

THE INTERACTION OF LEAD(II) WITH GLYCURONANS: U.V. ABSORPTION AND CIRCULAR DICHROISM SPECTRA

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(Received August 29th, 1986; accepted for publication in revised form, February 15th, 1988)

ABSTRACT

U.v. absorption and circular dichroic spectra of lead(II) complexed by uronates and polyuronates in aqueous 0.05M NaClO₄ are presented for the first time. It is shown that not only the carboxylate spectral bands are substantially modified by the interaction, but also the bands of the cation, shifted to higher wavelengths, become optically active. The sign of some bands reflects the stereochemistry of the sugar unit(s) in the complex. On increasing the ion-to-uronate molar ratio, R , the intensity of some bands, changes in a discontinuous mode, suggesting that two types of complex are formed at low and at high values, respectively, of R . The relevance of this structural information to the model of chain dimerization occurring with other divalent cations is discussed.

INTRODUCTION

a. Interaction between charged polysaccharides and cations. — The complexation of metal ions by ionic polysaccharides has been the subject of extensive investigations from many points of view. One of the major scientific interests is the role of some ions in gelation, which is believed to occur after some bridging of partially ordered polysaccharidic chains. Although, for biological and biotechnological reasons, the system polyuronate–calcium has received^{1–6} the greatest attention, other divalent ions (*e.g.*, Cd²⁺, Cu²⁺, Sr²⁺, and Co²⁺) and some trivalent ions (Bi³⁺ and Cr³⁺) have also been investigated^{5–8}. These studies are aimed at the characterization of both the polymeric sites and the structures induced in complex-formation and of the physicochemical properties which arise from chain–ion and chain–chain interactions.

As far as the experimental techniques are concerned, the literature reveals a great variety of approaches, including circular dichroism (c.d.), n.m.r. spectroscopy, and thermodynamic studies, the latter mainly devoted to determination of the activity coefficients, or the selectivity constants, of the cations. More recently, the interaction between polyuronates and Ca²⁺ and Cu²⁺ have been studied in our laboratory by direct microcalorimetry^{9,10}, in addition to other techniques^{11–13}. In

particular, it has been reported that, when the enthalpy of interaction between ionic polysaccharides and divalent cations is exothermic, the polymeric chain is suspected to undergo a conformational transition induced by the binding of cations^{9,10,13-15}. It is of particular interest that the enthalpy of interaction of Pb^{2+} with both alginate and pectate is slightly more exothermic¹⁵ than that of Ca^{2+} , while for other divalent cations (e.g., Cd^{2+} and Zn^{2+}), it is in the range expected for merely electrostatic interactions.

b. Circular dichroic spectroscopy of interacting species. — A feature shared by a wide variety of biopolymers is the existence of helical structures which generate typical dichroic bands associated with the absorption bands. However, c.d. bands may be observed even in the absence of dissymmetric, ordered conformations, because of the intrinsic asymmetry of most biological monomeric residues. In addition, the electronic transitions of a nondissymmetric chromophore can be more or less extensively perturbed by the dissymmetric field of a chiral ligand. The result is that the absorption band of the chromophore becomes optically active (extrinsic Cotton effect).

In the case of polyuronates, the carboxylic chromophore is permanently subjected to an asymmetric neighboring (both the C-5 and C-4 atoms are "asymmetric"), giving rise to well known c.d. bands which are a function of the ionization state of the acidic groups^{16,17} and of the nature and amount of the counterions^{9,15,18,19}. Whatever may perturb the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ electronic transitions, it will therefore affect the energies (wavelength) and the probabilities (intensities) of these bands. These factors may include the addition of a co-solvent, or of specific cations, or both, and changes of temperature and of conformation.

In a way almost similar to the "chemical dissymmetric field", the application of a magnetic field is able to generate magnetic circular dichroism bands (m.c.d.), and, hence, magnetic optical rotatory dispersion (m.o.r.d.) curves, on any chromophore in solution, including (and more specifically) ions. In particular, the m.c.d. spectrum of Pb^{2+} in aqueous 0.1M HClO_4 has recently been reported by Mason²⁰. The absorption spectrum is split into positive and negative bands which cross zero intensity at 209 nm, very close to the maximum wavelength of the absorption band (208 nm) in the same solvent.

Despite that, we have found no data in the literature on the c.d. of Pb^{2+} ions complexed by low-molecular-weight or polymeric carbohydrate ligands. It has only been reported²¹ that, at high pectin concentration (0.5%), the solution dialyzed against PbCl_2 "becomes turbid at an early stage, and no further c.d. measurements were possible". Due to the high value of the extinction coefficient of aqueous Pb^{2+} ions ($9890\text{M}^{-1}\text{cm}^{-1}$), it is conceivable that measurements were not possible in that case, because of too large an absorption of the solution, which, under the experimental conditions used by the authors²¹, can be estimated to be >2 at an ion:carboxylate molar ratio equal to 0.01.

In view of these interesting features, we have therefore investigated the interaction of Pb^{2+} with various polyuronates, and we report here on the characterization of these systems by u.v. absorption and c.d. spectroscopy.

TABLE I

CHEMICAL COMPOSITION OF GLYCURONAN SAMPLES

<i>Compound</i>	<i>Composition, (%)</i>	<i>M_w (× 10⁻³)</i>
Alginate	mannuronic acid	55
	guluronic acid	45
Galacturonan	galacturonic acid	>95
Oligogulonate	guluronic acid	>90
Oligomannuronate	mannuronic acid	~95
C-6-oxyamylose	glucuronic acid	42
C-6-oxycellulose	glucuronic acid	84
C-6-oxyscleroglucan	glucuronic acid	40
O-(Carboxymethyl)amylose	glucose-CH ₂ -CO ₂ H	30
		260

EXPERIMENTAL

The polysaccharide samples were either commercial products, purified by precipitation with ethanol and already studied in our laboratory, or derivatives already described in previous studies. In particular, characteristics and properties of the alginate, pectate, oligogulonate, and oligomannuronate samples, already reported^{11-13,22}, are summarized in Table I. Monomeric uronic acids were commercial products (Sigma); they were used as received. The preparation and properties of the samples of selectively C-6-oxidized amylose (C6OA), cellulose (C6OC), and scleroglucan (C6OS) have been described^{17,23}. The sample of O-(carboxymethyl)-amylose (d.s. 0.3) was kindly provided by Dr. D. A. Brant.

The circular dichroic spectra were recorded at room temperature (~23°) with a J-500A Jasco Spectropolarimeter equipped with a DP-500 N Data Processor. Four runs were cumulated for each spectrum, carried out on either a 5- or 10-mm cell containing the ligand solution to which increasing amounts of Pb(ClO₄)₂ solution were carefully added under stirring. Measurements were made until the appearance of insoluble microgels was detected by eye and confirmed by an evident baseline shift at 260 nm.

The absorption spectra were similarly recorded with a Cary 219 spectrophotometer.

RESULTS AND DISCUSSION

The optical absorption of the u.v. band of lead(II) was measured in aqueous 0.05M NaClO₄ in the concentration range of 20 to 200 μM. A very small deviation from the Lambert-Beer law was found, and ascribed to partial hydrolysis occurring in the absence of added acid. In fact, if extrapolated to zero concentration, the extinction coefficient reached the value of 9890 L⁻¹.mol obtained in the presence of 0.05M HClO₄, as reported by Mason²⁰. A series of spectra was recorded in the presence of polymer at different molar ratios, *R*, of lead to polymer, in the range

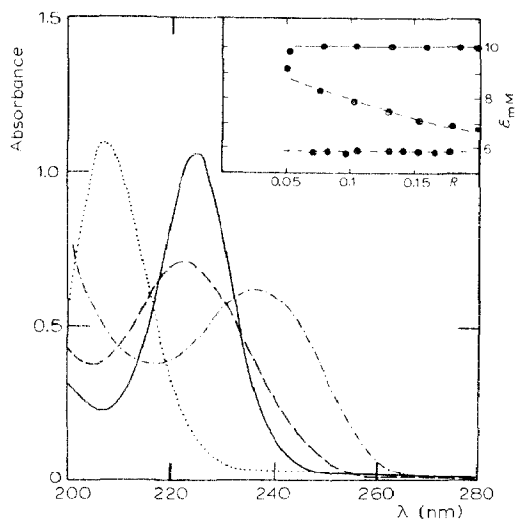


Fig. 1. U.v. absorption spectra of Pb^{2+} ($210\mu M$) in aqueous $0.05M$ $HClO_4$ (—); in the presence of pectate (---), alginate (-.-), and C6OA (.....). Polymer concentration is $mequiv.L^{-1}$; cell path-length 0.5 cm. The insert shows the dependence of the apparent extinction coefficient of Pb^{2+} in the presence of polyuronate as a function of R , the molar ratio of ion to polymer.

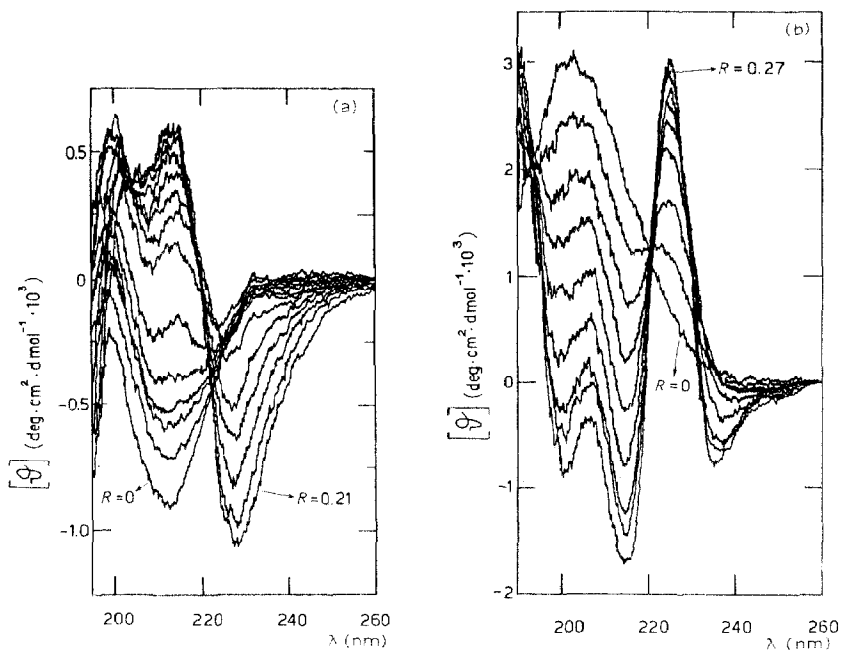


Fig. 2. Circular dichroism spectra of (a) aqueous alginate and (b) pectate upon addition of increasing amounts of Pb^{2+} , measured as ion-to-polymer molar concentration, R . Polymer concentration is $mequiv.L^{-1}$; cell path-length, 10 mm.

of R from 0 to ~ 0.3 . In Fig. 1, the u.v. absorption spectra of lead alone and in the presence of sodium polygalacturonate, alginate, and C6OA (at $R = 0.2$) are reported.

With alginate, the following perturbations of the spectra were observed: (i) a broadening of the absorption peak (the half-width changes from 17 to ~ 30 nm); (ii) a marked shift of the wavelength of the maximum, which furthermore shifts on increasing R ; and (iii) a strong dependence of the apparent extinction coefficient on the value of R (see insert in Fig. 1). In the case of pectate, only the shift of the peak maximum to higher wavelengths was observed.

The circular dichroic spectra were recorded in the range of 190–260 nm, in the presence of various polymeric and monomeric ligands, as a function of the ratio R . The c.d. spectra obtained with alginate and pectate upon addition of increasing amounts of lead perchlorate in the R range of 0–0.3 are shown in Fig. 2.

These spectra are clear evidence of the intrinsic Cotton effect(s) induced on the electronic transitions of lead(II) by the chiral ligands, and arise from the same source of perturbation as revealed by the absorption results. In the wavelength range explored in the present study, four bands (and the tail of a fifth one) are detected for the lead–pectate system: the two bands of opposite sign crossing at 220 nm may arise from a band-splitting, similar to that observed²⁰ in the m.c.d. band of Pb^{2+} ion. In fact, the zero-crossing wavelength corresponds to the maximum wavelength in the absorption spectrum. In that case, the sign of the split bands should reflect the dissymmetry of the chiral ligand. In agreement with the ab-

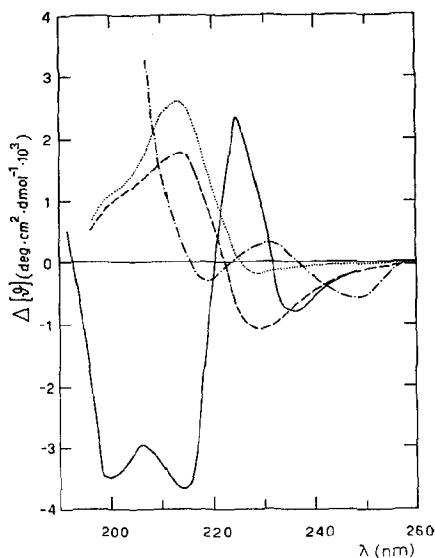


Fig. 3. Circular dichroism difference spectra of aqueous pectate (—), alginate (---), oligoguluronate (.....), and C6OA (-.-.-) in the presence of Pb^{2+} ($210\mu\text{M}$). Polymer concentration is $\text{mequiv}\cdot\text{L}^{-1}$.

TABLE II

SIGN OF THE C.D. BANDS^a OF THE LEAD-SACCHARIDE COMPLEXES

<i>Band λ (nm)</i> <i>(approximate value)</i>	<i>IV</i> <i>200</i>	<i>III</i> <i>215</i>	<i>II</i> <i>225</i>	<i>I</i> <i>240</i>
Substrate				
Alginate	+	+	—	—
Galacturonan	—	—	+	—
Oligoguluronate	+	+	—	—
Oligomannuronate			(—)	
C-6-oxyamylose	+	—	+	—
C-6-oxycellulose			—	
O-(Carboxymethyl)amylose			+	
Galacturonic acid		(—)	+	

^aThe bands have been assigned starting from that located at longer wavelength. The parentheses indicate low intensity of the band.

sorption bands, the presence of an isodichroic point in the lead(II)–pectate system indicates an equilibrium between two dissymmetric species, whereas more than two species seem to contribute to the c.d. spectrum of lead alginate and oligoguluronate.

The differential c.d. spectra of four polyuronates at similar R values are shown in Fig. 3, while the signs of the uronate–Pb²⁺ c.d. bands are reported at four wavelength ranges in Table II. They were detected (where possible) in the presence of the various ligands, from which the corresponding sodium polyuronate spectra have been subtracted. The following features are interesting. (1) Polygalacturonate and monomeric galacturonate have all the bands opposite in sign with respect to alginate and oligoguluronate, except for that centered at ~ 240 nm. (2) C6OA and (carboxymethyl)amylose show the band at 200 nm with the same sign of that of alginate, while the bands at 212.5, 225, and 240 nm have the opposite sign with respect to the corresponding ones of alginate. Polygalacturonate shows the same sign for bands at 200, 212.5, and 225 nm. (3) The only band clearly detected for C6OS, C6OC, oligomannuronate, and glucuronic acid occurs at 225 nm. Although very small in intensity, it is negative in all cases.

The intensity change of two significant bands for alginate, oligoguluronate, polygalacturonate, and C6OA is reported as a function of R in Fig. 4. These data, although not normalized for the actual amount of ion bound to the polymer, clearly show that, in some cases, the band emerges at a later step of the process of complex-formation.

The trends reported for the first three polymers in Fig. 4a disclose a similar behavior at low R , but diverging at high R , in agreement with the content of reactive ligand (which may be assumed to be 90% in oligoguluronate, $\sim 95\%$ in polygalacturonate²², and 45% in the alginate sample¹¹). Limiting the following discus-

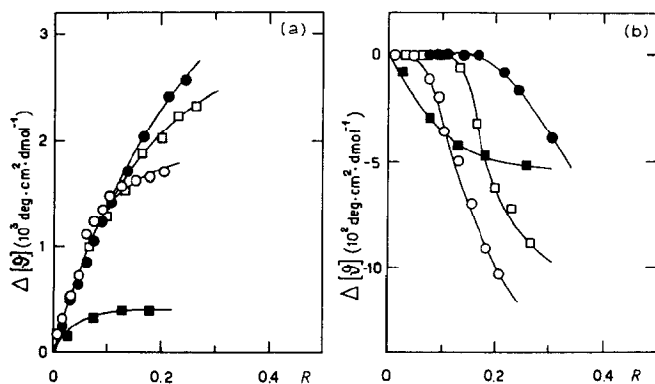


Fig. 4. Molar ellipticity change as a function of R (Pb²⁺: polymer molar concentration ratio) of aqueous pectate (□), oligoguluronate (●), alginate (○), and C6OA (■). (a) $\lambda = 215 \text{ nm}$; (b) $\lambda = 235 \text{ nm}$.

sion to the three aforementioned polymers, the typical, Langmuir type of behavior of these curves may allow it to be assumed that the intensity of c.d. bands in the range 215–225 nm is a measure of the amount of ion bound in the dissymmetric field of carboxylate. On the contrary, the behavior shown by the bands at $\sim 235 \text{ nm}$ is quite different, because an “induction” range is observed before the intensity starts changing. The development of this band can be associated to the well known process of dimerization promoted by divalent ions (such as calcium^{2–6,24}) through a conformational transition of the polyuronate^{9,13}. Although the process involving lead and the polyuronates is “cooperative” from the thermodynamic point of view^{15,24}, the changes of c.d. intensity may therefore be related in one case to the total amount of lead bound (see Fig. 4a) and in the other case to the amount of lead bound in the chain dimers (see Fig. 4b).

To elucidate, at least partially, this aspect, the scanty structural information obtained on the basis of studies carried out on the complex of Pb²⁺ with gluconate by polarography²⁵, n.m.r. spectroscopy²⁶, potentiometry²⁷, optical rotation²⁵, and X-ray diffraction²⁸ may be referred to. All studies in solution refer mainly to strongly alkaline conditions, and provide evidence for a complex that involves α -, β -, and γ -alcoholic (ionized) oxygen atoms of the gluconate anion. The only known solid-state gluconate–lead complex shows the presence of two five-membered rings involving the oxygen atoms of the carboxylate and of the α -hydroxyl groups of two gluconate moieties²⁸. The cation coordinates a total of six oxygen atoms, with a geometry of a very distorted octahedron (oxygen–lead distances are 243, 248, and 271 pm, respectively). The fact that, in our solution study, c.d. bands with different energies (*i.e.*, λ) are implicated to various extents in the binding process suggests that the external orbitals of lead are perturbed to a different extent and at different R values. In the oligoguluronate–calcium complex, the geometry proposed for the guluronate ligand surrounding the cation involves O-3, O-2, and the carboxylate group of the neighboring unit, thus providing for a distorted octahedron ligand

within the dimeric polymer chains. Although not strictly necessary, a similar chain-topology may occur²⁹ for oligogulonate and the guluronate segments of alginate with lead(II) and with other cations. With other polyuronates, the stereochemical patterns exhibited must be compatible with the conformational energies and the packing of the chains. A twofold chain-symmetry is conformationally compatible, for example, for polygalacturonate³⁰, which, except for O-3, may be similar to the mirror image of that proposed for polyguluronate by Mackie *et al.*³¹. This structure may be further stabilized by the additional hydrogen-bond between O-3 and O-5', although only four sugar oxygen atoms are able to coordinate.

We conclude that the sugar stereochemistry has been experimentally verified to affect, largely, the c.d. bands of the lead(II)-uronate complexes. The intensity of these bands is also a function of the binding affinity of the complex (that is, of the strength of the "chelation", in addition to the specific "rotatory strength"), while the sign of four of the bands is related to the configuration of the sugar involved in the chelate. The selective dependence of the c.d. bands of lead upon the sugar configuration suggests that this system may be fruitfully used as a configurational probe of glycuronans.

ACKNOWLEDGMENTS

This work was supported by the University of Trieste and the "Progetto Finalizzato Chimica Fine e Secondaria", C.N.R. (Rome), and also by Chimica del Friuli S.p.A. through a Fellowship to A.F.

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