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COMPOSITION AND PROPERTIES OF *OPUNTIA FICUS-INDICA* MUCILAGE

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Abstract—The mucilage isolated from *Opuntia* is shown to contain arabinose, galactose, galacturonic acid, rhamnose and xylose. It has a uronic acid content of *ca* 10% and a MW of 4.3×10^6. It equilibrates to 175% of its dry wt at 100% relative humidity. Its possible role in the physiology of the plant is discussed.

INTRODUCTION

Mucilage cells and mucilage production are characteristic of the Cactaceae family [1, 2]. The chemical composition of *Opuntia* mucilage has been the subject of repeated studies. Most of the work was carried out on the mucilage from *O. fulgida* (cholla gum). A good deal of contradiction is found in the published data. Some reports describe the mucilage as neutral and consisting mainly of D-galactose [3, 4], while others claim that it consists of D-galactose and L-arabinose [5, 6]. Later work demonstrated that the mucilage is acidic and contains arabinose, galactose, rhamnose and galacturonic acid [7, 8]. Xylose was also said to be present [9]. Studies on the oligosaccharides obtained by enzymatic digestion were done by Andrews *et al.* [10]. A detailed structural and chemical analysis showed that the mucilage is built of a backbone of β (1 → 3)-linked galactose units with branchings on C_6, the branches being of D-galacturonic acid, D-galactose, D-xylose and L-rhamnose in the pyranose form and L-arabinofuranose units [11–13].

The mucilages of other *Opuntia* species have also been studied. In the case of *O. megacantha* it was found [14] that the backbone of the molecule is (1 → 3)-, (1 → 4)- and (1 → 6)-linked galactopyranose units, the branches consisting of arabinose, galactose, rhamnose and glucuronic acid. In contrast, *O. dillenii* mucilage was found to be an arabinogalactan, the backbone being (1 → 4)-linked galactose units with side-chains of (1 → 4, 5)-linked arabinose [15]. The mucilages of *O. monacantha* and *O. nopalea coccinillifera* were rather similar to that from *O. fulgida*, being acidic polymers composed of arabinose, galactose, rhamnose, xylose and galacturonic acid [16], and the same basic compounds were also found in the mucilage of *O. aurantiaca* and *O. brasiliensis* [17]. In a comparative study of the composition of different mucilages from members of the Cactaceae other than *Opuntia* [17–19] the common components found were again L-arabinose, D-galactose, L-rhamnose and D-galacturonic acid [16–18]. The mucilages of other higher plants seem to have a rather different composition [2, 19]. Two studies of the mucilage of *O. ficus-indica* Mill. show appreciable differences. Amin *et al.* [20] found that the mucilage was a neutral polysaccharide of *ca* 55 sugar residues containing no uronic acids and composed of arabinose, galactose, rhamnose and xylose. In contrast, the water-extractable polysaccharides of *O. ficus-indica* cv. Burbank's spineless contained both neutral fractions consisting of glucans and glycoproteins and acidic fractions containing arabinose, galactose, rhamnose, xylose and galacturonic acid [21].

Some of the investigations previously published [3–6, 16, 21] took insufficient precautions in isolating the mucilage fraction, before analysis. Probably some of the apparent contradictions are due to contamination of the mucilage with other cell compounds, e.g. those originating in the cell walls. In addition there may be varietal differences in the chemical composition of mucilages.

The aim of the present study was to isolate, purify and characterize a mucilage preparation suitable for biophysical studies of its water-absorbing capacity, and other biophysical properties.

RESULTS AND DISCUSSION

Histochemical studies at the light and electron microscope level [22] showed that mucilage is located only in mucilage cells which in their final state do not contain proteins, but a large amount of acidic polysaccharides. From electron probe analysis of sections from freeze substituted tissue blocks it appears that the mucilage is present as its Ca and Mg salt [33].

Since both ethanolic and aqueous extracts are frequently contaminated with cytoplasmic and wall proteins, the original extract was treated with TCA. This precipitated contaminating proteins and gave a purer mucilage fraction. Indeed, elemental analysis showed C, 42.57%; H, 6.31%; O, 51.2%; Ca, 0.022% and Mg, 0.013%; and no N or S, indicating the absence of proteins and amino acid. From the elemental analysis a molecular formula $(CH_2O)_n$ together with Ca and Mg was deduced. The Ca and Mg content was low, perhaps due to decomposition of the salt during TCA treatment.

Determination of sedimentation and diffusion coefficients by means of analytical centrifugation showed a single sharp, narrow peak indicating a pure preparation.

The MW of the mucilage was determined by ultracentrifugation. The mucilage moved as a single,

sharp peak in the ultracentrifuge. The physical characteristics found were $S_{20°,w}$ 21.3 S and $D_{20°,w}$ 0.31 10^{-7} cm^2/sec. The MW was calculated as 4.3×10^6 assuming a partial specific volume of 0.6. The MW did not change in the presence of Na^+ or Mg^{2+} nor did the nature of the buffer affect it. This indicates that no intermolecular bonding occurs in the presence of Mg^{2+}.

TLC of the acid hydrolysate of the mucilage revealed only four neutral sugars: arabinose, galactose, rhamnose and xylose. Uronic acids were not detected, probably due to decarboxylation of the uronic acid during hydrolysis. The neutral sugars as determined by GC were found in the following ratio: arabinose, 24.6%; galactose, 40.1%; rhamnose, 13.1%; and xylose, 22.2%. The uronic acid content of the purified mucilage was determined by three methods; the carbazole method gave a uronic acid content of 19.5%, the *m*-hydroxydiphenyl method 12.7% and direct titration 46 nmol carboxyl/mg sugar or 10.7%. The purified mucilage had a p*K* of 4.8 as determined by titration. The carbazole method is known to overestimate uronic acid since the reagent also gives colour with hexoses and pentoses.

Plants were fed with [^{3}H]myo-inositol, which is known to be a specific precursor for pectic substances, and the mucilage was purified, hydrolysed and analysed by TLC as described. The spots from the TLC plates were removed, and counted in a liquid scintillation counter. The results showed that 67.3% of the radioactivity was in arabinose, 6.3% in galactose, 5.4% in rhamnose and 20.4% in xylose. This indicates that much of the uronic acid was present as galacturonic acid, since this yields arabinose on decarboxylation.

Since it has been claimed that polysaccharides contribute to the drought resistance of cacti [23, 24] we determined the water-holding capacity of the purified mucilage, both as the free acid and as its Ca salt. The results are shown in Fig. 1. It can be seen that the increase in water content at 100% RH is 75.5%, which is fairly low. No difference was observed between the behaviour of the free acid mucilage and its Ca salt. The hydration curve

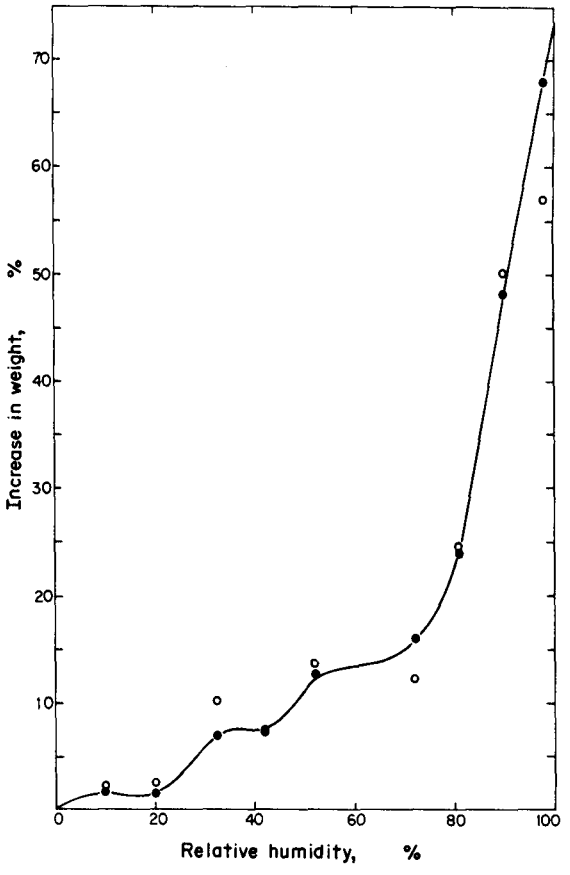

Fig. 1. Increase in weight of mucilage equilibrated at different relative humidities. Results as % increase. ●——●, Acid form; ○——○, Ca salt.

may show three phases. The mucilage lost water without any indication of transitions (Fig. 2). Again the acid and Ca salt behaved in the same manner. The water-holding

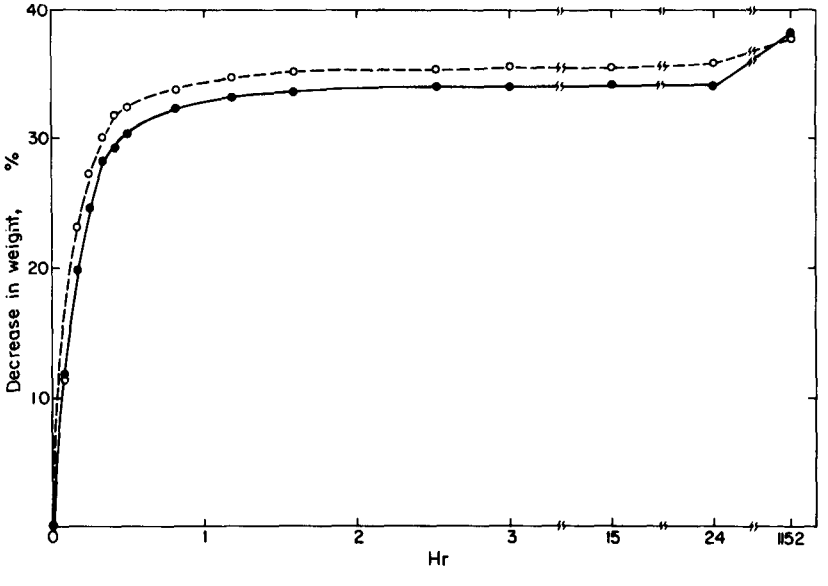

Fig. 2. Rate of drying of mucilage equilibrated at 98% relative humidity and placed over P_2O_5. Results as % decrease. ●——●, Acid form; ○——○, Ca salt.

Table 1. Elemental analysis (%) of purified *Opuntia* mucilage (for purification see Experimental)

Ca	Mg	Na	C	H	O	N	S
0.022	0.013	—	42.57	6.31	51.12	—	—

Table 2. Distribution of radioactivity between neutral sugars in hydrolysed mucilage after administration of [^{3}H]myo-inositol to intact tissue

Sugar	%
Arabinose	67.25
Galactose	6.27
Rhamnose	5.43
Xylose	20.41

capacity is not such as to explain drought resistance, but the mucilage might contribute towards frost hardiness and wound healing as has been suggested in general for arabinogalactans [25]. However, the water-holding capacity may be modified by the pH in the cell and the presence of sugars and different cation concentrations. The properties of arabinogalactans have been ascribed to the ratio of rhamnose to uronic acid [26] the former contributing to hydrophobic, the latter to hydrophilic properties. In the *Opuntia* mucilage the ratio was *ca* 1.0 and this may account for hydration and dehydration characteristics.

O. ficus-indica thus contains a high MW polysaccharide which differs from those described previously in the literature. Its properties are not such as to clearly indicate its physiological function.

EXPERIMENTAL

Materials. Terminal *O. ficus-indica* stems from the previous year were collected from the eastern slope of Mt Scopus, Jerusalem. The stems were washed in H_2O, dried and weighed. Weighed samples were taken for density measurements, by submerging in H_2O. The density was 0.926 ± 0.02 g/ml.

Extraction. Stems were homogenized in double dist. H_2O in a blender; the homogenate was filtered through cheese-cloth, and the debris resuspended and filtered ×3. The filtrate was centrifuged at 5000 *g* and the pellet collected, suspended and centrifuged again ×3. The extract was frozen and lyophilized, suspended in a small amount of H_2O and TCA was added to a final concn of 5%. The suspension was centrifuged, in the cold, at 10000 *g*. The ppt. was resuspended and centrifuged again. The TCA-treated supernatant was dialysed at 4° against 2× dist. H_2O for 72 hr. 3 vol. EtOH were added to the dialysed soln which was centrifuged at 15000 *g*. The ppt. was suspended in H_2O and pptd again ×3. The ppt. was then lyophilized and kept in a desiccator over P_2O_5. The yield was 1.124 mg dry wt/ml of tissue or 0.123 mg/g fr wt.

Hydrolysis. 10 mg of mucilage were suspended in 10 ml 2 N H_2SO_4 in a sealed tube at 98° for 15 hr. The soln was neutralized, filtered and evapd *in vacuo* to 1 ml.

TLC was carried out on 0.1 mm cellulose. The chromatographic solvents being, in one direction, EtOAc–pyridine–HOAc–H_2O (36:36:7:21) and in the 2nd direction EtOAc–pyridine–H_2O (2:1:2, upper phase). The sugars were detected by spraying the plates with *p*-anisidine phthalate 0.1 M in 96% EtOH and heating at 100° for 10 min [27].

Uronic acids were determined using the carbazole method [28], or the *m*-hydroxydiphenyl method [29] using, in both cases, galacturonic acid as standard.

[^{3}H]*Myo-inositol administration.* Tissue blocks, 1 cm^3 in size, were kept in closed Petri dishes, over wet filter paper in the light. A drop of 10 μCi [^{3}H]myo-inositol was spread over the surface of the block and the tissue incubated for 12 hr at room temp. The blocks were washed and the mucilage isolated as before.

GC was carried out according to ref. [30]. Methylation was carried out using dimethylformamide, MeI and Ag_2O ×2 for 24 hr. Methanolysis was with 4% HCl in MeOH [31].

Analytical ultracentrifugation was carried out on a Beckman E analytical centrifuge equipped with a Schlieren optical system. Sedimentation and diffusion coefficients were measured for a decreasing series of mucilage concns. Measurements were carried out on samples dissolved in 50 mM Pi–citrate buffer, pH 6.8, containing 100 mM NaCl and 20 mM Tris–HCl buffer, pH 7.4, containing 10 mM $MgCl_2$.

Hydration studies. Weighed samples of mucilage dried over P_2O_5 were kept in vials over satd solns of different salts at 20° [32]. Samples were weighed at intervals until equilibrium was reached.

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