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Lectin-like Properties Associated with Mucus and Mucilage of Diverse Biological Origin

D.W. Fountain

Botany and Zoology Department, Massey University, Palmerston North, New Zealand

High viscosity slimes, mucins and mucilages are produced by a great diversity of living organisms. I report here that such materials obtained from a variety of sources ranging from a prokaryotic organism to man, exhibit properties akin to those of the class of agglutinating proteins or glycoproteins known as lectins. Lectins bind specific carbohydrates and were initially associated with plant sources but are now known from diverse sources within the plant and animal kingdoms [1–3]. Table 1 shows details of lectin-like properties of several slime materials chosen from organisms well-known for their slime accumulation or mucus production. Each preparation shows the red cell agglutination specificity typical of soluble lectins. Each agglutinated rabbit red cells and those of *Nostoc, Narcissus* and human nasal mucus also agglutinated sheep red cells. None of the materials agglutinated human types A, B or 0 or ox cells. Each positive agglutination response was tested for sugar competition by performing the agglutination assay (with rabbit red cells) in the presence of 25 m*M* saccharide solutions. All showed saccharide inhibition characteristics suggestive of lectin-induced agglutination. The activities were heat-sensitive. These characteristics suggest that the agglutinating activity of the materials is mediated through specific carbohydrate-binding sites similar to those exhibited by soluble lectins. The values in parenthesis in Table 1 are the hemagglutination titer and refer to the least protein concentration [4] capable of producing a positive agglutination pattern. The titers generally fall across the ranges normally associated with purified and partially purified lectins.

The mucilage and mucins examined are of obvious diverse nature. Glycoprotein comprises the major part of saliva protein [5] and mucins from gastric and respiratory tract origin [6]. Plant mucilages [7] and the slime of eel skin [8] are chiefly carbohydrate but little account has been taken of their protein content. The self-aggregating systems of slimes, mucilages and mucins are incompletely understood. Intermolecular disulphide bonding is detectable in mucins from mammalian sources [6, 9] and a variety of other animal origins [9, 10] and also from bacteria [9]. Other as yet unknown non-covalent cross-links however have been postulated for animal mucus substances [6]. Here the glycoprotein components appear to be responsible for their special rheological properties [6]. In plant mucilages, the carbohydrate fraction exhibits gelling properties and is likely to be chiefly responsible for the viscous property [7].

Of the organisms studied here, two soluble lectins are already known. The human 0-specific lectin of eel serum [11] however does not share agglutination specificity with the slime activity reported, while the sugar but not the red cell specificity of *Helix* mucus is similar to the well-known lectins of the albumin gland of *H. pomatia* [12] and *H. aspersa* [13].

On the basis of the detection of a common lectin-like activity associated with these mucins and mucilages from such diverse sources I propose the hypothesis that lectins may form at least one of the crosslinking mechanisms for biological slime and

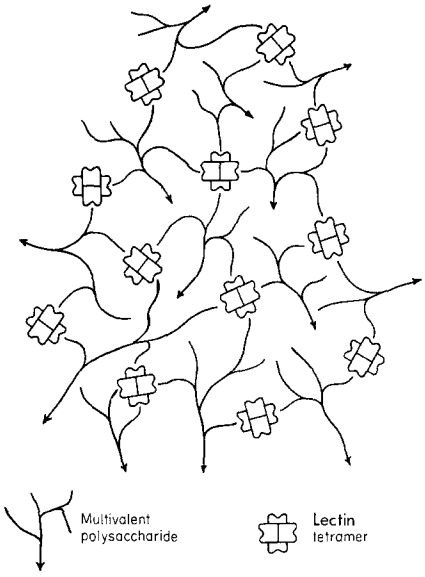

Fig. 1. Hypothetical macromolecular structure of biological mucilages and mucins consistent with evidence of exposed lectin-like activity associated with these materials. Multivalent lectin molecules interact with their specific glycosyl residues of polysaccharide or glycoprotein moieties to form a macromolecular structure. Binding sites not occupied would then be available for binding to the surface glycoproteins of red blood cells were they introduced to the complex. This would allow detection of the "lectin" by agglutination testing. This figure is reproduced with modification and outside its original context from a recent review of lectins [3] by kind permission of the authors and publisher, Academic Press, New York

Table 1. Agglutination by mucins and mucilages. Materials were cell-free and were dispersed into buffered-saline pH 7.2. Agglutination was assayed as described previously [14]. Saccharide specificity was determined by inclusion in the hemagglutination assay of the following sugars at 25 m*M* final concentration: D-glucose, D-galactose, D-mannose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, D-xylose and L-arabinose or lactose. Titer in μg/ml protein

	Material	Hemagglutination		Inhibitor
		Rabbit	Sheep	
Nostoc pruniforme	mucilage	+(<1)	+(≪1)	D-Gal=D-GalNAc
Narcissus sp.	leaf exudate	+(0.75)	+(3)	D-Gal=D-GalNAc
Tradescantia fluminensis	stem exudate	+(29)	–	D-Gal
Helix aspersa (snail)	irritation mucus	+(6.7)	–	D-GalNAc>D-GlcNAc≫D-Gal
Anguilla australis (eel)	skin secretion	+(<1)	–	D-Gal=D-GalNAc
Homo sapiens	salivary mucus	+(5)	–	D-Gal=D-GalNAc
	nasal mucus	+(10)	+(80)	D-Gal=D-GalNAc

mucins. I envisage the crosslinking to be similar in nature to the postulated [3] "chain-end mechanism" of polysaccharide precipitation by the soluble lectin concanavalin A (Fig. 1).

This hypothesis attributes a basic structural role to lectin-like molecules in biological slimes. Much evidence has been advanced for lectin mediation in many recognition and binding phenonema in biology (see [1–3]). Perhaps these represent the evolution of degrees of sophistication from a more primitive and widespread binding phenomenon.

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Electric Field-induced Release of Chloroplasts from Plant Protoplasts

U. Zimmermann, G. Küppers and N. Salhani

Arbeitsgruppe Membranforschung am Institut für Medizin der Kernforschungsanlage Jülich, D-5170 Jülich

Electrical breakdown of the cell membrane is induced in cells exposed to field pulses of high field strength (kV/cm range) and short duration (μs range) [1, 2]. The electrical breakdown is associated with a reversible increase in membrane conductance and permeability.

The phenomenon of electrical breakdown has been used to entrap drugs in living cells (in order to guide drugs to selected sites in the body) [3, 4], to fuse cells [5–8] and to study the mechanism of exocytosis which is still relatively unknown [9]. In this communication we describe release of organelles (chloroplasts) in response to electrical breakdown of the cell membrane without apparent deterioration of the cellular functions or of the membrane integrity.

Field-induced release of chloroplasts was studied on mesophyll cell protoplasts of *Avena sativa* prepared in the usual manner [10]. For field application the same electrode arrangement was used as in the case of electric field-induced fusion of cells [5–8]. Two cylindrical platinum electrodes (diameter 200 μm) were glued onto a microslide at a distance of 300 μm and connected to a pulse generator (Hewlett Packard 214B). Protoplasts suspended in a 0.5 *M* mannitol solution were injected into the electrode gap which could be observed under a microscope. The Ca^{2+} concentration in the mannitol solution was adjusted between 10^{-3} and 10^{-8} mol/l by treatment of the solutions with chelex beads (immobilised IDAA) [11]. Because of their density chelex beads can easily be separated from the protoplasts. For experiments in which the Ca^{2+} concentration of the external solution was kept at an extremely low level, a few chelex beads were also introduced into the electrode gap in order to prevent the external calcium level from changing as a result of irreversible destruction of some cells which occurred occasionally after the field application.

The field strength required for the electrical breakdown of the membranes of the protoplasts of *Avena sativa* is 700 V/cm (corresponding to a breakdown voltage of about 1 V, radius 20 μm) [6]. The pulse length was adjusted to 20 μs. With an external Ca^{2+} concentration of 10^{-3} *M* and field strengths in the range of 3.5 to 4.5 kV/cm, chloroplasts were seen to pass out of some of the protoplasts without these being destroyed. The resealing of the membrane occurred within a few seconds at room temperature [2].

Below a Ca^{2+} concentration of 10^{-5} *M* release of chloroplasts was observed in a great many protoplasts (Fig. 1). The application of several pulses at intervals of a few seconds led to gradual release of up to 7 chloroplasts (Fig. 1). The chloroplasts adhere to the external surface of the outer membrane; however, they can be removed very easily, in particular by the application of shearing forces (i.e. by sucking the cells several times through the tip of a micropipette). The cells remain completely intact in this procedure. Even after several days, no irreversible changes can be detected in the cells under the microscope, if the cells were previously transferred to solutions containing electrolytes in addition to the mannitol [5].

The application of field strengths exceed-

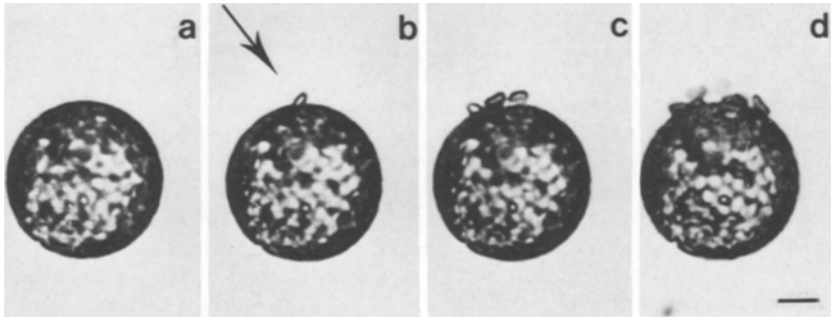

Fig. 1. Field-induced release of chloroplasts from a mesophyll protoplast of *Avena sativa* a) before application of the field, b) after injection of a field pulse of 4 kV/cm strength and 20 μs duration, c) and d) after injection of a second and third pulse, respectively, of the same strength and duration. The time interval between subsequent pulses was about 5 s. Bar = 10 μm

