Biosynthesis of Pellicle Glycoproteins of *Leptomonas collosoma*

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When washed suspensions of the trypanosomatid flagellate *Leptomonas collosoma* are incubated in salts solution containing [1-¹⁴C]glucosamine, radioactivity is incorporated into trichloroacetic acid-precipitable material. The presence of glucose abolishes this incorporation.

Subsequent fractionation of the cells reveals that 20–26% of the cold-trichloroacetic acid-precipitable radioactivity is soluble in trichloroacetic acid at 80°C. Of the remaining radioactivity 29–47% is retained by Amberlite IRC-50 after hydrolysis. This fraction has been shown to co-chromatograph with glucosamine. Negligible further radioactivity is removed by Amberlite IR-120 (H⁺ form), indicating little or no incorporation into amino acids. The remaining radioactivity is not retained by the resins and its identity is being studied. The cold-trichloroacetic acid-soluble material consists of two major components that co-chromatograph with glucosamine and UPD-*N*-acetylglucosamine.

Cells have been labelled in this way for various times and the radioactivity has been 'chased' into different fractions by subsequent incubation of the cells in non-radioactive medium. Differential centrifugation has been used to divide cell homogenates into a pellet that sediments on centrifugation at 4000g for 10min, a pellet that sediments on centrifugation at 39000g for 30min and a supernatant. Only 48% of the radioactivity is sedimentable after 4h of incubation in labelled medium, but if this is followed by an 8h 'chase' 82% of the radioactivity sediments. With a 'chase' more of the radioactivity sediments in the 4000g pellet than in the 39000g pellet. With no 'chase' the opposite is true.

The sedimentable material has been fractionated on sucrose density gradients into a band that has been shown by electron microscopy to be mainly pellicle with associated microtubules, and a less dense band that is composed of membranes with no associated microtubules, and small vesicles. With a 4h incubation in labelled glucosamine, but no 'chase' with non-radioactive medium, only 36% of the radioactivity is recovered in the pellicle band and the other 64% is in the less dense band. After a 4h 'chase' in non-radioactive medium 74% of the radioactivity is recovered in the pellicle band.

Membranes from these fractions have been analysed by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis. All the radioactivity in the less-dense fraction is associated with one periodate-Schiff-staining band that runs with the tracking dye.

In the pellicle fraction both this low-molecular-weight glycoprotein and a higher-molecular-weight glycoprotein are observed. After a 4h 'chase' in non-radioactive medium a larger proportion of the radioactivity was found in the higher-molecular-weight glycoprotein than with no 'chase'.

Preliminary Investigations on the Production, Purification and Properties of an Extracellular Xylanase from *Bacillus polymyxa*

By W. M. FOGARTY and P. J. GRIFFIN (*Department of Industrial Microbiology, University College, Dublin 4, Irish Republic*)

Studies on the extracellular starch-degrading and protein-degrading enzymes of *Bacillus polymyxa* have been reported previously (Griffin & Fogarty, 1971a,b). In a survey of 17 strains of *B. polymyxa* for the production of enzymes that attack the pentosans of wheat flour it has been recorded (Simpson, 1955) that they all produced pentosanases but that only six possessed this enzyme constitutively. In the present study we have investigated factors relating to the elaboration of an extracellular xylanase (EC 3.2.1.8) by *B. polymyxa* (N.C.I.B. 8158) and we have also examined some of the properties of the enzyme.

The bacterium was grown in a basal salts medium (Griffin & Fogarty, 1971c). Maximum xylanase activity was detected in the stationary phase of growth in the presence of an inducible substrate. Cellobiose or xylan resulted in the production of the highest yields of the enzyme. The proteolytic enzyme in the cell-free culture supernatant was removed by centrifugation after treatment with basic lead acetate. The xylanase activity in the supernatant was precipitated by careful addition of cold acetone (–10°C). The active fraction was dissolved in 20mM-phosphate buffer, pH 6.5, and dialysed for 18h against water. A 90-fold increase in specific activity with an overall yield of 55% was thus achieved.

The xylanase was found to be most stable at its optimum pH, which was established as 6.5. The optimum temperature for activity was 45°C. When exposed to different temperatures at pH 6.5 the enzyme was quite stable up to 25°C, but on increasing the temperature the activity decreased rapidly.

Metal ion-chelating agents EDTA, *o*-phenanthroline, $\alpha\alpha'$ -bipyridyl and the metal ions Ca²⁺, Mn²⁺, Mg²⁺ and Zn²⁺ had no effect on enzyme activity. Fe²⁺ (1mM), Hg²⁺ (0.2mM) and Cu²⁺ (0.2mM) caused decreases in activity of 12, 65 and 75% respectively.

Sodium arsenate, *p*-chloromercuribenzoate and GSSG at concentrations of 0.1mM, and iodine, *N*-bromosuccinimide and iodoacetate at 0.4mM, completely inhibited the enzyme.

Histidine at 1 mM had no effect on enzyme activity but at 10 mM it caused 50% increase in activity. At a concentration of 1 mM histidine reversed the inhibitory effect of iodine and *N*-bromosuccinimide. The effects of a variety of analogues of histidine on enzyme activity were also examined.

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Polygalacturonate Lyase of a *Bacillus* sp. Associated with Increased Permeability of Sitka Spruce (*Picea sitchensis*)

By O. P. WARD and W. M. FOGARTY (*Department of Industrial Microbiology, University College, Dublin 4, Irish Republic*)

Water storage of Sitka spruce causes destruction of the tori and bordered pit membranes of the sapwood as a result of invasion by bacteria. Breakdown of the pits brings about an increase in permeability of the wood to preservatives. Since the pit membranes in sapwood contain considerable pectic material, pectinolytic enzymes may be important in degrading these structures (Ward & Fogarty, 1971a). A *Bacillus* sp. isolated from stored Sitka spruce has been shown to produce high extracellular polygalacturonate lyase activity, even when glucose is used as sole carbon source (Ward & Fogarty, 1971b). We have purified this enzyme and investigated some of its characteristics.

The cell-free supernatant of 48 h cultures grown on a glucose-basal salts medium was fractionated with $(\text{NH}_4)_2\text{SO}_4$. The 60–90%-saturated- $(\text{NH}_4)_2\text{SO}_4$ fraction was applied to a CM-cellulose column and the polygalacturonate lyase was eluted with 35 mM-phosphate buffer, pH 6.5. This preparation was free of amylase, protease and xylanase activities. Further, the enzyme had a 59-fold increase in specific activity over the starting material.

The purified enzyme showed higher activity towards acid-soluble pectic acid (McReady & Seegmiller, 1954) than it did towards sodium polygalacturonate, and the former was used as substrate in subsequent investigations. Addition of Ca^{2+} caused a fivefold increase in activity above the 'basal' value and Sr^{2+} had a slight activating effect. Most bivalent ions not only inhibited the 'basal' activity in the

absence of added Ca^{2+} , but also depressed the activating effect of Ca^{2+} on the enzyme. EDTA caused complete inhibition.

The purified enzyme appeared to be highly stable. At 30°C it retained 100% activity over the pH range 3.4–8.8 for 24 h. It also retained full activity in deionized water at the same temperature for 24 h. The enzyme showed considerable resistance to inactivation by a range of bacterial, animal and plant proteases.

Recent work has shown that polygalacturonate lyase activity is associated with increased permeability of Sitka spruce when stored in water (W. M. Fogarty & O. P. Ward, unpublished work). Permeability increase is a relatively slow process (Dunleavy & Fogarty, 1971) during which the pH falls from 7 to 5. The stable nature of this polygalacturonate lyase must surely be highly advantageous to an enzyme involved in such a prolonged process.

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The Structure of Secreted Proteins

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Fisher (1964), Bigelow (1967) and Gates & Fisher (1971) have developed interesting approaches to the prediction of protein structure from a knowledge of the composition of individual proteins. A very simple model of the structure of globular proteins was adopted, consisting of a hydrophobic core surrounded by a shell of hydrophilic amino acid residues. On the basis of this model Bigelow (1967) showed that globular proteins had components with average hydrophobicities ($H\phi_{av.}$) close to 4.6 kJ (1.1 kcal)/mol of residue, and Fisher (1964) demonstrated that the hydrophobic core is inordinately diminished in small or asymmetric globular proteins.

Attached saccharides and disulphide bridges are structural features peculiar to secreted proteins (cf. Leaback, 1970). In order to assess, if possible, the properties conferred by these components on the structure of proteins, Fisher's (1964) 'polarity ratio' (p), Bigelow's (1967) average hydrophobicity, and a new parameter χ termed the 'cross-link' index (defined