Host Specificity of DNA in *Haemophilus influenzae*: Similarity between Host-specificity Types of *Haemophilus influenzae* Re and Rf

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SUMMARY

Strain Re of *Haemophilus influenzae* carries two genetically distinct host-specificity systems E1 and E2 each of which is able to restrict *Haemophilus* phage HP1c1 and each of which confers a specific modification upon phage grown in strain Re. These two systems are apparently identical to the host-specificity systems of *H. influenzae* Rf F1 and F2.

INTRODUCTION

Piekarowicz & Glover (1972) have shown that rough derivatives of the *Haemophilus influenzae* serotypes sa, sb, sd, se and sf are each able to restrict and host-modify *Haemophilus* phages HP1 and S2. The specificities of restriction and modification were different for each of the strains Ra, Rb and Rd; however, Re and Rf appeared to be of the same specificity type but different from Ra, Rb and Rd.

Haemophilus influenzae Rf carries two genetically distinct host-specificity systems, one giving a high level of restriction and the other a low level (Piekarowicz, Kauc & Glover, 1974). It was of interest to determine whether *H. influenzae* Re has two host-specificity systems.

METHODS

All *Haemophilus influenzae* strains, bacteriophages and methods are described in the accompanying paper (Piekarowicz *et al.* 1974).

RESULTS

The analysis of restriction and modification in Haemophilus influenzae Re

Genetical analysis of *Haemophilus influenzae* Rf (Piekarowicz *et al.* 1974) has shown that this strain carries two distinct host-specificity systems. The F1 system restricts phage HP1c1.O with an efficiency of plating (e.o.p.) of 10^{-6} while the other system, F2, restricts this phage with e.o.p. of 10^{-3} . Preliminary results (Piekarowicz & Glover, 1972) demonstrated that strain Re like strain Rf has an extremely high level of restriction. Comparison of the e.o.p. of unmodified phages HP1c1 and S2 on *H. influenzae* Re and Rf shows that strain Re restricts these phages slightly less efficiently than strain Rf (Table 1). On the other hand, phage HP1c1.Re can form plaques on strain Rf with an e.o.p. of 1.0; likewise HP1c1.Rf plaques on strain Re with an e.o.p. of 1.0. These results indicate that the host specificity determined by Re and Rf may well be the same.

Table 1. *Efficiency of plating of HP1c1 and S2 phages on strains of Haemophilus influenzae*

Previous host for phage HP1c1	Host strain		
	rd30*	Re	Rf
HP1c1.Rd30	1	1×10^{-8}	1×10^{-9}
HP1c1.Re	2×10^{-1}	1	1
HP1c1.Rf	2×10^{-1}	1	1
S2.Rd30	1	1×10^{-8}	1×10^{-9}
S2.Re	2×10^{-1}	1	1

* Rd is an r⁻m⁻ strain of *Haemophilus influenzae* rd (Glover & Piekawicz, 1972).

Table 2. *The efficiency of plating of HP1c1 phage carrying F1 or F2 types of modification on Haemophilus influenzae Re and Rf strains*

Previous host for phage HP1c1	Host-specificity phenotype*	Host strain	
		Re	Rf
HP1c1.Rf	r _{F1} ⁺ m _{F1} ⁺ r _{F2} ⁺ m _{F2} ⁺	1	1
HP1c1.Rf101	r _{F1} ⁻ m _{F1} ⁻ r _{F2} ⁺ m _{F2} ⁺	1×10^{-4}	1×10^{-6}
HP1c1.Rf102	r _{F1} ⁻ m _{F1} ⁺ r _{F2} ⁺ m _{F2} ⁺	1	1
HP1c1.Rf232	r _{F1} ⁺ m _{F1} ⁺ r _{F2} ⁻ m _{F2} ⁻	1×10^{-1}	1×10^{-3}
HP1c1.Rf217	r _{F1} ⁺ m _{F1} ⁺ r _{F2} ⁻ m _{F2} ⁺	1	1

* Piekawicz, Kauc & Glover (1974).

The restriction and modification properties of Haemophilus influenzae Re as assayed with phage HP1c1 carrying F1 or F2 modification

If the host specificities of strain Re are exactly the same as Rf, it is to be expected that wild-type Re will restrict phage HP1c1 carrying F1 and F2 modification with the same efficiency as strain Rf. However, the results presented in Table 2 show that there are some apparent differences between these two strains. Phage HP1c1 carrying either F1 or F2 modification only is less restricted by strain Re than by Rf wild-type. Nevertheless, it seems clear that *Haemophilus influenzae* Re carries restriction and modification systems similar to those in Rf.

Isolation of restriction-deficient mutants of Haemophilus influenzae Re

To obtain more information about the host-specificity systems possessed by *Haemophilus influenzae* strain Re, restriction-deficient mutants were isolated following NTG mutagenesis (Piekawicz *et al.* 1974). Table 3 shows the properties of restriction-deficient mutants of strain Re. Just like the previously isolated mutants of *H. influenzae* Rf, none of the restriction-deficient mutants of strain Re was completely permissive for phage HP1c1.O. The properties of these mutants indicate that they are phenotypically r[±]m⁺ or r[±]m[±]. The simplest explanation of these results is that strain Re possesses more than one independent system of restriction.

Two restriction and modification systems in Haemophilus influenzae Re

To test that *Haemophilus influenzae* Re, like *Haemophilus influenzae* Rf, possesses two separate restriction and modification systems, phage HP1c1 was grown on each of the

Table 3. *Restriction and modification properties of restriction-deficient mutants of Haemophilus influenzae Re*

Expt no.	Mutant no.	Restriction e.o.p. of HP1c1.O on mutant	Modification e.o.p. of progeny phage HP1c1 mutant on Re
I	Re101	1×10^{-7}	I
	Re146	1×10^{-7}	I
	Re118	1×10^{-7}	1×10^{-1}
	Re153	4×10^{-5}	5×10^{-2}
	Re171	1×10^{-3}	I
	Re152	1×10^{-3}	I
	Re120	1×10^{-3}	3×10^{-6}
II	Re244	1×10^{-6}	5×10^{-2}
	Re340	1×10^{-7}	1×10^{-1}
	Re337	4×10^{-4}	I
	Re364	1×10^{-1}	I
	Re316	1×10^{-7}	I
	Re501	1×10^{-3}	1×10^{-4}
	Re640	2×10^{-6}	I
III	Re608	5×10^{-5}	I
	Re626	2×10^{-5}	I
	Re679	2×10^{-5}	I
	Re662	2×10^{-5}	I
	Re609	1×10^{-3}	I
	Re610	3×10^{-4}	I
	Re615	1×10^{-7}	I

The mutants were isolated after NTG treatment as described previously (Piekarowicz *et al.* 1974).

Expt I: NTG 200 µg/ml, treatment for 30 min at 37 °C, 0.8 % survivors, 7 mutants among 150 colonies tested were isolated.

Expt II: NTG 200 µg/ml, treatment for 30 min at 37 °C, 0.8 % survivors, 6 mutants among 150 colonies tested were isolated.

Expt III: NTG 200 µg/ml, treatment for 30 min at 37 °C, 0.1 % survivors, 9 mutants among 220 colonies tested were isolated.

Table 4. *The efficiencies of plating on restriction-deficient mutants of Haemophilus influenzae Re of phage HP1c1 previously grown on the mutants*

Previous host for phage HP1c1	Host strain									
	Re146	Re153	Re118	Re340	Re120	Re244	Re501	Re610	Re615	Re364*
HP1c1.Re146	I	I	I	I	I	I	I	I	I	I
HP1c1.Re153	I	I	I	I	I	I	I	I	I	I
HP1c1.Re118	I	I	I	I	I	I	I	I	I	I
HP1c1.Re340	I	I	I	I	I	I	I	I	I	I
HP1c1.Re120	1×10^{-4}	1×10^{-3}	1×10^{-3}	1×10^{-4}	I	2×10^{-4}	I	I	1×10^{-3}	I
HP1c1.Re244	I	I	I	I	I	I	I	I	I	I
HP1c1.Re501	1×10^{-4}	3×10^{-4}	2×10^{-4}	4×10^{-5}	I	3×10^{-4}	I	I	5×10^{-4}	I
HP1c1.Re610	I	I	I	I	I	I	I	I	I	I
HP1c1.Re615	I	I	I	I	I	I	I	I	I	I
HP1c1.Re364	I	I	I	I	I	I	I	I	I	I
HP1c1.Re152	I	I	I	I	I	I	I	I	I	I
HP1c1.Re171	I	I	I	I	I	I	I	I	I	I
HP1c1.Re337	I	I	I	I	I	I	I	I	I	I

* Plus two additional mutants.

Table 5. *The efficiency of plating of phage HP1c1 previously grown on strains carrying Rf types of specificity on restriction-deficient mutants of Haemophilus influenzae Re*

Host strain	Previous host for phage HP1c1	
	HP1c1.Rf232 ($r_{F1}^+m_{F1}^+$)	HP1c1.Rf101 ($r_{F2}^+m_{F2}^+$)
Re340	2×10^{-1}	5×10^{-4}
Re244	2×10^{-1}	2×10^{-4}
Re153	1×10^{-1}	2×10^{-4}
Re120	1×10^{-1}	I
Re501	1×10^{-1}	I
Re610	1×10^{-1}	I
Re152	1×10^{-1}	I
Re364	1×10^{-1}	I
Re337	1×10^{-1}	I

Table 6. *The efficiency of plating on restriction-deficient mutants of Haemophilus influenzae Rf of phage HP1c1 previously grown on strains carrying Re type of host specificity*

Previous host for phage HP1c1	Host strain	
	Rf101	Rf232
HP1c1.Re501	I	1×10^{-4}
HP1c1.Re120	I	1×10^{-6}
HP1c1.Re153	I	2×10^{-1}
HP1c1.Re244	I	3×10^{-3}
HP1c1.Re118	I	1×10^{-1}

mutants and its e.o.p. on each of the other mutants was measured (Table 4). The analysis of the restriction and modification properties of these mutants shows that we were unable to isolate two classes of mutants each with a separate type of host specificity. Nevertheless, it seems clear that two mutants, Re120 and Re501, each possess only one complete system of host specificity and the phenotypes of these mutants can be written $r_{E1}^-m_{E1}^-r_{E2}^+m_{E2}^+$ for Re120 and $r_{E1}^-m_{E1}^+r_{E2}^+m_{E2}^+$ for Re501. Three other mutants, Re171, Re152 and Re610, are restriction-deficient in system E1 but since they are able to modify phage HP1c1 their phenotypes would be $r_{E1}^-m_{E1}^+r_{E2}^+m_{E2}^+$. All other mutants expressed phenotypes intermediate between + and - for restriction and some also for modification. None of the mutants isolated has the E1 type of host specificity alone, i.e. the phenotype $r_{E1}^+m_{E1}^+r_{E2}^-m_{E2}^-$. Phage grown on such mutants should be restricted by strains possessing E1 restriction specificity. The results summarized in Table 4 show that none of the mutants was of this type.

Comparison of the restriction and modification properties of restriction-deficient mutants of Haemophilus influenzae Re and Rf

The existence of two independent host-specificity types in *Haemophilus influenzae* Re can be confirmed by testing the e.o.p. of phage HP1c1 carrying F1 or F2 type of modification on restriction-deficient mutants of Re (see Table 5). If the host-specificity types F1 and F2 are identical with E1 and E2 then phages HP1c1.F1 and HP1c1.F2 should not be restricted by strains possessing restriction-specificity types E1 and E2 respectively. On the other hand phage HP1c1.F1 should be restricted by strains carrying E2 restriction specificity and phage

Table 7. Double restriction-deficient mutants isolated from *Haemophilus influenzae* Re501

Previous host for phage HP1c1	The efficiency of plating on <i>Haemophilus influenzae</i> Re and Rf						
	Re501-42	Re501-27	Re501	Re w.t.	Rf232	Rf101	Rf w.t.
HP1c1.Re501-42	1	1×10^{-1}	2×10^{-2}	3×10^{-6}	5×10^{-5}	3×10^{-3}	1×10^{-6}
HP1c1.Re501-27	1	1	1	—	—	—	—
HP1c1.O*	1	5×10^{-2}	1×10^{-3}	1×10^{-8}	1×10^{-6}	1×10^{-3}	1×10^{-9}

* Phage HP1c1 previously grown on strain Rd30 (Glover & Piekarowicz, 1972).

HP1c1.F2 by strains with E1 restriction specificity. It seems clear from these results that the host-specificity types E2 and F2 are identical. This hypothesis is further confirmed by the results presented in Table 6 which show that strain Rf101 $r_{E2}^+ m_{F2}^+$ does not restrict HP1c1 phage grown on Re120 and Re501. All other restriction-deficient mutants of Re are able to restrict phage HP1c1.Rf232, i.e. possessing F1 modification with the same efficiency as wild type of *H. influenzae* Re restricts this phage. These results suggest that all of the Re mutant strains have unchanged E2 host specificity, i.e. r_{E1}^+ , r_{E2}^+ phenotype and not r_{E1}^+ , r_{E2}^- phenotype. This conclusion is further confirmed by the finding that phage HP1c1 grown on Re153, Re244 and Re218 mutants is only restricted by Rf232 and not by Rf101 (Table 6).

One unexpected feature of the restriction-deficient mutants of Re and of the wild-type strain Re is that they restrict unmodified phage HP1c1 with a higher e.o.p. than the same phage grown on particular mutants of strain Rf which have only one host-specificity type. For example, HP1c1.F1.O phage is restricted with the e.o.p. of about 10^{-1} while phage HP1c1.O is restricted with the e.o.p. of about 10^{-3} . This cannot be due to an intermediate type of restriction deficiency in these mutants because we have isolated double mutants completely permissive for phage HP1c1.O.

Double mutants isolated for restriction-deficiency from *Haemophilus influenzae* Re

To confirm the hypothesis that *Haemophilus influenzae* Re possesses two independent host-specificity types, double mutants for restriction deficiency were isolated. Two such mutants were isolated following NTG treatment (Piekarowicz *et al.* 1974) and their restriction and modification properties are summarized in Table 7. The mutant Re501-42 was completely restriction-deficient for phage HP1c1. Phage HP1c1 grown on this mutant was restricted by strain Re501 as well as by the other one-step restriction-deficient mutant of Re and by strains Rf232 and Rf101. This means that this mutant was restriction- and modification-deficient in both of the host-specificity types carried by *H. influenzae* Re. In respect to modification this strain had an intermediate m_{E1}^+ phenotype like its parent strain Re501. This is shown by the fact that phage HP1c1.Re501-42 was less restricted by Re wild-type and Rf232 than unmodified HP1c1.O. The phenotype of this mutant can thus be written $r_{E1}^- m_{E1}^+ r_{E2}^- m_{E2}^-$. The other mutant isolated has intermediate restriction and modification properties and its phenotype is $r_{E1}^- m_{E1}^+ r_{E2}^+ m_{E2}^+$.

DISCUSSION

Analysis of the host-specificity phenotypes of restriction-deficient mutants of *Haemophilus influenzae* Re demonstrates the existence of two independent host-specificity types in this strain. The existence of the host-specificity system designated E2 was confirmed directly by the isolation after mutagenesis of mutants carrying only this one particular system. On the

Table 8. *Comparison of the restriction and modification properties of two host-specificity systems of Haemophilus influenzae Rf and Re*

Phage HP1c1 previously grown on	Host strain					
	Rf F1, F2	Rf F1	Rf F2	Re E1, E2	Re E1	Re E2
HP1c1.O	1×10^{-9}	1×10^{-6}	1×10^{-8}	1×10^{-8}	1×10^{-6}	1×10^{-8}
HP1c1.Rf F1, F2	1	1	1	1	1	1
HP1c1.Rf F1	1×10^{-8}	1	1×10^{-8}	1×10^{-1}	—	1×10^{-1}
HP1c1.Rf F2	1×10^{-6}	1×10^{-6}	1	1×10^{-4}	—	1
HP1c1.Re E1, E2	1	1	1	1	—	1
HP1c1.Re E1	—	—	—	—	—	—
HP1c1.Re E2	1×10^{-6}	1×10^{-6}	1	1×10^{-6}	—	1

other hand the existence of the second host-specificity system can be established indirectly. First of all, after a single mutagenesis we were unable to obtain mutants completely permissive for phage HP1c1.O. However, such mutants were obtained after a second mutagenic treatment (double mutant re501-42). Secondly, we have obtained mutants with the phenotype $r_{E1}^+ m_{E1}^+ r_{E2}^+ m_{E2}^+$; these mutants restrict phage HP1c1 grown on strains carrying specificity E2 alone. Our failure to isolate Re mutants carrying only the E1 system could be due to one or other of the following reasons. First, the screening procedure for isolating restriction-deficient mutants may not have been sensitive enough to detect such mutants. However, this seems rather unlikely because such mutants were obtained for *H. influenzae* Rf host specificity systems using the same procedures (Piekarowicz *et al.* 1974) and restriction-deficient mutants for the E2 system were isolated as double mutants from an $r_{E1}^- m_{E1}^- r_{E2}^+ m_{E2}^+$ parental strain. Secondly, the fact that neither $r_{E2}^- m_{E2}^+$ nor $r_{E2}^- m_{E2}^-$ mutants were obtained in strains which were wild-type for the E1 host-specificity system could be due to a functional relationship between these two systems permitting complementation by exchange of subunits between them.

The efficiency of plating of phage HP1c1.O on *Haemophilus influenzae* Re is reduced to 1×10^{-8} as a result of the activity of these two systems. E2 system reduces the e.o.p. to 1×10^{-3} while E1 system reduces the e.o.p. to 1×10^{-6} . This last value can be measured only indirectly because we have not isolated mutants carrying E1 system alone so we could not measure the e.o.p. of HP1c1.O on such a strain. However, the fact that phage HP1c1.F2 is restricted with an e.o.p. 1×10^{-6} by the wild-type of *H. influenzae* Re (as well as by Rf232 which carries the analogous system F1 alone) allows us to argue that unmodified phage is also restricted by this system with the e.o.p. 1×10^{-6} .

The phenotypes of restriction-deficient (r^-) E1 mutants fall into two roughly equal classes in respect of modification; those which are modification-deficient and those which are modification-proficient. These are the same phenotypes which have been obtained for other host-specificity types in *Haemophilus influenzae* (Glover & Piekarowicz, 1972; Piekarowicz & Glover, 1972; Piekarowicz *et al.* 1974) as well as in other bacteria, e.g. *Escherichia coli* K and B (Wood, 1966; Lederberg, 1966; Glover & Colson, 1969), *Staphylococcus typhimurium* (Colson, Colson & van Pel, 1969). The usual interpretation of these findings is that this type of host specificity is controlled by three cistrons *hss*, *hsr* and *hsm* (Arber & Linn, 1969; Hubacek & Glover, 1970; Boyer, 1971).

An unexpected property of the Re host-specificity system is that Re strains restrict HP1c1 phage carrying each of the F1 and F2 modifications less efficiently than completely unmodified phage. On the other hand, the Rf strains carrying F1 and F2 systems restrict

with equal efficiency unmodified phage and phage with E2 modification (Table 8). These two host-specificity systems in *r_f* and *r_e* strains appear to have the same specificities since the E2 system does not restrict phage grown on a strain with an F2 modification system and vice versa, nor does the F1 system restrict phage grown on a strain with an E1 modification system.

For these reasons it is difficult to explain why phage HP1c1 grown on strain *r_f* $r_{F1}^+m_{F1}^+r_{F2}^-m_{F2}^-$ is less restricted by strain *r_e* $r_{E1}^-m_{E1}^-r_{E2}^+m_{E2}^+$ than phage grown on strains without any modification. The same situation is observed for phage grown on strain *r_f* $r_{F1}^-m_{F1}^-r_{F2}^+m_{F2}^+$ which is less restricted by the wild-type of *r_e* than unmodified phage. In both cases this difference is about 100-fold.

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