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*)

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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

as the ratio of a protein's molecular weight to the number of covalent cross-links) were calculated for at least 20 highly characterized proteins in each of the following categories: (i) cytoplasmic proteins; (ii) eukaryotic secreted proteins without appended carbohydrate; (iii) animal glycoproteins; (iv) microbial secreted proteins.

In accord with Bigelow's (1967) findings, p was not a very reliable guide to protein structure, but it did indicate that the size of the hydrophilic shell increases in glycoproteins in parallel with the carbohydrate content. Proteins in all these categories showed typical $H\phi_{av}$ values, but markedly lower values were noted in small or asymmetric proteins secreted by animals. These proteins also showed very low χ values, so it appears that globular structure was probably stabilized by disulphide bridges.

Proteins secreted by prokaryotic micro-organisms have average hydrophobicities of about 4.4 kJ (1.05 kcal)/mol of residue, but rarely possess disulphide bridges, in spite of the ubiquitous distribution of the enzyme for disulphide-bridge formation (Williams *et al.*, 1969). A plausible explanation is that there is a requirement for deformability in such proteins to facilitate their passage through cell walls (cf. Pollock & Richmond, 1962). The stability of such proteins could be due to the development of an extraordinary 'tightness' of folded structure (cf. Ottensen *et al.*, 1970) as the result of the high evolutionary pressure to which micro-organisms are usually subject.

Thus disulphide bridges seem to have the function of stabilizing the globular structures of certain kinds of secreted protein, but there is no immediately apparent general function for appended carbohydrate.

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A New Theory Concerning the Biological Significance of Glycoproteins

By D. H. LEABACK (*Department of Biochemistry, Institute of Orthopaedics, Stanmore, Middlesex HA7 4LP, U.K.*)

That appended saccharides and disulphide bridges are characteristic of the proteins secreted by animal cells, and that the saccharides invariably have recognizable sugar sequence types and exhibit 'micro-heterogeneity', are features completely rationalizable in terms of established specificities and locations of ribosomes and enzymes in the pathways for the biosynthesis and secretion of the proteins (Leaback, 1970, 1972). It is difficult, however, to reconcile existing theories on the biological significance of glycoproteins (see Eylar, 1965; Soodak, 1965; Strumyer & Malin, 1970; Pazur *et al.*, 1970) with what is known of these pathways and of the properties of secreted proteins as a whole (Leaback, 1972).

Glycoproteins occur among the secreted products of plants, moulds, yeasts, protozoa and metazoa and in the membranes of animal viruses, but rarely, if at all, in products from bacteria. This phylogenetic distribution and the fact that viral glycoproteins are almost certainly produced by the host's biosynthetic machinery (Burge & Huang, 1970; Bächli *et al.*, 1969) suggest that proteins with attached saccharides are only characteristic of the secreted products of eukaryotes. This is hardly surprising, since prokaryotes lack cytoplasmic systems of membranes of the endoplasmic reticulum type. There are indications that there were probably membrane changes in the evolution of the earliest eukaryotes (Stanier, 1970), and that the cellular apparatus for the synthesis of the polysaccharides and proteins was probably 'internalized' in the process to give rise to the cytoplasmic system of eukaryotic membranes (D. H. Leaback, in preparation). It is therefore proposed that the advent of the endoplasmic reticulum made more probable the transfer of sugars to appropriate receptor sites on proteins (and other substances) in the course of secretion. It is envisaged that the carbohydrate moieties that became attached to some such proteins later assumed various specialized roles (e.g. that in collagen; Spiro, 1970), whereas in other cases no such roles developed. This theory offers an explanation in evolutionary terms for the many proteins (and other substances; see Smith & Williams, 1970) that have appended saccharides with no readily ascribable functions.

The essentials of this communication formed part of a presentation to the Mucopolysaccharide Club in London on 21 December 1971.

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