ENZYMATIC DEACETYLATION OF Vi-POLYSACCHARIDE BY Vi-PHAGE II

Karol Taylor

Biochemical Laboratory of the Institute of Marine Medicine

Gdańsk, Poland

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Serological studies suggest that acetylated polyaminogalacturonic acid /Vi-antigen, Vi-polysaccharide/ is localized on the surface of the bacterial cell. This polysaccharide, isolated from Salmonella typhi or Escherichia coli 5396/38 and adsorbed on the erythrocytes, adsorbs subsequently Vi-phage II in the cold /Koziński and Opara 1954, Taylor and Taylor 1965/. The adsorption of Vi-phage II is accomplished by the tail tip /Taylor and Kwiatkowski 1963/. Hence it may be assumed that Vi-polysaccharide represents a bacterial receptor for Vi-phage II.

Incubation of Vi-polysaccharide with Vi-phage II results in the loss of the receptor activity of the polysaccharide. After incubation, the infectivity of the phage as well as its ability to destroy the receptor activity of the new batch of the polysaccharide, are maintained /Koziński and Opara 1954, Taylor 1965/. Koziński and Opara /1954/ suggested the enzymatic character of the action of Vi-phage II on Vi-polysaccharide, however, they could not find any serological difference between Vi-antigen and the same substance deprived of receptor activity by the phage action. The experiments presented in this paper have shown that Vi-polysaccharide is enzymatically deacetylated by Vi-phage II, N-acetyl as well as O-acetyl groups being split off.

Vi-polysaccharide from E.coli 5396/38, purified by column chromatography on human erythrocyte stroma /Taylor and Taylor 1965/, was dialyzed against 0.01 M EDTA pH 7.0, then against bidistilled water saturated with chloroform, and lyophilized. Vi-phage II was purified by chromatography on ECTEOLA-cellulose and differential centrifugation /Taylor 1965/. For control experiments the phage preparation was inactivated by 18 hr. incubation with 5% formaldehyde at 37° and subsequent 2 min. heating at 100°. Vi-polysaccharide /2 mg/ml/ was incubated with Vi-phage II /$2x10^{12}$/ml/ in 0.01 M $MgSO_4$-0.05 M HCl-veronal buffer pH 7.6, containing 10^{-4}M p-chloromercuribenzoate, at 37°, for 4 hours. The phage was removed by 1 hr. centrifugation at 16000 g. The supernatant was dialyzed for 24 hours against 10 volumes of bidistilled water, the diffusate being kept for further experiments. Dialysis against 0.01 M EDTA pH 7.0 and against bidistilled water saturated with chloroform was followed by lyophilisation, yielding the preparation called "digested" polysaccharide.

The comparison of specific viscosity and the sedimentation analysis /in pH 4.7 and 7.8/ of both /initial and digested/ polysaccharide preparations excluded the depolymerization of the polysaccharide by the phage action. Free microelectrophoresis seemed to show that at pH 4.7 the digested polysaccharide moves a little more slowly than the initial one. At lower pH, in contrast to the initial preparation, the digested one dissolved poorly. The above-described behaviour of the digested polysaccharide could be explained by the decrease of the negative charge of the molecule. This effect could have been caused by the liberation of free amino groups, i.e. by splitting the N-acetyl groups off. The analyses presented in Tab.1 confirmed this supposition; moreover, it appeared that the product of phage action was practically deprived of O-acetyl groups. The loss of the ester bond /O-acetyl group/ was confirmed by the infrared absorption analyses of sodium salts of both preparations /Fig.1/.

	Vi-polysaccharide initial	digested
Receptor activity /RU/mg/	240	<1
Acetyl groups, total /%/	18.0	2.9
O-acetyl groups /%/	8.4	0.8
N /%/	4.9	6.5

Tab.1. Analyses of initial and phage-digested Vi-polysaccharide. Receptor activity /Taylor and Taylor 1963/, total acetyl groups /Pregl and Roth 1958/, O-acetyl groups /Hestrin 1949/, and nitrogen /Dumas/ were estimated.

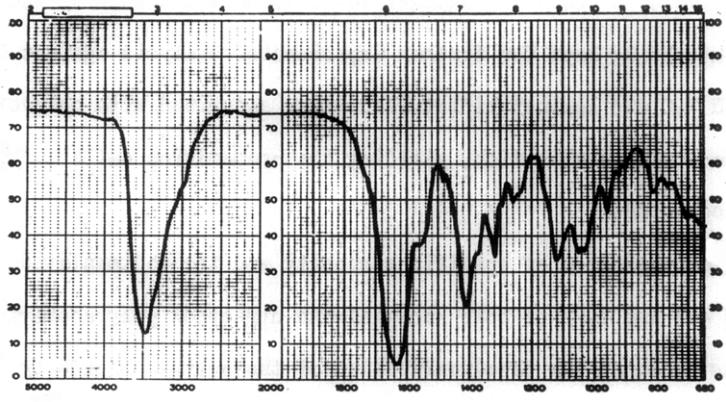

Fig.1. Infrared absorption spectra of initial /above/ and phage-digested /below/ Vi-polysaccharide preparations. Sodium salt of Vi-polysaccharide /0.8 mg/ was pressed with KBr and examined in Perkin-Enfracord SP-200 spectrophotometer.

In the spectrum of the digested preparation the absorption peaks at 1740 cm^{-1} and 1240 cm^{-1} were absent. The nitrogen contents were in agreement with the presented results : the value calculated for sodium salt of deacetylated polyaminogalacturonic acid amounted to 6.76%, and for sodium salt of polyaminogalacturonic acid containing two acetyl groups /one O- and one N-/ per one sugar residue - 4.98%.

The control phage preparation /formolized and heated/ did not cause deacetylation of Vi-polysaccharide.

Acetic acid, being split off by the phage and passing into the diffusate during the preparation of digested polysaccharide, was identified by chromatography as acetohydroxamate /Block, Durrum and Zweig 1955/ in the following solvent systems : 1/ amyl alcohol-formic acid-water /75:25:75, v/v/, 2/ amyl alcohol-acetic acid-water /4:1:5, v/v/, 3/ n-butanol-acetic acid-water /4:1:5, v/v/, 4/ phenol saturated with water. In the last system an additional unidentified spot, moving more slowly, was detected.

The phage-digested Vi-polysaccharide deprived of receptor activity was dissolved in formamide and acetylated by acetic anhydride in pyridine. The acetylated preparation possessed the receptor activity amounting to about 80% activity of the initial, undigested polysaccharide /Tab.2/.

The phage-digested polysaccharide, subsequently acetylated by acetic anhydride underwent another digestion /deacetylation/ by Vi-phage II /Tab.2/.

The enzymatic deacetylation of Vi-polysaccharide by Vi-phage II presents the known phenomena in a new light. The receptor activity of Vi-polysaccharide consists in the ability of the substrate to bind phage en-

	Vi-polysaccharide digested, acetylated	digested, acetylated, digested
Receptor activity /RU/mg/	200	<2
Acetyl groups, total /%/	18.3	5.4
O-acetyl groups /%/	8.3	1.7

Tab.2. Analyses of Vi-polysaccharide. On the left, the phage-digested preparation, acetylated by acetic anhydride, on the right the same preparation redigested by the phage.

zyme. The electron micrographs showing the phage adsorption on erythrocyte membranes coated with Vi-polysaccharide /Taylor and Kwiatkowski 1963/ represent the binding of the substrate with the enzyme, localized on the tip of the phage tail, probably in the spikes found by Kwiatkowski /personal communication/. The preparation of Vi-polysaccharide, deprived of O-acetyl groups and partially depolymerized, obtained by Webster, Landy and Freeman /1952/ did not possess receptor activity /Taylor and Taylor 1965/. It may be that the presence of O-acetyl groups is indispensable for the binding of phage enzyme with Vi-polysaccharide.

It follows from the quantity of Vi-II phages /10^{11}/ bound in the cold by 1 microgram of Vi-polysaccharide adsorbed on erythrocytes /Taylor and Taylor 1963/ that one phage is bound by a polysaccharide quantity corresponding to the mol.wt. $6x10^6$. As the range of the mol.wt. of Vi-polysaccharide is 10^6 /Webster, Sagin, Anderson, Breese, Freeman and Landy 1954/, the phage is bound by at least one polysaccharide particle. The phage-polysaccharide linkage lasts several minutes in the cold /Taylor and Taylor 1963/ and after phage elution the receptor activity of the polysaccharide is completely lost. Hence it seems that the phage, once bound with the polysaccharide particle, is detached only after having split all accessible acetyl groups off. It may be that during deacetylation Vi-phage II is moving along the polysaccharide chain; this phenomenon could have some significance in the process of phage penetration through the bacterial cell wall.

Further details of this study will be published elsewhere.

References

Hestrin S. /1949/. J.Biol.Chem. 180, 249.
Kosiński K.W. and Opara Z. /1954/. Bull.Acad.Polon.Sci.Cl.II 2, 39.
Pregl F. and Roth H. /1958/. "Quantitative organische Mikroanalyse", p.239 /Springer Verlag, Vien/.
Taylor A. and Taylor K. /1965/. Acta Biochim.Polon. 12, 123.
Taylor K. /1965/. Acta Biochim.Polon. 12, 157.
Taylor K. and Kwiatkowski B. /1963/. Acta Microbiol.Polon. 12, 107.
Taylor K. and Taylor A. /1963/. Acta Microbiol. Polon. 12, 97.
Webster M.E., Landy M. and Freeman M.E. /1952/. J.Immunol. 69, 135.
Webster M.E., Sagin J.F., Anderson P.R., Breese S.S., Freeman M.E. and Landy M. /1954/. J.Immunol. 73, 16.