IDENTIFICATION OF A HOST SPECIFICITY SYSTEM IN *Shigella*

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Investigations in the last ten years have shown the presence of a system of DNA host specificity in prokaryotes. The writers first showed the presence of such a system previously in bacteria of the genus *Shigella* [2] and in particular in strain *Shigella sonnei* 311.

This paper gives information on the presence of another host specificity system in recently isolated strain *Sh. sonnei* 47843, identified by cross titration of phage DDSh.

EXPERIMENTAL METHOD

Museum and freshly isolated bacterial strains of *Sh. sonnei*, *Sh. stutzeri* 2, and *Escherichia coli* B from the museum of the Tbilisi Institute of Vaccines and Sera, Ministry of Health of the USSR, and bacteriophages DDSh, T3, and R7, generously provided by Dr. D. Krüger [7], were used.

The exact titer of phage was determined on the bacterial test strain by the agar layer method [5]. The phage seeding efficiency on a given strain was estimated as the ratio of the titer on the test strain to the titer on the original culture. Adsorption of phage on the bacterial cell was studied by the standard method of Adams [1]. One-step growth experiments were carried out by the method of Ellis and Delbruck [4]. Preliminary determination of the lytic spectrum of the phages of the test strains and selection of strains for the scl-feature were carried out as described previously [2].

EXPERIMENTAL RESULTS

The results of the experiments for primary selection of r^+-strains for the scl-feature showed that phage DDSh has a very narrow lytic spectrum of action and can replicate only in cells of *Sh. stutzeri* 2, *Sh. sonnei* 47843, and *Sh. sonnei* 1188. It was shown previously that cells of *Sh. stutzeri* 2 and *Sh. sonnei* 1188 do not possess modification and restriction systems [2]. The object of the present investigation was to identify the host specificity system in cells of *Sh. sonnei* 47843 by the method of cross titration of phage DDSh with different phenotypes. As will be clear from Table 1, the efficiency of infection of *Sh. sonnei* 47843 cells with phage DDSh·st2 and phage DDSh·1188 was between four and five orders of magnitude lower in both cases than the efficiency of infection of *Sh. stutzeri* 2 and *Sh. sonnei* 1188 cells, respectively. Meanwhile, cells of *Sh. sonnei* 47843 did not restrict propagation of homologous phage DDSh with the 47843 phenotype. Since the time and magnitude of adsorption of phage DDSh in all the variants studied were practically identical, and amounted to 75-80% for all three phenotypes of phage DDSh it can be concluded that a modification–restriction system is present in cells of *Sh. sonnei* 47843.

The presence of a restriction system in cells of *Sh. sonnei* 47843 also was confirmed by cross titration of phage R7 with the ocr genotype [7], a mutant of phage T3. The results of cross titration and determination of the parameters of adsorption of phage R7 with different phenotypes are shown in Table 2. Data on the seeding efficiency of phage T3 on these strains are given for comparison.

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TABLE 1. Adsorption and Cross Titration of Phage DDSh with Different Phenotypes

Phenotypes of phage DDSh*	Bacterial strain of Shigella								
	Stutzeri 2			Sonnei 47 843			Sonnei 1183		
	seeding efficiency	adsorption		seeding efficiency	adsorption		seeding efficiency	adsorption	
		%	min		%	min		%	min
St2	1,0	95	6	$2,5 \cdot 10^{-5}$	75	8	0,9	86	6
47843	1,25	—	—	1,0	73	8	1,2	—	—
1188	1,1	—	—	$5,0 \cdot 10^{-5}$	79	8	1,0	—	—

*Phenotype of phage means last host in which phage was replicated.

TABLE 2. Cross Titration and Adsorption of Phage R7 with Different Phenotypes

Phage phenotype*	Seeding efficiency on		Adsorption on			
	Sh. sonnei 47 843	E. coli B	Sh. sonnei 47 843		E. coli B	
			%	min	%	min
R7·B	$5 \cdot 10^{-3}$	1,0	72	4	52	4
R7·47843	1,0	$2,0 \cdot 10^{-3}$	64	4	50	4
T3·B	$3,0 \cdot 10^{-3}$	1,0	—	—	—	—
T3·47843	1.0	0,95	—	—	—	—

*Nomenclature of phenotype as in Table 1.

TABLE 3. One-Step Growth of Phage DDSh·st2 on Different Hosts

Bacterial strain	Latent period, min	Time of lysis, min	Yield of phage, number of phage particles per cell
Sh. stutzeri 2	13	19	118
Sh. sonnei 47843	15	20	11

Legend. Mean results of six independent determinations are given.

As Table 2 shows, successful infection of *E. coli* B and *Sh. sonnei* 47843 cells with phage R7 depends on the phenotype of the phage. Since the completeness and time of adsorption of both R7B and R7 47843 phages coincided in the case of infection by both strains (Table 2) there are no grounds for doubt that the two species of cells possess active modification—restriction systems, which differ from one another. Phage T3 is known to synthesize a specific inhibitor of restriction endonucleases in *E. coli* B, *E. coli* K, and certain other cells [6, 7], belonging to class 1 enzymes according to Boyer's classification [3]. Meanwhile, this inhibitor did not act on the class 2 restriction system. Since the inhibitor of phage T3 did not act on the restricting capacity of *Sh. sonnei* 47843 cells, it can be assumed that the restriction endonucleases of this strain of *Shigella* belong to the class 2 enzymes.

Both strains of cells — *Sh. sonnei* 47843 and *Sh. stutzeri* 2 — are Gram-negative bacteria, fermenting glucose, arabinose, sorbitol, and mannitol. Strain *Sh. sonnei* 47843 exists in the R-form. Strain *Sh. stutzeri* 2 is characterized by colonies of the S-form. *Sh. sonnei* 47843 cells possess colicinogenic activity, but *Sh. stutzeri* 2 cells do not have this property. Both strains have low resistance to penicillin, tetracycline, and ampicillin. One-stage growth experiments were carried out for phages on both strains. As Table 3 shows, the latent period and time of lysis were practically identical for both strains, although the yield of phage on the strain possessing a modification—restriction system was almost one order of magnitude less.

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DISSOCIATION AND LOOSENING OF CHROMATIN BY HEPARIN IN A MEDIUM OF PHYSIOLOGICAL IONIC STRENGTH

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Natural polyanions play a very important role in the function of the genetic apparatus in eukaryotic cells. As a rule it is considered that polyanions can induce dissociation of repressor histone molecules from DNA, and can thereby influence gene expression [13]. Some workers, while not ruling out the dissociating effect of polyanions on chromatin, distinguish their modifying effect on the structure of chromosome fibrils [13]. Structural reorganization of chromatin by polyanions is not disputed, although some of its details are not yet clear. However, experimental confirmation of the dissociating ability of polyanions in a medium of physiological ionic strength is not to be found in the literature. We know [1] that dissociation of the histones of chromatin under the influence of tRNA or DNA, taking place in a solution of low ionic strength, is considerably weakened in intensity by an increase in the salt concentration in the medium. The presence of 40 mM NaCl or 1 mM $MgCl_2$ is sufficient to completely suppress dissociation of all histones except H1 by polyanions [1].

The object of this investigation was to study the possibility of dissociation of chromatin proteins under the influence of the natural polyanion heparin in medium with physiological ionic strength (0.15 M NaCl). Under these conditions evidence could be obtained on the loosening effect of heparin on the compact fibrils of chromatin isolated from cells.

EXPERIMENTAL METHOD

Chromatin was isolated from calf thymus. The minced tissue was homogenized in 0.75 M NaCl plus 0.025 M EDTA-Na_2, pH 8.0, and washed five times in the same medium and twice in 0.15 M NaCl plus 0.7 mM Na-phosphate buffer, pH 7.0. Dispersions of chromatin in the last medium with equal DNA concentration (C_{DNA} = 80 μg/ml, $C_{protein}/C_{DNA}$ = 1.1 to 1.3) were mixed with equal volumes of heparin solutions (from Polfa, Poland) in order to obtain mixtures with different heparin/DNA ratios. The heparin solutions were prepared by diluting the mother solution, obtained by dissolving a definite quantity of the dry preparation in water and subsequent adjustment of the ionic strength of the medium to physiological with concentrated salt solution. Mixtures of deoxyribonucleoprotein (DNP) and heparin were centrifuged for 1.5 h or 12 h at 114,000g (Beckman L2-65B centrifuge, USA). Concentrations of DNA [6] and protein [9] were determined in the supernatant. The presence of heparin did not affect the determination of DNA. The contribution of heparin was allowed for during determination of the protein concentration (1 g heparin corresponds to 0.008 g protein), assuming that all the heparin added to the suspension was present in the supernatant. Since the stoichiometry of binding of histones with heparin is characterized by a histone/heparin ratio by weight of 2.5:1 [7], this assumption is not essential for the conclusions drawn above. The composition of histones in the DNP-heparin mixtures was determined by electrophoresis [8, 6]. Densitograms of the gels were obtained on a Gilford spectrophotometer at 600 nm.

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