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OXIDATION OF 2-ACETAMIDO-2-DEOXY-D-GLUCOSE BY Cr^{VI} IN PERCHLORIC ACID

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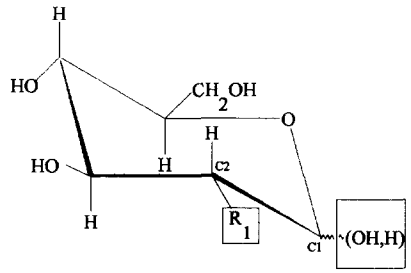
Abstract—The oxidation of 2-acetamido-2-deoxy-D-glucose by Cr^{VI} in perchloric acid has been found to follow the rate law: $-d[Cr^{VI}]dt = (a+b[H^+]^2+c[GlcNAc]^2[H^+]^2)\,[Cr^{VI}]_T$ where $a = 7.37\pm0.35\times10^{-5}$ s^{-1}; $b = 3.90\pm0.67\times10^{-4}$ M^{-2} s^{-1}; and $c = 1.18\pm0.01\times10^{-3}$ M^{-4} s^{-1}. This rate law corresponds to the reaction leading to the formation of 2-acetamido-2-deoxy-D-gluconic acid when a 20-fold or higher excess of aldose over chromium is employed. The results are discussed in terms of a possible mechanism with the associated reaction kinetics.

Chromium, in oxidation state 6, in many different compounds is a well-established carcinogen and mutagen.[1-4] There is, therefore, appreciable interest in determining the mode of action of chromium species derived from initial Cr^{VI} compounds taken into the body.[5] Until now, the major coordination sites involved in chromium binding in natural systems have been found to be hydroxyl and thiol donors, which form stable esters with chromate and stabilize Cr^{V} intermediates after further interaction with biological reductants.[6-10] For this reason, some sugars, or their derivatives, may play an important role in the chemistry of Cr^{VI}, especially in the environment.[11]

In previous work we have found that the number and arrangement of hydroxyl groups in the sugar molecules affect the chromic oxidation rate.[12-14] In any case, the first reaction step involves formation of a chromic ester right before the slow redox steps with C(1)—OH and C(2)—OH being the preferred coordination sites in the electron transfer precursor. This precursor behaves as a steady state for 2-deoxyaldoses,[15] whereas for aldoses and 2-aminoaldoses[16] (amino groups in 2-aminoaldoses seem to be as or more effective than the hydroxyl groups in binding Cr^{VI}) its formation has been interpreted as a rapid preequilibrium with kinetics giving typical saturation curves.

We decided to study oxidation of 2-acetamido-2-deoxy-D-glucose (GlcNAc) (Scheme 1) by Cr^{VI} in order to determine whether acetate acts as a blocking group compelling chromium to interact with the anomeric hydroxyl group only (in this case kinetic results should be similar to those obtained with 2-deoxyglucose) or if it would be able to behave as a binding site.



R_1=OH D-glucose
R_1=H 2-deoxy-D-glucose (deoxysugar)
R_1=NH_2 2-amino-2-deoxy-D-glucose (aminosugar)
R_1=$NHCOCH_3$ 2-acetamido-2-deoxy-D-glucose

Scheme 1.

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EXPERIMENTAL

Materials

2-Acetamido-2-deoxy-D-glucose (Sigma grade), potassium dichromate (Cicarelli c.a), perchloric acid (P.A. Merck), acrylonitrile (Aldrich grade) and sodium perchlorate (Sigma grade) were used without further purification.

Spectrophotometric measurements

Kinetic measurements were made at 350 nm by monitoring the absorbance changes on a Gilford Response II spectrophotometer with fully thermostated cell compartments. Rate constants deduced from multiple determination were within $\pm 5\%$ of each other. In most experiments the concentration of Cr^{VI} was kept constant at 6×10^{-4} M while the GlcNAc was varied from 0.036 to 0.144 M. Mixtures of sodium perchlorate and perchloric acid were used to maintain a constant ionic strength of 1.5 M.

Disappearance of Cr^{VI} was followed until at least 80% conversion. Reactants solutions were previously thermostated and transferred into a cell of 1.0 cm path length, immediately after mixing. Experiments were performed at 50°C unless otherwise mentioned.

Product analysis

Under conditions used in the kinetic measurements (ratios of GlcNAc over Cr^{VI} from 60:1 to 240:1) qualitative identification of 2-acetamido-2-deoxy-D-gluconic acid (Scheme 2) (acid GlcNAc) as the reaction product was made against an authentic sample using n-butanol/acetic acid/water (4:1:5) as eluant. Paper chromatograms were visualized by two kinds of development reagents: a three stage dip of silver nitrate, sodium hydroxide and sodium thiosulphate[17] and *p*-anisidine reagent.[18] Under these conditions carbon dioxide was never detected.

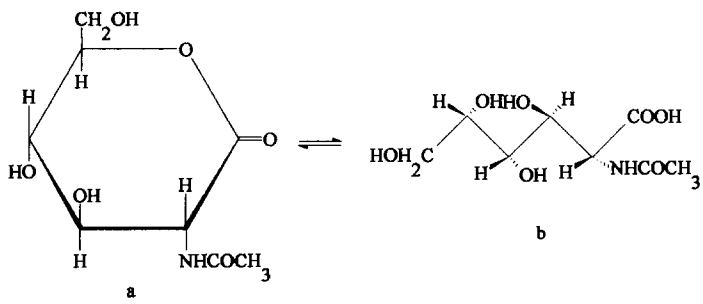
Substrate stability under experimental conditions was determined by comparing ^{13}C spectrum and paper chromatogram of a 0.75 M $HClO_4$ aqueous solution of GlcNAc heated for 3 h against those of unheated samples. In this way, no decomposition was observed.

Test for free radicals

A solution of potassium dichromate (3.97×10^{-3} M) in 2 cm^3 of 0.75 M $HClO_4$ was added to a mixture of 0.2 M GlcNAc in 0.75 M $HClO_4$ (2 cm^3) at 52°C. After 30 min a white precipitate of a polymer slowly appeared. Blank experiments in the absence of organic substrate or oxidant agent did not show formation of any such precipitate.

RESULTS AND DISCUSSION

Over the whole range of perchloric acid concentrations used in the kinetic measurements, UV–vis studies showed that reaction of GlcNAc with Cr^{VI} resulted in an absorbance band at 350 nm and a shoulder at 420–500 nm characteristic of the $Cr_2O_7^{2-}$ ion. It is known that Cr^V species, usually formed in these oxidation reactions, absorb at 350 nm and may superimpose Cr^{VI} absorbance yielding wrong interpretation at spectrophotometric absorbance decay values.[19] However, if Cr^V reacts as soon as it is formed, changes in absorbance at 350 nm essentially reflect changes in Cr^{VI} concentration. Several reasons indicate that this is the case. First, at 350 nm a monotonic decrease of absorbance is observed and it may be described by a single exponential decay. Reaction mixtures do not show an ESR signal even when saturated solutions of reactants were used nor the band at 750 nm, typical of Cr^V species, was observed under our acid conditions as it is in the case for oxidation reactions in



Scheme 2. 2-Acetamido-2-deoxy-D-gluconic acid (acid GlcNAc): (a) lactone form; (b) open chain form.

Table 1. Observed first-order rate constants for different $[Cr^{VI}]_o$. [GlcNAc] = 0.0896 M; $[H^+]$ = 0.75 M; I = 1.5; Temp. 50°C; λ = 350 nm

$10^4[Cr^{VI}]_o$ (M)	4.0	5.0	6.0	7.0	10.0	15.0	20.0
$10^3 k_{obs}(s^{-1})$	1.11	1.12	1.14	1.14	1.19	1.26	1.29

Table 2. Observed first-order rate constant for the oxidation of 2-acetamido-2-deoxy-D-glucose by Cr^{VI} at different $HClO_4$ concentration. $[Cr^{VI}]_o$ 6×10^{-4} M; I = 1.5 M, temp.: 50°C, λ = 350 nm

	$10^4 k_{obs}$ (s^{-1}) at $[HClO_4]^2$ (M)					
	0.16	0.25	0.423	0.563	0.723	1.00
[GlcNAc] (M)						
0.036	1.47	2.33	3.31	4.54	5.21	6.61
0.072	2.16	3.06	4.65	6.10	7.53	9.85
0.084	2.83	3.78	5.69	7.77	9.76	—
0.120	4.49	6.03	9.26	11.4	15.6	21.8
0.144	5.68	8.17	12.7	16.4	21.2	30.0

which Cr^V reduction occurs at a comparable or slower rate than that of Cr^{VI}.[13,19-21] Thus, it seems reasonable to think that Cr^V, if it is formed, exists in solution in a sufficiently small concentration causing no interfering absorbance at 350 nm.

In order to verify the first-order dependence of rate upon Cr^{VI} pseudo-first-order rate constants were calculated at various $[Cr^{VI}]_o$ from $(4-20) \times 10^{-4}$ M but at constant temperature, [GlcNAc], $[H^+]$ and I (Table 1). As expected on the basis of a rate law $-d(\ln[Cr^{VI}])/dt = k_{obs}$, where $k_{obs} = f([GlcNAc][H^+])$, k_{obs} was found to be essentially constant with increasing amounts of $[Cr^{VI}]_o$.

Table 2 summarizes values of k_{obs} for various concentrations of GlcNAc at fixed concentrations of perchloric acid. Plots of k_{obs} *vs* $[H^+]^2$ at constant [GlcNAc] gave straight lines (Fig. 1) with a positive intercept from which values of k_a and k_b were determined (Table 3). k_a $(7.37 \pm 0.35 \times 10^{-5}\ s^{-1})$ is independent of [GlcNAc] whereas plots of k_b *vs* $[GlcNAc]^2$ (Fig. 2) may be expressed as consisting of an independent and quadratic term:

$$k_b = k_1 + k_2[GlcNAc]^2 \quad (1)$$

where $k_1 = 3.90 \pm 0.67 \times 10^{-4}\ M^{-2}\ s^{-1}$ and

$$k_2 = 1.18 \pm 0.04 \times 10^{-1}\ M^{-2} s^{-1}.$$

The complete rate law is then given by:

$$-d[Cr^{VI}]/dt = k_{obs}[Cr^{VI}]_T$$

$$(k_o + k_1[H^+]^2 + k_2[GlcNAc]^2[H^+]^2)[Cr^{VI}]_T \quad (2)$$

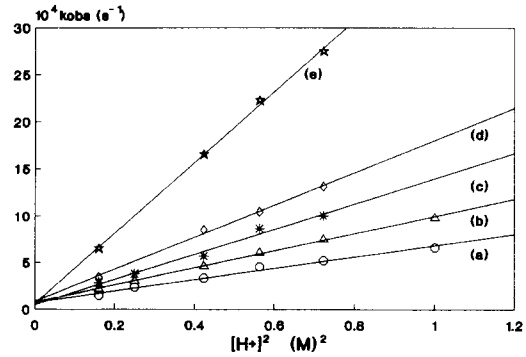


Fig. 1. k_{obs} as a function of $[H^+]^2$ at different [GlcNAc]. $[Cr^{VI}]_o = 6.0 \times 10^{-4}$ M; I = 1.5 M; λ = 350 nm; Temp. 50°C. (a) [GlcNAc] = 0.036 M, (b) [GlcNAc] = 0.072 M, (c) [GlcNAc] = 0.084 M, (d) [GlcNAc] = 0.120 M, (e) [GlcNAc] = 0.144 M.

where $[Cr^{VI}]_T$ represents the total Cr^{VI} concentration.

Mechanism

A mechanism consistent with all experimental data is given in the scheme

$$GlcNAc + Cr_2O_7^{2-} \underset{}{\overset{K_1}{\rightleftharpoons}} \underset{A^{2-}}{[GlcNAc\text{---}Cr_2O_7]^{2-}} \quad (3)$$

Table 3. Calculated k_2 for the oxidation of 2-acetamido-2-deoxy-D-glucose by Cr^{VI}. $[Cr^{VI}]_o$ 6×10^{-4} M; I = 1.5 M, temp.: 50°C, λ = 350 nm

	[GlcNAc] (M)				
	0.036	0.072	0.084	0.120	0.144
$10^5 k_1$ ($M^{-2} s^{-1}$)	7.53	7.73	7.02	7.16	7.43
$10^4 k_2$ ($M^{-2} s^{-1}$)	6.08	9.21	12.4	20.6	28.8

$$A^{2-} + GlcNAc \overset{K_2}{\rightleftharpoons} [(GlcNAc)_2 \cdots Cr_2O_7]^{2-} \quad B^{2-} \tag{4}$$

$$A^{2-} \xrightarrow{k_3} \text{acid GlcNAc} + Cr^{IV} \tag{5}$$

$$A^{2-} \underset{k_4}{\overset{H^+}{\longrightarrow}} HA^- \underset{k_5}{\overset{H^+}{\longrightarrow}} H_2A \xrightarrow[k_6]{} \text{acid GlcNAc} + Cr^{IV} \tag{6}$$

$$B^{2-} \underset{k_7}{\overset{H^+}{\longrightarrow}} HB^- \underset{k_8}{\overset{H^+}{\longrightarrow}} H_2B \xrightarrow[k_9]{GlcNAc} \text{acid GlcNAc} + Cr^{IV}. \tag{7}$$

By this mechanism two intermediate complexes between GlcNac and Cr^{VI} are formed. The anionic complex A^{2-}, where only one GlcNAc molecule binds Cr^{VI}, decomposes directly to the products or may be doubly protonated to H_2A to yield acid GlcNAc and finally Cr^{IV}. Complex A^{2-} is in equilibrium with B^{2-} where a second GlcNAc molecule binds Cr^{VI}. Complex B^{2-} yields acid GlcNAc and Cr^{IV} in the presence of an additional GlcNAc molecule by an acid catalysed pathway.

Oxidation paths are proposed to be two-electron transfer steps. However, at present, two one-electron steps may not be disregarded since the presence of Cr^{IV} or Cr^{V} could not be evidenced.

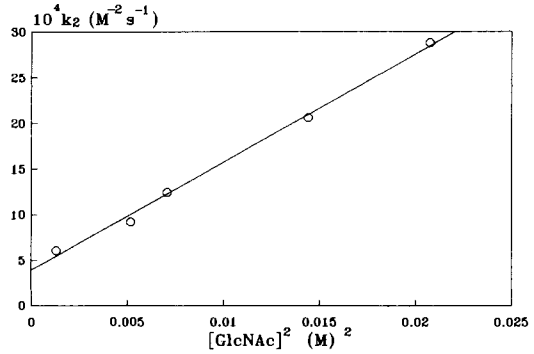


Fig. 2. k_2 as function of $[GlcNAc]^2$. $[Cr^{VI}]_o = 6.0 \times 10^{-4}$ M; I = 1.5 M; λ = 350 nm; temp. 50°C.

In the proton concentration range studied here, Cr^{VI} exists mainly as $Cr_2O_7^{2-}$.[22] On the other hand, the N-atom has a great tendency to complex with transition metal ions, and, if there is a free hydroxyl group in the vicinity, bidentate complexes are formed that are much stronger than those N-free sugars.[23] Several 2-acetamidoaldoses appear to complex at the acetamido group.[24] Besides, in blocked amino acids the acetamido group appears to play a role in the interaction with ions as shown by ^{13}C NMR where the acetamido methyl group signal is broadened.[25,26] Thus, the first step of the proposed mechanism may be interpreted as the formation of a monochelate, with GlcNAc binding $Cr_2O_7^{2-}$ through C(1)—OH and C(2)—NAc (eqs 8 and 9):

$$GlcNAc + Cr_2O_7^{2-} \rightleftharpoons GlcNAc \cdots Cr_2O_7^{2-} \tag{8}$$

or

$$GlcNAc + Cr_2O_7^{2-} \rightleftharpoons GlcNAc \cdots CrO_3H^- + HOCrO_3^-. \tag{9}$$

Also since the observed rate constants are independent of the initial chromium concentration, it is possible that the reactive species is the bischelate and that all the Cr^{VI} is converted into the monochelate in the first step:

$$[(GlcNAc)_2Cr_2O_7 \cdot 2H_2O]^{2-} \rightleftharpoons 2[GlcNAcCrO_3H \cdot H_2O]^-. \tag{10}$$

After the slow steps, reactions (11), (12) and (13) may take place:

$$GlcNAc + Cr^{IV} \xrightarrow{fast} GlcNAc^{\cdot} + Cr^{III} \tag{11}$$

$$GlcNAc^{\cdot} + Cr^{VI} \xrightarrow{fast} \text{acid GlcNAc} + Cr^{V} \tag{12}$$

$$GlcNAc + Cr^{V} \xrightarrow{fast} \text{acid GlcNAc} + Cr^{III}. \tag{13}$$

Cr^{IV} formed in the slow steps yields the final Cr^{III} and the GlcNAc· radical by a later fast step. GlcNAc· radical formation is supported by the observed polymerization after addition of acrylonitrile. These radicals may react with Cr^{VI} to yield the oxidation product and Cr^{V}, which reacts with GlcNAc to produce acid GlcNAc and Cr^{III} in the two-electron step.

According to the proposed mechanism, the full rate law for the oxidation of GlcNAc by Cr^{VI}, under conditions studied in the kinetic measurements, can be written as follows:

$$-d[Cr^{VI}]/dt = (k_3K_1[GlcNAc] + k'K_1[H^+]^2[GlcNAc] + k''K_2[H^+]^2[GlcNAc]^3)[Cr^{VI}] \quad (14)$$

where $k' = k_4k_5k_6$ and $k'' = k_7k_8k_9$.

Since

$$[Cr^{VI}]_T = [Cr^{VI}](1 + K_1[GlcNAc] + K_1K_2[GlcNAc]^2), \quad (15)$$

replacing $[Cr^{VI}]$ in eq. (14) gives

$$-d[Cr^{VI}]/dt = (k_3K_1[GlcNAc] + k'K_1[H^+]^2[GlcNAc] + k''K_2[H^+]^2[GlcNAc]^3)[Cr^{VI}]_T/(1 + K_1[GlcNAc] + K_1K_2[GlcNAc]^2). \quad (16)$$

Thus, if K_1 [GlcNAc] is the largest term in the denominator, eq. (16) will be simplified to the experimentally observed form.

It has been found that aldoses 6-deoxyaldoses[12] and 2-deoxy-2-aminoaldoses[16] are oxidized by Cr^{VI} to the corresponding aldonic acid from precursor chromic esters by slow redox steps. In every case, plots of k_{obs} *vs* [ald.] show saturation at high [ald.] which fit kinetic laws of the type: $(a + b[H^+]^2)$ [ald.] $[Cr^{VI}]_T/(1 + K[ald.])$, with K values of 2–6 for aldoses (T = 33°C) and 25 for 2-aminoaldoses (T = 50°C). On the other hand, for 2-deoxyaldoses the chromic ester behaves as a steady state with a kinetic law of the type $(a + b[H^+]^2)$ [ald.]$[Cr^{VI}]_T$. Besides, none of these oxidation processes shows a substrate second-order kinetic term.

In this work K_1, taken at the largest [GlcNAc], has to be at least ≈700, a value which is much larger than that found for aldoses. However, oxidation rates are of the same order. Thus, it seems reasonable to suggest that Cr^{VI} binds aldoses and aminoaldoses through $OH(NH_2)$ at C(1) and C(2) to form ald.–Cr^{VI} species yielding the redox products. Lack of C(2)—OH in 2-deoxyaldoses compels Cr^{VI} to bind the anomeric hydroxyl group only and, consequently, a weaker ester is formed and rapidly oxidized to products. Protection of C(2)—NH_2 in GlcNAc could be thought to prevent N—Cr^{VI} binding and to afford a kinetic result similar to that of 2-deoxyaldoses. However, it is not the case. On the contrary, our kinetic analysis of curves seems to show that the 2-NAc group participates in binding Cr^{VI} to give larger K values and even a bischelate intermediate ester. In summary, relative formation rates of Cr^{VI} redox precursor complexes with aldoses and related sugars may be outlined as follows: 2-deoxyaldoses < aldoses ≈ 2-aminoaldoses. At present, new examples are being studied to confirm this observation.

It must be noted that the above discussion refers to the formation of the redox steps precursor complex, even though several linkage isomers might be formed by coordination of the sugar with Cr^{VI} *via* any pair of properly disposed hydroxyl (amino; acetamido) groups.

CONCLUSIONS

The above results indicate that 2-acetamido group favours chromic ester rate of formation and that acetate does not act as a protective group preventing nitrogen to bind Cr^{VI} in previous electron transfer steps. Nitrogen has already been found to participate in the formation of chromic ester, e.g. in the methionine[27] and cysteine[28] oxidation, where oxidation products were explained by formation of a Cr^{VI} complex with substrates acting as tridentate ligands through, O, N, S donor sites.

The present results, together with the behaviour of 2-aminoaldoses comparable with that of aldoses, suggest that nitrogen might play an important role in the Cr^{VI} metabolism. Until the present, attention has been focused especially on oxygen and sulphur as playing the role of binding or reducing Cr^{VI} (Cr^{V}). Consequently, the data presented here suggest that coordination sites involved in the chromium binding in natural systems should be alcohols, thiol and nitrogen-containing groups.

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