Evidence for a Radical Reaction between Chlorine and Glycosides in the Dark and its Retardation by Chlorine Dioxide

PETER S. FREDRICKS,
BENGT O. LINDGREN and
OLOF THEANDER

Träkemiska avdelningen, Svenska Träforskningsinstitutet, Stockholm Ö, Sweden

It has been known for nearly a decade that the presence of small amounts of chlorine dioxide decreases the degradation of cellulose during chlorine bleaching.[1] Recently an extensive investigation of this effect has been made by Croon and Dillén.[2] The present communication describes some preliminary results which suggest that the mechanism of the chlorine oxidation of glycosides in acid aqueous solution follows a predominantly free radical chain mechanism and that the effect of chlorine dioxide is due to its functioning as a radical scavenger.

Extensive investigations have shown that chlorine in acid aqueous solution attacks a glycoside rather randomly. In the initial reactions the glycosidic linkages are cleaved and the primary and secondary hydroxyl groups are oxidised to aldehyde and keto groups, respectively.[3-5] The oxidation seems to follow a more specific course under non-aqueous conditions, where the cleavage of the glycoside linkages strongly predominates as shown by Whistler *et al.*[6] They have suggested a mechanism for this cleavage, which involves non-radical intermediates. The kinetics of the hypochlorite oxidation (pH 5—10) of cellulose and glycosidic model compounds have been studied by several workers [7-9] and the occurrence of radical reactions suggested.[7,8] However, no reaction mechanism has been generally accepted and the oxidation at a pH around 2, which is important commercially in the chlorination of pulp, has received little attention with regard to mechanistic studies.

In the present preliminary investigation we have determined the rates of chlorine oxidation of a number of model compounds at pH 2.2 and 50°. The effect of the presence of small quantities of chlorine dioxide on the rates was also examined. For experimental details, see Table 1.

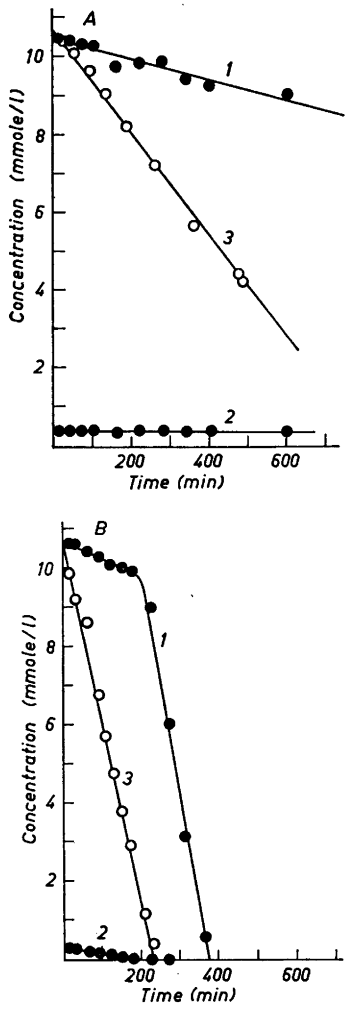

Fig. 1. The oxidation of methyl β-D-glucoside with chlorine in the presence and in the absence of chlorine dioxide. The concentrations of total amount of chlorine and of chlorine dioxide *vs.* reaction time. A. The reaction in darkness. B. The reaction under illumination. Curves 1: $[Cl_2]$ + [HOCl] in the expt. with ClO_2. Curves 2: $[ClO_2]$ in the expt. with ClO_2. Curves 3: $[Cl_2]$ + [HOCl] in the expt. without ClO_2.

As shown in the table and Fig. 1 A, the oxidations of methyl β-D-glucoside under

Table 1. The initial rates of chlorine oxidation of some carbohydrates.

Carbohydrate	The initial rate[a] $10^7 \times$ M/sec	
	Chlorine alone	Chlorine + ClO_2
Methyl β-D-glucopyranoside	2.04 ± 0.05 (5)[b]	0.45 ± 0.06 (4)
Methyl α-D-glucopyranoside	1.87 ± 0.04 (2)	0.45 ± 0.12 (5)
D-Glucose	2.45 ± 0.05 (5)	1.19 ± 0.10 (3)
Gluconic acid[c]	4.54 ± 0.33 (3)	3.55 ± 0.35 (2)
3-Keto-methyl β-D-glucopyranoside [d]	5.81 (1)	1.98 (1)
6-Aldehydo-methyl α-L-glucopyranoside[e,f]	5.42 (1)	2.52 (1)
Cellobiose	4.14 ± 0.87 (3)	0.95 ± 0.07 (3)
Cellobiitol	1.72 (1)	0.47 (1)

[a] The oxidation conditions were: 50°C; pH 2.2 (buffer of 0.25 M H_3PO_4 and 0.25 M KH_2PO_4); initial carbohydrate concentration, 10.3 mM; and initial chlorine concentration, 10.5 ± 0.5 mM. The concentration of chlorine dioxide, if present, was 0.3 mM.
[b] Standard deviation and the number of experiments performed.
[c] This substance was equilibrated in the buffer at reaction temperature before the run.
[d] Correct nomenclature methyl β-D-*ribo*-hexopyranosid-3-ulose.
[e] Correct nomenclature methyl α-L-*gluco*-hexodialdo-1,5-pyranoside.
[f] This substance was more available than the D-glucoside. The initial chlorine concentrations used were about 10 % lower than in the other experiments.

these conditions and in the dark underwent a marked retardation in the presence of a small amount of chlorine dioxide. The initial rate was diminished by a factor of about 5. The retardation was about the same over the concentration range from 0.003 to 0.03 mole of chlorine dioxide per mole of chlorine. At about 0.0003 mole of chlorine dioxide a considerably weaker retardation was observed.

These results suggested to us that chlorine dioxide was acting as a radical scavenger in a chain mechanism. Some effort was devoted to finding another substance which would retard this reaction. The limitations of solubility in water and unreactivity towards molecular chlorine eliminated many of the common radical scavengers. Ferric chloride, however, known as an inhibitor in styrene and acrylonitrile polymerisations,[10] also caused a marked retardation of the reaction when present in a concentration of 0.05 mole per mole of chlorine. Both dimethyl sulphoxide and methylamine hydrochloride showed mildly retarding effects.

Further evidence of a free radical mechanism has been obtained by studying the effect of light on the reaction. Light is known to accelerate chlorine oxidations but does not markedly alter the products of reaction.[6,11] The rapid light induced reaction of methyl β-glucoside was also shown to be retarded by chlorine dioxide (see Fig. 1B). Chlorine dioxide is, however, consumed fairly rapidly, presumably largely by photochemical decomposition [12] and, probably to a smaller extent, by reaction with chlorine atoms formed by the photolysis of chlorine molecules.[13,14] As soon as all the chlorine dioxide had disappeared the reaction rate rapidly increased and then continued at a rate close to that of the normal light induced reaction. The retardation of both the light-catalysed reaction, which undoubtedly involves radical processes initiated by photolysis,[13] and the dark reaction, constitutes the strongest evidence in favour of the latter also being predominantly free radical in nature.

The oxidation of the glucosides in acetic acid solution, however, is not retarded by chlorine dioxide as observed by us. Under these conditions the ionic mechanism suggested by Whistler *et al.*[6] may be the predominant one.

The keto and aldehydo methyl glucosides, glucose, and gluconic acid are among the primary chlorine oxidation products from the methyl glucosides.[3-5] Table 1 shows that these primary reaction products react more rapidly than the glucosides. The rates in the table are the initial ones.

Chlorine dioxide retards the chlorine oxidation of all the compounds in Table 1; in some cases, however, the retardation was rather weak. The oxidations of the methyl glucosides, cellobiose, and cellobiitol are strongly retarded and that of gluconic acid is weakly retarded. The other compounds lie in between these extremes. The amount of retardation may indicate the degree by which the carbohydrate reacts by a radical mechanism.

The reaction of chlorine, under these conditions, with some simpler substances, *e.g.* dioxane, was also found to be retarded by chlorine dioxide.

The mechanism of the initiation of the dark reaction is obscure. Walling and Mintz have recently shown that molecule induced homolyses take place readily between some aldehydes and ethers, and *t*-butyl hypochlorite.[15] An analogous mechanism involving the substrate and chlorine or hypochlorous acid could be suggested for the present reaction. A radical chain could then be considered to be carried by chlorine atoms or, less probably, hydroxyl radicals. It is known that chlorine dioxide, at least in the gas phase, reacts with chlorine atoms.[14]

Acknowledgement. The financial support from *Cellulosaindustriens stiftelse för teknisk och skoglig forskning samt utbildning* is gratefully acknowledged.

1. "The Bleaching of Pulp" *Tappi Monograph* No. 27 (1963), p. 63.
2. Croon, I. and Dillén, S. *Tappi* **50** (1967) 138 A.
3. Lindberg, B., Dyfverman, A. and Wood, D. *Acta Chem. Scand.* **5** (1951) 253; Lindberg, B. and Wood, D. *Acta Chem. Scand.* **6** (1952) 791; Dyfverman, A. *Acta Chem. Scand.* **7** (1953) 280.
4. Theander, O. *Svensk Papperstid.* **61** (1958) 581.
5. Theander, O. *Tappi* **48** (1965) 105.
6. Whistler, R. L., Mittag, T. W., Ingle, T. R. and Ruffini, G. *Tappi* **49** (1966) 310.
7. Epstein, J. A. and Lewin, M. *Textil-Rundschau* **16** (1961) 494.
8. Wolfrom, M. L. and Lucke, W. E. *Tappi* **47** (1964) 189.
9. Sarkanen, K. V. and Bhargawa, D. P. *3rd Intern. Pulp Bleaching Symp.*, Seattle Aug. 19–20, 1964.
10. Walling, C. *Free Radicals in Solution*, Wiley, New York 1957, p. 165.
11. Uchino, N. and Whistler, R. L. *Cereal Chem.* **39** (1962) 477.
12. Bowen, E. J. and Cheung, W. M., *J. Chem. Soc.* **1932** 1200.
13. Young, K. W. and Allmand, A. J. *Can. J. Res.* **B 27** (1949) 318.
14. Clyne, M. A. A. and Coxon, J. A. *Trans. Faraday Soc.* **62** (1966) 1175.
15. Walling, C. and Mintz, M. J. *J. Am. Chem. Soc.* **89** (1967) 1515.

Received November 18, 1967.

The Zymogen of Phospholipase A^2 in Rat Pancreatic Juice

BO ARNESJÖ, JAMES BARROWMAN* and BENGT BORGSTRÖM

Division of Physiological Chemistry, Chemical Center, University of Lund, Lund, Sweden

The presence of phospholipase A^1 and A^2 activities in pancreatic tissue, pancreatic juice and duodenal contents is well established.[1-4] Porcine pancreatic lipase has been shown to hydrolyse lecithin, preferentially at the α-fatty acid ester linkage.[5] Recently, de Haas [6] has presented evidence for the existence of precursor of phospholipase A in porcine pancreas. This "prophospholipase", subjected to tryptic digestion can be converted to its active form by splitting off a heptapeptide.

This investigation was undertaken in order to determine whether this zymogen form of phospholipase also exists in pancreatic juice and the relationship of its active form to other phospholipid splitting enzymic activities in rat pancreatic juice. Pancreatic juice from 3 to 4 male Sprague-Dawley rats was collected into a container chilled with dry ice after cannulation of the pancreatic ducts. The juice was subsequently lyophilised and used in this study within one week. The experiments were performed with the powder dissolved in ice cold water to the original concentration. Phospholipase, lipase and activity against micellar monoglyceride were assayed as previously described,[2,7] with the exception that the incubation period for phospholipase assay was shortened from 2 h to 10 min. Tryptic digestion was carried out as follows:

* In receipt of a Wellcome-Swedish Travelling Research Fellowship.