BINDING OF CADMIUM CATIONS TO PECTIN

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Using two experimental techniques (dialysis and polarography) the binding of cadmium ions to the pectin carboxylic groups was studied as a function of their degree of esterification E in the range of E 0 to 84·2%. Stability constants for the binding of cadmium ions to magnesium pectinates (ionic strength $I = 0{\cdot}30$ mol l^{-1}) were determined by the dialysis technique, the constants for the binding of cadmium to potassium pectinates by the polarographic technique. In both cases stoichiometric binding of cadmium ions was demonstrated using the theory of multiple equilibria. The stability constants K of cadmium pectinates depend considerably on E. With the decrease of the degree of esterification the value of K increases similarly as in the case of the binding of Ca^{2+} and Sr^{2+} ions to pectin.

The utilization of pectin as a natural non-toxic ligand in the prophylaxis against intoxication by heavy metal cations is still actual. Recently much attention has been devoted to the application of pectin in order to protect people working with lead[1].

Cadmium belongs to the group of deleterious cations occurring as environmental industrial contaminants. Till now the binding of cadmium cations to biopolymers, alginates, chitosan as well as to cellulose derivatives[2] was mainly investigated. Only a few reports or short remarks about the binding of cadmium cations to pectin, especially in studies dealing with the binding of various divalent cations to polygalacturonic acid and pectic acid (fully deesterified pectin) can be found[3-5]. Cd^{2+} ions are relatively tightly bound to these polyuronic acids. Therefore the insoluble pectic acid can be used to eliminate cadmium ions from solutions or outlet waters. Markova and coworkers[6] investigated the effect of various sorts of apple pectin on the secretion of cadmium in the excrements of laboratory animals intoxicated by cadmium. Up to now the binding of cadmium to pectin has not been studied.

In connection with our previous work on the binding of lead and chromium cations to pectin[1] we try in this paper to clarify the effect of the degree of esterification of pectin on the interaction of the cadmium ions with its carboxylic groups.

EXPERIMENTAL

Material

Pectin samples, in which the carboxylic groups have been esterified by methanol to various degrees were prepared from a purified citrus pectin (Genu Pectin, Pektinfabrik, København,

Denmark) by a controlled alkaline deesterification[7]. Highly esterified pectin (sample 6) was prepared from a purified pectin preparation by the action of 1M-H_2SO_4 in methanol at +3°C. The esterification proceeded over two weeks[7]. Potassium pectate was prepared by alkaline deesterification in suspension using KOH in 60% (V/V) ethanol[8]. The samples were characterized as described previously[7].

The reagents used were of analytical grade. Specific conductivity of the redistilled water was less than $2 \cdot 10^{-4}$ S m^{-1}. Solutions of 0·05M-NaOH and 0·05M-KOH were carbonate free. Dialysis tubing from Kalle (Wiesbaden, FRG) was used.

Analytical Methods and Other Procedures

The equilibrium establishment of the ion exchange $Cd^{2+} \rightarrow Mg^{2+}$ using pectins with various esterification degrees was followed by the dialysis technique at room temperature as described earlier[9]. The solutions of the potassium pectate and pectinate at c (COOK) = 0·02—0·04 mol . l^{-1} were dialyzed against 0·1M solutions containing $Cd(NO_3)_2$ and $Mg(NO_3)_2$ at different ratios (solution ionic strength $I = 0{\cdot}30$ mol l^{-1}; pH 6·0). After dialysis against redistilled water the cadmium and magnesium bound to pectin were removed by dialysis against 0·1M-HNO_3. Respective dialysis steps (10 to 16 hours) were repeated four times. The concentration of cadmium ions in the dialysate was determined by polarography in acid media using a polarograph Radelkis OH 105 (Budapest) with a dropping mercury electrode.

The equilibrium exchange $Cd^{2+} \rightarrow 2K^+$ of pectin was studied by a direct polarographic method in solutions at c(COOK) = 3·0 mmol l^{-1} at an ionic strength $I = 0{\cdot}02$ mol l^{-1} (with KNO_3 as supporting electrolyte), see further.

The concentration of free carboxylic groups in the initial potassium pectate or potassium pectinate solution was determined by the method of precipitation of insoluble pectates or pectinates of copper[10,11]. The concentration of Cd^{2+} and Mg^{2+} respectively in the initial solutions of $Cd(NO_3)_2$ and $Mg(NO_3)_2$ was measured by chelatometry using Eriochrome Black T with spectrophotometric indication of the equivalence point (Complexon IV; interference filter Zeiss Jena, IF 650 nm).

The stability (binding) constant (K) of the cadmium pectate and cadmium pectinates was calculated using the equation resulting from the theory of multiple equilibria[12]:

$$r^{-1} = (nK[Cd^{2+}])^{-1} + n^{-1}, \qquad (1)$$

where r is the number of Cd^{2+} ions bound to the repeating macromolecular segment with n binding sites and $[Cd^{2+}]$ is the equilibrium concentration of free Cd^{2+} ions in solution. A segment of the macromolecule containing 2 free carboxylic groups binding stoichiometrically 1 Cd^{2+} cation (a 1 : 1 complex, $n = 1$) was chosen as the binding unit.

RESULTS AND DISCUSSION

The cation binding to pectin and exchange $Cd^{2+} \rightarrow Mg^{2+}$ and $Cd^{2+} \rightarrow 2\,K^+$, respectively, as a function of the degree of esterification E of its carboxylic groups by methanol in the range of E 0 to 84·2% was investigated using the dialysis method and polarography. The characteristics of the samples used for the study are presented in Table I. $[\eta]$ is the intrinsic viscosity; mean relative molecular weight determined from viscosimetry $\overline{M}_r$ was calculated according to Owens and coworkers[13].

The binding and exchange $Cd^{2+} \rightarrow Mg^{2+}$ was followed by the dialysis technique. This method can be used only in the case of ions of equal valence. The magnesium ions were chosen, because they are most loosely bound to carboxylic groups of pectin in the whole series of divalent cations[14]. As the Cd^{2+} ions are relatively strongly bound to pectin carboxylic groups, we studied the $Cd^{2+} \rightarrow Mg^{2+}$ exchange in the region of low cadmium salt concentrations, at Cd^{2+} ion mole fraction $X \leq 0{\cdot}22$. The pH of solutions was adjusted to 6·0 using very diluted nitric acid, because at higher pH values undesirable precipitation of basic cadmium salts could occur.

The binding and exchange $Cd^{2+} \rightarrow Mg^{2+}$ was evaluated using the equation deduced from the theory of multiple equilibria. In addition to the stability constant of the cadmium pectate and pectinates the number of binding sites in a binding unit (segment) n can be determined. In Fig. 1 the function $r^{-1} = f([Cd^{2+}]^{-1})$ for the system $Cd^{2+} \rightarrow Mg^{2+}$ is plotted. It can be stated that the interaction of Cd^{2+} ions with free pectin carboxylic groups follows the theory of multiple equilibria with a linear course of the above mentioned function. The value of $n \approx 1$ determined by the experiments in all systems studied is considered as an evidence for stoichiometric binding of Cd^{2+} ions to magnesium pectate and magnesium pectinates. The values of the stability constants ($\log K$) of cadmium pectinates are summarized in Table I.

In contrast to the stoichiometric binding of Cd^{2+} ions in the solutions of macromolecular pectin confirmed by our results, Makridou and coworkers[15] assume the formation of a complex $(CdA)^+$ (A^- being anion of the uronic acid) on interaction of Cd^{2+} ions with monomeric D-galacturonic acid. By a protometric method the stability constant for this complex was determined $\log K = 1{\cdot}15$ (25°C, $I = 1{\cdot}0$ mol . l^{-1}); ref.[15].

TABLE I

Characteristics of pectin samples and stability constants K of cadmium pectate and cadmium pectinates

Sample	E %	Polyuronide content % (m/m)	$[\eta]$ $m^3\ kg^{-1}$	$\bar{M}_r$	log K	
					$Cd^{2+} \rightarrow Mg^{2+}$ $I = 0{\cdot}30$ mol l^{-1}	$Cd^{2+} \rightarrow 2\,K^+$ $I = 0{\cdot}02$ mol l^{-1}
1	0·2	88·2	0·125	28 000	2·49	4·60
2	25·1	87·3	0·208	40 000	2·32	4·05
3	39·5	91·3	0·297	53 000	2·19	3·64
4	49·6	89·8	0·305	54 000	1·85	3·10
5	63·3	86·8	0·298	53 000	1·67	2·57
6	84·2	94·4	0·087	21 000	1·42	2·42

By the dialysis technique the stability constant K of cadmium pectate (E 0%) for the binding of cadmium ions to calcium pectate in $Cd(NO_3)_2$ and $Ca(NO_3)_2$ solutions under conditions of a large Ca^{2+} excess ($I = 0{\cdot}30$ mol l^{-1}) was measured. The stability constant $\log K = 1{\cdot}38$ proves a certain degree of preferential binding of Cd^{2+} ions in comparison with Ca^{2+} ions, which is important with regard to the application of this substance in the prophylaxis against cadmium intoxications.

Binding and exchange $Cd^{2+} \rightarrow 2\,K^+$. Due to different valence of the respective cations the dialysis technique cannot be used for the studies of the binding and exchange of the cadmium ions to potassium pectinates. The polarographic method appears to be more suitable for this purpose[16]. Lapanje and Oman[17] used the polarographic method to characterize the binding of cadmium ions to polystyrenesulphonate Introducing several approximations they developed a modified polarographic method, which found good application in studies of ion binding in polyelectrolyte solutions[18–21]. The application of this method is based on the assumption that only free ions in solution are polarographically active. The analytical limiting polarographic current must be solely a diffusion current. A kinetic current must not occur in the system under investigation. Undesirable migration current should be mostly eliminated by the addition of sufficient amount of an indifferent electrolyte (KNO_3, KCl).

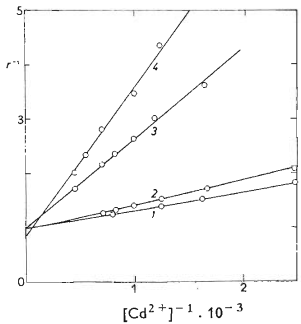

FIG. 1

Binding of Cd^{2+} ions to magnesium pectinates. The function $r^{-1} = f([Cd^{2+}]^{-1})$. 1–4 degree of esterification of pectin E 0, 25, 50 and 84%. $I = 0{\cdot}30$ mol l^{-1}

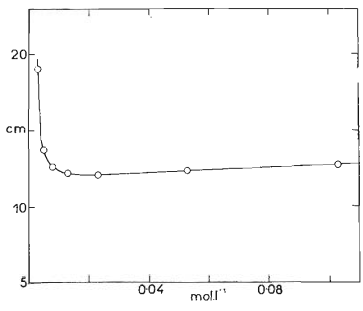

FIG. 2

The height of the polarographic wave h of Cd^{2+} ions as a function of ionic strength I. $c(Cd(NO_3)_2) = 1 \cdot 10^{-3}$ mol l^{-1}

Therefore we investigated the height of the polarographic wave h of a solution of $c(Cd(NO_3)_2) = 1 \cdot 10^{-3}$ mol l^{-1} as a function of the solution ionic strength I (supporting electrolyte KNO_3) – Fig. 2. The course of the curve reveals that the intensity of the polarographic current is practically constant starting with solution ionic strength $I > 0{\cdot}02$ mol l^{-1}. In this ionic strength region the migration current is already fully eliminated.

In order to make sure that the polarographic current measured is really a diffusion current exclusively, the dependence of the polarographic current on the mercury column height in the solution of monomeric cadmium D-galacturonate and in the solution of cadmium pectinate (E 50%) at various ionic strengths (I = 0·01 to 0·5 mol . l^{-1}, KNO_3) was tested. In all cases the height of the polarographic wave was a linear function of the second root of the mercury column height so that under given experimental conditions the polarographic current proved to be a pure diffusion current. As the cadmium ions are relatively firmly bound to the pectinates and cannot be quantitatively released by addition of a supporting electrolyte according to the original polarographic procedure[17], we determined the concentration of free Cd^{2+} ions using calibration curves constructed separately for each measurement under equal experimental conditions.

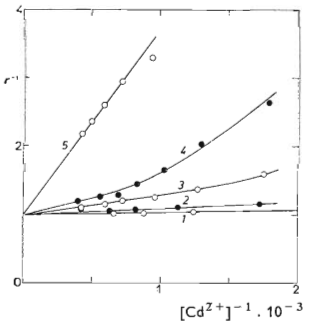

FIG. 3

Binding of Cd^{2+} ions to potassium pectinates. The function $r^{-1} = f([Cd^{2+}]^{-1})$. *1–5* degree of esterification of pectin E 0, 25, 40, 50 and 84%. $I = 0{\cdot}02$ mol l^{-1}

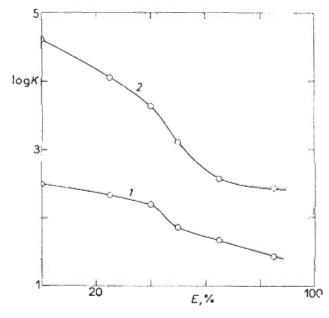

FIG. 4

Stability constant K of cadmium pectinates as a function of the degree of esterification of pectin E. *1* binding of Cd^{2+} ions to magnesium pectinates ($Cd^{2+} \rightarrow Mg^{2+}$; $I = 0{\cdot}30$ mol l^{-1}), *2* binding of Cd^{2+} ions to potassium pectinates ($Cd^{2+} \rightarrow 2\,K^{+}$; $I = 0{\cdot}02$ mol l^{-1})

The binding of cadmium ions was studied polarographically in the potassium pectate and potassium pectinate solutions ($c(COOK) = 3{\cdot}00$ mmol l^{-1}). The binding was again evaluated using the theory of multiple equilibria (Fig. 3). In this case, however, the function $r^{-1} = f([Cd^{2+}]^{-1})$ was not always linear for various degrees of pectin esterification. At a high esterification degree E 84·2% only electrostatic binding of Cd^{2+} to free carboxylic groups takes place and the function is linear in the whole range of cadmium salt concentrations. We assume further that with increasing concentration of Cd^{2+} ions in the esterification degree range E 40–50% the electrostatic binding of Cd^{2+} ions in a molecularly dispersed system changes gradually into stronger chelate bonds with parallel aggregation of the macromolecules. Therefore the function mentioned earlier deviates from linearity in these cases. At low esterification degree E Cd^{2+} ions are exclusively bound by chelate bonds and the function is again linear. Stability constants K for the binding of Cd^{2+} ions to potassium pectate and potassium pectinates were calculated from the linear part of the function $r^{-1} = f([Cd^{2+}]^{-1})$.

The results of the studies of the interaction of cadmium ions with carboxylic groups of pectin obtained by both experimental techniques are summarized in Table I and Fig. 4. Markedly higher stability constants K of cadmium pectinates when cadmium is bound to potassium pectinates as compared with the values obtained with magnesium pectinates are connected not only with different affinity of Mg^{2+} and K^+ ions to carboxylic groups of pectin, but also with different ionic strength of the solutions I. (See[22] concerning the effect of ionic strength on the binding Ca^{2+} ions to pectins, for instance).

Similarly to the binding of Ca^{2+} (ref.[22,23]) or Sr^{2+} (ref.[23]) ions to pectin, also in the case of Cd^{2+} binding the esterification degree affects strongly the binding of these cations to carboxylic groups of pectin. On the other hand Pb^{2+} ions show a different behavior, their binding being relatively little affected by the esterification degree of pectin even in the region of a high esterification degree[1]. More pronounced changes of the stability constants of cadmium pectinates ($\log K$) occur in the region of esterification degree E 40–55% (Fig. 4), which is most probably due to the transition from electrostatic binding of Cd^{2+} ions to chelate bonds with simultaneous formation of gel coagulate.

Pronounced dependence of cadmium ion binding on the esterification degree E leads to the conclusion that fully deesterified (E 0%) pectin should be used in the cases of acute cadmium intoxications. This preparation shows not only markedly higher affinity to cadmium ions, but also its binding capacity (the content of free carboxylic groups) is several times higher than that of commercial alimentary pectin or that of pectin contained in consumed fruits and vegetables.

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