# Calcium Complexes of Uronic Acid Monomers

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*Summary* Stability constants are reported for calcium complexes with anomeric forms of two uronic acids, and complex stability is related to structure and biological function.

THE interaction of alkaline-earth cations with acidic polysaccharides is considered to be of major significance in cellular structure of both land plants and algae,[1] and also in cation regulation in mammalian bone formation.[2] Kohn *et al.*,[3] in a study of the cation exchange properties of alginates, report, however, that no detectable complexing occurs between calcium ions and uronic acid monomers. We have investigated the interaction between calcium(II) and D-glucuronate and D-galacturonate ions, and have measured stability constants which, although small, vary significantly with the structure of the acid. We have not detected complexes of higher order than 1 : 1.

Free alduronic acids in aqueous solution rapidly establish equilibrium between $\alpha$- and $\beta$-pyranose forms, so a stability constant measured for their complexes with a bivalent ion will be essentially of the form:

$$K' = \frac{[ML_\alpha^+] + [ML_\beta^+]}{[M^{2+}]\{[L_\alpha^-] + [L_\beta^-]\}}$$

where the subscripts represent the two modifications of the ligand, and it is assumed that activity coefficients for the singly charged species effectively cancel one another. Stability constants were also measured for the calcium complexes of $\alpha$-D-methylglucuronoside and $\beta$-D-methylgalacturonoside, substances which cannot undergo mutarotation.

Stability constants were estimated at various ionic strengths by three methods. In the first, the fall in calcium ion activity on the addition of a potassium uronate solution to a solution of calcium nitrate was followed potentiometrically using an Orion liquid ion exchange membrane electrode (No. 9220). The second was the standard pH method, using a glass electrode. The third method was polarimetric, making use of the difference in molecular rotation between potassium uronate alone and in the presence of a large excess of calcium ions, to determine the degree of complexing at intermediate molar ratios. In the latter two cases, single-ion activity coefficients were applied to the calculated free metal concentrations. The results are summarised in Table 1.

The molecular rotation of the methyl glycosides is not affected by the presence of calcium ions. Since their calcium complexes are of a similar strength to those of the free uronates, the shift in rotation in the latter case must be

due to a shift in the equilibrium between the α- and β-forms of the anion. Equilibrium constants of the form:

$$k_L = \frac{[L_\alpha^-]}{[L_\beta^-]} \qquad k_{ML} = \frac{[ML_\alpha^+]}{[ML_\beta^+]}$$

were evaluated. Those for glucuronate were estimated from the relative sizes of the $^1H$ n.m.r. signals of the anomeric protons in a deuterium oxide solution, and those for galacturonate were calculated from the data of Isbell and Frush.[4] Using these constants, it is possible to convert values of $K'$ into true stability constants for complexes of the two anomeric forms of the ligand. These are given in Table 2, and may be compared with those for the glycoside complexes in Table 1.

TABLE 1. *Measured stability constants for calcium complexes* (K′ *or* K; $M^{-1}$)

| Ligand | Method 1 | Method 2 | Method 3 |
|---|---|---|---|
| Glucuronate .. .. .. | 32 | 33 | 30 |
| Galacturonate .. .. | 64 | 72 | 73 |
| α-Methylglucuronosidate .. | 40 | (a) | (b) |
| β-Methylgalacturonosidate .. | 56 | (a) | (b) |

(a) Not measured; (b) method not applicable.

The greater strength of the galacturonate complexes can be related to the hydroxyl group on C(4), which is axial in galacturonate and equatorial in glucuronate. A smaller enhancement of the stability may be associated with the axial hydroxyl group at C(1) in the α-anomers. These axial groups allow for greater interaction with a cation, and cause galacturonic acid to be a significantly weaker acid than glucuronic.[5]

TABLE 2. *Constants from mutarotation data*

| Ligand | $k_L$ | $k_{ML}$ | $K_\alpha$ | $K_\beta$ |
|---|---|---|---|---|
| Glucuronate | 0·72 | 0·94 | $37M^{-1}$ | $28M^{-1}$ |
| Galacturonate | 0·27 | 0·44 | $101M^{-1}$ | $61M^{-1}$ |

The results also suggest structure-function relationships for α-linked polygalacturonic acid in the tissues of higher plants. Similarly, the β-linked poly-L-guluronic acid in brown seaweeds has been shown to have the $1C$ conformation with three axial oxygen atoms,[6] while the D-mannuronic acid residues also present have only one. It is significant that an enzymic conversion of mannuronic into guluronic acid residues has been reported, in which the presence of calcium ions is required.[7] The considerably stronger binding of calcium by the guluronate groups is indicated, as the process would otherwise be thermodynamically unfavourable.[8]

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