Kinetics and mechanism of the oxidation of disaccharides by Cr^{VI}

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Abstract: The oxidation of D-lactose, D-maltose, D-melibiose, and D-cellobiose by Cr^{VI} yields the corresponding aldobionic acid and Cr^{3+} as final products when an excess of reducing disaccharide over Cr^{VI} is used. The rate law for the Cr^{VI} oxidation reaction is expressed by $-d[Cr^{VI}]/dt = k_H$ [disaccharide][Cr^{VI}], where the second-order kinetic constant, k_H, depends on [H^+]. The relative reactivity of the disaccharides with Cr^{VI} is expressed as follows: Mel > Lac > Cel > Mal, at 33°C. In acid medium, intermediate Cr^V forms and reacts with the substrate faster than Cr^{VI}. The EPR spectra show that five- and six-coordinate oxo-Cr^V intermediates are formed, with the disaccharide acting as bidentate ligand. Five-coordinate oxo-Cr^V species are present at any [H^+], whereas six-coordinate ones are observed only at pH < 2, where they rapidly decompose to the redox products. In the pH 3–7 range, where hexa-coordinate oxo-Cr^V species are not observed, Cr^V complexes are stable enough to remain in solution from several days to several months.

Key words: chromium, saccharides, kinetics, EPR.

Résumé : Lorsqu'on utilise un excès de du disaccharide réducteur par rapport au Cr(VI), l'oxydation du D-lactose, du D-maltose, du D-melibiose et du D-cellobiose par le Cr(VI) conduit à la formation de l'acide aldobionique correspondant et de Cr^{3+} comme produits finaux. La constante de vitesse de la réaction pour la réaction d'oxydation par le Cr(VI) peut être exprimée par l'équation $-d[Cr(VI)]/dt = k_H$ [disaccharide] [Cr(VI)], dans laquelle la constante cinétique du deuxième ordre, k_H, dépend de [H^+]; à 33°C, les réactivités relatives des disaccharides vis-à-vis du Cr(VI) sont les suivantes: Mel > Lac > Cel > Mal. En milieu acide, il se forme du Cr(V) comme intermédiaire qui réagit avec le substrat plus rapidement que le Cr(VI). Les spectres de RPE montrent qu'il se forme des intermédiaires penta- et hexacoordinés d'oxo-Cr(V) dans lesquels le disaccharide agit comme ligand bidentate. Les espèces pentacoordinées oxo-Cr(V) sont présentes à toutes les [H^+] alors qu'on n'observe les espèces hexacoordinées qu'à des pH < 2 et que dans ces conditions elles se décomposent rapidement en produits d'oxydoréduction. À des pH allant de 3 à 7 où on n'observe pas de formation des espèces hexacoordinées d'oxo-Cr(V), les complexes de Cr(V) sont suffisamment stables pour être observés en solution pour des périodes allant de plusieurs jours à plusieurs mois.

Mots clés : chrome, disaccharides, cinétique, RPE.

[Traduit par la Rédaction]

Introduction

Compounds of Cr^{VI} represent a potential environmental hazard because of their mammalian carcinogenicity and toxicity (1, 2), and they are known to bioaccumulate in flora and fauna, creating ecological problems (3–5). The observation of Cr^V and Cr^{IV} intermediates in the selective oxidation of organic substrates by Cr^{VI} and their implication in the mechanism of Cr-induced cancers (1, 6–7) has generated a considerable amount of interest in their chemistry and biochemistry (8–10).

The major coordination sites involved in Cr binding are hydroxo, alcoholato, carboxylato, and thiolato donor groups (2, 11–12). Five-membered, *O*-donor, chelate ligands, such as 1,2-diols and 2-hydroxy acids, are effective as non-enzymatic reductants (at low pH values) and chelates for high oxidation states of Cr (13–19). Carbohydrates constitute 5–20% (average 10%) of soil organic matter, and they

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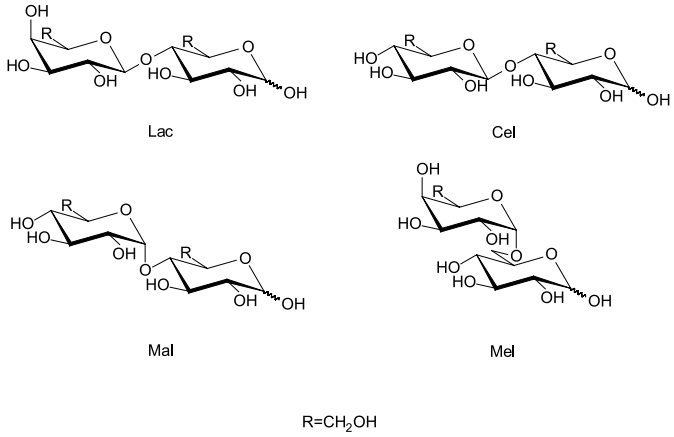
constitute more than 50% of dry matter in plants (20). For this reason, it is interesting to examine the ability of saccharides and their derivatives to reduce or stabilize high oxidation states of Cr, to understand their potential roles in the biochemistry of Cr.

We are studying the possible fate of Cr^{VI} and Cr^{V} in biological systems by examining reactions of Cr^{VI} with low-molecular-weight molecules (21–37). Our studies on the reduction of Cr^{VI} and the intermediate Cr^{V} by aldoses (21–26), deoxyaldoses (27–28), sugar acids (31, 32, 35), and methyl glycosides (29–30) showed that the relative redox reactivities of these saccharides toward chromate are based on the relative rates of the oxidation vs. complexation processes. In view of some preliminary results obtained on the milk–Cr^{VI} system suggesting the formation of long lived lactose–Cr^{V} species generated by direct reaction of Cr^{VI} with lactose (5), we decided to evaluate the kinetics of the reaction of Cr^{VI} with four disaccharides: D-lactose (Lac), D-maltose (Mal), D-cellobiose (Cel), and D-melibiose (Mel), to determine the influence of the glycoside moiety attached to the C^6 or C^4 of glucose (glc) on the chelating and (or) redox reactivity of the saccharide with high oxidation states of chromium.

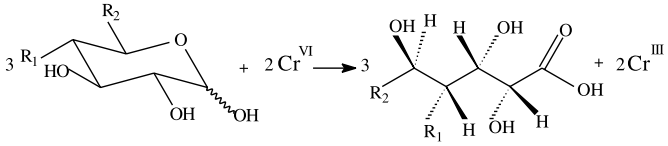


Lac, the most important representative of the β-galactosides, is found in the milk of all mammals up to a concentration of approximately 5% and in some plants in low concentrations. Mal is a product of enzymatic hydrolysis of starch; Mel is a constituent of the trisaccharide raffinose; and Cel is the basic repeating unit of cellulose and lichetin (38). In this work, we study the Cr^{VI} oxidation of these four disaccharides and provide information on the mechanism of oxidation as well as on the structure and stability of the Cr^{V} species formed as redox intermediates.

Experimental

Materials

D-Lactose monohydrate (Anedra, RA-ACS grade), D-maltose monohydrate (Sigma grade, 99%, less than 1.0% of maltotriose and less than 0.3% of D-glucose), D-melibiose



$R_1 = C_6H_{11}O_6$ (Lac, Cel, Mal)
CH_2OH (Mel)

$R_2 = CH_2OH$ (Lac, Cel, Mal)
$C_6H_{11}O_6$ (Mel)

(Sigma grade), D-cellobiose (Pfanshtiehl 99.9%), potassium dichromate (Cicarelli c.a.), potassium chromate (Aldrich grade), glutathione (Sigma grade), acrylonitrile (Aldrich grade), perchloric acid 70% (Merck P.A.), phosphoric acid (Anedra P.A.), and sulfuric acid (Merck P.A.) were used without further purification. Water was purified by deionisation, followed by double distillation from a potassium permanganate solution.

In experiments performed at constant ionic strength (I = 1.0 M) and in different hydrogen ion concentrations, mixtures of sodium perchlorate solutions and perchloric acid solutions were used. Sodium perchlorate solutions were prepared from sodium hydroxide and perchloric acid solutions. The concentration of stock solutions of perchloric acid was determined by titration using standard analytical methods.

The stability of the organic substrate under conditions used in the kinetic studies was tested by paper chromatography and HPLC at given hydrogen ion concentrations.

Caution: Cr^{VI} compounds are human carcinogens, and Cr^{V} complexes are mutagenic and potential carcinogens (39). Contact with skin and inhalation must be avoided. Acrylonitrile is a carcinogen and must be handled in a well-ventilated fume hood (40).

Spectrophotometric measurements

Kinetic measurements were performed by monitoring absorbance changes using a Jasco V-530 spectrophotometer with a fully thermostated cell compartment (±0.2°C). The reactions were followed under pseudo-first-order conditions, using an excess of disaccharide over Cr^{VI}. Reactant solutions were previously thermostated and transferred into a 1-cm pathlength cell immediately after mixing. Experiments were performed at 33°C unless otherwise stated.

The disappearance of Cr^{VI} was followed at 350 nm until at least 80% of the Cr^{VI} was consumed. In the kinetic measurements, the concentration of Cr^{VI} was kept constant at 8.0×10^{-4} M and the disaccharide concentration was varied from 0.08 to 0.36 M. Mixtures of sodium perchlorate and perchloric acid were used to maintain a constant ionic strength (I) of 1.0 M.

The observed pseudo-first-order rate constants (k_{obs}), determined from the slopes of plots of $\ln(A_{350} - A_8)$ vs. time, were averages of at least three determinations and were within ±5% of each other. The first-order dependence of the rate upon $[Cr^{VI}]$ was verified by calculating the k_{obs} values at various $[Cr^{VI}]_0$ ($4.0 \times 10^{-4} - 8.0 \times 10^{-4}$ M) at constant

Table 1. Observed pseudo-first-order rate constants (k_{obs})[a] for different concentrations of disaccharide in 0.2 M $HClO_4$.

	10^5 k_{obs} (s^{-1}) for							
	Lac		Mal		Cel		Mel	
[disaccharide] (M)	λ_{350}[b]	λ_{570}[c]	λ_{350}[b]	λ_{570}[c]	λ_{350}[b]	λ_{570}[c]	λ_{350}[b]	λ_{570}[c]
0.02	—	—	1.30(3)	1.28(3)	1.35(4)	—	—	—
0.04	—	—	2.42(5)	2.36(7)	3.34(1)	—	—	—
0.06	—	—	3.09(1)	2.98(7)	4.6(1)	—	—	—
0.08	7.35(9)	7.03(7)	4.35(1)	4.42(4)	6.36(6)	6.77(6)	10.2(5)	—
0.12	—	—	—	—	8.10(3)	8.5(2)	—	—
0.125	—	—	6.16(3)	6.07(5)	—	—	—	—
0.16	14.0(1)	13.9(6)	—	—	12.2(3)	12.1(2)	19.8(1)	19.0(1)
0.20	16.5(1)	16.0(3)	—	—	15.4(2)	—	25.0(2)	25.0(5)
0.24	19.2(1)	19.4(2)	—	—	17.0(3)	—	28.0(9)	28.6(6)
0.28	24.2(2)	22.8(1)	13.9(5)	—	20.7(5)	—	—	—
0.32	28.6(2)	27.0(3)	—	—	—	—	38.5(6)	38.8(2)
0.36	31.4(1)	31.6(6)	—	—	—	—	—	—
10^4 k_H (M^{-1} s^{-1})	8.6(1)	5.2(1)	7.4(1)	12.1(2)				

Note: T = 33°C; I = 1 M.
[a]Mean values from multiple determinations.
[b]$[Cr^{VI}]_0 = 8 \times 10^{-4}$ M.
[c]$[Cr^{VI}]_0 = 8 \times 10^{-3}$ M.

temperature, initial disaccharide concentration, acidity, and ionic strength.

The formation of Cr^{III} was monitored at 570 nm ($[Cr^{VI}]_o = 8.0 \times 10^{-3}$ M) in the presence of excess disaccharide (0.08 to 0.36 M) in 0.20 M $HClO_4$. Under these conditions, the first-order dependence of the rate upon $[Cr^{VI}]$ was verified and k_{obs} were obtained following the Cr^{III} growth at 570 nm. Initial reaction rates were obtained by fitting the time-dependent data for the absorbance at 570 nm to a polynomial expression (41) and calculating the slope of the tangent at time zero (r_i). First-order rate constants were calculated from these initial rates using the equation $-r_i/(A_o - A_f)$, with average values of the initial rate and calculated estimates of $(A_o - A_f)$. The results at 570 nm are in total agreement with those obtained at 350 nm (Table 1). At the end of the reaction, the two *d-d* bands ascribed to Cr^{III} were observed at λ_{max} = 410 nm (ε = 18 M^{-1} cm^{-1}) and λ_{max} = 570 nm (ε = 15 M^{-1} cm^{-1}). Both visible and UV spectral maxima and intensities are in close agreement with those observed for the $[Cr(H_2O)_6]^{3+}$ ion. These bands are attributable to the octahedral $^4A_{2g} \rightarrow {}^4T_{1g}$ and $^4A_{2g} \rightarrow {}^4T_{2g}$ transitions in O_h symmetry, and are distinctive of the free Cr^{III} aqua complex (42). However, in experiments performed at 20°C the reaction of Cr^{VI} with Lac (33:1 ratio, $[Cr^{VI}] = 12.7 \times 10^{-3}$ M) in 0.2 M $HClO_4$ affords two *d-d* bands at λ_{max} = 409 nm (ε = 26.8 M^{-1} cm^{-1}) and 574 nm (ε = 21.9 $M^{-1}cm^{-1}$) with higher absorbance than expected for $[Cr(OH_2)_6]^{3+}$. These two bands agree closely with those of the acid solutions of the Cr^{III} – aldobionic acid complex, which was independently prepared. The intensity of these *d-d* bands slowly decreases with time toward the two bands with the molar absorption coefficients corresponding to the $[Cr(H_2O)_6]^{3+}$ ion. This behaviour has been observed previously, i.e., when a Cr^{III} complex forms as the final redox product and then hydrolyses to the Cr^{3+} ion (29, 31, 43, 44). The hydrolysis of the Cr^{III} – aldobionic acid complex in 0.2 M $HClO_4$ precludes its separation, and consequently, its structural characterization. At pH > 2, where these kind of complexes are stable enough toward hydrolysis to be characterized (44, 45), the redox reaction between Cr^{VI} and the disaccharide is extremely slow at the temperature used in this work and does not reach completion, even after 1 year. The reaction of chromate with saccharides is completed in shorter periods only when the reaction is allowed to proceed under reflux (44, 45).

Chromate esters were investigated by UV–vis spectrophotometry in the 350–400 nm region in which these esters show characteristic absorption bands. Reactions were performed at pH 4.7, where the redox reaction is slow enough to enable the observation of the ester formation. The instruments were zeroed to both the reference and sample beams passing through matched cuvettes, both containing Cr^{VI} in H_2O at pH 4.7. The solution in the sample cell was replaced with the reaction solution containing 8×10^{-4} M Cr^{VI} and 0.12–0.32 M Lac at pH = 4.7, I = 1.0 M, and T = 33°C. Spectra obtained within 30 min after mixing revealed a distinctive absorption band at 377 nm.

The formation of Cr^{II} was examined by periodic scanning of solutions containing 0.21 M Lac and 0.016–0.09 mM Cr^{VI} in 0.1–0.2 M $HClO_4$ in the presence of dioxygen. Under aerobic conditions if Cr^{II} forms it rapidly converts to CrO_2^{2+}, which has characteristic absorption bands at 290 and 245 nm (33, 46) and can be detected at low $[Cr^{VI}]$. No new bands were detected at these wavelengths in the reaction of Cr^{VI} with Lac. The presence of Cr^{II} was also examined by carrying out experiments with Cr^{VI} and Lac in the presence of $[Co(NH_3)_5Cl]^{2+}$ (a Cr^{II} trapping agent) under anaerobic conditions (8, 47). A solution containing 0.3 M Lac, 4 mM Cr^{VI}, and 4 mM $[Co(NH_3)_5Cl]^{2+}$ in 0.2 M $HClO_4$, I = 1 M and T = 33°C, was left to react, and the Co^{II} content was analyzed after 10-fold dilution with concentrated HCl, centrifuging, and measuring the absorbance at 692 nm (the wavelength expected for the absorption band of $CoCl_4^{2-}$) in the supernatant. No Co^{II} was detected spectrophotometrically. These results suggest that the formation of Cr^{II} in the reaction of Cr^{VI} with Lac can be ruled out.

Product analysis

HPLC was employed to detect the reaction products under the conditions used in the kinetic measurements (excess disaccharide over Cr^{VI}). The chromatograms were obtained on a KNK-500A chromatograph provided with a 7125 HPLC pump. The effluent was monitored with refractive index (ERC-7522, ERMA INC) and UV (115 UV Gilson) detectors. The $[H^+]$ of the standard and the reaction mixture samples was adjusted to 0.1 or 0.2 M by addition of $HClO_4$, and then the samples were filtered through a 0.2 μm membrane prior to injection into the chromatographic system.

Standard solutions of the disaccharides and aldobionic acids were prepared individually in 0.10 M $HClO_4$ and chromatographed separately to determine the retention time of each sample. D-Maltobionic acid, D-cellobionic acid, and D-melibionic acid, used as standards, were synthesized according to the literature methods (48). The stability of the disaccharides and their respective acids was controlled by HPLC. Chromatograms registered after incubation of the standard samples in 0.2 M $HClO_4$ (the highest $[H^+]$ used in the kinetic measurements) at 34°C over 48 h were identical to those of freshly prepared samples.

Experimental conditions and retention times for the disaccharides and oxidation products are given as supplementary material.[3]

EPR measurements

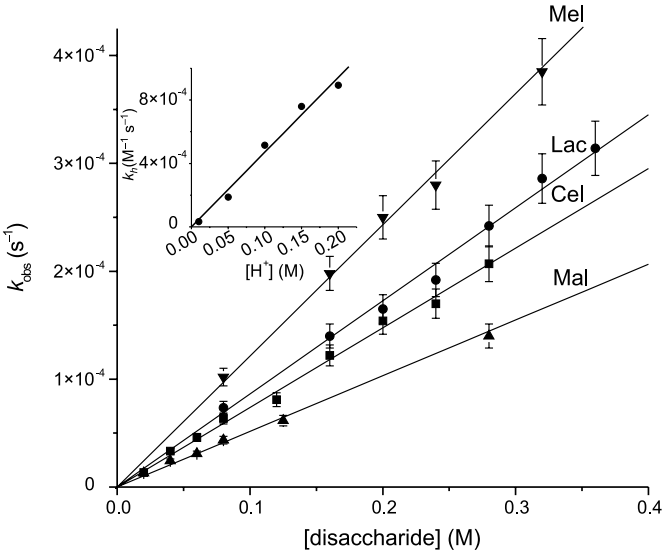
The EPR spectra were obtained on a Bruker ESP 300 E spectrometer. The microwave frequency was generated with a Bruker 04 ER (9–10 GHz) and measured with a Racal-Dana frequency meter. The magnetic field was measured with a Bruker NMR-probe gaussmeter. All of the EPR experiments were carried out at 20°C. Reactions were carried out by direct reaction of $K_2Cr_2O_7$ with 16- to 33-times excess disaccharide at a given pH or by addition of Na_2CrO_4 (8.3 mM) + glutathione (8.3 mM) to solutions of disaccharide (83 mM) at pH 5.0 and 20 ± 1°C.

EPR spectra were simulated using the program PEST WinSIM (49) with 100% Lorentzian lineshapes. The spectral parameters for each Cr^V species were consistent within all simulations, with maximum deviations between g_{iso} values being ±0.0001 units. In the simulations, values for ^1H a_{iso} were included only where the ^1H a_{iso} value was greater than the LW (line width) of the Cr^V species, since the signal is not significantly affected where the ^1H a_{iso} value is ≤LW.

Polymerization test

The presence of free radicals in the reactions of all of the disaccharides with Cr^{VI} was tested by the acrylonitrile polymerization test. In a typical experiment, acrylonitrile in 0.2 M $HClO_4$ (0.5 mL) at 33°C was added to a solution of 2.66×10^{-3} M $K_2Cr_2O_7$ and 0.04 M disaccharide in 0.2 M $HClO_4$ (2.0 mL). After a few minutes a white precipitate appeared. Control experiments (without $K_2Cr_2O_7$ or reductant present) did not show the formation of a precipitate. Possible reactions of Cr^V and Cr^{IV} with acrylonitrile were tested with $Na[Cr^VO(ehba)_2]$ (50) and $[Cr^{IV}O(ehba)_2]$ (ehba = 2-ethyl-2-hydroxybutanoic acid); the latter was generated in solution according to the literature method (51). No precipitation occurred on mixing the Cr^V or Cr^{IV} complexes with acrylonitrile under the same conditions as those used in the Cr^{VI} + disaccharide reactions.

Fig. 1. Effect of [disaccharide] on k_{obs} at 33°C and 0.20 M $HClO_4$. Inset: Effect of acidity on k_H, for Lac.



Results

Cr^{VI} oxidation of disaccharides

Over the whole range of perchloric acid concentrations used in the kinetic measurements, UV–vis studies showed that the reaction of each of the studied disaccharides with Cr^{VI} resulted in an absorbance band at 350 nm and a shoulder at 420–500 nm, characteristic of Cr^{VI} in acidic medium. At 350 nm a monotonic decrease in absorbance was observed and it could be described by a single exponential decay. Table 1 summarizes the values of the pseudo-first-order rate constants (k_{obs}), calculated from the slopes of the $\ln(A_{350} - A_8)$ vs. time data, for various concentrations of Lac, Mal, Cel, and Mel, in 0.20 M $HClO_4$. In every case, plots of k_{obs} vs. [disaccharide] show a first-order dependence on [disaccharide] (Fig. 1), and the values of the second-order rate constants (k_H) were determined from the slopes of the straight lines (Table 1, bottom). Consequently, at constant $[HClO_4]$, the rate law is expressed as:

$$[1] \qquad -d[Cr^{VI}]/dt = k_{obs}\,[Cr^{VI}]_T = k_H\,[\text{disaccharide}]\,[Cr^{VI}]$$

where $[Cr^{VI}]_T$ refers to the total Cr^{VI} concentration. For Lac, k_H shows first-order dependence with $[HClO_4]$ (inset of Fig. 1), which may be expressed as $k_H = k\,[H^+]$, with $k = 0.0047(1)$ M^{-2} s^{-1}.

As expected on the basis of the rate law, $-d(\ln[Cr^{VI}])/dt = k_{obs}$, where $k_{obs} = f([\text{saccharide}][H^+])$; k_{obs} was essentially constant with increasing $[Cr^{VI}]_0$.

[3] Supplementary data may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0S2, Canada (http://www.nrc.ca/cisti/irm/unpub_e.shtml for information on ordering electronically).

Fig. 2. Time evolution of the UV–vis spectrum of a mixture of Lac (0.32 M) and Cr^{VI} (8×10^{-3} M). [$HClO_4$] = 0.20 M; T = 33°C, I = 1.0 M over a period of ca. 250 min.

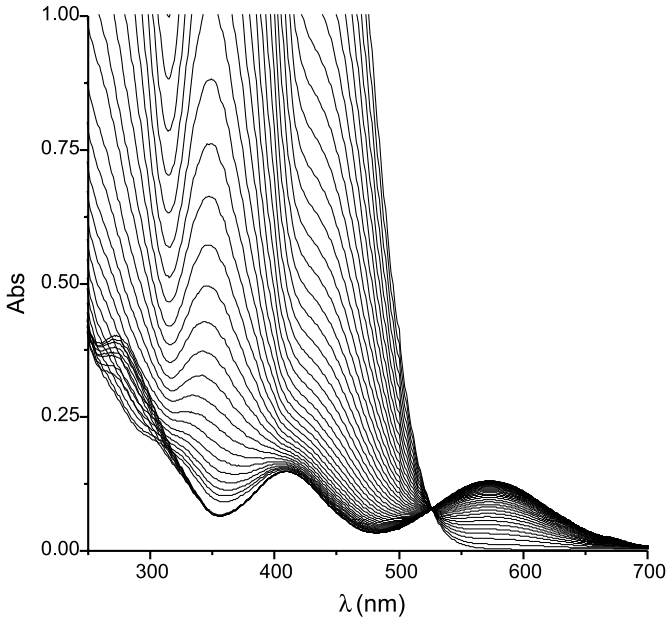
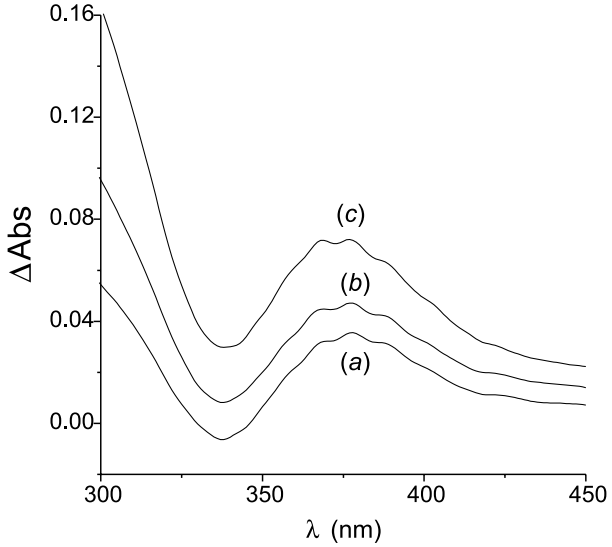
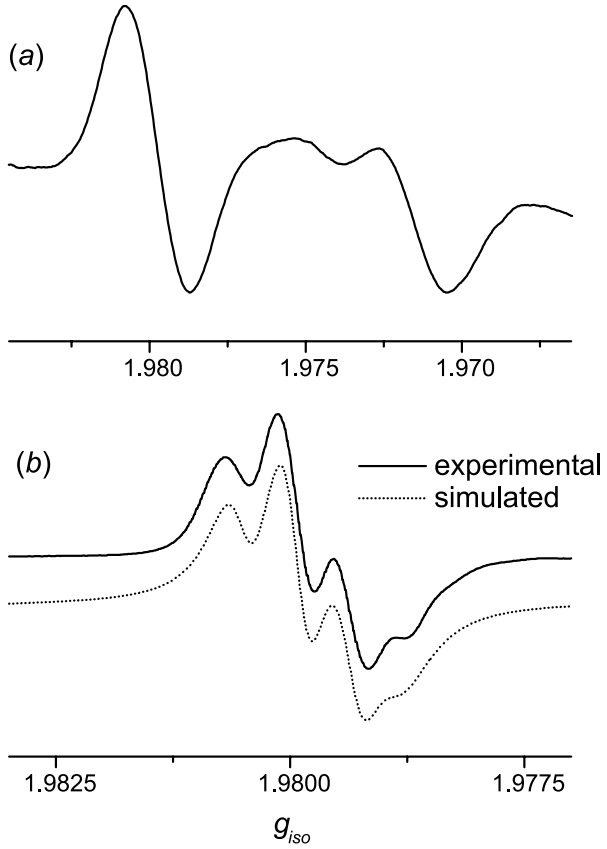


Fig. 3. UV–vis difference spectra of Cr^{VI}–Lac solutions at pH 4.7, showing the increasing band at 377 nm with increasing [Lac]: (*a*) 0.12, (*b*) 0.20, and (*c*) 0.32 M. [Cr^{VI}] = 8×10^{-4} M, T = 33°C, I = 1 M. Spectra taken after 2 min.



We followed the formation of Cr^{III} at 570 nm for various [disaccharide] in 0.20 M $HClO_4$ to determine the rate of Cr^{III} formation relative to that of Cr^{VI} consumption. The pseudo-first-order rate constants obtained at this wavelength (Table 1) are coincident with those obtained from the Cr^{VI} decay at 350 nm, with the same dependence on [disaccharide]. This means that Cr^{III} forms as fast as Cr^{VI} is consumed; this is consistent with the observation of an isosbestic point at 525 nm (Fig. 2). This result was independently confirmed by following the redox reaction by EPR spectroscopy. The rate

Fig. 4. X-band EPR spectra of mixtures of (*a*) Cr^{VI}–Lac = 1:33, [H^+] = 0.20 M, t = 6 min, modulated amplitude (mod. ampl.) = 4 G; (*b*) Cr^{VI}–Lac = 1:19, pH = 5.0, t = 24 h, mod. ampl. = 0.4 G. I = 1.0 M, T = 20°C, frequency ≈ 9.7 GHz.



constant of the reduction of Cr^{VI} by the saccharide (k_6) was found to be much lower than that of the reduction of Cr^V (k_5). The fact that $k_5 >> k_6$ implies that the slow redox step involves the reduction of Cr^{VI}.

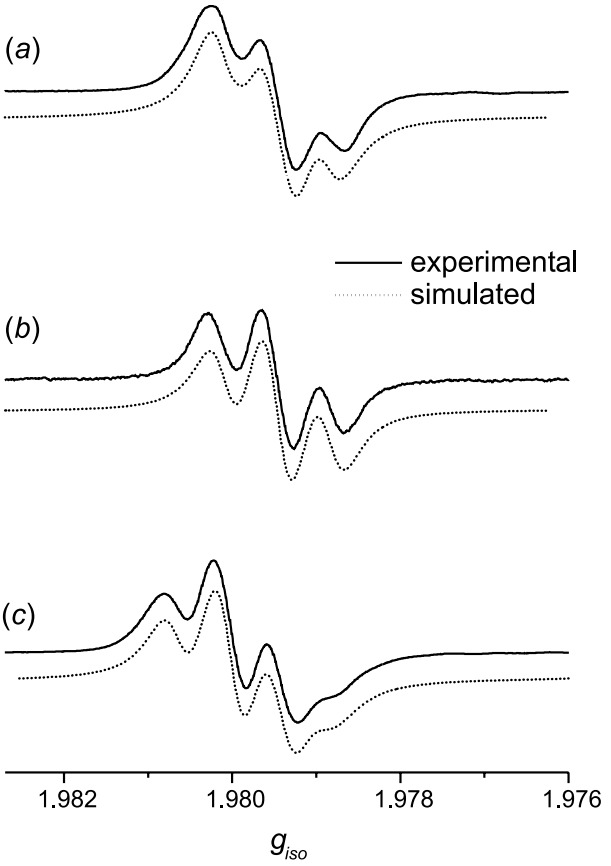
Detection of the intermediate Cr^{VI} ester

Differential UV–vis spectra of mixtures of Cr^{VI} and Lac exhibited an absorption band with λ_{max} = 377 nm (Fig. 3) consistent with that ascribed to Cr^{VI} oxy-esters (52, 53). At pH 4.7, the redox reactions of the studied disaccharides proceed very slowly with negligible reduction of Cr^{VI} within the first hour. Thus, at this pH the ester formation step can be distinguished clearly from the electron transfer reaction. Spectra obtained within 2 min after mixing revealed a distinctive absorption band at 377 nm. Continued scanning for 30 min showed no further change in the spectra. Varying the excess concentration of disaccharide at pH = 4.7 showed that the absorbance at 377 nm increased with increasing concentration of disaccharide (Fig. 3), probably as a result of a shift toward the ester in the esterification equilibrium.

Intermediacy of Cr^V

Using EPR spectroscopy, we have detected the formation of intermediate Cr^V species in the reaction of the disaccharides with Cr^{VI}. The EPR spectra of mixtures of Lac and Cr^{VI} in 0.2 M $HClO_4$ show the formation of several intermediate Cr^V species (Fig. 4*a*). At this [H^+], the EPR spec-

Fig. 5. Experimental and simulated X-band EPR spectra of mixtures of (*a*) Cr^{VI}–Mal = 1:19; (*b*) Cr^{VI}–Cel = 1:16; (*c*) Cr^{VI}–Mel = 1:19. [Cr^{VI}] = 0.015 M, I = 1.0 M, pH 5.0, t = 24 h, T = 20°C, frequency ≈ 9.7 GHz, mod. ampl. = 0.4 G.



tra consist of one signal at g_{iso} = 1.9797, which persists at higher pH, and two additional signals at g_{iso} = 1.9743 and 1.9715. At this [H^+], the EPR signals are not resolved because of the high modulation amplitude required to observe the Cr^V species. The signals at lower g_{iso} are not observed at pH > 2, where the electron transfer reaction becomes extremely slow; by contrast, the Cr^V species with g_{iso} = 1.9797 remains stable in solution for a long time.

At the [H^+] used in the kinetics measurements, the Cr^V EPR signal intensities grow and decay rapidly. For the reduction of Cr^{VI} by Lac ([H^+] = 0.2 M, [Lac] = 0.424 M, $[Cr^{VI}]_0$ = 0.0127 M, T = 20°C), the Cr^V EPR signal grows and decays within 3 h, while the total conversion of Cr^{VI} into Cr^{III} requires 20 h. Under these conditions, changes in the EPR signal intensity give values for the rate constant of the disappearance of Cr^{VI} (k_6), which is ~50-times higher than the corresponding rate constant for the disappearance of Cr^V (k_5). The k_6 values obtained from EPR data agree with the k_{obs} values calculated spectrophotometrically at 570 nm under the same conditions. Thus, the fact that $k_5 > k_6$ means that changes in absorbance at 350 nm reflect changes in Cr^{VI} concentration.

We have also performed the reaction of the disaccharides and Cr^{VI} at a lower [H^+] than was used in the kinetic measurements to obtain information on the structure of Cr^V species formed as intermediates in the reduction of Cr^{VI} to Cr^{III}.

Table 2. EPR spectral parameters for Cr^V intermediates in the reactions.[a]

Cr^V species	g_{iso}	a_{iso} (10^4 cm^{-1}) (N)
$[Cr(O)(cis\text{-}O,O\text{-Lac})_2]^-$	1.98	0.93 (2)
	1.9794	0.85 (2)
$[Cr(O)(O^1,O^2\text{-lactobionato})(H_2O)_3]^+$	1.9743	ND
$[Cr(O)(cis\text{-}O,O\text{-Lac})(H_2O)_3]^+$	1.9715	ND
$[Cr(O)(O^1,O^2\text{-Mal})_2]^-$	1.9795	0.76 (2)
	1.9794	0.92 (2)
$[Cr(O)(O^1,O^2\text{-Cel})_2]^-$	1.9793	0.97 (2)
$[Cr(O)(cis\text{-}O,O\text{-Mel})_2]^-$	1.9802	0.88 (2)
	1.9798	0.98 (2)

[a] N: number of equivalent protons. ND: not determined.

In the pH 3–7 range and with a Lac–Cr^{VI} ratio of 19:1, the EPR spectrum, taken 24 h after mixing, is composed (signals were deconvoluted by fitting the spectra to Lorentzian derivatives) of two triplets at g_{iso1} = 1.9800 and g_{iso2} = 1.9794 in an ~1:1 proportion. Each of these triplets shows four weak ^{53}Cr (9.55% abundance, I = 3/2) hyperfine peaks at 16.5(3) × 10^{-4} cm^{-1} spacing (Fig. 4*b*, Table 2).

In the pH 5–7 range, the EPR spectrum of a 19:1 Mal–Cr^{VI} reaction mixture affords two triplets at g_{iso1} = 1.9795 and g_{iso2} = 1.9794 in a 1:1 ratio (Fig. 5*a*). In the same pH range, a 16:1 Cel–Cr^{VI} reaction mixture yields one triplet at g_{iso} = 1.9793 (Fig. 5*b*, Table 2).

Mel behaves similarly to Lac. The best fit of the EPR spectrum of a 19:1 Mel–Cr^{VI} mixture in the pH 3–7 range taken 24 h after mixing affords two triplets at g_{iso1} = 1.9802 and g_{iso2} = 1.9798 (Fig. 5*c*, Table 2). These two signals are present in a 1:1 ratio independent of pH.

The ultimate fate of the chromium in these reactions is a Cr^{III} species, and a typical broad Cr^{III} EPR signal, centred at g ~ 1.98, is always observed at later times.

At room temperature and pH 5.0, the reaction of chromate with glutathione (1:1 ratio) in the presence of a 10-times excess disaccharide over Cr^{VI} affords Cr^V EPR spectra identical to those obtained by direct reaction of the disaccharide with Cr^{VI}.

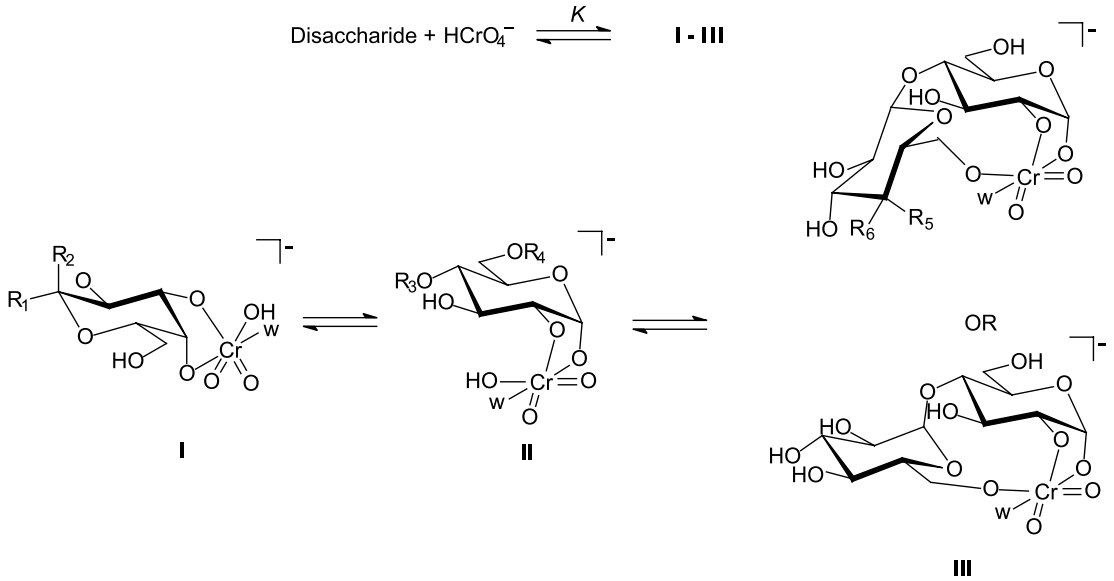
Discussion

Cr^{VI} oxidation of disaccharides

For the ranges of substrate and acid concentrations used in the kinetics measurements, the oxidation of the disaccharides by Cr^{VI} is a complex reaction, yielding $[Cr(H_2O)_6]^{3+}$ and the aldobionic acid as the final redox products. We propose a mechanism that takes into account: (*a*) the kinetic results; (*b*) the polymerization of acrylonitrile added to the reaction mixture; (*c*) the detection of an intermediate Cr^{VI} ester; (*d*) the detection of intermediate of oxochromate(V) species by EPR; and (*e*) the formation of the aldobionic acid as the only organic reaction product.

At the [H^+] and [Cr^{VI}] used in the kinetics studies, Cr^{VI} may exist as $HCrO_4^-$ (54), and this species is proposed as the reactive form of Cr^{VI}, in agreement with the first-order dependence of the reaction rate on [Cr^{VI}]. The chromic oxidation of alcohols and glycols are preceded by the formation of a chromate ester (52, 53). The observation of the

Scheme 1. Cr^{VI}–Mel: $R_1 = O^5$-glc, R_2 = H; Cr^{VI}–Lac: R_1 = H, $R_2 = O^4$-glc. Cr^{VI}–Lac(Mal, Cel): R_3 = glycoside, R_4 = H; Cr^{VI}–Mel: R_3 = H, R_4 = glycoside. Cr^{VI}–Lac: R_5 = OH, R_6 = H; Cr^{VI}–Cel: R_5 = H, R_6 = OH. AldA = aldobionic acid; w = water.



$$\mathbf{II/III} \xrightarrow{k,\ H^+} \text{AldA} + Cr^{IV} \quad [2]$$

$$Cr^{IV} + \text{Disaccharide} \longrightarrow \text{Disaccharide}\bullet + Cr^{III} \quad [3]$$

$$\text{Disaccharide}\bullet + Cr^{VI} \longrightarrow \text{AldA} + Cr^{V} \quad [4]$$

$$\text{Disaccharide} + Cr^{V} \longrightarrow \text{AldA} + Cr^{III} \quad [5]$$

absorbance band, characteristic of chromate oxy-esters, at approximately 377 nm (a few minutes after mixing the disaccharides and Cr^{VI} under conditions where the redox reaction is extremely slow) reveals that such an intermediate Cr^{VI} complex is rapidly formed prior to the redox steps. Thus, the first step of the mechanism proposed in Scheme 1 is the formation of the disaccharide–Cr^{VI} mono-chelate. The disaccharide acts as a bidentate ligand bound to Cr^{VI} via any pair of properly disposed hydroxyl groups. While there is no direct evidence for bidentate chelation of the sugar to Cr^{VI}, it is likely that this is the case, especially since Cr^{VI} chelates have been observed by NMR spectroscopy in the oxidations of another bidentate oxygen donor (18). The chelates proposed in Scheme 1 are those with the ligand bound to Cr^{VI} at the *cis*-$O^{3'}$,$O^{4'}$-diolato (**I**) and *cis*-O^1,O^2-diolato (**II**) moieties of Lac and Mel and the *cis*-O^1,O^2-diolato (**II**) moiety of Mal and Cel. These are proposed on the basis that α-glc forms the Cr^{VI}–glc chelate faster than the β-D-anomer — a fact attributed to the *cis*-1,2-diolato moiety present only in the α-anomer — (21) and the observation of the marked preference of the CrO^{3+} ion for binding to *cis*- rather than *trans*-diol groups of cyclic diols (21, 22, 29, 55). For Mal, Lac, and Cel, **II** is proposed to be in equilibrium with Cr^{VI}–O^1,O^2,$O^{6'}$-disaccharide chelates (**III**). Molecular models show that Lac, Mal, and Cel can afford Cr^{VI}–chelates with the disaccharide bound to Cr^{VI} at O^1,O^2,$O^{6'}$ and with retention of the $^4C_1$ chair conformation of the glycoside moiety. Taking into account that in the redox reaction only the anomeric hydroxyl group is oxidized, it seems reasonable to hypothesize that the complexes with the primary hydroxyl group bound to Cr^{VI} (**II**, **III**) should be the precursors of the slow redox steps.

The slow redox step in the mechanism might take place by either a one-electron or a two-electron transfer (56). The slow step proposed in Scheme 1 involves the intramolecular transfer of two electrons to yield Cr^{IV} and the aldobionic acid (eq. [2]). This is based on the mechanism reported for aldoses, which are also selectively oxidized by Cr^{VI} at the hemiacetalic group with an initial two-electron transfer slow step (21–28). After the slow redox step, Cr^{IV} is predicted to react with excess saccharide to yield Cr^{III} and a disaccharide radical in a fast step (eq. [3]). The latter is supported by the observed polymerization of acrylonitrile when it is added to the reaction mixture. The rapid reaction of the disaccharide radical with Cr^{VI} affords Cr^{V} (eq. [4]), which can further oxidize the disaccharide to yield Cr^{III} and the aldobionic acid as the final oxidation product (eq. [5]).

The k_H in the rate law corresponds to $kK[H^+]$ in this mechanism. This means that the relative reactivity of the disaccharides may be interpreted by taking into account both the rate of formation of the Cr^{VI} ester and its redox rate. Our experimental data show that the disaccharide–Cr^{VI} chelate formation is much faster than the redox steps. For certain aldoses the rate constant for the formation of the aldose–Cr^{VI} chelate (K) in 0.75 M $HClO_4$ has been determined and was found to be 500- to 2000-times higher than the redox

constant (k). Thus, the redox rate should depend, essentially, on the energy barrier the Cr^{VI} chelate has to overcome to attain the five-membered cyclic transition state for the intramolecular H transfer (22). The kinetics data show that the relative reactivity of the disaccharides toward Cr^{VI} is Mel > Lac > Cel > Mal. If we compare the reactivity of these saccharides with glc, which is oxidized by Cr^{VI} with a rate constant of $k_H = 9.9 \times 10^{-4}$ M^{-1} s^{-1} in 0.2 M $HClO_4$ at 33°C (22), we can observe that substitution of the O^4H group of glc by the glycoside moiety results in the retardation of the redox rate, while substitution of the primary O^6H accelerates the redox reaction. The retardation of the redox reaction observed for Mal, Cel, and Lac is probably related to the formation of the Cr^{VI}–O^1,O^2,$O^{6'}$-disaccharide chelates (III). The probability for Mel to act as a tridentate ligand is very low because of the less favourable spatial orientation of the galactoside moiety; therefore, it shows the highest reactivity in the series.

Cr^V intermediates

The most common means of characterizing Cr^V complexes in solution is EPR spectroscopy. The EPR spectral parameters, g_{iso} and A_{iso}, together with the proton superhyperfine (shf) coupling, are useful in determining the binding modes of sugars to the Cr^V center (1, 22, 24, 26, 27, 57, 58). An empirical relationship between the nature and number of donor groups and the EPR spectral parameters of Cr^V complexes has been established (1, 57). Five-coordinate Cr^V species show higher g_{iso} and lower ^{53}Cr A_{iso} values than the corresponding six-coordinate species (57, 59, 60). Thus, the assignment of the structures of new oxo-Cr^V species in solution was made according to the isotropic EPR parameters (g_{iso} and A_{iso} values) and the superhyperfine (shf) pattern of the signal (1, 59).

The EPR spectrum of a Cr^V-diolato species of six-membered ring *cis*-diols yields a doublet, since only one proton is in the plane of the unpaired electron density of the Cr^V ion, while the EPR spectrum of a Cr^V-diolato species of six-membered ring *trans*-diols (with no protons lying in the ligand plane) yields a singlet (55). Consequently, the EPR spectral multiplicity of the bis-chelate Cr^V-diolato$_2$ species formed between Cr^V and pyranosic *cis*- and *trans*-diols will exhibit a triplet and a singlet, respectively, which arise from the orientation of the ring protons of the coordinated diol groups with respect to the basal plane of the complex (1, 55, 57). Based on this, the isotropic EPR parameters g_{iso} and A_{iso} were used to deduce the coordination number and nature of the donor groups of the Cr^V species formed, either for the reaction of Cr^{VI} with the disaccharides or for the reaction of Cr^{VI} with glutathione in the presence of excess disaccharide, according to a described empirical method (1, 57, 59, 60).

The *cis*-diolato moieties of the disaccharides under study are potential binding sites to give five-membered Cr^V species. The five-membered Cr^V chelates are the most thermodynamically favoured, and CrO^{3+} shows a marked preference for binding to *cis*- rather than *trans*-diol groups of cyclic diols (21, 22, 29, 55). Based on this, the EPR spectral features of disaccharide–Cr^V mixtures were interpreted in terms of Cr^V-diolato species involving binding mainly through the *cis*-O^1,O^2-diolato of Mal and Cel and the *cis*-O^1,O^2-diolato and *cis*-$O^{3'}$,$O^{4'}$-diolato of Lac and Mel. The contribution to the EPR signals from other species, involving *trans*-diolato moieties, should be small.

Lactose–Cr^V

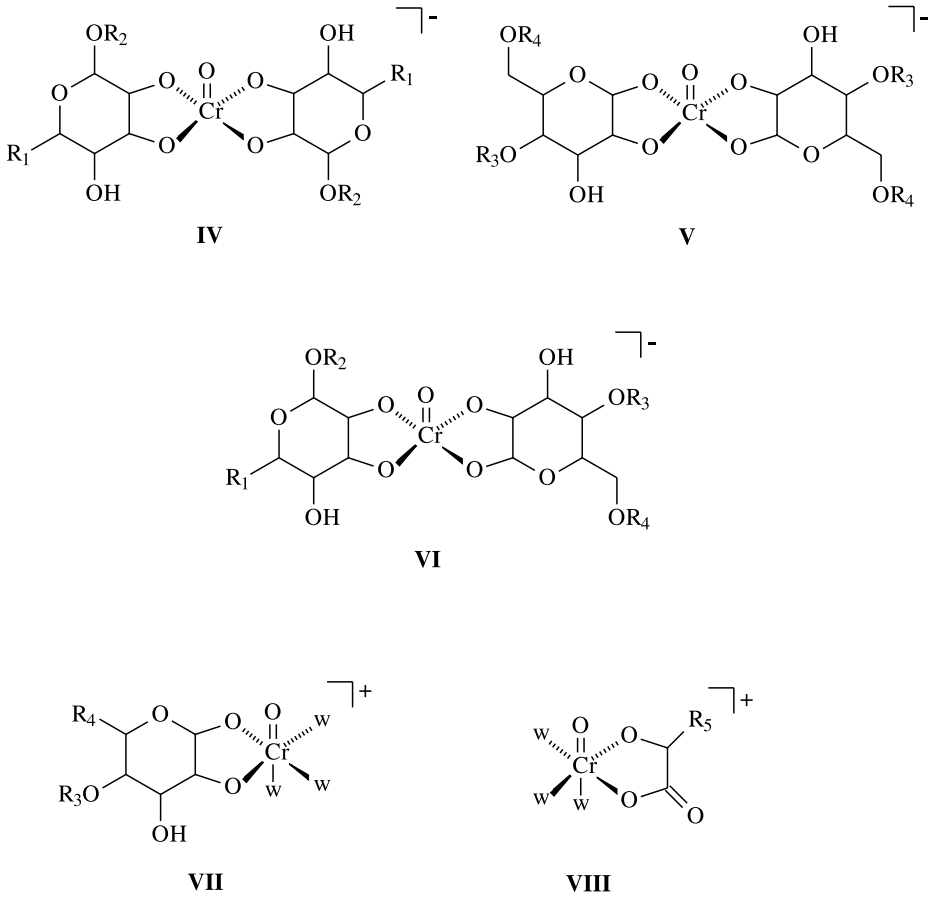
The reaction of Cr^{VI} with a 20-times excess of Lac in the pH 3–7 range affords an EPR spectrum dominated by a single detectable signal at $g_{iso} = 1.9797$ with the four weak ^{53}Cr (9.55% abundance, $I = 3/2$) hyperfine peaks at 16.5×10^{-4} cm^{-1} spacing; the product remains in solution from several days to several months. When the modulation amplitude is lowered to 0.4 G, the EPR signal resolves into two triplets at $g_{iso1} = 1.9800$ and $g_{iso2} = 1.9794$ ($A_{iso} = 16.5(3) \times 10^{-4}$ cm^{-1}, ~50:50 g_{iso1}–g_{iso2} ratio) (Fig. 4*b*, Table 2). The g_{iso} and A_{iso} values are consistent with those calculated for five-coordinate oxochromate(V) complexes with four alcoholato donors ($g_{calc} = 1.9800$, $A_{calc} = 16.5 \times 10^{-4}$ cm^{-1}) (1, 57). The shf pattern indicates that the EPR signal is a composite of two oxo-Cr^V-*cis*-diolato$_2$ species with two (one from each chelate ring) carbinolic protons coupled to the Cr^V electronic spin. The two components can be attributed to linkage isomers of the $[Cr(O)(cis\text{-}O,O\text{-Lac})_2]^-$ (61). Even when three different linkage isomers $[Cr(O)(cis\text{-}O^{3'},O^{4'}\text{-Lac})_2]^-$, $[Cr(O)(cis\text{-}O^1,O^2\text{-Lac})_2]^-$, and $[Cr(O)(cis\text{-}O^1,O^2\text{-Lac})(cis\text{-}O^{3'},O^{4'}\text{-Lac})]^-$ (**IV**–**VI** in Fig. 6) are possible, only two species are required to simulate the EPR signal. It seems reasonable to assume that the angles between the carbinolic protons and the Cr^V–ligand plane in two of the linkage isomers of $[Cr(O)(cis\text{-}O,O\text{-Lac})_2]^-$ are very similar, as we cannot distinguish between them.

A more complex EPR spectral pattern is observed in a 0.2 M $HClO_4$ solution. At this $[H^+]$ and a 33:1 ligand–Cr^{VI} ratio, two additional signals at $g_{iso3} = 1.9743$ and $g_{iso4} = 1.9715$ appear, together with the signal at $g_{iso} = 1.9797$ (Fig. 4*a*, Table 2). The shf pattern of these two new signals was not resolved, but the signal at $g_{iso} = 1.9715$ suggests the presence of six-coordinate oxo-Cr^V species, possibly $[Cr(O)(cis\text{-}O,O\text{-Lac})(H_2O)_3]^+$ (**VII**). The calculated g_{iso} value for a six-coordinate oxo-Cr^V species with two alcoholato donors and three water molecules ($g_{clc} = 1.9724$) is in reasonable agreement with the observed g_{iso4} value. The positive charge of this species is also consistent with its appearance at high $[H^+]$. The signal at $g_{iso3} = 1.9743$ may be tentatively attributed to another positively charged six-coordinate oxo-Cr^V mono-chelate $[Cr(O)(O^1,O^2\text{-lactobionato})(H_2O)_3]^+$ (**VIII**, $g_{clc} = 1.9735$), with Cr^V bound to the 2-hydroxyacid moiety of the oxidized product and to three water molecules. Coordination to the oxidized ligand is consistent with the fact that Cr^{VI} is a stronger oxidant in more acidic medium. The six-coordinate oxo-Cr^V mono-chelate **VII** is possibly responsible for the higher rates observed for the Cr^V–disaccharide redox reactions at pH < 2. At higher pH, where Cr^V mono-chelates are not observed, Cr^V complexes are stable enough to remain in solution from several days to several months.

Maltose–Cr^V

For the reaction of Cr^{VI} with Mal, in the pH 5–7 range and with a 19:1 ligand–metal ratio, the EPR spectra (Fig. 5*a*, Table 2) consist of two triplets at $g_{iso1} = 1.9795$ and $g_{iso2} = 1.9794$. The g_{iso} values and the shf pattern indicate these signals may be attributed to two geometric isomers (typical

Fig. 6. Structures of Cr^V complexes with disaccharide ligands. R_1 = CH_2OH, R_5 = $C_{10}O_9H_{19}$, w = water. Cr^V–Lac: R_2 = O^4-glc; Cr^V–Mel_2: R_2 = O^6-glc; Cr^V–Lac_2 (Cr^V–Mal_2, Cr^V–Cel_2): R_3 = glycoside, R_4 = H; Cr^V–Mel_2: R_3 = H, R_4 = glycoside.



Δg_{iso} for geometric isomers are $1 \times 10^{-4} - 2 \times 10^{-4}$) (27, 30) of the five-coordinate oxo-Cr^V complex $[Cr(O)(O^1,O^2\text{-Mal})_2]^-$ (**V**), with Mal bound to Cr^V via the 1,2-*cis* diolato moiety.

Cellobiose–Cr^V

Intermediate Cel–Cr^V species formed in the reaction of Cr^{VI} with Cel, in the pH 5–7 range and with a 14:1 ligand–metal ratio, yield EPR spectra (Fig. 5*b*, Table 2) consisting of one triplet at g_{iso1} = 1.9793. The g_{iso} value corresponds to that calculated for a five-coordinate oxochromate(V) complex with four alcoholato donors, and the shf pattern indicates this signal may be assigned to the $[Cr(O)(O^1,O^2\text{-Cel})_2]^-$ chelate (**V**), with Cel bound to Cr^V via the 1,2-*cis* diolato moiety.

Melibiose–Cr^V

In the reaction of Cr^{VI} with Mel at a pH 3–7 range and a 19:1 ligand–metal ratio, the EPR spectra of the intermediate Cr^V species show two triplets at g_{iso1} = 1.9802 and g_{iso2} = 1.9798 (~50:50 g_{iso1}–g_{iso2} ratio). The g_{iso} values and the shf pattern reveal that these signals correspond to different linkage isomers of the five-coordinate oxo-Cr^V complex $[Cr(O)(O,O\text{-Mel})_2]^-$. As in the case of Lac, the three linkage isomers $[Cr(O)(O^{3'},O^{4'}\text{-Mel})_2]^-$ (**IV**), $[Cr(O)(O^1,O^2\text{-Mel})_2]^-$ (**V**), and $[Cr(O)(O^{3'},O^{4'}\text{-Mel})(O^1,O^2\text{-Mel})]^-$ (**VI**) are possible, but only two Cr^V species are required to simulate the EPR signal, probably for the same reasons as for the Lac–Cr^V system.

EPR spectra identical to those shown in Figs. 4*b* and 5*a*–5*c* are obtained when Cr^V is generated by the reaction of Cr^{VI} with glutathione (1:1 ratio) at pH = 5.0 and stabilized by the presence of a 10-times excess of disaccharide over Cr^{VI}. This result indicates that Cr^V species formed at this pH effectively correspond to chelates formed with the disaccharide and not with the oxidized product (aldobionic acid).

Conclusions

Disaccharides form stable high-valent chromium chelates at pH > 2, while at higher $[H^+]$ their oxidation by Cr^{VI} and (or) Cr^V is favoured. The oxidation occurs selectively at the hemiacetalyc C^1OH group, with first-order kinetics for both $[Cr^{VI}]$ and [disaccharide]. The relative ability of the studied disaccharides to reduce Cr^{VI} — Mel > Lac > Cel > Mal — can be attributed to the increasing stability of the intermediate disaccharide–Cr^{VI} chelates formed with Mel through Mal. The redox and complexation chemistry observed for the disaccharide–Cr^{VI} systems parallels that of aldoses. The glycoside moiety can retard the redox rate of the aglycone when additional binding to Cr^{VI} by the glycoside moiety is possible. In the case of Mal, this effect reduces k_H to one half of the value obtained for glc.

At pH 3–7, redox reactions between Cr^V and disaccharides are extremely slow, since once the bis-chelates are formed they are long-lived. A different reactivity pattern is observed at high [H^+], where intramolecular electron-transfer processes take place and mono-chelates are observed. The observation of six-coordinate $[Cr^VO(O,O\text{-disaccharide})(OH_2)_3]^+$ at only high [H^+], together with the selectivity of the redox reaction, suggest that the six-coordinate Cr^V mono-chelate **VII** could be the precursor of the intramolecular electron-transfer reaction step in the redox reactions.

Acknowledgments

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