Carbohydrate Research, 203 (1990) 19–32
Elsevier Science Publishers B.V., Amsterdam

Structural and conformational effects on the complexation of calcium by 2,3-dicarboxy derivatives of β-cyclodextrin (cyclomaltoheptaose), amylose, and cellulose

Michiel Floor, Joop A. Peters, Herman van Bekkum*, Antonius P. G. Kieboom,
Laboratory of Organic Chemistry, Delft University of Technology, Julianalaan 136, 2628 BL Delft (The Netherlands)

Jan H. Koek, Frans L. M. Smeets, and Rob E. Niemantsverdriet
Unilever Research Laboratories, P.O. Box 114, 3130 AC Vlaardingen (The Netherlands)

(Received July 15th, 1989; accepted for publication, November 7th, 1989)

ABSTRACT

The conformational effects upon complexation of calcium (II) and lanthanide (III) ions by 2,3-dicarboxy-β-cyclodextrin (DC-βCD) have been studied by ^{1}H-, ^{13}C-, and ^{17}O-n.m.r. spectroscopy. The complexation of Ca^{2+} by DC-βCD involves conformational transitions at metal/ligand ratios (ρ) of 0.25 and 0.5 with predominant changes in the resonances of C-1,4,5. For the La^{3+}–DC-βCD complex (up to ρ 0.3), a structure involving two La^{3+} per DC-βCD macrocycle, each coordinated to 8 oxygen atoms, is proposed. Optical rotation data indicated similar conformational behaviour on complexation of Ca^{2+} with DC-βCD and dicarboxyamylose (DCA), whereas dicarboxycellulose (DCC) showed a substantially larger change in conformation. Static and dynamic light scattering indicated aggregation of DCA and DCC on complexation with Ca^{2+}, with a concomitant reduction of the hydrodynamic radius (R_h).

INTRODUCTION

The 2,3-dicarboxy derivatives of starch and cellulose are potential substitutes for phosphate in laundry detergents owing to their excellent calcium-complexing properties[1–3]. Dicarboxy derivatives (**3**) can be prepared[4,5] by periodate oxidation of (1→4)-glucans (**1**), followed by chlorite–hydrogen peroxide oxidation of the resulting dialdehyde (**2**).

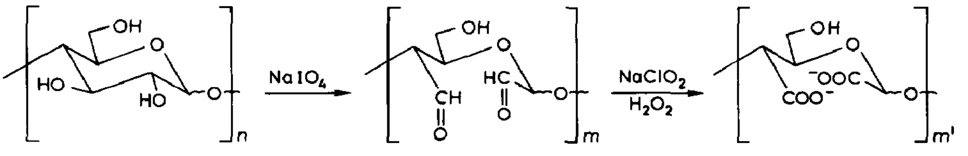
The $^-OOC–C–O–C–COO^-$ moiety of dicarboxy polysaccharides can form[6,7] complexes with Ca^{2+}. The calcium-complexing properties of dicarboxystarch and dicarboxycellulose depend on the molecular weight[5], being poor below $\overline{M}_w$ 10^4 (d.p. ~50), increasing significantly from $\overline{M}_w$ 10^4 to 10^5, and reaching a constant level at $\overline{M}_w > 10^5$.

Some conformational aspects of the interaction of Ca^{2+} with dicarboxyamylose (DCA) and dicarboxycellulose (DCC) have been reported[8–10]. It was concluded from inflections in the ^{1}H-n.m.r., microcalorimetric, and conductometric titration curves that

---

* Author for correspondence.

0008-6215/90/$ 03.50



1 2 3

various complexes were formed, but the prevalent stoichiometry was one Ca^{2+} ion per two dicarboxy units. Nieuwenhuizen *et al.*[3] also found this preferred stoichiometry and tentatively proposed the formation of helix structures with Ca^{2+} being coordinated by two dicarboxy units in subsequent turns of the helix.

We now report on the preparation of dicarboxy-β-cyclodextrin (DC-βCD) from β-cyclodextrin (cyclomaltoheptaose, βCD) and on its complexation with Ca^{2+} and La^{3+}. Ca^{2+} and La^{3+} complexes generally show structural similarities and n.m.r. spectroscopy has proved to be a valuable tool in the elucidation of the structure of La^{3+} complexes[11].

EXPERIMENTAL

Amylose and βCD were gifts from AVEBE (Veendam, The Netherlands).

Preparation of dicarboxy-β-cyclodextrin (DC-βCD). — Sodium periodate (16.0 g, 75 mmol) was added to a stirred suspension of βCD (9.0 g, 56 mmol of "anhydroglucose" units) in water (150 mL) at 5° in the dark. After 48 h, the solution was treated twice with 1 equiv. of Dowex MR-3 mixed-bed resin, filtered, and either freeze-dried or used immediately for the chlorite oxidation.

To a solution of the dialdehyde (8.0 g, 0.10 mol of aldehyde groups) in water (350 mL) was added a solution of sodium chlorite (80%, 40.0 g, 0.35 mol) and acetic acid (13.2 g, 0.22 mol) in water (100 mL) during 1 h at 10°. The solution was allowed to attain room temperature (the pH remained at ~4.5) and, after 24 h, nitrogen was blown through in order to remove chlorine dioxide, the pH was adjusted to 9 (2.5M NaOH), the solution was poured into aqueous 96% ethanol (2 vol.), and the insoluble material was centrifuged. The viscous pellet was washed with ethanol–water (2:1, 50 mL), and a solution in water (20 mL) was freeze-dried to yield DC-βCD as a white powder (Table I). ^{13}C-N.m.r. data (D_2O): δ 62.5 (C-6), 79.3 (C-5), 80.9 (C-4), 101.3 (C-1), 175.4 (C-3), 177.1 (C-2) (assignment of peaks according to ref. 9).

Preparation of dicarboxyamylose (DCA) and dicarboxycellulose (DCC). — Amylose and cellulose were converted into their respective dialdehydes by conventional periodate oxidation[4].

To a stirred suspension of each dialdehyde (4.0 g, 50 mmol of aldehyde groups) in water (200 mL) was added 2.5M NaOH to maintain the pH at 5.0 whilst aqueous 35% hydrogen peroxide (5.0 g, 50 mmol) and Na_2EDTA (100 mg, 0.27 mmol) were added.

The reaction was started by the dropwise addition of aqueous 80% sodium chlorite (5.66 g, 50 mmol) and acetic acid (1.8 g, 30 mmol) in water (15 mL) during 30 min. The dialdehyde gradually dissolved and the evolution of oxygen was measured volumetrically after absorption of CO_2. When the reaction was complete (~8 h), the product was precipitated by the dropwise addition (30 min) of aqueous 96% ethanol (2 vol.). The gummy precipitate was washed with ethanol–water (2:1, 50 mL), and a solution in water (20 mL) was freeze-dried to yield DCA or DCC as a white powder (Table I).

The products contained $<1\%$ of NaCl and were analysed by h.p.l.c. after hydrolysis[5].

Determination of the carboxylic acid contents of DC-βCD, DCA, and DCC. — A solution of each freeze-dried product (0.5 g) in water (20 mL) was treated twice with an excess of Dowex AG50W-X8 (H^+) resin and freeze-dried in order to remove the HCl, and a solution of the residue in water (50 mL) was back-titrated with 0.1M HCl after the addition of 2.5M NaOH (1.0 mL). The results are expressed as mol of dicarboxy units.L^{-1}. The molecular weight of a dicarboxy unit is corrected for the degree of oxidation and is given by $1/0.5 \times$ content of COONa in mol.g^{-1}.

N.m.r. spectroscopy. — The ^{1}H- (400 MHz; internal ^{t}BuOH, δ_{Me} 1.2), ^{13}C- (101 MHz; internal ^{t}BuOH, δ_{Me} 31.2), and ^{17}O-n.m.r. spectra (54 MHz; external D_2O) were recorded with a Varian VXR-400 S spectrometer for solutions in D_2O. The metal-ion-induced shifts were measured after the addition of small amounts of the metal chloride (^{13}C, ^{17}O) or 0.1M $CaCl_2$ (^{1}H).

Light scattering. — Stock solutions of DCA or DCC were prepared, without prior fractionation or dialysis, in 0.5M NaCl at pH 10.8 (10 mL of aqueous 25% NH_3.L^{-1}). The dilutions (0.5–4.0 g.L^{-1}) were made with the same solution of NaCl. Samples were filtered (6 times) through 0.22 μm Millipore GVWP filters directly into the light-scattering cells.

Static light scattering was measured with a FICA 50 apparatus at λ 436 nm and angles of 30°–150° at 298 K. The refractive index increment (*dn/dc*) was determined using a Brice-Phoenix BP 2000 V differential refractometer (298 K, 436 nm). Since the polymer solutions were not dialyzed against the solvent prior to light scattering, *dn/dc* was determined at constant ionic strength rather than at constant chemical potential of the salt[12]. The correction required[13] is small for NaCl.

Dynamic light scattering was measured[14] at angles of 30°–90° at 298 K with a Spectra-Physics 2020 Ar-Laser (λ 514.5 nm). The data were evaluated by the method of cumulants[15].

Calcium complexation. — A Philips IS 561 Ca^{2+}-selective electrode, an HNU ISE-40-01-100 single-junction reference electrode, a Metrohm 654 pH meter, and a 655 dosimat (automatic burette) were used. The automatic titration was controlled by a Spectra-Physics SP 4270 integrator. The data were processed with a Lotus-1,2,3 spreadsheet.

Calibration of the electrode was performed using 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}M $CaCl_2$ buffered at pH 10 (NH_3) at ionic strength (μ) 0.02 (NaCl). A linear calibration plot was obtained up to 10^{-6}M Ca^{2+}, provided that doubly distilled water and polyethylene storage bottles were used for the 10^{-5} and 10^{-6}M solutions.

To a solution of the complexant [0.1 g.100 mL^{-1} of water, pH 10.8 (NH_3), μ_0 0.02, 61 mg of NaCl] was added 0.1M $CaCl_2$ (8 mL) at intervals of 2 min (40 additions, each of 200 μL). The activity of uncomplexed Ca^{2+} was measured before each addition.

For the calculation of the complexation constant as a function of the fraction of combined Ca^{2+}-complexing sites, a Ca^{2+}/2 COO^- stoichiometry was assumed (*see below*).

RESULTS AND DISCUSSION

Preparation of dicarboxy polysaccharides. — βCD, amylose, and cellulose were each oxidized with periodate. The dialdehyde from βCD was oxidized with sodium chlorite to yield dicarboxy-βCD (DC-βCD, Table I). The dialdehydes from amylose and cellulose were oxidized with sodium chlorite and hydrogen peroxide[5] to give dicarboxyamylose (DCA) and dicarboxycellulose (DCC), respectively, of high molecular weight in good yield (Table I).

TABLE I

Properties and characterization of DC-βCD, DCA, DCC oxydiacetate[a] (ODA), and sodium tripolyphosphate (STP)

Complexant	*COONa content (mmol. g^{-1})*	*Product composition*			*log $\bar{M}_w$*	SC[b] (*mmol.g^{-1}*)	*log K_{int}*	*Slope a*
		COONa (%)	*CHO (%)*	*CHOH (%)*				
DC-βCD	8.27	97	3	0	3.2	2.09	6.96	−2.48
DCA	6.85	74	25	1	5.0	2.39	6.68	−1.54
DCC	7.40	82	16	2	4.7	2.20	6.85	−2.30
ODA	11.24	-	-	-	-	0.24	3.90	−0.39
STP	-	-	-	-	-	2.00	-	-

[a] $HOOCH_2$-O-CH_2COOH. [b] Calcium sequestering capacity.

Calcium complexation. — An aqueous solution of each dicarboxy polysaccharide was titrated with 0.1M calcium chloride, and the uncomplexed Ca^{2+} was measured with a Ca^{2+}-ion-selective electrode. The calcium sequestering capacity (SC) is defined as the mmol of Ca^{2+} that can be added to 1 g of complexant until the concentration of non-complexed Ca^{2+} reaches 10^{-5}M (at pCa 5, the fraction of uncomplexed Ca^{2+} is only 0.5% for DCA). This value is the upper limit for Ca^{2+} during the washing process[16].

The calcium complexation curves (Fig. 1) show that, on a weight basis, DC-βCD,

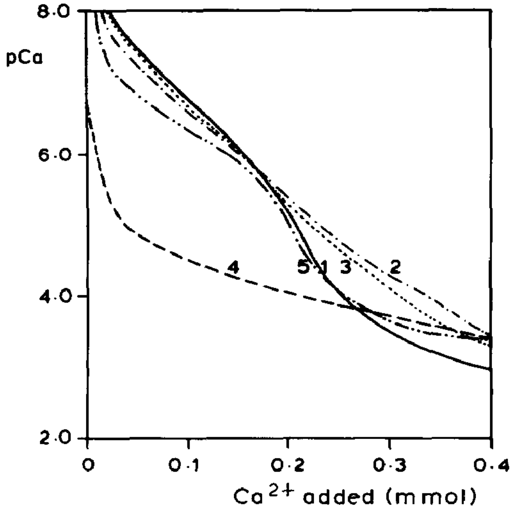


Fig. 1. Ca^{2+}-selective electrode titration curves for DC-βCD (1), DCA (2), DCC (3), oxydiacetate (ODA), (4), and tripolyphosphate (STP) (5).

DCA, and DCC are better complexants than sodium tripolyphosphate (STP, Table I) and far superior to oxydiacetate [$O(CH_2COOH)_2$, ODA, Table I].

According to the theory of multiple equilibria[17], the complexation of Ca^{2+} by the polycarboxylates, assuming that identical, electrostatically interacting complexing sites are involved, can be described by:

$$K = \frac{Z}{[\mathrm{Ca}](1-Z)} = K_{\mathrm{int}} e^{cZ},$$

where Z is the fraction of the sites in the polymer that are co-ordinated to metal ions. The intrinsic complexation constant at $Z = 0$, K_{int}, decreases exponentially on association due to decreasing electrostatic interaction of the polyelectrolyte and Ca^{2+}. Conversion into logarithmic form (with $a = c.\log e$) yields:

$$\mathrm{pCa} + \log \frac{Z}{1-Z} = aZ + \log K_{\mathrm{int}}.$$

Plots of the Ca^{2+} titration data according to this equation allow comparison of the complexation properties of the dicarboxy polysaccharides as a function of Z. Calculation of the fraction of combined sites Z was based on a complex stoichiometry of Ca^{2+} COO^-, as indicated by the Job's plots[18], given for DC-βCD in Fig. 2. In accordance with the theory, the curves are approximately linear (up to $Z = 0.8$) with slopes a and intercept log K_{int} (Fig. 3, Table I). At $Z > 0.8$, precipitation complicates the titration. The inflection in the DC-βCD curve at $Z = 0.5$ indicates a conformational transition that affects the electrostatic interaction of the polyelectrolyte and the Ca^{2+}. As expected for the 1:1 complex of ODA, the curve is approximately horizontal ($\log K = 3.90$).

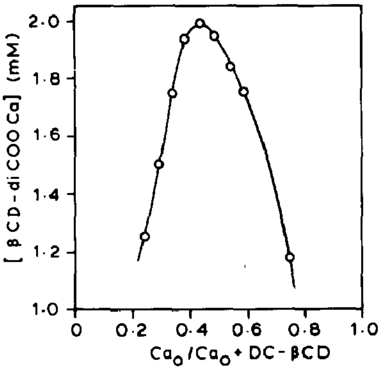


Fig. 2. Job's plot for DC-βCD. Solutions of DC-βCD (5mM dicarboxy units, pH 10.8, μ 0.02) and 5mM $CaCl_2$ were mixed at different ratios. The concentration of the complex was determined from the concentration of uncomplexed Ca^{2+} as measured with a Ca^{2+}-ion-selective electrode.

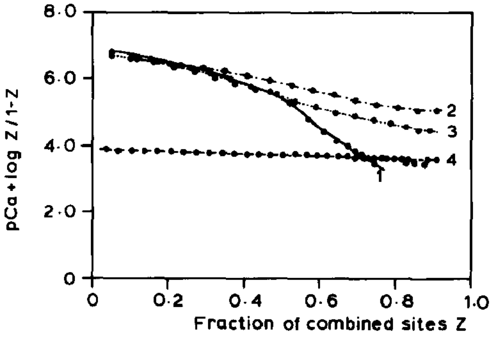


Fig. 3. Dependence of the complexation constant on the fraction of combined Ca^{2+} complexing sites Z for DC-βCD (1), DCA (2), DCC (3), and ODA (4).

Conformational effects. — (a) Complexation of Ca^{2+} and Ln^{3+} ions by DC-βDC. The number of metal-ion-coordinated polycarboxylate ligands has been determined by ^{17}O-n.m.r. spectroscopy in the presence of Dy^{3+}. By comparison of the Dy^{3+}-induced shifts of the ^{17}O water resonance in the presence and absence of DC-βCD, the number of coordinated water molecules was determined (Fig. 4) to be 0.8 at a metal:ligand ratio (ρ) of 0.10 (ρ = mol of metal ion/mol of dicarboxy units) and 1.3 at $\rho = 0.27$ (just before precipitation occurred). From the difference with the assumed Dy^{3+} coordination number[19] of 9, an effective complexation of Dy^{3+} by ~8 DC-βCD oxygens was calculated. This result implies the presence of effective coordinating sites within the DC-βCD macrocycle, since the formation of *inter*molecular complexes is entropically and electrostatically unfavourable at low metal:ligand ratios. Although the curve of Fig. 4 is not linear, its slope is identical at $\rho = 0$ and 0.3. The inflection of the S-curve around $\rho = 0.2$ might be related to a change of the conformation of the complex.

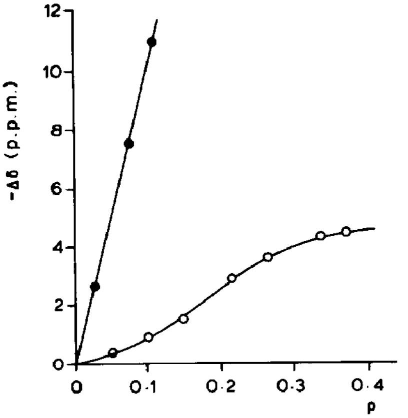


Fig. 4. Dy^{3+}-induced shifts of the ^{17}O water resonance without (●) and with 0.25M DC-βCD added (o) at pH 7.5, 303 K; ρ = mol of Dy^{3+} per mol of dicarboxy units.

The changes in chemical shifts of the ^{13}C resonances of DC-βCD upon the addition of metal ions also indicate changes in conformation. In Fig. 5, the $\Delta\delta$ values $(\delta_{\rho=\rho} - \delta_{\rho=0})$ are plotted as a function of ρ for the addition of La^{3+} and indicate a change at $\rho = 0.15$, corresponding to complexation of one La^{3+} per DC-βCD macrocycle. Addition of La^{3+} up to $\rho = 0.3$ might lead to incorporation of another La^{3+} into the macrocycle. Molecular models show that two La^{3+} ions can be accommodated inside the DC-βCD macrocycle. Folding of the molecule creates two eight-coordination sites that involve carboxylate, acetal, and 6-CH_2OH groups (Fig. 6). This hypothesis is supported by the immediate precipitation of the complex upon further addition of La^{3+} at $\rho > 0.3$, presumably via disruption of the intramolecular complex and formation of intermolecularly La^{3+}-bridged structures.

Unfortunately, relaxation studies revealed extensive exchange broadening (Yb^{3+} and Tm^{3+}) or slow exchange (Gd^{3+}), and the natures of the complexes remain to be elucidated.

With Ca^{2+} as the coordinating cation, changes in conformation were observed at $\rho = 0.25$ and 0.4–0.5 as shown by the changes ($\Delta\delta$) in chemical shifts and line-widths of the ^{13}C resonances on the addition of Ca^{2+} (Fig. 7). The ^1H-n.m.r. data also point to changes in conformation at $\rho = 0.2$–0.3 and 0.5 (Fig. 8).

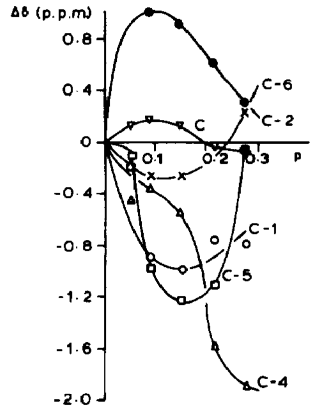


Fig. 5. La^{3+}-induced shifts of the ^{13}C resonances of DC-βCD (0.25M, pH 7.5, 303 K); $\Delta\delta = \delta_{\rho=\rho} - \delta_{\rho=0}$, ρ = mol of La^{3+} per mol of dicarboxy units.

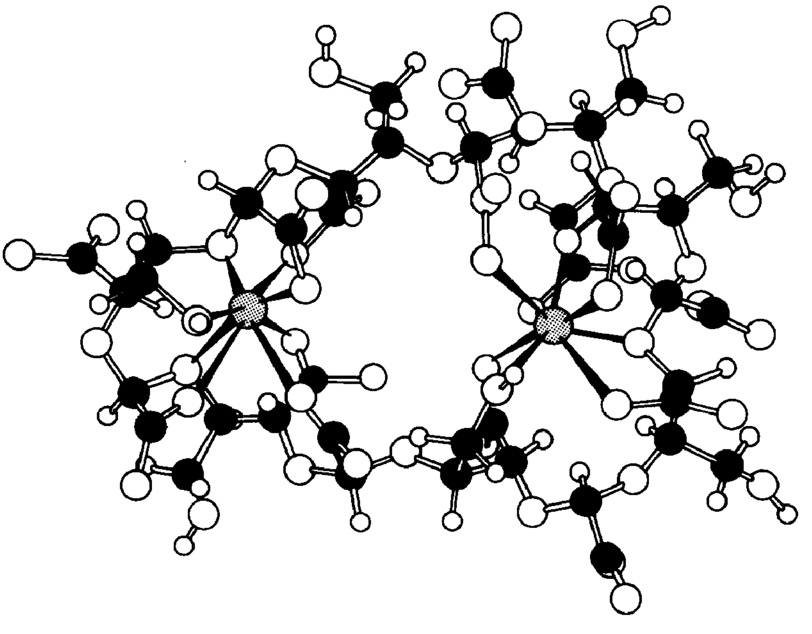


Fig. 6. Structure tentatively proposed for the $(La^{3+})_2$–DC-βCD complex.

With both La^{3+} and Ca^{2+}, the major changes were observed for C-1,4,5 resonances. These atoms of the macrocyclic structure are expected to be involved in torsional adaptation of the macrocycle to a more favourable conformation for complexation. The different location of the first inflection points (La^{3+}, $\rho = 0.15$; Ca^{2+}, $\rho = 0.25$) indicates a somewhat different geometry of the complexing site of DC-βCD for Ca^{2+} and La^{3+} ions. Nevertheless, by analogy with the complexation of Ln^{3+}ions by 8 ligand oxygens, the high complexation constant for Ca^{2+}at $Z = 0$–0.5 ($\log K = 7.0$–5.5)

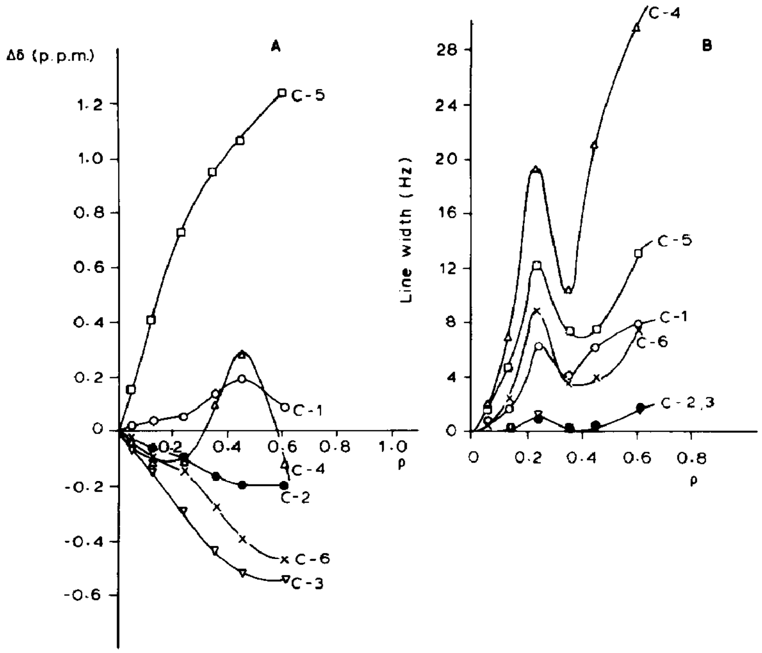


Fig. 7. Ca^{2+}induced shifts (A) and line widths (B) of the ^{13}C resonances of DC-βCD (0.25M, pH 7.5, 303 K); p = mol of Ca^{2+} per mol of dicarboxy units.

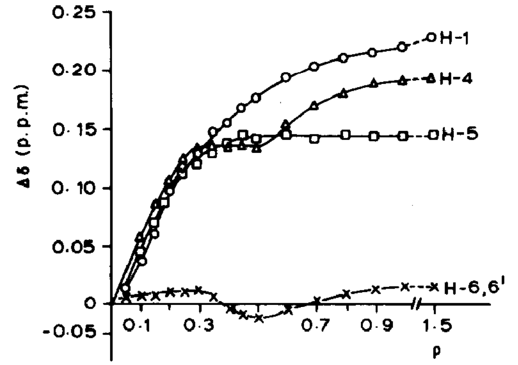


Fig. 8. Ca^{2+}-induced shifts of the ^{1}H resonances of DC-βCD (4mM, pH 7.5, 303 K); p = mol of Ca^{2+} per mol of dicarboxy units.

indicates coordination that presumably involves at least four carboxyl groups (apart from other ligands), as may be concluded from comparison with complexation constants of the 1:1 complexes of Ca^{2+} with glycol disuccinate (GDS; 4 COO^-, $\log K = 5.1$), carboxymethyl oxysuccinate (CMOS; 3 COO^-, $\log K = 4.3$), and ODA (2 COO^-, $\log K = 3.9$). The COO^- groups of the macrocycle are oriented so that complexation with Ca^{2+} is favoured, which explains the outstanding complexation of DC-βCD in

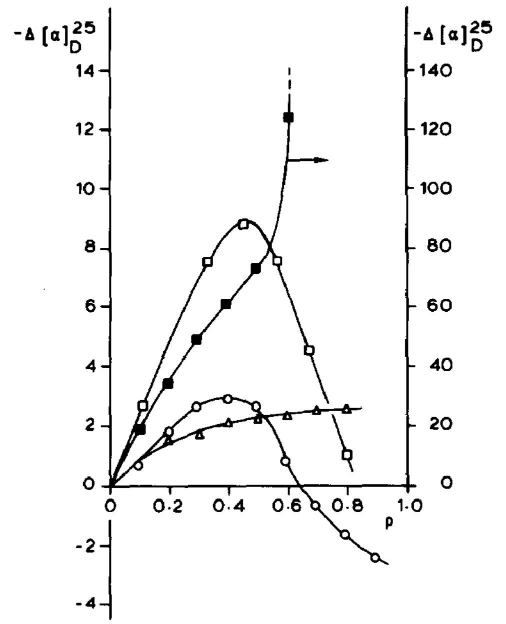


Fig. 9. Variation of the $[\alpha]_D$ values of dicarboxyglucans (0.1M, pH 10.8) upon addition of Ca^{2+}; $\Delta[\alpha]_{D,p=p} - [\alpha]_{D,p=0}$, p = mol of Ca^{2+} per mol of dicarboxy units; DC-βCD (o), DCA (□), DCC (■), and DCA upon addition of NaCl (Δ).

comparison with that of linear dicarboxyglucans of the same molecular weight. The latter can adopt electrostatically favourable extended conformations that do not have effective complexing sites.

The change in conformation at $p = 0.25$, which corresponds to one Ca^{2+} per 4 dicarboxy units, does not affect the electrostatic interaction of DC-βCD and Ca^{2+} since there is no inflection at $Z = 0.25$ in the curve of Fig. 3. The change in conformation at $p = 0.5$, on the other hand, leads to a rapid decrease of log K to values below 4 at $Z > 0.5$ and marks the transition of complexes of an average stoichiometry of $Ca^{2+}/4\,COO^-$ to a fully saturated complex with a final stoichiometry of $Ca^{2+}/2\,COO^-$.

(b) Complexation of Ca^{2+} by DCA and DCC. The changes in conformation observed for DC-βCD at $p = 0.5$ are similar to the results[8–10] for DCA and DCC at $p = 0.5$, but no conformational transitions were observed at $p = 0.25$. The differences in the chemical shifts of the H-1 resonances are identical for DCA[10] and DC-βCD (Fig. 7); however, H-1 is insensitive to the transition at $p = 0.25$.

The optical rotation can be sensitive to changes in conformation of a solute[20,21]. The difference of the optical rotation ($\Delta[\alpha]_D = [\alpha]_{D,p=p} - [\alpha]_{D,p=0}$) of DCA, DCC, and DC-βCD as a function of the amount of Ca^{2+} added is given in Fig. 9. The α-like structures DCA and DC-βCD showed a maximum for $-\Delta[\alpha]_D$ at $p = 0.4$–0.5 (but no inflection at $p = 0.25$) indicative of some similarity in conformation. The β-linked structure DCC, on the other hand, showed a ten-fold higher change and no maximum, which indicated a different change in conformation on the addition of Ca^{2+}. Apparently, the different configuration at C-1 induces a relatively unfavourable conformation for complexation of Ca^{2+} as compared to DCA. However, the energy barrier for the formation of complexes by DCC is sufficiently low to allow good complexation.

In contrast to DC-βCD, both DCA and DCC retain a high complexation constant at $Z > 0.5$ (*e.g.*, Fig. 3, $Z = 0.75$; DC-βCD, $\log K = 3.5$; DCA, $\log K = 5.2$). This phenomenon is probably related to the reduced translational and rotational freedom of the DC-βCD macrocycle as compared to that of the backbone of DCA and DCC which apparently hampers the formation of complexing sites on saturation with Ca^{2+}.

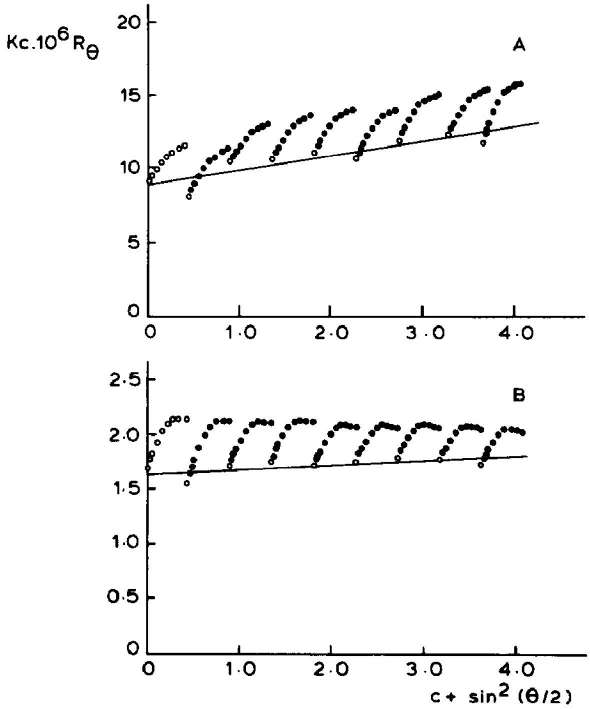


Fig. 10. Zimm plots (A, $\rho = 0$; B, $\rho = 0.65$) of the static light-scattering measurements of DCA in 0.5M NaCl (pH 10.8 at 298 K), ρ = mol of Ca^{2+} per mol of dicarboxy units.

For a further comparison of DCA and DCC, preliminary experiments on static and dynamic light scattering were performed both in the presence and absence of Ca^{2+}. From the static Zimm plots (Fig. 10) of DCA (0.5–4.0 g.L^{-1}, scattering angles 30°–150°) without and with Ca^{2+} [$\rho = 0.40$ and 0.65 (0.10 below the ρ value where precipitation occurred)], the molecular weight ($\overline{M}_w$), radius of gyration (R_g), and second virial coefficient (A_2) were calculated (Table II). Results from a similar experiment with DCC [$\rho = 0$ and 0.40 (0.1 before precipitation)] are included in Table II. The six-fold increase of $\overline{M}_w$ for DCA on the addition of Ca^{2+} ($\rho = 0.65$) points to the formation of aggregates.

TABLE II

Molecular weight ($\overline{M}_w$, radius of gyration (R_g), second virial coefficient (A_2), and refractive index increment (dn/dc) of DCA and DCC obtained from static light-scattering by the Zimm plot method

Dicarboxyglucan	$\overline{M}_w$	R_g (*nm*)	A_2 (*mol.mL.g*$^{-2}$)	dn/dc (*mL.g*$^{-1}$)
DCA	9.8×10^4	30	2.95×10^{-4}	0.133
DCA + Ca^{2+}, $\rho = 0.40$	2.5×10^5	39	1.52×10^{-4}	0.088
DCA + Ca^{2+}, $\rho = 0.65$	6.0×10^5	41	2.30×10^{-5}	0.056
DCC	5.2×10^4	48	7.54×10^{-4}	0.131
DCC + Ca^{2+}, $\rho = 0.40$	7.5×10^4	47	-8.33×10^{-4}	0.088

Association is observed well before complete saturation of the polycarboxylate with Ca^{2+}, *i.e.*, when considerable interchain electrostatic repulsion still exists. With DCC, chain association occurred to a much lesser extent (50% increase in $\overline{M}_w$). The negative value of A_2 for DCC ($\rho = 0.40$) indicates poor solvation of the complexed polycarboxylate, with substantial interaction of segments of the polymer chain. The significant change of the refractive index increment upon addition of Ca^{2+} is indicative of a change in conformation of the macromolecule. A similar effect was found for the acidic polysaccharide of the marine alga *Emiliana huxleyi*[22].

Experiments on dynamic light scattering were performed with DCA (1.0 g.L^{-1}) at ρ in the range 0–0.88. The translational diffusion coefficient (D) and the hydrodynamic radius (R_h, calculated from D by application of the Stokes–Einstein equation) are given in Table III. On the addition of Ca^{2+}, R_h decreased from 12.3 ($\rho = 0$) to 8.7 nm ($\rho = 0.88$), and indicated a contraction of the polyelectrolyte.

In principle, information on the size and shape of the scattering particle can be obtained from an analysis of the angular dependence of the scattering function in the Zimm plots. The rather low $\overline{M}_w$ of DCA and DCC, however, does not allow such detailed evaluation of the present results[23]. Nevertheless, interpretation of the data provides some qualitative information on the changes in conformation upon complexation.

TABLE III

Diffusion coefficient (D) and hydrodynamic radius (R_h) of DCA at various calcium concentrations (ρ = mol of Ca^{2+} per mol of dicarboxy units) as obtained by dynamic light-scattering at 298 K

ρ	D ($\times 10^7$) ($cm^2 \cdot s^{-1}$)	R_h (*nm*)
0.00	2.00	12.3
0.22	2.53	9.70
0.44	2.71	9.05
0.66	2.77	8.86
0.88	2.82	8.70

In the absence of Ca^{2+} and at the ionic strength applied (0.5M NaCl), DCA and DCC are each expected to be present in a random coil conformation. On the addition of Ca^{2+}, the R_h gradually decreases for DCA, which indicates contraction of the coils in accordance with a decreasing electrostatic repulsion between the segments of the polymer. The six-fold increase of $\overline{M}_w$ as calculated from the Zimm plots points to intermolecular association of the Ca^{2+}-polycarboxylates. The considerable changes of the conformation and dimensions required for simultaneous increase of $\overline{M}_w$ and decrease of R_h is indicated by the change of the structure-sensitive parameter[24] R_g/R_h from 2.43 ($\rho = 0$) to 4.61 ($\rho = 0.65$). The formation of helices, as suggested by Nieuwenhuizen *et al.*[3], is improbable, since this would require R_h to increase sharply upon complexation with Ca^{2+}.

ACKNOWLEDGMENTS

Light-scattering measurements were performed at Leiden University (The Netherlands) with the kind cooperation of Drs. F. A. Varkevisser and C. J. Bloys van Treslong. We thank Professor T. Odijk (Delft University of Technology) for helpful discussion and the Netherlands Technology Foundation (STW) under the auspices of the Netherlands Organization for Scientific Research (NWO) for financial support.

REFERENCES

1 I. A. Eldib, U. S. Pat. 3,629,121 (1971); *Chem. Abstr.*, 76 (1972) 115 231; S. C. Bright, V. Lamberti, and P. J. Powers, Br. Pat. 1,330,122 (1973); *Chem. Abstr.*, 80 (1974) 61392; S. C. Bright, V. Lamberti and P. J. Powers, Br. Pat. 1, 330, 123 (1973) *Chem. Abstr.*, (1974) 61393.
2 M. Diamantoglou, H. Mägerlein, and R. Zielke, *Tenside Deterg.*, 14 (1977) 250–256.
3 M. S. Nieuwenhuizen, A. P. G. Kieboom, and H. van Bekkum, *Staerke*, 37 (1985) 192–200.
4 G. Tegge, *Staerke*, 12 (1960) 321–325.
5 M. Floor, L. P. M. Hofsteede, W. P. T. Groenland, L. A. Th. Verhaar, A. P. G. Kieboom, and H. van Bekkum, *Recl. Trav. Chim. Pays-Bas* 108 (1989) 384–392.
6 M. S. Nieuwenhuizen, A. P. G. Kieboom, and H. van Bekkum, *J. Am. Oil Chem. Soc.*, 60 (1983) 120–124.
7 M. S. Nieuwenhuizen, A. P. G. Kieboom, and H. van Bekkum, *Tenside Deterg.*, 22 (1985) 247–251.
8 B. Casu, S. V. Meille, A. Naggi, P. Su, G. Torri, G. Zoppetti, and G. Allegra, *Carbohydr. Polym.*, 2 (1980) 283–287.
9 B. Casu, U. Gennaro, S. V. Meille, M. Morrone, A. Naggi, M. S. Occhipinti, and G. Torri, *Int. J. Biol. Macromol.*, 6 (1984) 89–92.
10 V. Crescenzi, M. Dentini, C. Meoli, B. Casu, A. Naggi, and G. Torri, *Int. J. Biol. Macromol.*, 6 (1984) 142–144.
11 J. A. Peters and A. P. G. Kieboom, *Recl. Trav. Chim. Pays-Bas* 102 (1983) 381–392.
12 M. Nagasawa and A. Takahashi, in M. B. Huglin, (ed.), *Light Scattering from Polymer Solutions*, Academic Press, London, 1972, pp. 671–723.
13 T. A. Orofino, and P. J. Flory, *J. Phys. Chem.*, 63 (1959) 283–290.
14 S. Sasaki and M. Mandel, *J. Phys. E.*, 17 (1984) 738–740.
15 D. E. Koppel, *J. Chem. Phys.*, 57 (1972) 4814–4820.
16 M. W. Hollingworth, *J. Am. Oil Chem. Soc.*, 55 (1978) 49–51.
17 C. Tanford, *Physical Chemistry of Macromolecules*, Wiley, New York, 1961, pp. 526–586.
18 P. Job, *Ann. Chim. (Paris)*, 9 (1928) 113–203.

19 C. A. M. Vijverberg, J. A. Peters, A. P. G. Kieboom, and H. van Bekkum, *Recl. Trav. Chim. Pays-Bas*, 99 (1980) 403–409.
20 R. Kohn, *Pure Appl. Chem.*, 42 (1975) 371–397.
21 D. A. Rees, *Pure Appl. Chem.*, 53 (1981) 1–14.
22 A. H. Borman, D. J. Kok, E. W. de Jong, P. Westbroek, F. A. Varkevisser, C. J. Bloys van Treslong, and L. Bosch, *Eur. Polym. J.*, 22 (1986) 521–523.
23 P. Kratochvíl, ref. 12, pp. 333–384.
24 W. Burchard, M. Schmidt, and W. H. Stockmayer, *Macromolecules*, 13 (1980) 1265–1272.