Pectate Lyase and Polygalacturonate Lyase Activity in a Vi Antigen-Degrading Enzyme Preparation*

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ABSTRACT: The Vi antigen-degrading enzyme makes an eliminative attack on native Vi antigen. It releases a series of uronic acid oligomers containing a 4–5 double bond at the nonreducing end of the molecule. These end products show an absorption maximum at 240 mμ and react with quinine sulfate, thiobarbituric acid, and *o*-phenylenediamine to give reaction products characteristic of α,β-unsaturated carboxylic acids. The enzyme was also able to degrade O-deacetylated Vi antigen, pectin, and polygalacturonic acid. The enzymatic attack was eliminative and the end products were analogous to those formed from Vi antigen. Attempts to demonstrate more than a single enzyme species in the preparation were unsuccessful. The Vi antigen was not degraded by a commercial Pectinase having a hydrolytic mechanism of action. However, it was attacked by a polygalacturonate lyase produced by a strain of *Bacillus polymyxa* induced with pectin.

The Vi antigen is a surface polysaccharide formed by some members of the Enterobacteriaceae. It is a viscous, acidic substance containing O- and N-acetylated α-1→4-linked 2-amino-2-deoxy-D-galacturonic acid (Heyns and Kiessling, 1967; Heyns *et al.*, 1959; Baker *et al.*, 1959). Although recent studies have suggested that interchain ester bonds are present (Jarvis *et al.*, 1967), no other structural features are known to exist.

Past structural studies have used severe methods, involving prolonged heating in concentrated mineral acid or base, to isolate and degrade the Vi polymer. It was possible, therefore, that more labile components exist in the native structure. To test this possibility, Baker *et al.* (1959) developed an extremely mild procedure for extracting Vi antigen from cells. Baker and Whiteside (1965) also isolated a soil microorganism, *Bacillus sphaericus*, which makes an inducible enzyme capable of degrading the Vi antigen under mild conditions. The work to be presented in this paper was undertaken to examine the enzymatic degradation of the Vi antigen and to determine whether nonuronic acid substances were present in this material.

Initial spectrophotometric and paper chromatographic analysis of the end products of Vi enzyme degradation suggested the presence of $\Delta^{4,5}$ unsaturated uronic acids. Sugars of this type were first isolated and identified by Linker *et al.* (1956) from the degradation of hyaluronic acid by various bacterial hyaluronidases. These compounds have absorption maxima in the low-ultraviolet region near 235 mμ; condense directly with thiobarbituric acid to give chromogens; condense with *o*-phenylenediamine to give hydroxyquinoxaline derivatives; release oxalic acid upon ozonolysis; and react with such reagents as quinine sulfate, H_2, and HBr (Linker *et al.*, 1956; Fuchs, 1965). Unsaturated sugars are released by various microbial and plant enzymes active against such uronic acid containing polymers as chondroitin (Suzuki, 1960), the chondroitin sulfates (Hoffman *et al.*, 1957), heparin and heparitin sulfate (Hoffman *et al.*, 1957), alginic acid (Preiss and Ashwell, 1962), pectin (Albersheim *et al.*, 1960b), and polygalacturonic acid (Nagel and Vaughn, 1961).

The pectic substances, variably methoxylated polymers of α-1→4-linked D-galacturonic acid, are structurally similar to the Vi antigen. It therefore seemed possible that the pectic substances could serve as substrates for the Vi antigen-degrading enzyme. This paper will present data demonstrating that pectin, polygalacturonic acid, and deacetylated Vi antigen are subject to eliminative cleavage by the Vi antigen-degrading enzyme. In addition, the degradation of Vi antigen by a polygalacturonate lyase will be discussed.

Materials and Methods

Enzyme Substrates. Vi antigen was isolated from *Escherichia intermedium* strain 481 by the method of Baker *et al.* (1959). O-Deacetylated Vi antigen was prepared by treatment with 0.1 N NaOH or 0.1 N acetic acid (Whiteside and Baker, 1960). Polygalacturonic acid was obtained from Nutritional Biochemicals Corp. and citrus pectin from Eastman Chemicals.

Enzymes. The Vi antigen-degrading enzyme was isolated from cultures of *B. sphaericus* ATCC 17932, by the method of Baker and Whiteside (1965). Vi enzyme assays were carried out at 40° with the substrate in a standard buffer containing 0.033 M Tris (pH 8.2) and 1 mM $CaCl_2$. Bovine serum albumin fraction V (1 mg/ml; Nutritional Biochemicals) was present when native Vi antigen was the substrate.

Enzyme activity was followed as an increase in ultraviolet-absorbing material or reducing substance or a decrease in viscosity. Ultraviolet absorption was determined in a Beckman

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TABLE I: Paper Chromatography of Vi Enzyme-Degraded Vi Antigen.[a]

Substance	Spot	R Values		Staining Reactions			
		R_F	$R_{galuron}$	Aniline–Trichloroacetic Acid	Bromocresol Green	Fluor. in Ultraviolet Region	Quinine Sulfate
Galacturonic acid	a	0.67	1.00	+	+	–	–
Glucuronic acid	b	0.71	1.06	+	+	–	–
Vi antigen + albumin	c	0	0	+	+	–	–
Enzyme-treated Vi	d_1	0.66	0.99	+	+	+	+
	d_2	0.76	1.13	+	+	+	+
	d_3	0	0	+	+	–	–

[a] Descending chromatography on Whatman No. 1 paper with ethyl acetate–water–glacial acetic acid (5:3:2.5) as the solvent. Run length: 18 hr at room temperature.

DB spectrophotometer with a cell path length of 1 cm. The reference solution was a reaction mixture to which no enzyme had been added. Reducing material was determined by the Nelson arsenomolybdate method (Ashwell, 1957). D-Galacturonic acid was used for the standard curve and the results are expressed as micromoles of galacturonic acid per milliliter. The blank for these determinations was a non-enzyme-treated buffer–substrate mixture. Both of these assay methods gave high zero-time intercepts, due in part to the high concentration of protein and carbohydrate in the enzyme preparation (3 units = 0.9 mg of Lowry protein and 0.03 mg of anthrone carbohydrate). Relative viscosity was measured in an Ostwald viscometer. A unit of enzyme was that amount required to release 0.05 optical density unit/min from a 1 mg/ml of Vi antigen solution under the standard conditions outlined above.

Polygalacturonate lyase was prepared from a strain of *Bacillus polymyxa* obtained through the courtesy of Dr. R. H. Vaughn, Department of Food Science and Technology, University of California, Davis, Calif. This enzyme was prepared as a crude culture supernatant liquid following the method of Nagel and Vaughn (1961). It was assayed using the conditions described above for the Vi enzyme.

A hydrolytic *Pectinase* was obtained from Nutritional Biochemicals Corp. and assayed as described by Albersheim *et al.* (1960b).

Paper Chromatography. All chromatograms were done on Whatman No. 1 paper without preirrigation, using the solvent systems described in the results. The sheets were air dried and stained by dipping, using bromocresol green to detect acids (McCready and McComb, 1953), aniline–trichloroacetic acid to detect reducing substances (McCready and McComb, 1953), and quinine sulfate to detect α,β-unsaturated carboxylic acids (Block *et al.*, 1955). Galacturonic acid was included on all chromatograms as a reference, and mobilities were reported as R_F's or $R_{galuron}$'s (the distance traveled by the unknown divided by the distance traveled by galacturonic acid), depending upon the length of the run. The exhaustively degraded substances used for chromatography were 1 mg/ml of substrate in buffer treated with four doses of three enzyme units each over a 48-hr period.

Condensations. Thiobarbituric acid condensations were done using the method of Albersheim *et al.* (1960a). The method of Fuchs (1965) was used for *o*-phenylenediamine condensation.

Serology. Rabbit anti *E. intermedium* serum was made using the schedule of Baker *et al.* (1959). Slide immunodiffusion was done by the method of Crowle (1961).

Results

Analysis of Enzymatically Degraded Vi Antigen. Exhaustively degraded Vi antigen was examined by paper chromatography. In a descending system with ethyl acetate–water–glacial acetic acid (5:3:2.5) (McCready and McComb, 1953) intact Vi antigen and albumin did not migrate. Degraded antigen gave two substances, staining as acidic reducing sugar, and some material remaining at the origin (Table I). There was no evidence of a nonuronic acid sugar.

It seemed possible that the material remaining at the origin represented a core structure not attacked by the enzyme, since Baker and Whiteside (1965) observed that the enzyme did not bring about complete hydrolysis. Exhaustively degraded Vi antigen was concentrated on an Amicon filter. The filtrate contained the two substances observed in Table I. The material held back by the filter would not migrate in this solvent system, and it contained no serological activity tested by capillary precipitin or Crowle slide diffusion tests with anti *E. intermedium* serum.

When the chromatograms summarized in Table I were examined under ultraviolet light the two new substances in the enzyme-treated material gave a bright violet fluorescence. This fluorescence was not observed in the standards or the intact control. In view of this observation, the ultraviolet absorption spectrum of the enzymatic degradation products was measured (Figure 1) against an untreated blank. There was a definite absorption maximum at 240 mμ. The course of the reaction could be followed by measuring the rate of release of ultraviolet-absorbing material, and this release was proportional to the release of reducing sugar.

Groups absorbing in the low ultraviolet often contain a C=C group conjugated to an aldehyde, ketone, or carbox-

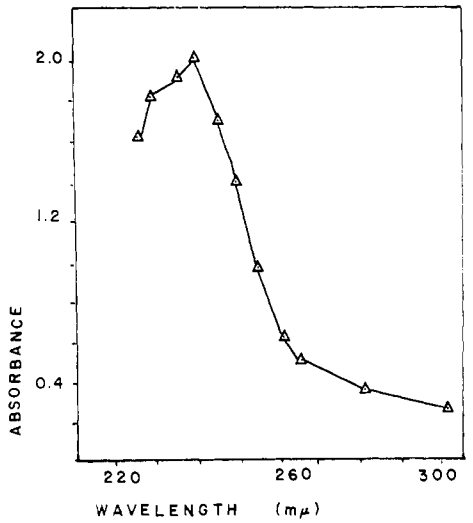

FIGURE 1: Ultraviolet absorption spectrum of enzymatically degraded Vi antigen. Vi antigen (1 mg/ml) with bovine serum albumin (1 mg/ml) in the standard reaction buffer was exhaustively degraded with Vi enzyme. The absorption of this treated mixture was read against a blank containing all reagents but the enzyme.

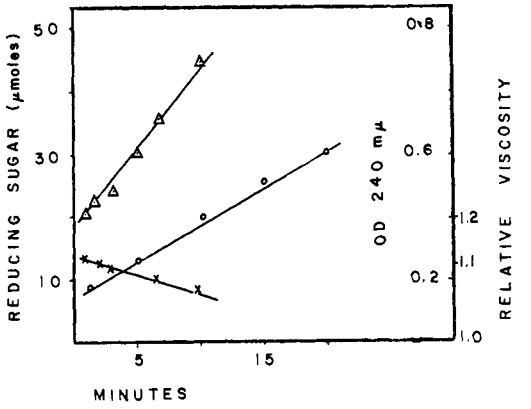

FIGURE 2: O-Deacetylated Vi antigen as a substrate for the Vi antigen-degrading enzyme. A 1-mg/ml solution of O-deacetylated Vi in the standard reaction buffer was treated with 3 units of Vi enzyme. The reaction rate could be followed as an increase in optical density at 240 mμ (△—△), increase in reducing sugar per milliliter (○—○), or decrease in viscosity (×—×). The blanks for these assays contained all reagents but the enzyme.

ylic oxygen. Therefore, the chromatograms of degraded Vi antigen were sprayed with quinine sulfate, a reagent which detects α,β-unsaturated acids (Hasegawa and Nagel, 1962). The enzymatically treated substances do react with this reagent (Table I).

O-Deacetylated Vi As a Substrate for the Vi Antigen-Degrading Enzyme. Since some carbohydrases are quite sensitive to the degree of acetylation of their substrate (Linker and Jones, 1966) it was of interest to see whether the Vi enzyme would degrade O-deacetylated Vi antigen. It was possible to measure a decrease in viscosity, an increase in reducing sugar, and an increase in ultraviolet-absorbing material upon incubation of O-deacetylated Vi with the Vi enzyme (Figure 2). The absorption maximum of the end products was 235 mμ, and the ultraviolet curve of Figure 2 was read at this wavelength. Interestingly enough, albumin was not required for this activity, even though Ca^{2+} was an absolute requirement (L. A. McNicol and E. E. Baker, manuscript in preparation). Albumin is necessary for the degradation of the intact polymer. The end products of enzymatic degradation of the O-deacetylated polymer gave paper chromatograms analogous to those reported for native Vi antigen.

Pectic Substances As Substrates for the Vi Antigen-Degrading Enzyme. Since the pectic substances are quite similar in structure to the O-deacetylated Vi antigen, they were tried as substrates for the Vi-degrading enzyme. An increase in reducing sugar and ultraviolet-absorbing material was seen when pectin and polygalacturonic acid were incubated with the Vi antigen-degrading enzyme (Figure 3). The absorption maximum of the end products was 236 mμ for degraded polygalacturonic acid and 239 mμ for degraded pectin. The ultraviolet curves in Figure 3 were read at 235 mμ.

As was the case with O-deacetylated Vi, albumin was not necessary for the enzymatic degradation of the pectic substances, although Ca^{2+} was. A decrease in the viscosity of enzyme-treated pectin and polygalacturonic acid could also be used to measure enzymatic activity.

Mechanism of Action of the Vi Antigen-Degrading Enzyme. The Vi antigen-degrading enzyme released reaction products with absorption maxima in the low ultraviolet and with an oxygen-conjugated double bond, as evidenced by the positive staining with quinine sulfate. Therefore, it seemed that the enzyme had an eliminative mechanism of action, releasing $\Delta^{4,5}$-galactoseenuronic acids. Sugars of this type condense directly with thiobarbituric acid to form chromogens (Albersheim *et al.*, 1960b). When enzymatically degraded Vi antigen and polygalacturonic acid were treated with the thiobarbituric acid reagent, the condensation products gave an absorption spectrum in the visible range (Figure 4). The intact polymers

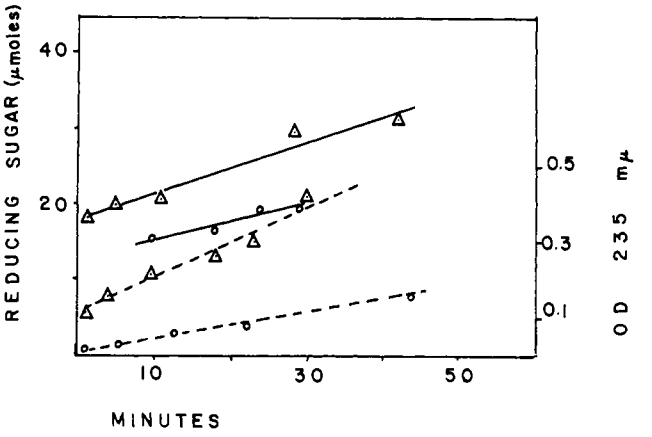

FIGURE 3: Pectic substances as substrates for the Vi antigen-degrading enzyme. Solutions (2.5 mg/ml) of pectin and of polygalacturonic acid in the standard reaction buffer were treated with 2.4 units of Vi enzyme. The reaction rate was followed as increased optical density at 235 mμ ((△—△) polygalacturonic acid and (○—○) pectin) or increased reducing sugar per milliliter ((●---●) polygalacturonic acid and (○---○) pectin). The blanks for these assays were reaction mixtures minus enzyme.

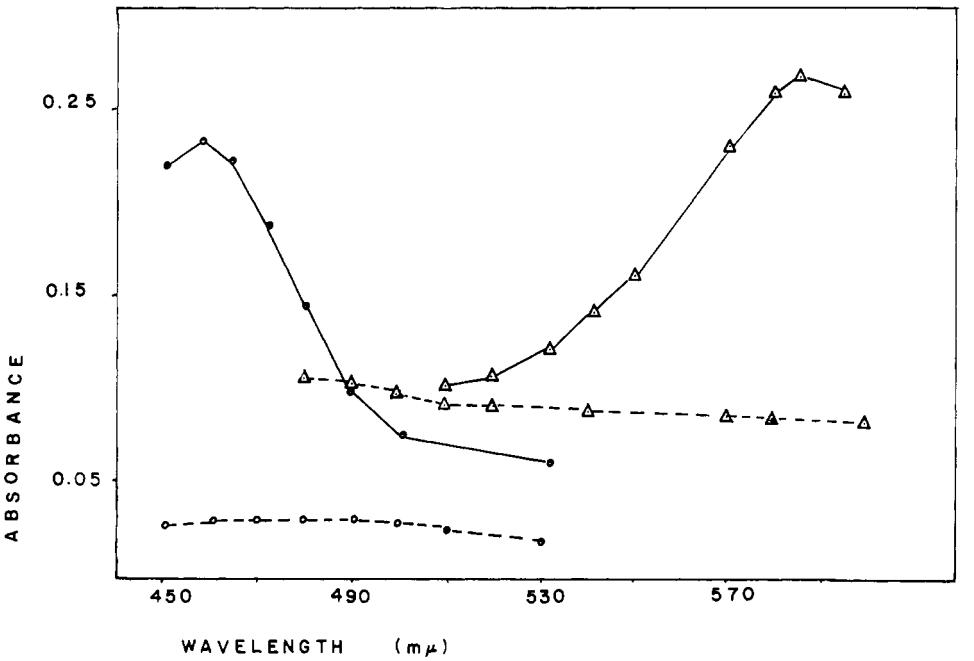

FIGURE 4: Absorption spectra of the thiobarbituric acid condensation products of Vi enzyme-degraded Vi antigen and polygalacturonic acid. Vi antigen (2.5 mg/ml) with bovine serum albumin (1 mg/ml) in the standard reaction buffer and polygalacturonic acid (2.5 mg/ml) in the standard buffer were exhaustively degraded with Vi enzyme. This degraded material (1 ml) was boiled with the thiobarbituric acid reagent for 30 min. After cooling, this mixture was read against a blank containing the thiobarbituric acid reagent. (⊙—⊙) Enzyme-degraded Vi antigen, (⊙---⊙) non-degraded Vi antigen, (△—△) enzyme-degraded polygalacturonic acid, and (△---△) nondegraded polygalacturonic acid.

TABLE II: Time Course Study of the Vi Enzyme Degradation of Polygalacturonic Acid.[a]

Substance	Spot	Av R_F	Av $R_{galuron}$	Aniline–Trichloro-acetic Acid	Quinine Sulfate
Galacturonic acid	a	0.67	1.00	+	−
Polygalacturonic acid, 4-hr degradation	b_1	0.74	1.12	+	+
	b_2	0.85	1.28	+	−
Polygalacturonic acid, 48-hr degradation	c_1	0.74	1.12	+	+
	c_2	0.85	1.28	+	−
	c_3	0.65	0.98	+	+

[a] Descending chromatography on Whatman No. 1 paper with ethyl acetate–water–glacial acetic acid (5:3:2.5) as the solvent. Run length: 18 hr at room temperature. Identical patterns were seen in material which had been degraded for 1, 2, 4, or 8 hr.

give flat curves upon treatment with this reagent. These enzymatic end products gave absorption maxima of 458 mμ for Vi antigen and 587 mμ for polygalacturonic acid. Enzyme-treated pectin gave a maximum at 587 mμ and O-deacetylated Vi antigen 549 mμ upon treatment with the thiobarbituric acid reagent.

Another property of $\Delta^{4,5}$-unsaturated uronides is their ability to condense with *o*-phenylenediamine to give products with the characteristic absorption spectrum of an hydroxyquinoxaline—an absorption maximum at 340 mμ (Fuchs, 1965). The products of enzymatically degraded Vi antigen and pectin gave such a spectrum when condensed with the *o*-phenylenediamine reagent (Figure 5). As a control, the lack of a hydroxyquinoxaline spectrum when undegraded Vi antigen is treated with the reagent is included on Figure 5. Enzymatically degraded polygalacturonic acid and O-deacetylated Vi gave curves with similar maxima.

To determine further the action of the Vi enzyme on pectin and polygalacturonic acid, degraded material was examined by paper chromatography. A 1-mg/ml solution of polygalacturonic acid was treated with 3 enzyme units for 4 hr. Upon chromatography in a descending system with ethyl acetate–water–glacial acetic acid (5:3:2.5), two substances could be seen (Table II). Both stained as reducing sugar but only the faster component contained unsaturation, judged by a positive quinine sulfate staining. When the polygalacturonic

TABLE III: Paper Chromatography of Vi Enzyme-Degraded Vi Antigen and Polygalacturonic Acid.[a]

Substance	Spot	Av $R_{galuron}$	Aniline–Trichloroacetic Acid	Bromocresol Green	Ultraviolet
Polygalacturonic acid	a_1	1.33	+	+	+
	a_2	1.05	+	+	+
	a_3	0.95	+	+	−
	a_4	0.76	+	+	−
Vi antigen	c_1	1.33	+	+	+
	c_2	1.05	+	+	+

[a] Descending chromatography on Whatman No. 1 paper with pyridine–ethyl acetate–water–glacial acetic acid (5:3:3:1) as the solvent. Run length: 18 hr at room temperature.

acid was exhaustively degraded, these same two substances were observed. In addition, however, there was a third unsaturated species with a slower mobility.

The end products of Vi enzyme degradation were further analyzed using a solvent system which has been employed to separate $\Delta^{4,5}$-unsaturated galacturonide oligomers, pyridine–ethyl acetate–water–glacial acetic acid (5:3:3:1) in a descending system (Hasegawa and Nagel, 1962). The exhaustively degraded polygalacturonic acid gave four substances, two of which were saturated (Table III). A similar chromatographic pattern was seen with enzymatically degraded pectin. The published value for the $R_{galuron}$ of $\Delta^{4,5}$-unsaturated galacturonic acid (1.68) does not agree with the mobility found for any of the substances in Table III. Presumably, the unsaturated species have a degree of polymerization that is higher than 1.

In this solvent system, the Vi antigen gave only two spots, both of which were unsaturated. From their mobilities, they were similar to the substances released from polygalacturonic acid.

Vi Antigen As Substrate for Pectic Enzymes. Since the Vi enzyme preparation contained pectate and polygalacturonate lyase activity, it seemed of interest to determine whether known pectinolytic enzymes could degrade Vi antigen. A commercial pectinase with a hydrolytic mechanism of action was ineffectual. However a polygalacturonate lyase induced from *B. polymyxa* by growth on pectin was active, and released reducing sugars and ultraviolet-absorbing material from Vi antigen (Figure 6). For reference, the release of

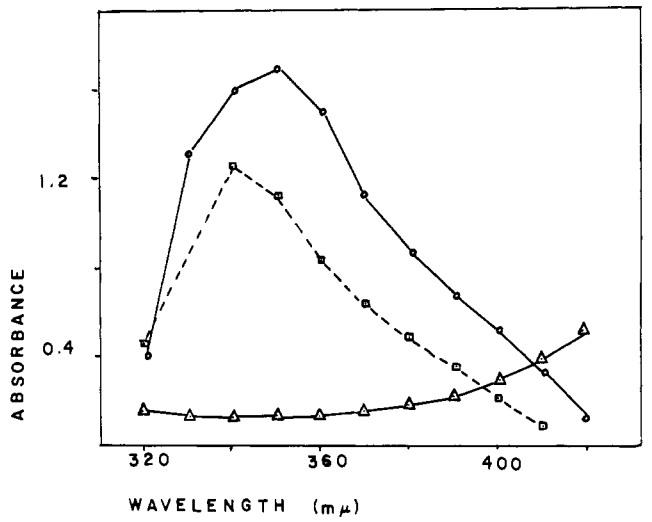

FIGURE 5: Absorption spectra of the *o*-phenylenediamine condensation products of enzymatically degraded Vi antigen and pectin. Vi antigen (2.5 mg) with bovine serum albumin (1 mg/ml) in the standard buffer and pectin (2.5 mg/ml) in standard buffer were exhaustively degraded with Vi enzyme. The *o*-phenylenediamine reagent (1 ml) was mixed with 2 ml of this material and its absorption spectrum was read against *o*-phenylenediamine reagent as the reagent blank. (⊙—⊙) Enzyme-degraded Vi antigen; (⊡—⊡) enzyme-degraded pectin; (△—△) Vi antigen without enzyme.

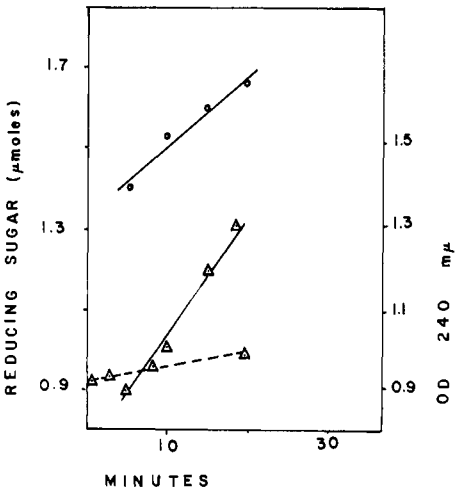

FIGURE 6: Vi antigen as a substrate for *B. polymyxa* polygalacturonic acid lyase. A mixture (10 ml) of Vi antigen (1 mg/ml) with bovine serum albumin (1 mg/ml) in the standard reaction buffer was mixed with crude *B. polymyxa* enzyme (0.5 ml). The reaction could be followed by an increase in reducing sugar (△—△) or increase in optical density at 240 mμ (△- - -△). For reference, the release of ultraviolet absorbing material from a solution of 1 mg/ml of polygalacturonic acid in the standard reaction buffer is included (⊙—⊙).

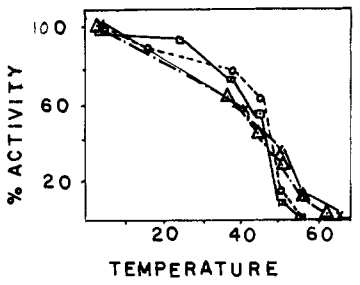

FIGURE 7: Heat stability of pectinolyase and Vi antigen-degrading activities in the Vi enzyme preparation. Aliquots (1 ml) of enzyme were heated at various temperatures for 20 min. After immediate cooling, the enzyme was assayed for residual activity by measuring the rate of release of ultraviolet-absorbing substance. Substrates: Vi antigen (⊙---⊙), O-deacetylated Vi antigen (⊡—⊡), polygalacturonic acid (●—●—●), and pectin (△—△). These substrates were 1-mg/ml solutions in standard reaction mixture buffer. Bovine serum albumin (1 mg/ml) was present in assays involving Vi antigen.

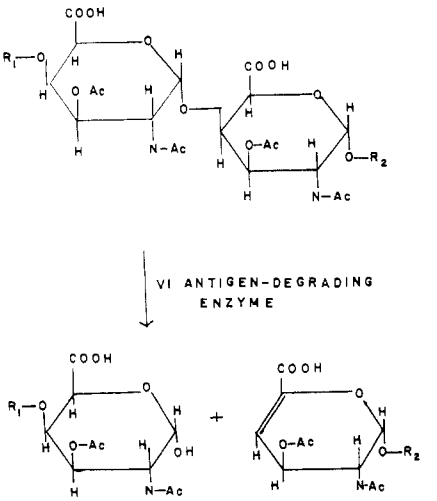

FIGURE 8: Mechanism of action of the Vi antigen-degrading enzyme. The substrate shown here is Vi antigen.

reducing substance from polygalacturonic acid by this enzyme is included in the figure. These reducing sugar values are much lower than those measured in Figures 2 and 3. This was an unpurified enzyme taken from a culture supernatant liquid and it was quite dilute. A decrease in the viscosity of the enzyme-treated Vi antigen could also be measured.

Interestingly enough, the *B. polymyxa* enzyme could degrade Vi antigen only in the presence of albumin. No such requirement was seen when O-deacetylated Vi antigen or the pectic substances were used as substrates for this enzyme. It did require Ca^{2+} for activity, however.

Attempts to Separate Polysaccharidase Activities in the Vi Enzyme Preparation. In view of the fact that the enzyme preparation was able to degrade O-deacetylated Vi antigen and both of the pectic substances in addition to the native Vi antigen, there was a question whether a single enzyme species was attacking all four substances. The Vi enzyme used in this study was not a purified material. The preparation contained three antigenically distinct species, and column chromatography on Sephadex G-200 revealed the presence of two protein peaks. Initial attempts at purification were discontinued because the *B. sphaericus* grew poorly and the amounts of enzyme which could be isolated were insufficient for purification procedures.

Since purification was impractical, we attempted to separate the various enzyme activities on a functional basis, looking for major differences in such properties as pH optimum, temperature optimum, and heat stability which would point to the presence of different proteins. However, these characteristics were identical for each substrate. With all four substances the pH optimum of degradation was 8.1 and the temperature optimum 40°. Figure 7 illustrates the heat stability curves for these substrates. The temperature of 50% inactivation was 44° in all cases.

Discussion

Recent investigations on the bacterial metabolism of uronide polysaccharides have shown that eliminative attack is the usual form of depolymerization (Linker and Hovingh, 1965). In keeping with this generalization we have found that the Vi antigen-degrading enzyme also has an eliminative mechanism of action (Figure 8). Such enzymatic attack releases oligomers bearing a $\Delta^{4,5}$-unsaturated uronic acid on the nonreducing end of the molecule. The presence of such sugars among the products of Vi enzyme degradation has been demonstrated using such criteria as absorption maxima from 235 to 240 mμ, condensation with thiobarbituric acid, formation of hydroxyquinoxalines upon condensation with *o*-phenylenediamine, and positive staining with quinine sulfate.

Although the Vi enzyme preparation was induced with Vi antigen as a carbon source, we have observed that the enzyme preparation is active against O-deacetylated Vi, pectin, and polygalacturonic acid as well as the native Vi antigen. Moreover, a *B. polymyxa* enzyme induced with pectin was also found to have the same substrate range. In view of the structural similarities between these substances it is not unreasonable to expect that a single enzyme could attack all four. We attempted to demonstrate the presence of different enzyme species in the Vi enzyme preparation, but the pH optima, temperature optima, and heat stability of all four activities were identical. It is likely that a single enzyme is present, although absolute proof must await purification.

From the variety of end products observed on the paper chromatograms, the Vi enzyme appears to be an endopolysaccharidase, giving rise to a series of oligomeric end products. In the degradation of the pectic substances, the end products contain saturated oligomers as well as unsaturated species. Since no unsaturated monomer was found, the enzyme may not attack units smaller than a tetramer.

In the case of the Vi antigen the pattern of end products is a bit different, with no saturated species being released. This result is unexpected since the rapid loss of viscosity seen in enzymatically treated Vi antigen, coupled with the concomitantly slow increase in reducing substances and ultraviolet-absorbing material, suggests an endolytic form of attack. It is possible, however, that the solvent systems used do not permit efficient separation of the saturated and unsaturated acetylated oligomers.

In none of the chromatograms of enzymatically degraded

Vi antigen was there evidence of a nonuronic acid component. The conditions used to degrade the Vi antigen were so mild that it is unreasonable to assume that any labile substances could have been destroyed in the process. Therefore, we conclude that the native Vi antigen contains only N- and O-acetylated galacturonic acid residues.

Acknowledgments

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Immunochemical Studies of the Reaction between a Mouse Myeloma Macroglobulin and Dextrans*

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ABSTRACT: The immunoglobulin (IgM) secreted by the mouse plasmacytoma MOPC 104E, reacts with a number of dextrans in the manner characteristic of antibody–antigen reactions, *viz.*, precipitation of soluble dextran, inhibition of precipitation by small oligosaccharides, agglutination of dextran-coated erythrocytes, and fixation of complement. Fixation is more efficient at 37° for 1 hr than at 4° for 18 hr. The order of efficiency for inhibition of dextran–IgM reactions by α-linked glucose oligosaccharides is 1,3 ≫ 1,6 > 1,2 > 1,4. Nigerotetraose and nigeropentaose are the best inhibitors of the nigerodextrin series.

Affinity between IgM and various dextrans is determined by measuring the amount of nigerose required to inhibit precipitation of a constant amount of IgM. This affinity is directly proportional to activity of the dextran in microcomplement fixation.

Myeloma proteins have proved valuable in studies of the primary amino acid sequence, subunit structure, and molecular morphology of immunoglobulin molecules. The myeloma proteins which display antibody activity toward known antigens are of great potential value in studying antibody functions and the nature of the binding site.

The first reported mouse myeloma with antibody activity showed specificity for the C-polysaccharide of the pneumococcus (Cohn, 1967). Shortly thereafter activity of other mouse myeloma proteins was demonstrated for the ligands dinitrophenol and trinitrophenol (Eisen *et al.*, 1968) and 5-AcU, purine, and AMP (Schubert *et al.*, 1968).

We undertook (with Dr. M. Potter) a program of screening murine myeloma sera for reactivity with polysaccharides,

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