures and functions, the OmpC protein is very closely related to another outer membrane protein, OmpF, which was not recognized by Ox2h10 or Ox2h12. An examination of the OmpC amino acid sequence, in the regions where it differs from that of OmpF, revealed that one region shares considerable homology with a region of the OmpA protein which most likely is required for phage Ox2 receptor activity.

The outer membrane of *Escherichia coli* K-12 contains several proteins which serve as, or are components of, the receptors for bacteriophages. Evidence for this has been obtained by in vivo and in vitro studies (see reference 21 for a review). Inactivation in vitro of phages often requires both the outer membrane protein and lipopolysaccharide (LPS). This is the case for the OmpA, OmpC, and OmpF proteins and the T-even-like phages which utilize these polypeptides (8). In such phage-protein systems, it is therefore difficult to know which outer membrane component is the primary site recognized by the phage.

A surprising finding was that phages have been isolated which appear to be able to use two or more outer membrane proteins as receptors. A derivative of the OmpF-specific phage TuIa, called Tp1, can also use the LamB protein (25). Other phages of this parentage can utilize the OmpC, OmpF, and LamB (Tp5); the OmpC or OmpF (Tp2); and OmpC proteins only (Tp6 [16]). Tp1 most likely is a two-step host range mutant (16). Another example of this type of extended host range behavior is exhibited by phage SS1, a natural isolate, which can use the LamB or OmpC proteins (2). Recent work by Gabay and Schwartz (9) has shown that monoclonal antibodies to the LamB protein could inhibit adsorption of phage λ but had no effect on Tp1 adsorption. The results contradict the genetic evidence which suggests that Tp1 recognizes the same site on LamB as does λ (25). It seems as though Tp1 uses the LamB protein in an indirect manner. A similar situation may exist for phage T4. In *E. coli* K-12, sensitivity to T4 is dependent on both the OmpC protein and the nature of the LPS. The OmpC protein is not required when the LPS is of the *E. coli* B type (12, 26). In summary, in the cases of host range mutants which appear to be able to use more than one protein as receptor, it is not entirely clear whether the parental phages do specifically recognize a polypeptide or LPS, or both. Although there is no doubt that the mutants recovered did change their receptor specificity, one may wonder, particularly in view of Tp1 and T4, if this change always exclusively involved an extension of this specificity from one protein to another.

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Since for a number of OmpA-specific phages there is little doubt, if any, that this protein serves as the primary receptor (17), we have questioned whether such phages also can give rise to host range mutants of the type just discussed. We isolated from these phages, Ox2, an extended host range mutant most likely able to use the OmpA or OmpC proteins as receptors.

MATERIALS AND METHODS

Bacterial and phage strains; isolation of host range mutants. All bacterial strains are described in the accompanying paper (17) and elsewhere (3, 5, 6, 19, 22). Phage Ox2 (10, 20) was propagated on the *E. coli* K-12 strain P400 (22). Host range mutants were isolated by plating 10^8 to 10^9 PFU together with about 2×10^8 cells of the selection strain in a soft LB agar overlay. They were isolated by collecting single plaques on the selection strain and propagated once on the same strain. All incubations were at 37°C.

Test for sensitivity or resistance to phages and isolation of phage-resistant mutants. Testing was performed as described previously (17); in brief, serial 10-fold dilutions of a lysate (~10^9 PFU/ml) were spotted onto a lawn of cells in LB soft agar and incubated overnight. Phage-resistant mutants were isolated by plating 10^8 to 10^9 PFU together with about 2×10^8 cells on LB agar. Generally, 10 independently grown cultures were used. Four colonies from each plate were purified and tested with OmpA-specific phages (17), the OmpF-specific phage TuIa, the OmpC-specific phage TuIb (8), and, as required, with a crude preparation of colicin L (17).

Phage DNA isolation and analysis. Purification of phages on a CsCl step gradient and subsequent DNA extraction was essentially as described elsewhere (20). Digestion with restriction enzyme *Taq*I and DNA hybridization (23) with [α-^{32}P]dATP-labeled probes were also performed as described previously (20).

RESULTS AND DISCUSSION

The *ompA* mutant P400-1.2, having suffered an amino acid substitution replacing the glycine residue at position 70 by an aspartic acid residue, is resistant to phage Ox2 (17). At a low

TABLE 1. Activity of phages Ox2 and Ox2h5 on *ompA* mutants[a]

ompA allele or plasmid	strain	Mutational alteration	Sensitivity to:	
			Ox2	Ox2h5
Allele				
Wild type	P400		S	S
1	P460	no OmpA	R	R
725	P2899	Gly-65 → Asp	R	S
2009	P400-K3	Gly-65 → Arg	R	S
2008	P400-M1	Glu-68 → Gly	R	R
2005	P400-2.2	Glu-68 → Lys	R	R
2004	P400-1.2	Gly-70 → Asp	R	10^{-1}
2003	P692-4gII	Gly-70 → Val	R	10^{-1} (t)
2001	P692-2eI	—[c]	S	S
2000	P530-1cII	Val-110 → Asp	10^{-1}	S
Plasmid				
pTU115	UH201.3	Gly-70 → Arg	R	R
pTU105	UH201.3	—[b]	R	10^{-3} (t)
pTU202	UH201.3	Gly-154 → Ser	R	R
pTU106	UH100	Promoter[d]	R	R[e]
pTU107	UH100	Promoter[d]	R	R[e]

[a] Sensitivity to phage was determined in comparison with strain UH201.3 carrying pTU201 (harboring the complete *ompA* gene in pBR325 [4]) for the *ompA* alleles 105, 115, and 202 located on a plasmid present in that strain (6); sensitivity was compared with strain W620 (*ompA*$^+$) for the *ompA* alleles 106 and 107 present on a plasmid in strain UH100, an *ompA* derivative of W620 (3). In all other cases the control strain was P400 (22). S, Efficiency of plating of 1; R, complete resistance; t, turbid plaques.

[b] Duplication of 24 base pairs between the codons for amino acid residues 63 and 64 (6).

[c] Deletion of 6 base pairs causing Ala-Asp-105-Thr-Lys → Ala-Lys (17).

[d] These mutations affect the *ompA* promoter (5), resulting in very low levels of ompA protein which still allow growth of the host range mutant K3h1 (14).

[e] No plaques formed but the growth of the tester strain was somewhat inhibited when a high titer lysate was applied.

frequency (10^{-6}), plaques were found on this strain when Ox2 was plated with it. The activity spectrum of one of these phages, designated Ox2h5, is shown in Table 1. It was still dependent on the OmpA protein, but could grow with full or greatly increased efficiency of plating on many mutants which are resistant to Ox2. The extended host range of Ox2h5 does not appear to be of the trigger-happy type (1, 7, 14). Host range mutants, e.g., K3h1, of another OmpA-specific phage, K3, have been described (14) which most likely are of this type because they grow well on *ompA* mutants possessing greatly reduced amounts of protein (14). Ox2h5, unlike K3h1 cannot grow on two such mutants (the *ompA* alleles present on plasmids pTU106 and pTU107; Table 1 [5]).

Plating of Ox2h5 with two strains devoid of OmpA protein (UH201-3 or P460) again resulted in turbid plaques at low frequencies (~10^{-6}), and one of these phages, isolated on UH201-3, was designated Ox2h10. It therefore appeared that Ox2h10 can utilize an outer membrane component other than OmpA as receptor. A search revealed that Ox2h10 was unable to grow on an *ompA ompC* double mutant (P400.6 h1^r). It could grow on an *ompC* mutant (P400.6) or on the *ompA ompC* double mutant when this strain was lysogenized with a λ*ompC*$^+$ transducing phage (Table 2).

To confirm that Ox2h10 could use either OmpA or OmpC as receptor components, mutants resistant to this phage were isolated in strain P400.6 (*ompC*). Several clearly were *ompA*, i.e., they were resistant to all other OmpA-specific phages and also to colicin L (4). Mutants resistant to Ox2h10 were also isolated in strain UH201.3 (*ompA*). A number of mutants were recovered which were resistant to the OmpC-specific phage TuIb (8), but sensitive to the OmpF-specific phage TuIa (8), thus showing that they are of the *ompC* type. In addition, many mutants were recovered from both selections which appeared to be of the LPS core sugar-defective type (*rfa*). Indeed, when Ox2h10-resistant mutants were selected in strain P400 (*ompA*$^+$ *ompC*$^+$), the isolates proved to be either fully or only partially resistant to Ox2h10, and most had aquired full resistance to phage TuIa, and some were also resistant to phages TuIb or P1. These observations are consistent with the fact that, as mentioned earlier, both the OmpC and OmpF proteins inactivate their respective phages only in the presence of LPS (8); it is also known that selection for resistance to various T-even-type phages very frequently results in the isolation of mutants defective in LPS structure (10, 11, 18). In summary, there is little question that selection for resistance to Ox2h10 can yield *ompA* or *ompC* mutants, and mutants with defects in LPS core sugar biosynthesis.

Since Ox2h10 formed turbid plaques on P460 at a reduced efficiency of plating (Table 2) we tried to isolate a derivative of it which could use the OmpC protein better. Clear plaques were readily found when Ox2h10 was plated on P460. These plaques were relatively frequent (10^{-3}), suggesting that some selection for this type of phage had occurred during plaque purification on strain UH201.3. One plaque was isolated and the resulting phage, Ox2h12, showed an increased efficiency of plating on P460 compared with Ox2h10 (Table 2). The ability of Ox2h12 to plate on P400.6 (*ompC*), compared with that of Ox2h10, had decreased (Table 2). This suggests that this phage had a decreased ability to infect via the OmpA protein, whereas its adaption to the OmpC protein had increased, (Ox2h10 and Ox2h12 adsorb to P400.6 and P460, respectively, at similar rates [data not shown]).

The isolation of multiple host range mutants involves the danger of recovering a contaminating phage not derived from the original parent. All OmpA-specific phages studied so far exhibit different electrophoretic patterns of DNA fragments obtained by digestion with the restriction enzyme, *Taq*I (20).

TABLE 2. Activity of Ox2 host range mutants on *omp* mutants[a]

Strain	Protein absent	Sensitivity to:				
		Ox2	Ox2h5	Ox2h10	Ox2h12	TuIb
P400		S	S	S	S (st)	S
P460	OmpA	R	R	10^{-1} (t)	S	S
P400.6	OmpC	S	S	S	10^{-1} (t)	R
P400.6h1^R	OmpA, OmpC	R	R	R	R	R
P400.6h1^R (λ540)	OmpA, OmpC	R	NT	R	R	R
P400.6h1^R (λ540 *ompC*$^+$)	OmpA	R	NT	10^{-1} (t)	S	S
UH201.3	OmpA	R	R	0.3 (st)	NT	S

[a] All strains, except UH201.3 (6), are derivatives of P400 (22). Strain P400.6h1^R is an *ompA* mutant of P400.6 (*ompC* [19]) obtained by selection with phage K3h1 (14). It was lysogenized with λ540 or an *ompC*$^+$ transducing derivative (C. Schnaitman, personal communication). t, Turbid plaques; st, semiturbid plaques, NT, not tested; numbers, efficiency of plating.

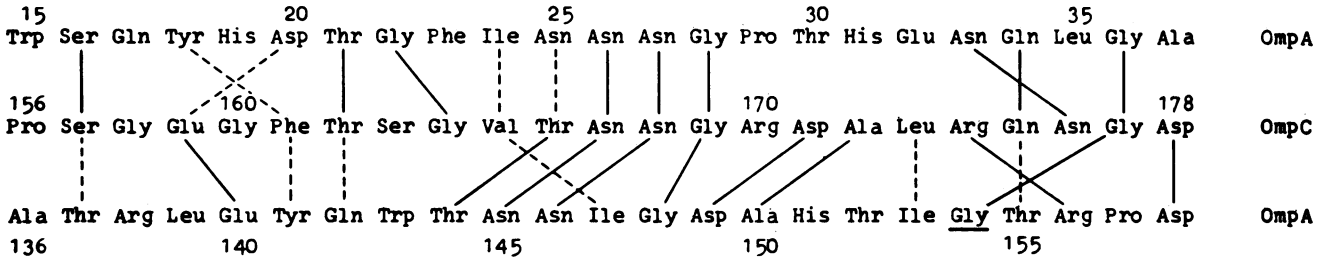

FIG. 1. Amino acid sequence homologies between the OmpA and OmpC proteins. Two regions of the OmpA protein thought to be cell surface exposed and to be required for Ox2 receptor activity (17) are compared with part of region C of the OmpC protein (15). A mutation replacing the glycine residue at position 154 (underlined) of the OmpA protein with a serine residue results in resistance to phage Ox2 (17). Amino acids 156 to 169 of the OmpC protein are deleted in the OmpF protein (15). Solid lines connect identical amino acids; dashed lines connect functionally similar amino acids.

Beyond this property, the best diagnostic tool to identify a phage is DNA hybridization with probes representing the gene of the most distal polypeptide of the long tail fiber. About 50% of this gene of phage Ox2 has been cloned (I. Riede-Kainrath, M.-L. Eschbach, and U. Henning, unpublished data) and was used to probe (23) electrophoretically separated *Taq*I digests of phages Ox2, Ox2h10, and Ox2h12. The resulting radioactive patterns were identical (data not shown).

We cannot be absolutely sure that mutants Ox2h10 and Ox2h12 do use the OmpC protein as a receptor; theoretically, instead, they could use LPS. Since, however, the parent phage Ox2 undoubtedly recognizes the OmpA protein (17, 24), in the latter case one would have to invoke a change in specificity from a protein to probably a carbohydrate—a rather remote possibility. There is however additional, albeit circumstantial, evidence that indeed a switch in specificity from the OmpA to the OmpC protein has occurred. At first sight such a switch appears quite remarkable because the two proteins have very different properties, both functionally and structurally (13). This led us to consider that there may yet be similarities between the two polypeptides discernible at the primary structure level. If so, such a similarity in the OmpA protein may correspond to one of the regions in the OmpA protein that we have identified to be involved in phage Ox2 receptor activity (17), and it should not be present in proteins similar to OmpC, e.g., the OmpF protein (13, 15).

Mizuno et al. (15) have identified six regions which differ substantially in the otherwise closely related outer membrane proteins OmpC, OmpF, and PhoE. One of these regions (region C) is missing in the OmpF protein. The amino acid sequence in this region of OmpC does indeed show considerable homology to the area around residue 154 of the OmpA protein (Fig. 1) where we have located a mutational alteration (Gly-154→Ser) causing resistance to phage Ox2 and a few related phages (17). We noted that this region also exhibits some similarity to an area around residue 25 of the OmpA protein, an area which was also proposed to be needed for phage Ox2 adsorption (17). A similarity of this OmpA area with region C of OmpC is also discernible although it is less extensive (Fig. 1). We speculate that the presence of region C in the OmpC and its absence in the OmpF protein is the cause for Ox2h10 or h12 recognizing the former but not the latter polypeptide.

We envisage the following events leading from Ox2 to Ox2h12. The initial mutation in Ox2 to yield Ox2h5 perhaps relaxed the phage's dependence on a region around residue 70 of the OmpA protein (17). The Ox2h10 phage then arose by a mutation in its receptor binding site(s), allowing it to interact with a site (region C?) on the OmpC protein. Ox2h12 then arose by a mutation increasing the ability of the phage to infect via the OmpC protein. It is likely (and hoped) that at least one of the mutational alterations is located in the tail fiber protein mentioned above; this should enable us to find it by sequence analysis of the cloned gene.

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LITERATURE CITED

1. **Arscott, P. G., and E. B. Goldberg.** 1976. Cooperative action of the T4 tail fibers and baseplate in triggering conformational change and in determining host range. Virology **69**:15–22.
2. **Beher, M. G., and A. P. Pugsley.** 1981. Coliphage which requires either the LamB protein or the OmpC protein for adsorption to *Escherichia coli* K-12. J. Virol. **38**:372–375.
3. **Bremer, E., E. Beck, I. Hindennach, I. Sonntag, and U. Henning.** 1980. Cloned structural gene (*ompA*) for an integral outer membrane protein of *Escherichia coli* K-12. Localization on hybrid plasmid pTU100 and expression of a fragment of the gene. Mol. Gen. Genet. **179**:13–20.
4. **Chai, T., and J. Foulds.** 1974. Demonstration of missing outer membrane protein in *tolG* mutants in *Escherichia coli*. J. Mol. Biol. **85**:465–474.
5. **Cole, S. T., E. Bremer, I. Hindennach, and U. Henning.** 1982. Characterization of the promoters for the *ompA* gene which encodes a major outer membrane protein of *Escherichia coli*. Mol. Gen. Genet. **188**:472–479.
6. **Cole, S. T., U. Chen-Schmeisser, I. Hindennach, and U. Henning.** 1983. Apparent bacteriophage-binding region of an *Escherichia coli* K-12 outer membrane protein. J. Bacteriol. **153**:581–587.
7. **Crawford, J. T., and E. B. Goldberg.** 1977. The effect of baseplate mutations on the requirement for tail-fiber binding for irreversible adsorption of bacteriophage T4. J. Mol. Biol. **111**: 305–313.
8. **Datta, D. B., B. Arden, and U. Henning.** 1977. Major proteins of the *Escherichia coli* outer cell envelope membrane as bacteriophage receptors. J. Bacteriol. **131**:821–829.
9. **Gabay, J., and M. Schwartz.** 1982. Monoclonal antibody as a probe for structure and function of an *Escherichia coli* outer membrane protein. J. Biol. Chem. **257**:6627–6630.
10. **Hancock, R. E. W., and P. Reeves.** 1975. Bacteriophage resistance in *Escherichia coli* K-12: general pattern of resistance. J. Bacteriol. **121**:983–993.
11. **Hancock, R. E. W., and P. Reeves.** 1976. Lipopolysaccharide-deficient, bacteriophage-resistant mutants of *Escherichia coli* K-12. J. Bacteriol. **127**:98–108.
12. **Henning, U., and K. Jann.** 1979. Two-component nature of bacteriophage T4 receptor activity in *Escherichia coli* K-12. J.

Bacteriol. **137**:664–666.
13. **Lugtenberg, B., and L. van Alphen.** 1983. Molecular architecture and functioning of the outer membrane of *Escherichia coli* and other gram-negative bacteria. Biochim. Biophys. Acta **737**:51–115.
14. **Manning, P. A., A. Puspurs, and P. Reeves.** 1976. Outer membrane of *Escherichia coli* K-12: isolation of mutants with altered protein 3A by using host range mutants of bacteriophage K3. J. Bacteriol. **127**:1080–1084.
15. **Mizuno, T., Chou, M.-Y., and M. Inouye.** 1983. A comparative study of the genes for three porins of the *Escherichia coli* outer membrane. J. Biol. Chem. **258**:6932–6940.
16. **Moreno, F., and C. Wandersman.** 1980. OmpC and LamB proteins can serve as substitute receptors for host range mutants of coliphage Tula. J. Bacteriol. **144**:1182–1185.
17. **Morona, R., M. Klose, and U. Henning.** 1984. An *Escherichia coli* K-12 outer membrane protein (OmpA) as a phage receptor: analysis of mutant genes expressing altered proteins. J. Bacteriol. **159**:570–578.
18. **Picken, R. N., and I. R. Beacham.** 1977. Bacteriophage-resistant mutants of *Escherichia coli* K12. Location of receptors within the lipopolysaccharide. J. Gen. Microbiol. **102**:305–318.
19. **Schmitges, U., and U. Henning.** 1976. The major proteins of the *Escherichia coli* outer cell membrane. Heterogeneity of protein I. Eur. J. Biochem. **63**:47–52.
20. **Schwarz, H., I. Riede, I. Sonntag, and U. Henning.** 1983. Degrees of relatedness of T-even type *E. coli* phages using different or the same receptors and topology of serologically cross-reacting sites. EMBO J. **2**:375–380.
21. **Schwartz, M.** 1980. Interaction of phages with their receptor proteins. *In* L. Randell (ed.), Virus receptors, series B, vol. 7. Chapman and Hall, London.
22. **Skurray, A. R., R. E. W. Hancock, and R. Reeves.** 1974. Con$^-$ mutants: class of mutants in *Escherichia coli* K-12 lacking a major cell wall protein and defective in conjugation and adsorption of a bacteriophage. J. Bacteriol. **119**:726–735.
23. **Southern, E. M.** 1975. Detection of specific sequences among fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–518.
24. **Sukupolvi, S.** 1984. Role of lipopolysaccharide in the receptor function for bacteriophage Ox2. FEMS Microbiol. Lett. **21**:83–87.
25. **Wandersman, C., and M. Schwartz.** 1978. Protein Ia and the *lamB* protein can replace each other in the constitution of an active receptor for the same coliphage. Proc. Natl. Acad. Sci. U.S.A. **75**:5636–5639.
26. **Yu, F., and S. Mizushima.** 1982. Roles of lipopolysaccharide and outer membrane protein OmpC of *Escherichia coli* K-12 in the receptor function for bacteriophage T4. J. Bacteriol. **151**:718–722.