BINDING OF CALCIUM IONS TO 2,3-DICARBOXY DERIVATIVES OF STARCH AND AMYLOSE

Rudolf KOHN and Karol TIHLÁRIK

Institute of Chemistry, Centre of Chemical Research,
Slovak Academy of Sciences, 842 38 Bratislava

A series of 2,3-dicarboxy derivatives of oxidation degree $DO_{(d.e.)}$ 0·13 to 0·90 was prepared by a two-stage oxidation of corn starch and amylose. The activity coefficient $\gamma_{Ca^{2+}}$ of calcium counterions was estimated in solutions of calcium salts of these carboxy derivatives in a 3·00 mmol $(COOCa_{0.5})\,l^{-1}$ concentration by a metallochromic indicator (tetramethylmurexide) method. A loss of electrostatic free enthalpy, $\Delta(G_{el}/N)_K^{Ca}$, due to the exchange of $Ca^{2+} \rightarrow 2\,K^+$ cations was determined by potentiometric titrations of the investigated polyacids and pectinic acids of various linear charge density of the macromolecule employing potassium and calcium hydroxides. 2,3-Dicarboxy derivatives of starch and amylose show a considerably strong binding of calcium ions even at a low oxidation degree of the starting polysaccharides in contrast to solutions of the corresponding pectinates and carboxymethyl derivatives of cellulose and amylose. The strong bond of calcium ions to 2,3-dicarboxy derivatives of starch and amylose is ascribed to a favourable spatial arrangement of two neighbouring carboxyl groups at $C_{(2)}$ and $C_{(3)}$ of the oxidized D-glucose units due to a flexibility of the macromolecule chain at sites of the pyrane ring cleavage.

Carboxy derivatives of starch and amylose, similarly as carboxymethyl derivatives of these polysaccharides, are widely used in chemical and food industries, and in medicine. Recently, these derivatives were found useful as sequestration components of detergents. Carboxy derivatives of starch can here substitute polyphosphates, which display an extraordinarily good sequestration effect, but, nevertheless, they contribute to entrophy of waste-water (*e.g.* ref.[1-3]). Another feature of these starch derivatives is their bactericide and antiviral effect (*e.g.* ref.[4-7]). Their calcium salt is recommended for curing hypocalcemia of animals[8]. Carboxy derivatives of starch are effective sorbents of ammonia and urea in curing uremia[9,10], components of drugs enhancing sorption of antibiotics[11] *etc.*

2,3-Dicarboxy derivatives of starch and amylose are important among other carboxy derivatives of starch, since they are suitable models for theoretical studies of cation binding to polyelectrolytes. The macromolecule chain becomes fairly flexible as a result of cleavage of the pyrane ring between $C_{(2)}$ and $C_{(3)}$ of the oxidized D-glucose unit analogously, as found with periodate oxidation of alginate molecule causing its flexibility in solution[12]. Carboxyl groups at a various oxidation

degree of starch are always present as couples which can approach each other very closely due to flexibility of the macromolecule in contrast to other natural polyelectrolytes (pectic substances, alginates). This particular arrangement of carboxyl groups can, however, markedly influence also the binding and exchange of cation of the respective carboxy derivatives of polysaccharides.

2,3-Dicarboxy derivatives of starch and amylose are prepared by a selective oxidation of these polysaccharides with periodic acid or its salts *via* corresponding 2,3-dialdehyde, which undergoes further oxidation with sodium chlorite to furnish the desired products. Physicochemical properties of these compounds have only sparingly been reported as yet. According to Casu and coworkers[13], the Ca^{2+} ions are bound to dicarboxyamylose in form of several complexes of various composition, the most stable of which comprises 4 carboxyl groups per one Ca^{2+} counterion. The ^1H NMR spectra of dicarboxyamylose (DCA) and dicarboxycellulose (DCC) show[14] that the ionization change of carboxyl groups leads to a change of conformation of the polyelectrolyte chain, at least at level of the $C_{(4)}H/C_{(5)}H$ dihedral angle. The ^1H NMR titration curve of sodium DCC with Ca^{2+} ions displays an inflection after addition of roughly one Ca^{2+} ion per dicarboxylic monomeric unit[14]. Like results obtained Crescenzi and coworkers[15] who ascertained by a microcalorimetric method and CD spectra that the Ca^{2+} binding to DCA and DCC resulted in conformation change of the polyelectrolyte chain.

This paper concerns the binding of calcium ions to 2,3-dicarboxy derivatives of starch and amylose of various oxidation degrees. The Ca^{2+} ion binding is considered according to both activity coefficients of calcium counterions $\gamma_{Ca^{2+}}$ bound to carboxyl groups and change of the electrostatic free enthalpy associated with the exchange of $Ca^{2+} \rightarrow 2 K^+$ cations on polyelectrolytes under study.

EXPERIMENTAL

Materials

The starting materials were: corn starch (Slovenské škrobárne, Boleráz), amylose (a technical product of AVEBE, Holland) and citrus pectin (Genu Pectin, Medium Rapid Set, Pektinfabrik, Copenhagen, Denmark). The amylose was twice precipitated from dimethyl sulfoxide *via* a butanol complex, pectin was purified by washing with acidified 60% ethanol (5 ml of concentrated HCl in 100 ml of 60% ethanol), 60% and 90% ethanol as already reported[16].

The 0·05M-KOH and 0·05M-NaOH were carbonate-free; further were employed: a saturated 0·021M-Ca(OH)$_2$ solution, 0·01M-Complexon IV, tetramethylmurexide synthesized in our Laboratory[16,17], and redistilled atmospheric carbon dioxide free water.

Starch and Amylase 2,3-Dialdehydes

Solution of NaIO$_4$ (15 g) in water (200 ml) was successively added to a stirred suspension of an air-dried starch (10 g) in water (100 ml). After a 3 h-stirring the solid was filtered off and washed

untill the absence of of IO_3^- ions. The product was disperged in acetone, washed with the same solvent and dried in the air for 24 h and then at 70°C and water pump vacuum for 5 h. Yield 9 g; the aldehyde group content corresponded to 98% of the calculated value.

Preparations with a lower content of aldehyde groups were obtained by oxidation of the starting polysaccharides at a suitably chosen ratio of the reacting components, *i.e.* starch and periodate. The same procedure was applied for preparation of amylose 2,3-dialdehydes of various oxidation degrees.

Starch and Amylose 2,3-Dicarboxy Derivatives

Sodium chlorite (15 g) in water (50 ml) was added to a suspension of starch dialdehyde (5 g) in water (50 ml) and the mixture was kept at about 10°C. At this temperature 50% acetic acid (10 ml) was successively added and after 30 min the pH of the mixture was adjusted by addition of acetic acid to approximately 3·3. The mixture was left to react for 3 h, gaseous products were removed by airing with nitrogen for 30 min, and the dicarboxy derivative was precipitated by addition of a two-fold volume of methanol, filtered off, dissolved in a little amount of water, and the carboxyl groups were neutralized with sodium hydroxide. The solution was then dialyzed against distilled water for 24 h, concentrated and the dicarboxy derivative of starch was precipitated by addition of methanol. The preparation was filtered off, washed with methanol, diethyl ether, and dried at 40° and water pump vacuum for 5 h; yield 4·1 g.

Preparations with a lower content of carboxyl groups were prepared in an analogous way from the respective starch dialdehyde using a properly reduced amount of sodium chlorite. Amylose 2,3-dicarboxy derivatives of a lower oxidation degree were obtained by the same procedure. All carboxy derivatives were prepared as sodium salts.

Pectin of Various Esterification Degree

Pectin preparations of various esterification degrees E with a random distribution pattern of free carboxyl groups in the molecule were obtained by a partial alkaline deesterification of highly esterified citrus pectins in suspension in 60% ethanol. Highly esterified pectins (E 92 and 95%) were prepared from a purified pectin with methanolic $1M$-H_2SO_4 at +3°C for 3 weeks. Sample No 14 (Table II) is the purified commercial preparation of citrus pectin.

Analytical Methods

The aldehyde group content in starch and amylose dialdehyde derivatives was determined with hydroxylammonium chloride and alkalimetric estimation of the freed hydrochloric acid[18].

Free carboxyl groups in starch and amylose carboxy derivatives were determined alkalimetrically. Sodium salts of dicarboxy derivatives were transformed to corresponding polyacids by percolation of their solutions through cation exchanger (Dowex 50W2; H^+) and titrated potentiometrically with $0·05M$-KOH. The total content of carboxyl groups was determined analogously after opening the lactones present in the preparations by a little excess of hydroxide in 2—5 mmol NaOH l^{-1} medium at room temperature for 18 h. Excess of the hydroxide was removed by percolation of the solution through column of the afore-mentioned cation exchanger. The resulting polyacid was potentiometrically titrated with $0·05M$-KOH; all samples become water-soluble after treatment with alkali.

Procedures already reported[16] were employed for analysis of pectin (determination of the esterification degree E, of the polyuronide content in dry matter, of the limit viscosity number $[\eta]$). The limit viscosity number $[\eta]$ of starch and amylose carboxy derivatives was determined with Ubbelohde viscometer at 25·0°C in the $0·15M$-NaCl—$0·005M$-sodium oxalate medium.

The change of electrostatic free enthalpy, associated with the exchange of $Ca^{2+} \rightarrow 2\ K^{+}$ cations in starch and amylose carboxy derivatives and in pectin was determined by potentiometric titrations of the respective polyacids at a $3\cdot00$ mmol $(COOH)\ l^{-1}$ concentration with $0\cdot05M\text{-}KOH$ and $0\cdot021M\text{-}Ca(OH)_2$. The course of function $pH = f(DN)$ was plotted for both titrations; DN stands for the degree of neutralization. The change of the electrostatic free enthalpy of ion exchange $(\Delta(G_{el}/N)_K^{Ca};\ J\ site^{-1})$ is given by the area limited by both titration curves[19]. The area was determined planimetrically, solutions were titrated at $25 \pm 2°C$.

Activities of calcium ions $a_{Ca^{2+}}$ and their activity coeffcient $\gamma_{Ca^{2+}}$ in solutions of calcium starch and amylose 2,3-dicarboxylates were determined by the metallochromic indicator method (tetramethylmurexide) by a procedure already described[16,17]. Solutions of sodium salts of the dicarboxy derivatives investigated at a $c.$ 5 mmol $(COONa)\ l^{-1}$ concentration were transformed into the corresponding polyacids by passing through the cation exchanger and neutralized with calcium hydroxide to the point of equivalence. If the calcium salt precipitated during the neutralization process, the suspension was centrifuged at $20\ 000g$ for 15 min. The total content of calcium was determined in the supernatant, which was employed at the same time for activity $a_{Ca^{2+}}$ determination, by a chelatometric titration with Complexon IV and photometric indication of the point of equivalence (interference filter Zeiss, Jena, IF 600 nm, indicator murexide). Activity of calcium ions was determined in solutions of $3\cdot00$ mmol $(COOCa_{0.5})\ l^{-1}$ concentration without addition of an auxilliary electrolyte unless stated otherwise.

Further were used: pH-meter PHM 64, glass electrode, type 222B, saturated calomel electrode, type K 401 Radiometer (Denmark). Spectra of circular dichroism were recorded with a Dichrographe III, Jobin Yvon (France).

RESULTS AND DISCUSSION

Characterization of Starch and Amylose 2,3-Dicarboxy Derivatives

Samples of starch and amylose 2,3-dicarboxy derivatives of various content of carboxyl groups were prepared by a total oxidation of the respective samples of dialdehydes of these polysaccharides. Table I lists the mean oxidation degree $DO_{(d.c.)}$, which reflects the proportion of dicarboxylic monomeric units of the total number of saccharide units and the content of carboxyl groups in millimoles per 1 g of the sample. Symbol a refers to free carboxyl groups, symbol b to their total content. The circular dichroic spectra, measured in the $230 - 380$ nm region evidenced that the investigated dicarboxy derivatives do not contain any aldehyde group. The difference between the content of total carboxyl groups (b) and free carboxyl groups (a) corresponds to the presence of lactones, formed during preparation of dicarboxy derivatives. Excepting sample No 1, the content of lactone is within 5 to 15% of the total carboxyl groups.

Fig. 1 describes the dependence of the total carboxyl group content $(mmol\,(COOH)$ $g^{-1})$ of starch 2,3-dicarboxy derivatives $(Na^{+}$ form) upon the oxidation degree of the starting starch dialdehyde $(DO_{(d.a.)})$. Curve 1 expresses the theoretical dependence, curve 2 shows values determined experimentally. Content of carboxyl groupp is somewhat greater than the theoretical value, however, at the lowest oxidation

TABLE I
Binding of Ca^{2+} ions to 2,3-dicarboxy derivatives of starch and amylose

Sample No	$DO_{(d.c.)}$ a	b	$[\eta]$ ml g⁻¹ a	mmol (COOH) g⁻¹ a	b	$\gamma_{Ca^{2+}}$ a	γ a	$\Delta(G_{el}/N)_K^{Ca}$ J site⁻¹ a	b
				2,3-Dicarboxy derivatives of starch					
1	0.090	0.129	38	1.08 ± 0.00	1.51 ± 0.07	0.155 ± 0.001	0.175		−4 630 ± 130
2	0.234	0.260	28	2.60 ± 0.04	2.86 ± 0.07	0.103 ± 0.004	0.113		−5 650 ± 60
3	0.37	0.43	24	3.89 ± 0.05	4.42 ± 0.15	0.067 ± 0.003	0.073		−6 990 ± 320
4	0.55	0.68	22	5.40 ± 0.16	6.41 ± 0.09	0.055 ± 0.002	0.059		−7 690 ± 100
5	0.74	0.90	14	6.80 ± 0.08	7.85 ± 0.14	0.031 ± 0.005	0.033		−8 820 ± 60
				2,3-Dicarboxy derivatives of amylose					
6	c	0.23	—	—	2.59 ± 0.05	—	—	—	−4 080 ± 0
7	0.34	0.36	46	3.64 ± 0.12	3.82 ± 0.04	0.106 ± 0.009	0.118	−5 540 ± 120	−5 370 ± 310
8	0.45	0.52	28	4.58 ± 0.09	5.20 ± 0.22	0.093 ± 0.010	0.103	−6 630 ± 140	−6 110 ± 0
9	0.53	0.59	13	5.24 ± 0.05	5.74 ± 0.08	0.116 ± 0.006	0.128	−7 840 ± 140	−7 280 ± 110
10	0.67	0.74	10	6.36 ± 0.14	6.80 ± 0.05	0.112 ± 0.009	0.127	−8 800 ± 220	−8 710 ± 10

a,b See the text; c sample partially soluble, formation of a gel-like suspension.

degrees of starch dialdehyde only. Results in Fig. 1 evidence, in line with the CD spectra, that aldehyde groups were quantitatively oxidized to carboxyl groups.

The limit viscosity numbers $[\eta]$ determined in solutions of sodium salts of starch and amylose 2,3-dicarboxy derivatives (Table I) have very low values. The limit viscosity number $[\eta]$ drops strongly both with starch and amylose derivatives with the increase of oxidation degree. Low $[\eta]$ values can be due to a considerable enhancement of flexibility of the macromolecule chain after periodate oxidation even at a very low oxidation degree of the polysaccharide[12], as well as to cleavage of the macromolecule to shorter chains. Since carboxy derivatives of polysaccharides with a high linear charge density are involved, the linear segments of the starch molecule and the amylose chains are more or less stretched. It is, therefore, our opinion that the very low $[\eta]$ values indicate with a highest probability a considerable degradation of the macromolecule chain upon oxidation of the polysaccharide.

Opening of the lactone ring by alkali metal hydroxide was investigated in 0·1 to 0·15% solutions of starch and amylose 2,3-dicarboxy derivatives with 2·5 — 23 mM--NaOH (pH 11·2 — 12·3) at an ambient temperature for 18 h. Content of total carboxyl groups determined after opening of the lactone ring was found to be equal for all concentrations of hydroxide examined. The lactone ring should be opened at a lowest possible excess of the hydroxide (pH 11·3), because of the instability of 2,3-dicarboxy derivatives in alkaline medium. Degradation of the macromolecule of these derivatives takes place at pH > 11·5, namely at pH $\geq$ 12·0, this being manifested by a decrease of ability of these degradation products to bind calcium cations.

Degradation of starch and amylose 2,3-dicarboxy derivatives associated with a higher concentration of the sodium hydroxide can be identified according to some deformation of the potentiometric titration curve of neutralization of the carboxy derivatives with calcium hydroxide. Fig. 2a shows the change of course of the potentiometric titration curves of neutralization of 2,3-dicarboxyamylose (sample No 7, Table I) at a 5·5 mmol $(COOH)\, l^{-1}$ concentration with calcium hydroxide as evoked by treatment with sodium hydroxide. Opening of the lactone ring proceeded in 2·5, 5, 9 and 16 mmol $(NaOH)\, l^{-1}$ for 18 h (curves 1, 2, 3, 4). The course of neutralization of the original sample was virtually identical with curve 1. Fig. 2b traces the neutralization course of 2,3-dicarboxystarch (sample No 3, Table I) with calcium hydroxide in 6·0 and 4·8 mmol $(COOH)\, l^{-1}$ solutions (curves 6, 7) after treatment with 23 mmol $(NaOH)\, l^{-1}$. Curve 5 depicts the titration course of the original sample at approximately the same concentration. The activity coefficients $\gamma_{ca^{2+}}$ 0·070, 0·135 and 0·239 corresponding to curves 5, 6, 7 determined in 3·00 mmol $(COOCa_{0.5})\, l^{-1}$ solutions prove that the destruction of macromolecule is manifested by a substantially higher values of activity coefficients $\gamma_{Ca^{2+}}$ thus demonstrating a weaker binding of Ca^{2+} ions to carboxyl groups of the corresponding polysaccharides. Degradation of 2,3-dicarboxy derivatives of the investigated polysaccharides with alkali further depends, at a given pH on the ratio of the reacting components. At a higher concentration

of the alkali metal hydroxide $(pH \geqq 12\cdot0)$ a cleavage of the macromolecule occurs to give shorter chains, where the activity of calcium counterions already becomes a function of the chain length. The lactone ring of carboxy derivatives under study was, therefore, opened in further experiments at $pH \sim 11\cdot3$.

Binding of Ca^{2+} Ions to Starch 2,3-Dicarboxy Derivatives

Binding of Ca^{2+} ions to 2,3-dicarboxystarch of various oxidation degree was considered according to both activity coefficient of counterions Ca^{2+} $(\gamma_{Ca^{2+}})$ bound to free carboxyl groups of the original derivatives (a), and change of the electrostatic free enthalpy $(\Delta(G_{el}/N)_K^{Ca}, J \text{ site}^{-1})$ associated with the $Ca^{2+} \rightarrow 2\,K^{+}$ cation exchange of samples (b) having the content of lactones eliminated by the action of alkali. Value $\Delta(G_{el}/N)_K^{Ca}$ refers to 1 mol of carboxyl groups as binding sites. Results are

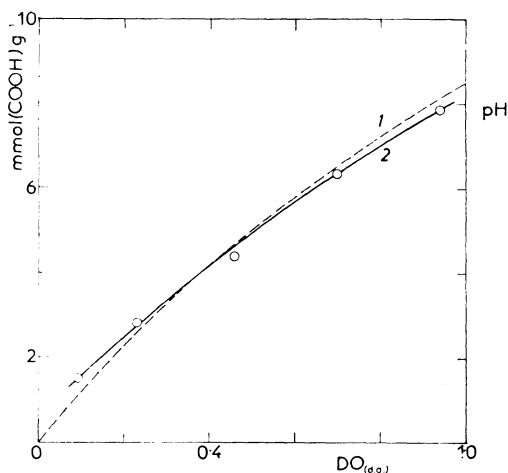
Fig. 1

The carboxyl group content in samples of 2,3-dicarboxystarch prepared from starch dialdehydes of various oxidation degree $DO_{(d.a.)}$. *1* The theoretical course of the function, *2* values experimentally determined
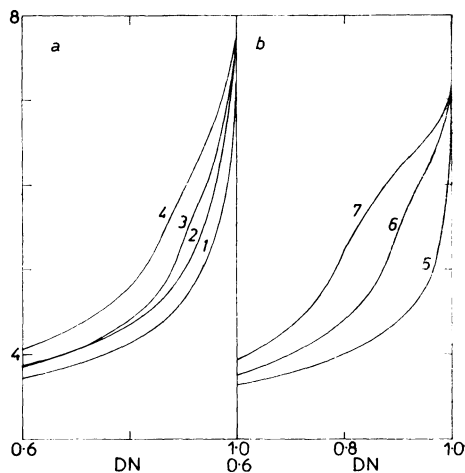
Fig. 2

Neutralization of 2,3-dicarboxy derivatives of amylose and starch with calcium hydroxide. The effect of alkaline medium on lactone ring opening upon degradation of carboxy derivatives of polysaccharides. *DN* neutralization degree; *a* 2,3-dicarboxyamylose (sample No 7, Table I); *b* 2,3-dicarboxystarch (sample No 3, Table I); *1, 2, 3, 4* 5·5 mmol (COOH) . $.\,l^{-1}$, medium 2·5, 5, 9, 16 mmol (NaOH) . $.\,l^{-1}$; *5* original sample, 5·0 mmol (COOH) . $.\,l^{-1}$; *6, 7* 6·0, 4·8 mmol (COOH) l^{-1}, medium 23 mmol (NaOH) l^{-1}

summarized in Table I. The activity coefficient $\gamma_{Ca^{2+}}$ decreases with the increasing oxidation degree and reaches markedly low values ($\gamma_{Ca^{2+}} = 0.031$) with derivatives with the highest content of carboxyl groups indicating a very strong binding of calcium ions to the polyelectrolyte. Similarly, an increase of oxidation degree is reflected with an enhanced loss of the electrostatic free enthalpy this documenting an increase of the high selectivity of 2,3-dicarboxy derivatives of starch towards calcium ions at a $Ca^{2+} \rightarrow 2\,K^+$ cation exchange.

2,3-Dicarboxy derivatives of starch represent a special case of a polyelectrolyte, where two carboxyl groups at $C_{(2)}$ and $C_{(3)}$ can approach each other very closely due to flexibility of the macromolecule chain resulting from the opening of the pyrane ring by periodate oxidation. To evaluate better the influence of such a spatial arrangement of carboxyl groups on the binding of calcium ions the exchange of $Ca^{2+} \rightarrow$ $\rightarrow 2\,K^+$ cations was examined with pectin preparations of various esterification degrees, too. This acid polysaccharide has not carboxyl groups in couples spatially close to each other, but they are randomly distributed along the linear macromolecule chain. A random distribution of free carboxyl groups in the molecule was achieved by a partial alkaline deesterification of highly esterified preparations of pectin. Pectin preparations are characterized in Table II. The term "polyuronide" refers to D-galacturonan partially esterified with methanol, E stands for esterification degree of carboxyl groups with methanol, substitution degree $DS_{(m.c.)}$ describes the proportion of D-galacturonic acid units with free (not esterified) carboxyl groups (m.c. monocarboxy derivative). The investigated pectin samples were examined for the $Ca^{2+} \rightarrow 2\,K^+$ cation exchange, characterized by the value $\Delta(G_{el}/N)_K^{Ca}$. The dependence of the activity coefficient $\gamma_{Ca^{2+}}$ in solutions of calcium pectinates upon their esterification degree E was elucidated earlier[20].

TABLE II

Binding of Ca^{2+} ions to pectin of various esterification degree E

Sample No	Polyuronide content %	$[\eta]$ ml g^{-1}	E %	$DS_{(m.c.)}$	$\Delta(G_{el}/N)_K^{Ca}$ J site^{-1}
11	83	117	0.0	1.00	$-3\,090 \pm 40$
12	86	211	20.6	0.79	$-2\,500 \pm 80$
13	84	327	46.1	0.54	$-2\,010 \pm 180$
14	86	503	63.7	0.36	$-1\,060 \pm 10$
15	91	232	86.1	0.14	-130 ± 70
16	91	158	91.7	0.08	[a]

[a] Within an experimental error range.

Pectin preparations revealed much higher values of limit viscosity number $[\eta]$ than starch and amylose 2,3-dicarboxy derivatives. As shown in our previous papers[21,22], the high relative molecular mass $(\overline{M}_r)$ of these pectin preparations is so high that the interaction of Ca^{2+} ions with carboxyl groups is here no more the function of chain length of the macromolecule. Selectivity of exchange of $Ca^{2+} \rightarrow 2 K^+$ cations decreases with the decreasing substitution degree $DS_{(m.c.)}$, i.e. with the increasing distance of neighbouring free carboxyl groups in the pectin molecule. Samples of highly esterified pectin (E 86·1 and 91·7%) are virtually no more Ca^{2+} ions selective.

To compare the selectivity of exchange of these cations with 2,3-dicarboxystarch and pectin, the dependence of $\Delta(G_{el}/N)_K^{Ca}$ on the mean oxidation degree $DO_{(d.c.)}$ of saccharide units of starch, or on the substitution degree $DS_{(m.c.)}$ of pectin with free carboxyl groups is presented. (The content of carboxyl groups of both investigated polysaccharides should be expressed equally; thus, for 2,3-dicarboxystarch $DS_{(m.c.)}$ $= DO_{(d.c.)} \times 2$). 2,3-Dicarboxy derivatives of starch (Fig. 3, curve 1) have a considerably higher selectivity of $Ca^{2+} \rightarrow 2 K^+$ ion exchange than pectin preparations (curve 2) of the same content of carboxyl groups in the molecule. E.g. the sample of 2,3-dicarboxystarch of the lowest oxidation degree $(DO_{(d.c.)} = 0·129)$ having the distribution of carboxyl groups expressed in Scheme I in Fig. 4 shows even greater selectivity of cation exchange $(\Delta G_{el}/N)_K^{Ca} = -4\ 630$ J site$^{-1})$ than the sample of fully deesterified pectin $(\Delta(G_{el}/N)_K^{Ca} = -3\ 090$ J site$^{-1})$, Scheme II.

A like difference in affinity of carboxyl groups towards Ca^{2+} counterions displays the dependence of activity coefficients $\gamma_{Ca^{2+}}$ as determined in solutions of cal-
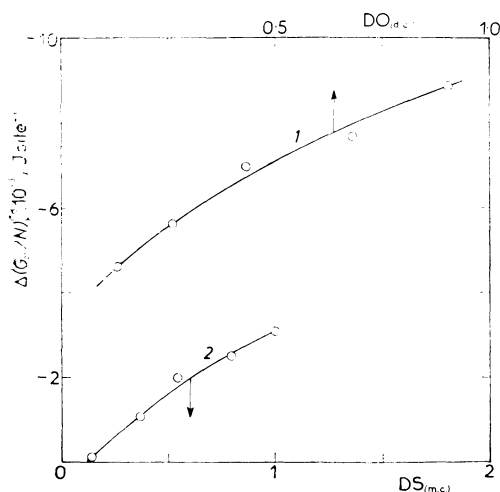

FIG. 3

Selectivity of $Ca^{2+} \rightarrow 2 K^+$ cation exchange of 2,3-dicarboxy derivatives of starch and pectin of various esterification degree E. $DO_{(d.c.)}$ the mean oxidation degree of 2,3-dicarboxystarch; $DS_{(m.c.)}$ the mean substitution degree of saccharide units of pectin by free carboxyl group; $\Delta(G_{el}/N)_K^{Ca}$ change of electrostatic free enthalpy at $Ca^{2+} \rightarrow 2 K^+$ cation exchange. 1 2,3-Dicarboxy derivatives of starch; 2 pectinates of various esterification degree E

cium salts of 2,3-dicarboxy derivatives of starch (Fig. 5, curve *1*) and pectinic acids[20] (curve *2*) upon the oxidation degree $DO_{(d.c.)}$, and substitution degree $DS_{(m.c.)}$, respectively. With an increasing substitution degree of pectin the intramolecular electrostatic bond of Ca^{2+} ions (region *A*) turns into intermolecular chelate binding (region *C*; region *B* is a transitive one). The anomalously low $\gamma_{Ca^{2+}}$ values for pectin of substitution degree $DS_{(m.c.)} > 0.7$ are due just to this intermolecular chelate binding[23]. For the intramolecular electrostatic bond of Ca^{2+} ions to polyuronate, the activity coefficients $\gamma_{Ca^{2+}}$ are markedly higher. Thus *e.g.* the $\gamma_{Ca^{2+}}$ value for highly substituted acetyl derivatives of pectic acid[24] was found to be 0·303 and for Ca-man-nuronan 0·281 (ref.[25]). A significant difference in the ion binding to both polysaccharides under study is manifested at a low oxidation or substitution degrees. As proved by experiments, the Ca^{2+} ions are extraordinarily firmly bonded to the couple of carboxyl groups at $C_{(2)}$ and $C_{(3)}$ of dicarboxy derivatives of starch.

Binding of Calcium Ions to Amylose 2,3-Dicarboxy Derivatives

Since 2,3-dicarboxy derivatives of starch represent molecules of a branched polysaccharide, it was useful to examine the binding of Ca^{2+} ions also to amylose 2,3-dicarboxy derivatives having a linear macromolecule. Results are summarized in Table I and in Fig. 6. The selectivity of the $Ca^{2+} \rightarrow 2 K^+$ cation exchange was investigated both with the original samples (*a*) and those after elimination of the lactones by alkali (*b*). The selectivity of $Ca^{2+} \rightarrow 2 K^+$ ion exchange of the original samples (curve *1*)
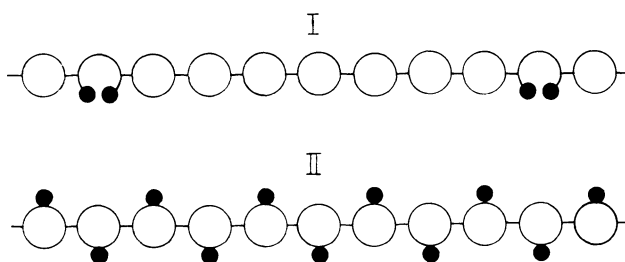

FIG. 4

Distribution of carboxyl groups in the 2,3-dicarboxystarch molecule of a low oxidation degree and in pectic acid molecule (*E* 0%). I 2,3-Dicarboxy derivative of starch of oxidation degree $DO_{(d.c.)}$ 0·125; II pectic acid (*E* 0%), $DS_{(m.c.)}$ 1·00; ○ D-glucose unit; Ω 2,3-dicarboxy derivative of D-glucose with an opened pyrane ring; ○ D-galacturonic acid unit; • carboxyl group

is a little higher than that of preparation (b) after treatment with alkali (curve **2**). This proves that degradation of the macromolecule took place to a very little extent as a result of the treatment with alkali applied for the opening of the lactone ring; this degradation leads to a lower affinity of carboxyl groups toward Ca^{2+} ions. Selectivity of the $Ca^{2+} \rightarrow 2 K^+$ cation exchange of amylose 2,3-dicarboxy derivatives is like to that found with starch 2,3-dicarboxy derivatives. The $\Delta(G_{el}/N)_K^{Ca}$ values determined for DCS of a low oxidation degree are a little higher than with the corresponding DCA samples; this could indicate a less regular distribution of oxidized D-glucose units in the starch macromolecule due to branching of the molecule.

The activity coefficients $\gamma_{Ca^{2+}}$ determined for 2,3-dicarboxy derivatives of amylose do not show, contrary to expectation, a dependence upon the oxidation degree $DO_{(d.c.)}$. The $\gamma_{Ca^{2+}}$ values corresponding to lower oxidation degrees (samples Nos 7 and 8) are close to $\gamma_{Ca^{2+}}$ ones as found for the corresponding starch derivatives.
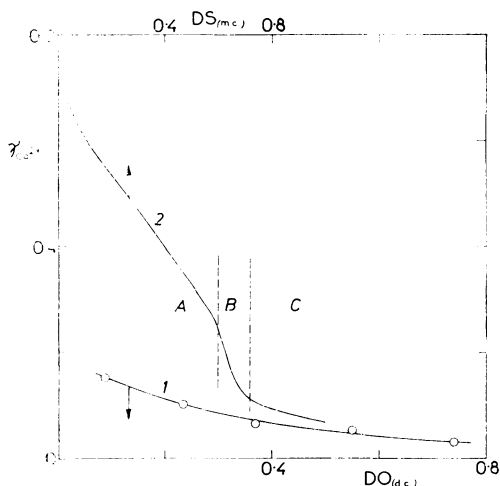

FIG. 5

Dependence of the activity coefficient $\gamma_{Ca^{2+}}$ in solution of calcium salts of 2,3-decarboxy derivatives of starch and calcium pectinates upon the oxidation degree $DO_{(d.c.)}$ and substitution degree $DS_{(m.c.)}$, respectively. *1* 2,3-Dicarboxy derivatives of starch; *2* calcium pectinates of various esterification degree E. *A* Region of intramolecular electrostatic bond of Ca^{2+} ions to carboxyl groups of pectin; *C* region of intermolecular chelate binding; *B* transitional region
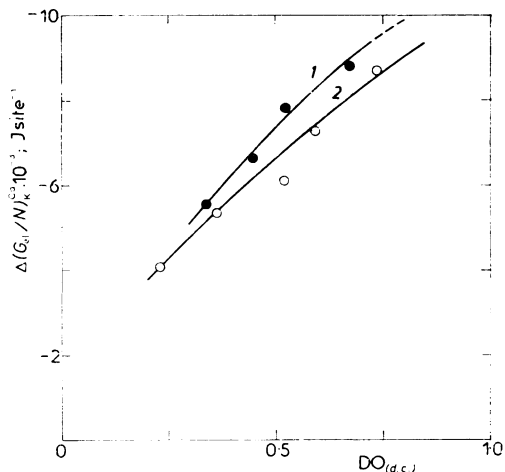
FIG. 6

Selectivity of $Ca^{2+} \rightarrow 2 K^+$ cation exchange of 2,3-dicarboxy derivatives of amylose of various oxidation degree $DO_{(d.c.)}$. $\Delta(G_{el}/N)_K^{Ca}$ as given for Fig. 3; *1* original samples; *2* samples after opening the lactone ring in 2·7 mmol (NaOH) l^{-1}

Values $\gamma_{Ca^{2+}}$ are markedly higher at a high oxidation degree (samples Nos 9 and 10) than those of a highly oxidized starch derivatives. (Even precipitation of calcium 2,3-amylosedicarboxylate occurred with samples No 10; activity $a_{Ca^{2+}}$ was determined in the supernatant at a $0.8-1.2$ mmol $(COOCa_{0.5})\,l^{-1}$ concentration). We pressume therefore, that a considerable degradation of the macromolecule to form short chains took place during preparation of amylose dicarboxy derivatives of a high oxidation degree; here the affinity of carboxyl groups towards Ca^{2+} ions already becomes a function of the chain length. Nevertheless, selectivity of $Ca^{2+}\rightarrow 2\,K^+$ ion exchange remains preserved.

Further, it seemed to be useful to compare the binding of Ca^{2+} ions to 2,3-dicarboxy derivatives of starch (DCS) and amylose (DCA) with that to carboxymethyl derivatives of cellulose (CMC) and amylose (CMA) using data already published[19]. This is why we present in Table I also the quantity γ, giving the ratio of free calcium ions to total concentration of calcium in solution $(\gamma = [Ca^{2+}]_{free}/c_{Ca})$. Contrary to 2,3-dicarboxy derivatives of polysaccharides, the pyrane ring of the substituted D-glucose units of carboxymethyl derivatives remained intact. Table III summarizes some results as obtained by Rinaudo and Milas[19] when studying binding of Ca^{2+} to carboxymethyl derivatives of cellulose and amylose under conditions close to experiments reported in this paper. These authors determined the activity $a_{Ca^{2+}}$ employing the ion-specific electrode Orion (USA) in solutions of 2 mmol $(COOCa_{0.5})\,.\,l^{-1}$ concentration, the selectivity of $Ca^{2+}\rightarrow 2\,Na^+$ cation exchange was determined

TABLE III
Binding of Ca^{2+} ions to carboxymethyl derivatives of cellulose and amylose and to 2,3-dicarboxy derivatives of starch and amylose

Sample	$DO_{(d.c.)}$	$DS_{(m.c.)}$	γ	$\Delta(G_{el}/N)^{Ca,K}_{Na}$ J site^{-1}
CMC[a]	—	1.0	0.345	$-$ 2 550 (Na^+)
		1.7	0.225	$-$ 4 520
		2.49	0.145	$-$ 6 590
		2.77	0.120	$-$ 8 270
CMA[a]	—	2.2	0.140	$-$ 8 670 (Na^+)
		2.5	0.126	$-$ 9 170
DCS	0.5	1.0	0.060	$-$ 7 100 (K^+)
	0.85	1.7	0.030	$-$ 8 700
DCA	0.5	1.0	0.115	$-$ 7 400 (K^+)
	0.85	1.7	—	~ -10 100

[a] Ref.[19].

at the same polyelectrolyte concentration by potentiometric titrations and procedure as already mentioned. The change of electrostatic free enthalpy of cation exchange, expressed in their paper in cal site^{-1} was transformed into J site^{-1}.

Values γ and $\Delta(G_{el}/N)_K^{Ca}$ at oxidation degrees $DO_{(d.c.)}$ 0·5 and 0·85 $(DS_{(m.c.)}$ 1·0 and 1·7) given for 2,3-dicarboxy derivatives of starch and amylose are presented just for comparison: the corresponding values were obtained by intra- and extrapolation of values listed in Table I. We presume that the difference in selectivity of $Ca^{2+} \rightarrow 2 Na^+$ and $Ca^{2+} \rightarrow 2 K^+$ cation exchange for the given sample will not be significant. Comparison of values γ and $\Delta(G_{el}/N)_{Na,K}^{Ca}$ (Table III) shows that 2,3-dicarboxy derivatives of polysaccharides of a low oxidation degree bind calcium ions by far firmer than carboxymethyl derivatives. The same concerns the selectivity of $Ca^{2+} \rightarrow 2 Na^+(2 K^+)$ cation exchange. The low γ values for CMC (similarly for CMA) were achieved even at a very high substitution degree $DS_{(m.c.)} = 2·2 - 2·77$. We ascribed the firm binding of Ca^{2+} cations to 2,3-dicarboxy derivatives of starch and amylose to a favourable spatial arrangement of two neighbouring carboxyl groups of oxidized D-glucose units due to flexibility of the macromolecule chain in sites of the pyrane ring opening.

REFERENCES

1. Wilham C. A., McGuire T. A., Mark A. M., Mehltretter Ch. L.: J. Amer. Oil Chem. Soc. *47*, 522 (1970).
2. Hills W. A., Volpp G. P.: Ger. Offen. 2 063 273 (22 Jul 1971), US Appl. (23 Dec 1969).
3. Eldib I. A.: US 3 629 121 (21 Dec 1971).
4. De Somer P., Claes P.: S. African 70 03,449 (13 May 1971), Brit. Appl. 30, 722 (17 Jun 1969).
5. Claes P., Billiau A., De Clercq E., Desmyter J., Schonne E., Vanderhaeghe H., De Somer P.: J. Virol. *5*, 313 (1970).
6. Billiau A., Desmyter J., De Somer P.: J. Virol. *5*, 321 (1970).
7. Eyckmans L., Billiau A., De Somer P.: Biomedicine *19*, (5), 187 (1973).
8. Gibbons R. A.: Brit. 1 137 540 (27 Dec 1968).
9. Giordano C., Esposito R.: Actual Nephrol. Hop. Necker *1972*, 281.
10. Meriwether L. S., Kramer H. M.: Kidney Int., Suppl. 7, 259 (1976).
11. Kaputskii V. E., Kaputskii F. N.: Tezisy Dokl. — Vses. Simp. Bioorg. Khim. *1975*, 72, (V. A., Rasskazov, Ed.). Akad. Nauk USSR, Dalnevost. Nauchn. Tsentr, Vladivostok, USSR; Chem. Abstr. *85*, 130 433 (1976).
12. Smidsrød O., Painter T.: Carbohyd. Res. *26*, 125 (1973).
13. Casu B., Gennaro U., Morrone M., Occhipinti M. S., Torri G.: Proceedings 4th Italian Symposium on Science of Macromolecules 1979, 271.
14. Casu B., Meille V., Naggi A., Su P., Torri G., Zoppetti G., Allegra G.: Carbohyd. Polym. *2*, 283 (1982).
15. Crescenzi V., Dentini M., Meoli C.: Carbohyd. Polym. *2*, 320 (1982).
16. Kohn R., Furda I.: This Journal *32*, 1925 (1967).
17. Kohn R.: Chem. Zvesti *28*, 625 (1974).

18. Gladding E. K., Purves C. B.: Paper Trade J. *116*, 26 (1943).
19. Rinaudo M., Milas M.: J. Polym. Sci. A-1, *12*, 2073 (1974).
20. Kohn R., Luknár O.: This Journal *40*, 959 (1975).
21. Kohn R., Luknár O.: This Journal *42*, 731 (1977).
22. Bock W., Anger H., Kohn R., Malovíková A.: Angew. Makromol. Chemie *64*, 133 (1977).
23. Kohn R.: Pure Appl. Chem. *42*, (3), 371 (1975).
24. Kohn R., Malovíková A.: This Journal *43*, 1709 (1978).
25. Kohn R., Larsen B.: Acta Chem. Scand. *26*, 2455 (1972).

Translated by Z. Votický.