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Binding of Calcium and Potassium Ions to Some Polyuronides and Monouronates

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The selectivity coefficients describing the calcium-potassium ion-exchange equilibrium were determined for pectate and for four samples of alginate of different uronic acid composition. Extrapolation of the results obtained with the alginates permitted calculation of the individual selectivity coefficients for polymannuronate and polyguluronate. The results showed that polygalacturonate and polyguluronate both have a much higher selectivity for calcium ions than has polymannuronate.

No binding of calcium ions to monomeric uronates was observed, and the calcium-binding properties of the polyuronides is therefore assumed to be due to their polymeric nature, and the differences between them to be caused by differences in their stereochemistry.

Polyuronides are present in most plant tissues and function as natural ion-exchange materials. Most important are pectin in higher plants and alginates in brown algae. Alginic acid is a linear heteropolysaccharide containing units of D-mannuronic and L-guluronic acid, while the basic skeleton of the pectin molecule is poly-D-galacturonic acid, the carboxyl groups being partially esterified with methanol.

The ion-exchange properties of alginates depend upon the uronic acid composition of the polymer. In exchange reactions between monovalent and divalent cations, alginates rich in guluronic acid units have a higher selectivity for the divalent ion than samples richer in mannuronic acid.[1,2] For the ion-exchange reactions Ca—Sr, Sr—Mg, Ca—Mg, and Co—Ca the selectivity of alginates is associated solely with the guluronic acid units that are present.[3]

The ion-exchange properties of pectin depend upon the degree of esterification of the carboxyl groups.[4,5] With increasing degree of esterification

the selectivity for calcium in the calcium-potassium ion-exchange reaction strongly decreases.

In the present investigation the selectivity coefficients in the calcium-potassium ion-exchange reaction were determined for pectate and alginates with different uronic acid compositions. By extrapolation of the results, the selectivities of polyguluronate and polymannuronate were estimated and compared to the selectivity of polygalacturonate. The calcium-binding properties of L-guluronate, D-mannuronate, D-galacturonate, and L-glucuronate were also compared by determining the calcium ion activity in calcium uronate solutions. The calcium ion activity was determined by using tetramethylmurexide as an auxiliary ligand.[4]

EXPERIMENTAL

Materials. D-Galacturonic and D-glucuronic acids were commercial products. D-Mannuronic and L-guluronic acids were prepared from alginic acid by hydrolysis and separation of the two uronic acids by chromatography on Dowex 1×8 anion exchange resin.[6]

Four samples of alginic acid were used. Their uronic acid composition (Table 1) was determined as described by Haug and Larsen.[7] The weight-average molecular weights were determined viscometrically,[8] using the constants in the Staudinger equation determined by light scattering.[9] The number-average molecular weights were found by determining the reducing end groups by Nelson's method.[10] Sample II and III were undegraded alginates prepared in the usual way,[6] while sample IV was prepared by soaking unripe receptacles of *Fucus vesiculosus* in distilled water, squeezing out the viscous solution, and preparing the alginate by precipitation with acid. Sample I was prepared by degradation of a sample of alginate for 2 h in 0.3 N hydrochloric acid at 100°, and subsequent fractionation of the insoluble material by precipitation with acid at pH 2.85 and with calcium and magnesium ions.[11] The sodium alginates were converted into alginic acid by washing first with acidic aqueous ethanol (5 ml 37 % hydrochloric acid added to 100 ml 60 % ethanol), and then with ethanol and ether. The samples were dried in air below 60°.

Commercial sugar-beet pectin (Svenska Sockerfabriks AB, Arlöv, Sweden) was used for the preparation of pectic acid. The purified pectin contained 81.3 % polyuronide, 0.3 % sulphate ash, L-rhamnose and D-galactose in a molar ratio of 11:9, and traces of L-arabinose and D-xylose. The degree of esterification was 27 %. Pectic acid was prepared by alkaline deesterification (pH 10.5, room temperature, 2—3 h) and precipitation by hydrochloric acid. This procedure was repeated four times. The pectic acid was washed with acidified aqueous ethanol, followed by dry ethanol and then ether. The sample was dried below 60°. The neutral monosaccharides present in hydrolysates of pectin were determined by paper chromatography,[4] and the viscosity-average molecular weight was calculated from the intrinsic viscosity.[15]

Tetramethylmurexide was prepared from caffein *via* alloxantine [12,13] and characterized as described previously.[4]

Methods. The content of carboxyl groups in the polyuronides and the degree of esterification of the pectin were determined by potentiometric titration.[4,14] Solutions of the potassium salts of the polyuronic acids were prepared by neutralizing the polyuronic acids with potassium hydroxide to pH 7.5. Free calcium in the solution was determined by the method of Raaflaub [16–17] using tetramethylmurexide as an auxiliary ligand (for a detailed description, see Refs. 4, 5) in solutions containing potassium salts of the polyuronic acids (4 mequiv./l), calcium chloride (4, 6, 8, and 10 mequiv./l) and tetramethylmurexide (4×10^{-5} mole/l). The ionic strength of the solution, μ 0.15, was adjusted by addition of potassium chloride. The pH was 7.0—7.5.

The absorbance at 490 and 530 nm was determined in a Uvispec-Hilger spectrophotometer.

Table 1. Composition of polyuronic acids.

Sample	Source	Uronic acid composition (%) D-Gal UA	L-Gul UA	D-Man UA	Molecular weight M_w	M_n
Pectic acid	Sugar beet	89.8	—	—	31 000	
Alginic acid I (degraded)	*Laminaria hyperborea*, stipes	—	91	9		15 000
Alginic acid II	*L. hyperborea*, stipes	—	72.5	27.5	500 000	
Alginic acid III	*L. digitata*		38.5	61.5	350 000	
Alginic acid IV	*Fucus vesiculosus*, receptacles		9	91	700 000	

Selectivity coefficients for the calcium-potassium ion-exchange reaction were calculated as

$$k_K^{Ca} = \frac{X_{Ca}^P \ (X_K^S)^2}{X_{Ca}^S \ (X_K^P)^2}$$

where X^P and X^S represent the equivalent fractions of the respective ions bound to the polyuronide (P) and present as free ions in solution (S).

Solutions of calcium uronates were prepared by titrating the uronic acids with 0.04 N calcium hydroxide. The calcium ion activity was determined in solutions of calcium uronate (3.0 mequiv./l) containing tetramethylmurexide (4×10^{-5} mole/l) as described previously.[4]

RESULTS AND DISCUSSION

a) Binding of calcium and potassium ions to polyuronides. The composition of the polyuronides is given in Table 1. The alginate samples contain only D-mannuronic and L-guluronic acids in varying proportions, while the pectic acid contains D-galacturonic acid as its only acidic constituent. In addition, the pectic acid sample contains small amounts of neutral sugar units, mainly L-rhamnose and D-galactose.

The selectivity coefficients for the calcium-potassium ion-exchange reaction was determined for the five samples of polyuronides investigated, and the results are shown in Table 2. The results are averages of measurements at four different calcium concentrations (see Experimental). In agreement with previous results,[1,2] the affinity of the carboxyl groups of alginate for calcium

Table 2. Selectivity coefficients (k_K^{Ca}) for polyuronides.

	k_K^{Ca}
Pectate	67.8 ± 3.6
Alginate I	57.0 ± 8.6
» II	37.3 ± 5.5
» III	15.1 ± 1.1
» IV	5.8 ± 0.9

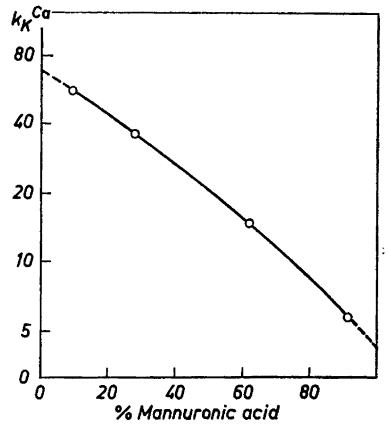

Fig. 1. Selectivity coefficients k_K^{Ca} of alginates with varying content of mannuronic and guluronic acid.

ions strongly increases with increasing content of L-guluronic acid units in the polymer. The dependence of the selectivity coefficient k_K^{Ca} on the uronic acid composition of the alginate is shown in Fig. 1. By extrapolation of the curve the values of the selectivity coefficient for polymannuronate and polyguluronate were estimated with the following results:

	k_K^{Ca}
Polymannuronate	4.2
Polyguluronate	70.8
Polygalacturonate	67.8

A clear distinction between polymannuronate on one hand and polyguluronate and polygalacturonate on the other hand was observed.

The polyuronide samples used in this investigation differ considerably in molecular weight (Table 1). The results given in Fig. 1 indicate that differences in molecular weight, within the range investigated here, do not influence the selectivity of the sample. This is in agreement with previous observations on pectin ranging from 20 000 to 110 000 in molecular weight.[5,19]

b) *Binding of calcium ions by uronic acids.* The remarkable difference in affinity of the three polyuronides for calcium ions may either be caused by the monomeric uronates having different affinities for calcium ions or it may be due to the arrangement of the monomers in the polymer chain.

Table 3. Activity of calcium ions in solutions of calcium uronates (1.5 mmole Ca/l).

	$a_{Ca^{2+}} \times 10^3$	$\gamma_{Ca^{2+}}$
D-Galacturonate	1.08 ± 0.03	0.72 ± 0.02
D-Glucuronate	1.04 ± 0.03	0.69 ± 0.02
D-Mannuronate	1.05 ± 0.01	0.70 ± 0.01
L-Guluronate	1.11 ± 0.01	0.74 ± 0.01
Calcium chloride	1.12	0.75

The affinity of D-galacturonate, L-guluronate, D-mannuronate, and D-glucuronate to calcium ions was investigated by determining the calcium ion activity in calcium uronate solutions. The result is given in Table 3, and, for comparison, a corresponding measurement of aqueous calcium chloride is included.[4] No significant differences between the calcium-binding properties were observed for the four uronates, and the calcium activity in a calcium chloride solution was very similar to the activity in a calcium uronate solution of the same molarity. Our results are in agreement with those of Buddecke and Drzeniek [18] who found the same stability constant for Ca-glucuronate and Ca-galacturonate and with Triffitt,[20] who found that monomeric L-guluronic acid has no detectable binding capacity for calcium and strontium.

A significant amount of lactones was present in the solution of D-mannuronic and L-guluronic acid. It has previously been demonstrated that the presence of neutral substances such as sucrose does not influence the calcium-binding properties of pectic acid.[19] It is therefore reasonable to assume that the presence of lactones does not influence the calcium-binding properties of the uronate.

The fact that no binding of calcium ions to any of the monomeric uronates was observed indicates that the calcium-binding properties of polyuronides are due to their polymeric nature, and that the differences between them in some way must be caused by differences in the steric arrangement of the active groups in the polymer chain.

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