

## INTRAMOLECULAR BINDING OF CALCIUM IONS TO L-GULURONAN AND D-GALACTURONAN

Rudolf KOHN and Anna MALOVÍKOVÁ

*Institute of Chemistry,*

*Slovak Academy of Sciences, 809 33 Bratislava*

Received October 22nd, 1980

The values of activity coefficients  $\gamma_{\text{Ca}^{2+}}$ , determined earlier in solutions of calcium oligoguluronates and oligogalacturonates of various polymerization degree  $n$ , were interpreted. Values  $\gamma_{\text{Ca}^{2+}}$  corresponding to an intramolecular  $\text{Ca}^{2+}$  bond to a polymeric chain of L-guluronan and D-galacturonan were obtained by extrapolation of functions  $\gamma_{\text{Ca}^{2+}} = f(1/n)$  and  $\gamma_{\text{Ca}^{2+}} = f((n-2)/n)$  to  $\lim n \rightarrow \infty$ . The  $\gamma_{\text{Ca}^{2+}}$  values were compared with those determined in solutions of calcium salts of O-acetyl derivatives of pectic acid and D-mannuronan. In molecular disperse solutions the  $\text{Ca}^{2+}$  ions are bound to L-guluronan and D-galacturonan by an electrostatic bond. The course of the above-mentioned functions leads to the conclusion that both terminal uronic acid units in polyuronates under study bind  $\text{Ca}^{2+}$  ions less firmly than do the inner units in the macromolecule chain. The linear course of the function  $\gamma_{\text{Ca}^{2+}} = f((n-2)/n)$  for L-guluronan proves the additivity of contribution of the terminal and inner uronic acid units to the total activity of  $\text{Ca}^{2+}$  counterions bound to L-guluronan.

Calcium ions are bound to carboxyl groups of L-guluronan and D-galacturonan by an intermolecular chelate bond under formation of aggregates of parallel oriented macromolecules, according to the "egg box" binding model<sup>1-5</sup>. On the other hand, the  $\text{Ca}^{2+}$  ions are bound to D-mannuronan<sup>4-6</sup> and carboxymethylcellulose<sup>4,7,8</sup> by an electrostatic bond in a molecular disperse solutions. The substantially higher selectivity of cation exchange in L-guluronan and D-galacturonan, when compared with that of D-mannuronan<sup>4,9</sup> is in connection with this different way of cation binding to polyuronates.

The question whether a chelate binding of  $\text{Ca}^{2+}$  ions takes place also in the case of their intramolecular binding to the single chains of the respective D-galacturonan, L-guluronan and pectate in molecular disperse solutions remained open. In connection with this problem we have proved by means of O-acetyl derivatives of pectic acid as model substances that the  $\text{Ca}^{2+}$  ions are bound to the isolated chains of pectate (D-galacturonan) by a pure electrostatic bond<sup>10</sup>, like to D-mannuronan and carboxymethylcellulose. Acetyl groups bound in the pectate molecule act here as a steric hindrance of aggregation of macromolecules.

This problem can be, nevertheless, solved by a principally different method involving the interpretation of the values of activity coefficients  $\gamma_{\text{Ca}^{2+}}$  determined

in calcium salts of oligomeric fragments of L-guluronan and D-galacturonan, where the  $\text{Ca}^{2+}$  ions are bound to carboxyl groups by an electrostatic bond in contrast to the corresponding macromolecular systems.

## EXPERIMENTAL

Interpreted were the  $\gamma_{\text{Ca}^{2+}}$  values determined in calcium oligogulonates<sup>6</sup> and oligogalacturonates<sup>5,11</sup> as already published. Oligogulonates of polymerization degree  $n = 1$  to 4 were homopolymeric and homodisperse; samples of a mean polymerization degree  $n \geq 9.5$  were homopolymeric with a very low scattering of molecular weights, roughly within  $\pm 5\%$ . Oligogalacturonates of  $n = 1$  to 7 were homopolymeric and homodisperse. The preparation of polymerization degree  $n = 7$  was kindly supplied by Dr K. Heinrichová of this Institute. The  $\gamma_{\text{Ca}^{2+}}$  value of this oligomer was determined in this paper.

The  $\text{Ca}^{2+}$  ion activities were determined in calcium oligogulonates of a 3.00 mmol ( $\text{COOCa}_{0.5}$ )  $\text{l}^{-1}$  concentration by the metallochromic indicator (tetramethylmurexide) method; for details see<sup>12,13</sup>.

## RESULTS AND DISCUSSION

Dilute calcium oligogulonate and oligogalacturonate solutions are molecular disperse. The  $\text{Ca}^{2+}$  ions are bound to these oligogulonates by a pure electrostatic bond. The strength of bond of  $\text{Ca}^{2+}$  ions to carboxyl groups of oligogulonates increases with the increasing polymerization degree  $n$  of these substances and consequently, the activity coefficient  $\gamma_{\text{Ca}^{2+}}$  of calcium counterions decreases. At a polymerization degree of oligogulonates higher than 18 an alteration of the electrostatic bond of  $\text{Ca}^{2+}$  ions to an intermolecular chelate binding<sup>6</sup> occurred in a relatively narrow interval of the polymerization degree, this being manifested by a considerable decrease of the  $\gamma_{\text{Ca}^{2+}}$  value. Oligogalacturonates display a similar feature<sup>5</sup> excepting that the chelate binding appears at already shorter chain of the molecule ( $n \geq 10$ ). At a polymerization degree of polyuronates greater than 30 the activity of  $\text{Ca}^{2+}$  counterions is virtually no more dependent on the macromolecule chain length<sup>5,6</sup>. To be able to judge, whether the  $\text{Ca}^{2+}$  ions are bound to these polyuronates by intramolecular electrostatic or chelate bonds, one needs to know the activity coefficients of counterions bound to the polymeric uronate in molecular disperse systems. This goal can be reached through the extrapolation of the function  $\gamma_{\text{Ca}^{2+}} = f(n)$ , determined in oligogulonate solutions to value  $n$  limiting to infinity.

Values  $\gamma_{\text{Ca}^{2+}}$  determined earlier<sup>5,6,11</sup> and listed in Table I were employed for this extrapolation. The extrapolation was first examined in the series of oligogulonates, which represents a set of  $\gamma_{\text{Ca}^{2+}}$  values for a broader interval of polymerization degree  $n$  applying the function  $\gamma_{\text{Ca}^{2+}} = f(1/n)$ . The course of this function shows (Fig. 1) that the  $\gamma_{\text{Ca}^{2+}}$  values of oligogulonates for  $n \geq 2$  lie on a line, whereas the value  $\gamma_{\text{Ca}^{2+}}$  corresponding to monomeric L-guluronic acid deviates aside. As it follows from the course of the function drawn in Fig. 1, both terminal uronic acid units

in the linear molecule of L-gulonate bind the  $\text{Ca}^{2+}$  ions less firmly as the inner units of the macromolecule chain. A similar course was found with oligogalacturonates, where the  $\gamma_{\text{Ca}^{2+}}$  values for oligomers ( $n = 3$  to 7) fit in a line. (The value  $\gamma_{\text{Ca}^{2+}}$  for the dimer is somewhat lower, as will be shown). The activity coefficients  $\gamma_{\text{Ca}^{2+}}$  in solutions of monomeric Ca-uronates under investigation ( $\gamma_{\text{Ca}^{2+}} = 0.740$  and  $0.730$ , resp.) are very close to that calculated according to Debye and Hückel ( $0.759$ ) for a solution of a strong electrolyte ( $\text{CaCl}_2$ ) of the same concentration.

For a polymeric chain ( $\lim n \rightarrow \infty$ ) the values  $\gamma_{\text{Ca}^{2+}} = 0.292$  for L-gulonatan and  $0.295$  for D-galacturonan (Table I) were obtained by the least squares method from the data lying within the linear course of the function; the corresponding correlation coefficients  $r$  were found to be  $0.9985$  and  $0.9991$ . (If the value  $\gamma_{\text{Ca}^{2+}}$  corresponding to the dimer is also taken for calculation of the linear course of this function for oligogalacturonates, the  $\gamma_{\text{Ca}^{2+}}$  and  $r$  values analogously are  $0.319$  and  $0.9951$ , respectively).

As shown by the study of circular dichroism in dilute solutions of sodium oligogalacturonates and D-galacturonan, the chromophoric carboxyl groups of the inner uronic acid units of the linear macromolecule are all in the same way mutually arranged in the space<sup>14</sup>. This phenomenon is caused by a considerable rigidity of the D-galacturonan molecule due to its *trans*-glycosidic diaxial  $\alpha(1 \rightarrow 4)$  bonds. The relation between the molar residual ellipticity  $[\Theta]_n/n$  and the polymerization degree  $n$

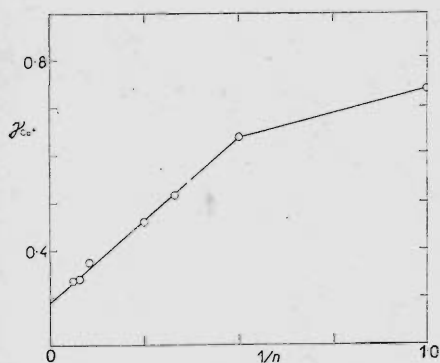


FIG. 1

Binding of  $\text{Ca}^{2+}$  ions to oligogalacturonates  
Function  $\gamma_{\text{Ca}^{2+}} = f(1/n)$ .  $n$  polymerization  
degree;  $(\text{COOCa}_{0.5}) = 3.00 \text{ mmol l}^{-1}$

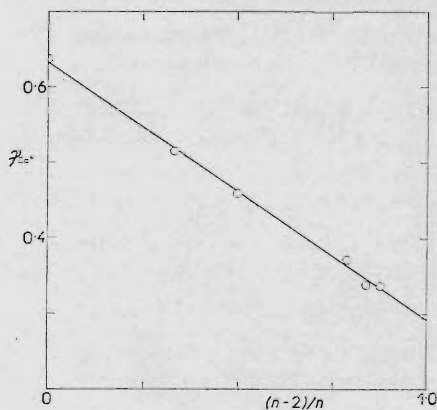


FIG. 2

Binding of  $\text{Ca}^{2+}$  ions to oligogalacturonates  
Function  $\gamma_{\text{Ca}^{2+}} = f((n-2)/n)$ ;  $(\text{COOCa}_{0.5})$   
 $= 3.00 \text{ mmol l}^{-1}$

could be expressed by the function  $[\theta]_n/n = f((n-2)/n)$  according to Freudenberg. The linear course of this relationship evidenced the validity of the additivity principle of the contribution of monomeric units in the D-galacturonan molecule to its total optical activity<sup>14</sup>. The linear dependence of ellipticity of pectinates upon the esterification degree of their carboxyl groups by methanol<sup>15</sup> also proves that the pectinate macromolecule is noticeably rigid and maintains more or less its regular conformation even in the solution. The course of the function  $\gamma_{Ca^{2+}} = f(1/n)$ , (Fig. 1) stimulated us to verify, whether the relationship  $\gamma_{Ca^{2+}}$  on the polymerization degree  $n$  also expresses the additivity of contributions of terminal and inner uronic acid units of oligogulonates to the final activity of  $Ca^{2+}$  counterions bound to L-gulonate.

The function  $\gamma_{Ca^{2+}} = f((n-2)/n)$  has a linear course for solutions of Ca-oligogulonates ( $n = 2$  to 16.3, Fig. 2). Ca-Oligogalacturonates of polymerization degree  $n = 3$  to 7 also evidence the linear course of this function (Fig. 3). The value  $\gamma_{Ca^{2+}}$  for the dimer is a little lower, as it follows from the linear course of the function ( $\Delta\gamma_{Ca^{2+}} = -0.037$ ). Extrapolated were only  $\gamma_{Ca^{2+}}$  values for oligomers of  $n = 3, 4, 5$  and 7.

TABLE I

Activity coefficients  $\gamma_{Ca^{2+}}$  determined experimentally in solutions of calcium oligogulonates and oligogalacturonates and calculated by extrapolation for the respective polyuronates;  $(COOCa_{0.5}) = 3.00 \text{ mmol l}^{-1}$

Polymerization degree $n$	L-GulUA <sup>a</sup>	D-GalUA <sup>b</sup>
1	0.740	0.730
2	0.638	0.648
3	0.515	0.556
4	0.459	0.491
5	—	0.456
7	—	0.405
9.5	0.373	—
12.5	0.339	—
16.6	0.337 <sup>c</sup>	—
$\infty^d$	0.292	0.295
$\infty^e$	0.292	0.295

<sup>a</sup> Ref.<sup>6</sup>, <sup>b</sup> ref.<sup>5,11</sup>; <sup>c</sup>  $(COOCa_{0.5}) = 2.40 \text{ mmol l}^{-1}$ ; <sup>d,e</sup> values calculated by extrapolation of functions  $\gamma_{Ca^{2+}} = f(1/n)$  and  $\gamma_{Ca^{2+}} = f((n-2)/n)$ , resp.

Both extrapolation functions were also tried to apply to values  $\gamma_{\text{Ca}^{2+}}$  determined in solutions of calcium oligo-D-mannuronates and calcium D-mannuronan ( $n = 1$  to 4000), where an intramolecular electrostatic bond of  $\text{Ca}^{2+}$  ions is involved in the whole range of the polymerization degree<sup>6</sup>. Both extrapolation functions do not reveal a linear course here, what might be associated with a greater flexibility of the D-mannuronan macromolecule given by its *trans*-glycosidic diequatorial  $\beta(1\rightarrow4)$  bonds.

Results of extrapolation are summarized in Table I; the correlation coefficients  $r$  are identical with those found when applying the extrapolation function  $\gamma_{\text{Ca}^{2+}} = f(1/n)$ . Both extrapolation equations lead to the same  $\gamma_{\text{Ca}^{2+}}$  values. Practically consistent values (0.292 and 0.295) were found for L-guluronan and D-galacturonan, respectively. This accordance is not surprising, since both polyuronates have the same type of glycosidic  $\alpha(1a\rightarrow4a)$  bonds and, as a consequence, also the same linear charge density of the macromolecule with the distance of adjacent carboxyl groups  $b = 0.435$  nm (ref.<sup>16-19</sup>).

Values  $\gamma_{\text{Ca}^{2+}}$  obtained by extrapolation (Table I) were compared with those determined earlier in solutions of calcium D-mannuronan<sup>6</sup> and O-acetyl derivatives of pectic acid<sup>10</sup>, where the  $\text{Ca}^{2+}$  ions are bound by an intramolecular electrostatic bond. (Pectic acid contained 90% of D-galacturonic acid units, traces of L-rhamnose and 10% of neutral saccharides as side chain components; the main chain of the macromolecule can be, therefore, denoted as D-galacturonan). Values  $\gamma_{\text{Ca}^{2+}}$  estimated by extrapolation well agree with that ( $0.302 \pm 0.002$ ) determined for O-acetyl derivatives of pectic acid<sup>10</sup> (D-galacturonan). The value  $\gamma_{\text{Ca}^{2+}} 0.281$  determined for calcium D-mannuronan is a little lower than that, which can be predicted on the basis of the difference in the distance between the neighbouring carboxyl groups determined

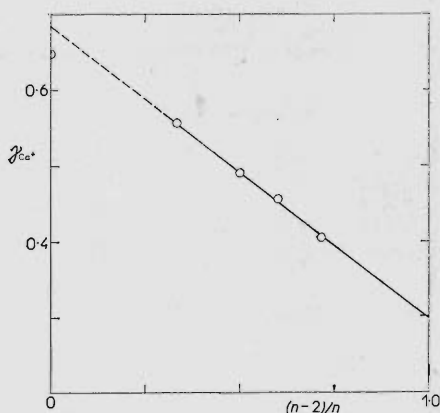


FIG. 3  
Binding of  $\text{Ca}^{2+}$  ions to oligogalacturonates  
Function  $\gamma_{\text{Ca}^{2+}} = f((n-2)/n)$ ;  $(\text{COOCa}_{0.5}) = 3.00 \text{ mmol l}^{-1}$

by an X-ray diffraction analysis of sodium and calcium salts of L-guluronan and D-galacturonan<sup>16-19</sup> ( $b = 0.435$  nm) on one hand and calcium D-mannuronan<sup>20</sup> ( $b = 0.500$  nm) on the other. The lower value  $\gamma_{Ca^{2+}}$  for D-mannuronan might be due to a greater flexibility of its linear macromolecule, so that in solutions of calcium D-mannuronan the carboxyl groups are little less remote than is the case with solid phase.

The accordance of  $\gamma_{Ca^{2+}}$  values calculated by extrapolation of functions  $\gamma_{Ca^{2+}} = f(1/n)$  and  $\gamma_{Ca^{2+}} = f((n-2)/n)$  with those of O-acetyl derivatives of pectic acid and D-mannuronan determined directly shows that the intramolecular bond of  $Ca^{2+}$  ions to L-guluronan and D-galacturonan in molecular disperse solutions is of electrostatic nature similarly, as is the case with D-mannuronan<sup>6</sup> and carboxymethylcellulose<sup>4,7,8</sup>. The chelation of  $Ca^{2+}$  ions takes place in an intermolecular bond only, at aggregation of macromolecules. The activity coefficient of counterions  $\gamma_{Ca^{2+}}$  is with a chelate binding of calcium to these polyuronates substantially lower (0.06 to 0.08, ref.<sup>6</sup>).

The additivity of contributions of terminal and inner uronic acid units to the final activity of  $Ca^{2+}$  counterions bound to carboxyl groups of a polyuronate was reliably evidenced by the Freudenberg's equation with L-guluronan (Fig. 2), where experimental data for a greater range of the polymerization degree were at our disposal. This phenomenon is due to a considerable rigidity of the linear L-guluronan macromolecule. The  $\gamma_{Ca^{2+}}$  values determined for oligogalacturonates ( $n = 3$  to 7; Fig. 3) can be interpreted in a similar way; the range of the polymerization degree is, however, too narrow and the conclusion cannot be, therefore, unequivocal. The substantially more flexible D-mannuronan macromolecule does not obey the additivity rule as expressed by the Freudenberg's equation.

#### REFERENCES

1. Rees D. A.: Chem. Ind. (London) 1972, 630.
2. Grant G. T., Morris E. R., Rees D. A., Smith P. J. C., Thom D.: FEBS (Fed. Eur. Biochem. Soc.) Lett. 32, 195 (1973).
3. Smidsrød O., Haug A., Whittington S. G.: Acta Chem. Scand. 26, 2563 (1972).
4. Kohn R.: Pure Appl. Chem. 42, (3), 371 (1975).
5. Kohn R., Luknár O.: This Journal 42, 731 (1977).
6. Kohn R., Larsen B.: Acta Chem. Scand. 26, 2455 (1972).
7. Rinaudo M., Milas M.: C. R. Acad. Sci., Ser. C 271, 1170 (1970).
8. Rinaudo M. in the book: *Polyelectrolytes* (E. Sélégny, Ed.), p. 157. Reidel, Dordrecht, Holland 1974.
9. Kohn R., Furda I., Haug A., Smidsrød O.: Acta Chem. Scand. 22, 3098 (1968).
10. Kohn R., Malovíková A.: This Journal 43, 1708 (1978).
11. Kohn R.: Carbohyd. Res. 20, 351 (1971).
12. Kohn R., Furda I.: This Journal 32, 1925 (1967).
13. Kohn R.: Chem. Zvesti 28, 625 (1974).

14. Bystrický S., Kohn R., Sticzay T.: *This Journal* 44, 167 (1979).
15. Plaschina I. G., Braudo E. E., Tolstoguzov V. B.: *Carbohydr. Res.* 60, 1 (1978).
16. Atkins E. D. T., Mackie W., Smolko E. E.: *Nature (London)* 225, 626 (1970).
17. Atkins E. D. T., Mackie W., Parker K. D., Smolko E. E.: *J. Polymer. Sci. B* 9, 311 (1971).
18. Palmer K. J., Hartzog M. B.: *J. Amer. Chem. Soc.* 67, 2122 (1945).
19. Sterling C.: *Biochem. Biophys. Acta* 26, 186 (1957).
20. Mackie W.: *Biochem. J. (London)* 125, (4), 89 (1971).

Translated by Z. Votický.