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Unusual characteristics of the receptor for the N sex factor-specific filamentous phage IKe

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SUMMARY

Plasmid-mediated sensitivity to filamentous phage IKe is shown to be a property exclusive to plasmids of the N incompatibility group. As with other sex factor-specific phages, IKe sensitivity results from the provision of a plasmid-encoded receptor. However, direct evidence for IKe adsorption to a sex pilus-like structure is so far lacking.

Mutations in an N plasmid were obtained which affected IKe infectability and N transfer frequency simultaneously, though to different extents. IKe receptors could be removed to a limited extent by high speed blending, but only under more extreme conditions (higher speed and in low ionic strength medium) than F pili. As with F-specific filamentous phages, IKe adsorption was partially blocked by Zn^{2+}.

We tentatively suggest that the results accord with the IKe receptor being a sex pilus rather different from F and I pili (possibly in being much shorter in liquid culture), but other interpretations of these data are possible.

1. INTRODUCTION

Sensitivity to the sex factor-specific filamentous phage, IKe (Khatoon, Iyer & Iyer, 1972), is conferred by plasmids belonging to the N incompatibility group (Datta & Hedges, 1971). Sex factor-specific filamentous phages whose receptors are known, adsorb to sex pili. However, although N^+ cells express IKe sensitivity constitutively and at a high level (Dennison, 1973), their donor ability in liquid is very poor. The disparity between the levels of expression of these two functions suggests that the IKe receptor does not correspond to the N conjugational organelle or sex pilus analogue.

Later work (Dennison & Baumberg, in press) has shown, however, that N plasmids determine high level constitutive fertility, but effective mating occurs only on solid medium.

Electron microscopic observations made on broth-grown N^+ cells revealed no structures resembling sex pili and IKe adsorption was apparently direct to the cell wall (Brodt, Leggett & Iyer, 1974). However, because of the similar dimensions of filamentous phages and conventional sex pili, the value of direct observation in this system is limited in the absence of specific label for phage or pilus. Even using specific labels it may not be possible to resolve short or partially retracted pili (Novotny & Fives-Taylor, 1974).

We therefore tried to determine whether the IKe receptor behaved physically and functionally like a conventional sex pilus. Two approaches were used: (*a*) IKe-resistant mutants of N plasmids were sought to determine whether IKe sensitivity is separable by mutation from donor functions; (*b*) the effect on IKe sensitivity was determined for treatments known to affect the functional integrity of F- and I-type pili.

2. MATERIALS AND METHODS

Bacteria, plasmids and phages used are listed in Table 1.

(i) *Media*

Minimal medium was that of Clowes & Hayes (1968). Nutrient broth was Oxoid no. 2. Drugs were kanamycin and nalidixic acid, used at 25 μg/ml and streptomycin used at 15 μg/ml.

(ii) *Mating*

Overnight broth cultures of recipient cells were diluted 100-fold in fresh broth and reincubated at 37 °C, with shaking, for 4 h. Overnight donor broths were diluted 10-fold in fresh broth and reincubated at 37 °C for 2 h. Liquid mating mixtures comprised 2 parts donor culture, 9 parts recipient culture and 9 parts fresh broth.

For matings on solid media, donor and recipient cultures were diluted serially in buffer; 0·1 ml vol. of each were spread together, at dilutions and in ratios equivalent to those used for measuring liquid mating, on plates selective for chromosomal markers of the recipient strain. Transconjugants (recipients acquiring the R factor) were selected by overlay with soft agar containing antibiotic(s) or by replica plating.

(iii) *IKe infective centre assay*

Adsorption mixtures comprised 0·2 ml phage suspension, containing IKe at *circa* 10^{12} pfu/ml and 0·2 ml of a 2 h culture (see Materials and Methods, section (ii)) of the R^+ host. Adsorption was for 15 min. The cells were pelletted by low-speed centrifugation and washed with 10 ml fresh broth. This washing procedure effectively eliminated free phage from the dilutions finally assayed. Dilutions of the post-adsorption cells were made in buffer and 0·1 ml aliquots spread on nutrient agar, in sets of 10. Plates were incubated overnight at 30 °C. Colonies were exposed to ether vapour, ventilated and carefully overlayered with IKe indicator in 0·3% top agar. Plates were incubated at 30 °C overnight. Infected colonies were then recognizable as those with a marginal halo where indicator growth was inhibited.

(iv) *High-speed blending for depiliation studies*

The blender was an M.S.E. 'Ato-mix', chamber capacity 200 ml, speeds 6000 and 12000 rev/min. Overnight broth cultures were diluted 5 ml in 45 ml fresh broth and reincubated at 37° C for 2 h. Bulk cultures were quickly chilled to 0 °C by immersion in an ice/water mixture, to reduce the probability of pilus

Table 1. *Bacteria, plasmids and bacteriophages*

	Strain designation	Relevant characters*	Reference or source
(*a*) Bacteria	*Escherichia coli* K12 J53	*pro*, *met*, *lac*$^+$, F$^-$	Clowes & Hayes (1968)
	Escherichia coli K12 J53-1	*pro*, *met*, *lac*$^+$, F$^-$, Nal^r†	Spontaneous mutant of J53
	Proteus morganii 2815	*nia*, *cys*, *pan*, *lac*	R. W. Hedges
(*b*) Plasmids	R46	ASTSu, *hspII*$^-$, *inc* specificity N	N. Datta
	R269N-1	ASTK, *hspII*$^+$, *inc* specificity N	R. W. Hedges
	F *lac*, *pro*	*proA*,*B*$^+$, *lac*$^+$, *inc* specificity F_I	A. E. Silverstone
(*c*) Bacteriophages	IKe	N sex factor-specific filamentous phage	R. V. Iyer
	M13	F donor-specific filamentous phage	P. Hofschneider

* Drug resistances: A = ampicillin; S = streptomycin; T = tetracycline; C = chloramphenicol. † Nalidixic acid resistant.

regeneration following blending (Novotny & Lavin, 1971). Cultures were sampled, then blended for 1 or 3 min at 6000 or 12000 rev/min, with the surrounding air temperature at 4 °C. Depiliation was assayed using male-specific filamentous phage infection (see Materials and Methods, section (iii)). All steps prior to plating were completed at 4 °C.

Table 2. *Plasmid specificity of IKe sensitivity in* Escherichia coli *K12*

Strain	Plasmid* genes	Plasmid incompatibility specificity	IKe increase
J53-1 (F*lac*)	*lac*+	F_I	–
J53(R1)	ASCKSu	F_{II}	–
712 (ColB-K98)	*col*+	F_{III}	–
J58(R124)	T	F_{IV}	–
J53(R64)	ST	Iα	–
J53(R483)	STp	Iβ	–
J53(R621a)	T	Iγ	–
AB712 (RM413)	A	Iϵ	–
J53 (JR66a)	SK	Iω	–
J53(RA1)	TSu	A	–
J53(R57b)	ACSuGK	C	–
J53(R27)	T	H	–
J53(R391)	K	J	–
J53(R387)	SC	K	–
J53(R471a)	A	L	–
J53(R446b)	ST	M	–
J53(R269N-1)	TASK	N	+
J53(R15)	SSu	N	+
AB712 (RM98)	ASSu	N	+
J53(R46)†	ASTSu	N	+
J53(R390)†	ASTCSu	N	+
J53 (N3-T)†	T	N	+
J53(R^{16})	ASTSu	O	–
J53(RP4)	ATK	P	–
J62-2(R478)	TCK	S	–
CSH-2(Rts-1)	K	T	–
J53-1 (S-a)	SCKSu	W	–
J53(R6K)	AS	X	–
J53(R441)	AK	Undesignated	–
J53(R695)	AK	Undesignated	–
J53 R^- control			–

All strains except AB712 (RM413) were from N. Datta; AB712(RM413) was from R. V. Iyer. Incompatibility specifications are the designations of N. Datta (personal communication).

* Drug resistances: C = chloramphenicol; Tp = trimethoprim; G = gentamycin.

† These strains were tested for IKe spot lysis only.

3. RESULTS

(i) *Basis and specificity of IKe sensitivity*

The basis of IKe sensitivity was shown to reside in the provision of a plasmid-encoded receptor, as with other sex factor-specific filamentous phages. Loss of plaque forming units (pfu) from supernatants of adsorption mixtures was first

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measured using the method of Tzagaloff & Pratt (1964), but no differential adsorption of IKe to N^+ and N^- K12 cells was detected.

Differential adsorption was detected, however, using a strain of *Proteus morganii* as host. We have found that N^+ derivatives of *P. morganii* fail to propagate IKe although N-linked IKe sensitivity is tranferable from the strain. Consequently, it is

Table 3. *Effect of blending on subsequent ability of cells for filamentous phage infection*

Host/phage	Treatment	No. cols tested	No. phage-infected cols	Phage-infected cols (%)	
J53-1(F*lac*)/M13	Unblended control	440	51	11·6	
	Blended 3 min at 6000 rev/min	1199	14	1·2	
J53(R269N-1)/IKe	Unblended control	1002	11	1·1	
	Blended 3 min at 6000 rev/min	1660	10	0·6	
	Unblended control	2553	24	1·0	
	Blended 3 min at 12000 rev/min	2492	23	0·9	
	Low ionic strength unblended control	1252	29	2·3	($\chi^2 = 3{\cdot}6$)
	Low ionic strength, blended 3 min at 12000 rev/min	3680	33	0·9	($\chi^2 = 11{\cdot}6$)

Statistical analysis of the data was by a version of the χ^2 test suitable for comparing two populations, to determine with what probablity they could have arisen from the same population by chance.

possible to measure adsorption following overnight incubation of phage and test strain. Under these conditions, with an input multiplicity of 10^{-7} to 10^{-6}, the reduction in IKe pfu from supernatants was reproducibly 6-fold for R269N-1^+ cells. No adsorption to N^- cells was detected. The reduction is comparable to that found for the F-specific filamentous phage, M13, using the method of Tzagaloff & Pratt (1964) (results not shown).

Conjugative plasmids which are known to produce similar sex pili do not necessarily belong to the same incompatibility group, although members of a given group usually produce similar pili. Thus F-like plasmids fall into 5 *inc* groups (Hedges & Datta, 1972; Datta, unpublished) and I-like plasmids fall into six *inc* groups (Hedges & Datta, 1973). If the IKe receptor is a sex pilus or functionally homologous structure, one might expect to find a similar divergence in *inc* specificity among plasmids determining IKe sensitivity.

Representative R factors from each of the known *inc* groups were tested for ability to permit IKe increase in overnight incubation with the K12 host. Input multiplicity was *c.* 10^{-5} to 10^{-4}. Since IKe sensitivity is known to be closely linked to the N sex factor region (Khatoon *et al.* 1972) all strains were tested for donor ability to ensure that they possessed sex factor activity. All were competent donors.

The only R factors which conferred IKe sensitivity by this test were N plasmids and all N plasmids tested possessed this property (Table 2). Note that the possibility that non-N plasmids may confer IKe sensitivity cannot be excluded entirely. Some F- and I-like plasmids which do not permit increase of the homologous phages nevertheless determine pili whose transfer functions are blocked by F- or I-type pilus antiserum (Harden & Meynell, 1972). It is also possible that the R factors studied here are unrepresentative of their *inc* group with regard to the possession of IKe sensitivity genes. With these reservations, we conclude that IKe sensitivity is N-specific.

(ii) *Characterisation of IKe^r mutants*

In an attempt to determine whether the same gene(s) encoded both IKe sensitivity and N plasmid transfer functions, a number of IKe^r mutants of J53(R269N-1) were isolated and characterized. Mutants were obtained by spotting a high-titre IKe suspension on lawns of indicator in kanamycin-containing top agar. Six mutants were obtained which gave no IKe increase and were not IKe carriers. On testing these for co-loss of other N plasmid functions it was evident that each had sustained extensive deletion(s).

Two possible point mutants, 17 and 21, which arose spontaneously during an experiment to determine the effect of SDS on N$^+$ populations (see Discussion), may provide more information about the nature of the IKe receptor. The mutant phenotypes were cotransferred with other R269N-1-linked genes, showing that the lesions were plasmid-located.

Experiments were carried out to determine whether the lesions simultaneously affected any other known R269N-1-linked genes, namely those encoding ampicillin, tetracycline, streptomycin and kanamycin resistance, *hspII* host specificity, stable replication, transfer and entry exclusion. Of these, only transfer was affected. However, transfer and IKe sensitivity were reduced disproportionately. Reduction in transfer frequency was 10- to 30-fold whereas reduction in phage increase ability was 10^5- to 10^6-fold.

To determine whether the IKe^r phenotype resulted from a changed receptor host range mutants of IKe able to plaque on 17 or 21 were sought but none was obtained.

(iii) *Comparison of the behaviour of IKe receptors and conventional pili*

(*a*) *Effect of high-speed blending on ability of cells to become infected by filamentous phages*

The extent of loss of function with blending at various speeds (the blending spectrum) differs characteristically for different cell appendages (Novotny *et al.* 1969*a*). The blending spectra for F pili and flagella fall in the same range, 1500–3500 rev/min. At higher speeds F pili are fragmented. The loss and reappearance kinetics of the three known functions of F pili, i.e. mating pair formation, ability to adsorb RNA-containing F-specific phages to the pili sides and DNA-containing filamentous phages to the tips are identical (Novotny, Carnahan & Brinton, 1969*b*).

If IKe receptors were filamentous appendages of similar gross structure to F pili, one might expect their blending spectra to fall in the same speed range. We followed the effect of blending J53-1 (F*lac*) and J53(R269N-1) cultures on their subsequent ability to become productively infected by the homologous filamentous phage.

Table 4. *Effect of Zn^{2+} at 10^{-3} g ions/l on M13 and IKe adsorption*

Host/phage	Zn^{2+} pre-treatment	M.o.i.	Proportion phage-infected cols	Phage-infected cols (%)
J53-1 (F*lac*)/M13	.	19	53/209	25·3 (χ^2 = 10·6)
	Phages	19	66/293	22·5
	Cells	26	30/352	8·5 (χ^2 = 9·4)
J53(R269N-1)	.	10	55/235	8·7
	Cells	32	0/70	< 1·4
J53(R269N-1)	.	22	276/1652	16·7 (χ^2 = 70·3)
	Cells	57	186/4671	3·9 (χ^2 = 197·5)
J53(R46)	.	10	146/1308	11·5 (χ^2 = 47·5)
	Cells	14	13/762	1·7 (χ^2 = 28·9)

The frequency of IKe receptor-bearing cells, in mid-exponential cultures assayed at 37 °C (8 %), was similar to that of M13 receptor-bearing cells of J53(R1*drd*19) (14 %). Under the same conditions 58·5 % F*lac*$^+$ cells became M13-infected; such cultures, brought quickly to 0°, contained 11·6 % phage-infectable cells (Table 3). Blending at 6000 rev/min for 3 min resulted in a 90 % reduction in the frequency of M13-infectable F*lac*$^+$ cells (Table 3). These results are comparable to those of Novotny *et al.* (1969*b*). The same treatment had no effect on the frequency of IKe-infectable N$^+$ cells. Blending for 3 min at 12000 rev/min was similarly ineffective. It would seem that the susceptibility to blending of IKe receptors is markedly less than that of F pili. However, it is possible that IKe receptors are sheared off, but subsequently resorb to the cell surface. F pili are capable of resorption and may function as phage receptors in this state (Novotny *et al.* 1969*a*). Resorption might depend upon ionic strength of the medium if, for instance, it results from protein–protein interactions. We therefore repeated the blending treatment of 3 min at 12000 rev/min under conditions where ionic strength was reduced 10^4-fold. A 2·5-fold reduction in proportion of IKe-infectable cells resulted (Table 3). Statistical analysis shows the result to be significant at the 0·5 % probability level.

(*b*) *Effect of Zn^{2+} treatment on IKe infection*

Adsorption to F pilus tips of filamentous phages M13 and f1 is reduced 4-fold in the presence of Zn^{2+} at 10^{-3} g ions/l, although that of RNA phages to pili sides is unaffected (Ou & Anderson, 1972). Plaque-forming ability of f1, however, is unaffected by Zn^{2+} treatment, suggesting that Zn^{2+} and phages compete for pili tips as these become available.

The effect of Zn^{2+} pretreatment of phages or cells was first repeated for the J53-1 (F*lac*)/M13 system. Cells were grown in Lennox broth (Lennox, 1955) and pretreatment with $ZnSO_4$ at 10^{-3} M, or deionized water as control, was for 15 min. As predicted, pretreated phages showed no loss in ability to productively infect J53-1 (F*lac*) cells, but pretreatment of cells resulted in a 2·7-fold reduction in the proportion of infectable cells (Table 4). The result is significant at the 0·5 % probability level.

Pretreatment of N^+ cells, on three different occasions, using two R factors, resulted in a mean reduction of 5·5-fold in the proportion which were IKe-infectable. This result is also significant at the 0·5 % probability level.

Since the specific inhibitory effect of Zn^{2+} arises from its interaction at F pilus tips, these results suggest that IKe adsorbs to analogous structures. In the absence of data on the effect of Zn^{2+} on adsorption of other phages, the interpretation is made with caution.

4. DISCUSSION

The main point emerging from this study is that IKe sensitivity results from N plasmid-directed synthesis of a receptor which, however, lacks some of the properties shared by F- and I-type pili.

There are, however, three independent experimental results which might possibly support the notion that the IKe receptor is a sex pilus analogue, albeit one which differs in gross structure from conventional sex pili; in all these cases other interpretations are possible. Firstly, independently isolated lesions in R269N-1 simultaneously affected IKe sensitivity and N transfer functions. In two mutants only IKe sensitivity and transfer ability were affected, but disproportionately so. That the loss of functions was only partial suggests that these lesions are single site mutations rather than deletions. Possibly the lesions affect a complex structure whose components are differentially used in these two processes. For instance, pilus retraction might be the affected function. Retraction of F pili is probably necessary for F-specific filamentous phage penetration (Jacobsen, 1972) but is not essentia for gene transfer (Ou & Anderson, 1970). A phenotype similar to that of these two mutants was described for mutants of the F8 sex factor (Tomoeda, Shuta & Inuzuka, 1972).

On the other hand, IKe sensitivity and N plasmid transfer might share a common regulatory mechanism, though not a common structure, in which case IKe receptor and conjugational organelle would not coincide. Another possibility is that the alteration in one surface component, e.g. the IKe receptor, may non-specifically influence the function of a second surface component, e.g. the conjugational organelle.

Secondly, IKe receptors were shown to be removable to some extent by blending under conditions of reduced ionic strength. The extent of removal was much less than for M13 receptors (F pili). These data suggest that in a proportion of cells at least, IKe receptors protrude beyond the general cell surface, though probably not to the same extent as do F pili. Reduced ionic strength may affect the extent of protrusion, facility of removal by blending or resorption of removed receptors.

Thirdly, the similar extent of Zn^{2+}-mediated inhibition of IKe and M13 infection suggests a similarity in their receptors. However, few data are available on the effect of Zn^{2+} on other types of receptor. The similar effect here might be fortuitous, although the lack of Zn^{2+} inhibition of MS2 adsorption to F pilus sides (Ou & Anderson, 1972) argues for a specific effect at F pili tips.

An obvious approach to the question of co-identity of IKe receptor and N conjugational organelle is to determine whether IKe and recipient cells compete for attachment to N^+ cells. However, the N system is refractory to study in this way since high-frequency mating occurs only on solid media (Dennison & Baumberg, in press) where blockage of pili by filamentous phage is not detectable (unpublished observations).

Since sex pili are known to sensitize their hosts to the lytic action of sodium dodecyl sulphate (SDS), treatment with the detergent tends to enrich for cells with no sex pili or reduced sex pilus expression (Salisbury, Hedges & Datta, 1972; Adachi *et al.* 1972). If the IKe receptor were borne on a sex pilus, SDS treatment should enrich for IKe^r mutants. However, this was not observed (results not shown). Possibly the structures conferring IKe and SDS sensitivity are not coincident.

It is possible that the inability to demonstrate sex pili of sorts on N^+ cells may result from their incomplete expression in liquid culture. We have shown that N plasmids transfer at high frequency on solid media, but hardly, if at all, in liquid (Dennison & Baumberg, in press). Mating-pair formation seems to be the limiting step. P plasmids (Datta *et al.* 1971) also confer this phenotype (Dennison & Baumberg, *op. cit.*). Sex pili determined by P plasmids have recently been sighted in the electron microscope (To & Brinton, submitted for publication; Bradley, 1974). They are very short (mean length 0·2 μm) compared to F pili (mean length 2·0 μm) and were probably recognized as such only because they were labelled with PRR1, the P-specific RNA phage (Olsen & Shipley, 1973). These pili are optimally expressed on solid medium (To & Brinton, *op cit.*).

If the N conjugational organelle, which seems poorly expressed in liquid culture, is also the IKe receptor, one must hypothesise that it functions as receptor in a state which is not easily removed by blending. Sex pili are capable of acting in this way. F pili caused to retract by NaCN treatment retain the ability to bind filamentous phages to their tips which are themselves invisible in electron micrographs (Novotny & Fives-Taylor, 1974).

Given the limitations of electron microscopy in this system, the question of the identity of the IKe receptor would now seem best resolved by an immunological approach.

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