NOTES

Bacteriophage Particles with Endo-Glycosidase Activity

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Some *Escherichia coli* K bacteriophage particles, capable of interacting specifically with bacterial polysaccharide capsules, carry an endo-glycosidase activity, probably located in the spikes.

The *Escherichia coli* capsule bacteriophages previously described (8, 10) lyse bacterial strains surrounded by a polysaccharide layer of approximately 400 nm in thickness. It appears very unlikely that these viruses, during the normal process of infection, either become fixed to the capsular glycans or eject their nucleic acid on contact with them. This is corroborated by the finding that, at 37°C, isolated *E. coli* capsular polysaccharides adsorb and inactivate only small amounts [about 10^4 plaque-forming units (PFU) per μg of glycan] of homologous K phages (9; S. Stirm, unpublished data). Since other bacterial surface components, e.g., *E. coli* cell wall peptidoglycan (5) and *Salmonella typhi* Vi antigen (11), are known to be enzymatically degraded by phage particles, it appeared possible that the K phages also have some effect on the chemical composition of the host capsules. This possibility was tested as follows.

The encapsulated host strains and the bacteriophages used, as well as the purification of the latter by isopycnic centrifugation, have been described previously. The capsular polysaccharides were obtained from the bacteria by phenol-water extraction and fractionated Cetavlon precipitation followed by ribonuclease treatment (4; L.-B. Nhan et al., Eur. J. Biochem., in press).

The purified glycans (2 mg/ml) were incubated with homologous capsule phage particles (10^{10} to 5×10^{10} PFU/ml, corresponding to 2 to 10 μg of phage protein as determined by the Folin reaction) in phosphate-buffered saline with 0.005 M Mg^{2+} . After 18 hr at 37°C, the mixture was dialyzed against distilled water, lyophilized, and weighed. Appreciable losses of material (i.e., above 10%) were not observed.

The phage-treated polysaccharides [PS (φ)] were then compared with the native materials (PS) by viscosimetry, analytical ultracentrifugation, and sugar component and acetyl analyses. The hexoses were determined by gas-liquid chromatography of the acetylated alditols obtained on hydrolysis, reduction with NaBH_4 , and acetylation (1, 7), hexuronic acid by the carbazole technique of Dische (2), and acetyl by the method of Ludowieg and Dorfman (6). The small contaminations with virus material were neglected throughout. The results are summarized in Table 1 and Fig. 1.

Together with the fact that phage treatment had not caused any appreciable loss of weight, the data of Table 1 and Fig. 1 show that the phage particles had not effected any gross chemical changes of the polysaccharides, except for the depolymerization of PS28 and PS29 by phages 28-1 and 29, respectively. In view of the Vi-deacetylating activity of Vi phage II (11), there remains some doubt, however, whether the differences in acetyl content between PS28 or PS42, on the one hand, and PS28 (φ 28-2) or PS42 (φ), on the other, are of any significance.

If, instead of isolated capsular polysaccharide, washed whole 09K29 bacteria (10^9 to 10^{10} cells/ml) were incubated with purified phage 29 particles in buffered saline under analogous conditions, the bacteria were decapsulated; i.e., they became agglutinable in an *E. coli* 09 serum and released large amounts of depolymerized capsular material into the medium.

The split products of the capsular polysaccharides produced by K phages 28-1 and 29 were further characterized by molecular weight determination and reducing end-group analysis.

TABLE 1. Comparison of native (PS) with phage-treated [PS(ϕ)] *Escherichia coli* capsular polysaccharides

Capsular polysaccharide	(η) ^c (ml/g)	Weight per cent of anhydro sugar					Weight per cent of O-acetyl
		Glucose	Galactose	Mannose	Fucose	Hexuronic acid	
<i>E. coli</i> K14a (09:K28:H ⁻) ^a							
PS28	460	22.3	21.0	4.3	11.6	19.0	2.87
PS28(ϕ 28-1) ^b	5	20.7	19.2	4.3	13.6	19.0	3.04
PS28(ϕ 28-2) ^b	410	22.1	21.2	3.7	12.4	19.5	1.16
<i>E. coli</i> Bi 161/42 (09:K29:H ⁻) ^a							
PS29	510	28.9	6.5	30.2		15.9	
PS29(ϕ) ^b	10	27.8	6.5	28.2		15.1	
<i>E. coli</i> A295b (08:K42:H ⁻) ^a							
PS42	620		21.4		15.9	25.4	2.35
PS42(ϕ) ^b	600		21.5		15.9	25.6	0.31

^a Designation and serotype of the host strain from which the capsular polysaccharide was isolated.

^b In the case of K28 polysaccharide, treatment with capsule phage 28-1 or 28-2, both lysing *E. coli* K14a, is distinguished. In the other cases, only one homologous capsule phage, numbered in accordance with the K antigen, was used. For further details, see references 8 and 10.

^c Limiting viscosity numbers, determined in an Ostwald viscosimeter, with physiological phosphate-buffered saline as a solvent.

Molecular weights were determined by the method of Yphantis (12). Since the native capsular polysaccharides are cross-linked by alkali-labile linkages, generally yielding at least two polymers with different sedimentation coefficients (Fig. 1), the molecular weights (MW) of alkali-treated materials are generally given (4; L.-B. Nhan et al., *Eur. J. Biochem.*, *in press*). In the phage 28 system, single polysaccharide chains having an MW of 259,000 were obtained by treatment of the native capsular glycan with 0.25 N NaOH at 56 C for 8 hr. Phage 28-1, on the other hand, split the native polymer to fragments of about 58,500 daltons. The K29 capsular polysaccharide has been reported (L.-B. Nhan et al., *Eur. J. Biochem.*, *in press*) to consist of chains having 332,000 daltons (about 300 repeating units); phage 29 hydrolyzed this large polymer into fragments with an MW of 18,500 (18 to 19 repeating units).

For determination of newly formed reducing groups, 1 μ mole of NaBT₄ (480 μ Ci) was added to 1 μ mole of PS(ϕ) in water and adjusted to pH 8 to 9. After 2 hr in the dark at room temperature and subsequent addition of NaBH₄ (12 μ moles), the mixture was left overnight. Removal of sodium borate, hydrolysis, deionization, and evaporation yielded a solution which was analyzed for radioactive hexitols by high-voltage paper electrophoresis, by using the 0.2 M arsenite (pH 9.6) buffer of Frahn and Mills (3). Samples were run together with labeled hexitol standards (3 hr at 62 v/cm), and the radioactivity of the

electropherograms was recorded with a Packard radiogram scanner. For comparison, native PS28 and PS29 were labeled, hydrolyzed, and analyzed in the same manner (Fig. 2). It can be seen that both phages mainly exert an endo-glucosidase activity but that K phage 28-1 splits some galactoside and K phage 29 splits some mannoside bonds as well. The latter thus cleaves the K29 polysaccharide preferentially at either or both of the sites indicated by arrows on its structure, which was elucidated by Nhan et al. (*Eur. J. Biochem.*, *in press*).

As described previously (S. Stirn et al., *Virology*, *in press*), K phage 29 induces the production of a spike-formed "enzyme" by its host, which causes the formation of halos around the plaques and which also splits the capsular glycan to form preferentially reducing glucose end groups. The possibility that this enzyme is the cause of the polysaccharide degradation here ascribed to the virus particles themselves is excluded by the facts that enzyme and phages band at 1.08 and 1.51 g/ml, respectively, in isopycnic centrifugation and that as little as 10⁶ to 10⁷

FIG. 1. Typical ultracentrifugation patterns of native (PS) and phage-treated [PS (ϕ)] *Escherichia coli* capsular polysaccharides. (A) PS29, 0.4%, after 57 and 89 min; (B) PS29 (ϕ), 0.5%, after 22 and 54 min (over-layer technique); (C) PS42, 0.4%, after 102 and 134 min; (D) PS42 (ϕ), 0.3% after 80 and 112 min. All runs at 60,000 rev/min in a Spinco model E analytical ultracentrifuge with buffered saline as a solvent.

purified phages are sufficient to cause a measurable depolymerization.

It is thus concluded that some *E. coli* capsule

FIG. 2. Radioactive hexitols obtained from native (PS) and phage-treated [PS (ϕ)] *Escherichia coli* capsular polysaccharides on reduction with $\text{NaBT}_4/\text{NaBH}_4$ and hydrolysis. Separation from neutral sugars and from each other by high-voltage (62 v/cm) paper electrophoresis in a pH 9.6, 0.2 M arsenite buffer (3). (A) Labeled standards; (B) PS28 (ϕ 28-I); (C) PS29 (ϕ); (D) PS28; (E) PS29.

phages carry endo-glycosidases that depolymerize the homologous host polysaccharide capsules. These enzymatic activities are probably located in the virus spikes, since these appear to be their only organs of attachment as reported previously and since spike-formed and equally depolymerizing particles can be isolated from some K phage lysates (S. Stirm et al., *Virology*, *in press*).

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