PERIODATE OXIDATION OF BIOPOLYMERS

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Introduction

Periodate oxidation of carbohydrates is a valuable tool for structural studies in which the quantitative determinations of the consumption of the oxidant and concomitant liberation of formic acid and formaldehyde are correlated with certain structural features (see, for example, Bobbitt, 1956). However, the information requires careful scrutiny, particularly where over-consumption of periodate occurs due to secondary reactions of the oxidized products, for example, in polyuronides. This "over-oxidation" often results in spurious yields of formic acid and correspondingly approximate deductions, such as in the determination of the average chain length of glycogen (Montgomery). Furthermore, in the presence of protein these results have little meaning since several residues in the protein are oxidized by periodate. Such oxidations of proteins and glycoproteins continue for many months at 5° and the titrimetric determination of the formic acid liberated is confused by the changing pK_a values of the oxidized protein residues. Information can be obtained

however for such biopolymers by following the course of the oxidation with analyses for those residues that have resisted oxidation.

Experimental

Oxidation was carried out with 0.05 M $NaIO_4$ at 5° in the dark using at least five times excess of oxidant and approximately a 0.5% initial concentration of biopolymer. The rate of periodate consumption was determined by the arsenite method of Fleury and Lange (1933). At intervals, the periodate in samples of the reaction solution was reduced with ethylene glycol and the oxidized biopolymer was isolated, either by dialysis or by passing the solution through a column of Sephadex G-25 (Porath and Flodin, 1959) which had been equilibrated with either 0.02 M Na_2SO_4 or 10^{-4} N HCl. The oxidized biopolymer is reduced with $NaBH_4$, either before or after the isolation step. The product is analyzed quantitatively for the original components.

D-Mannose and D-galactose, after hydrolysis of the samples with N H_2SO_4 for 4 to 6 hrs. at 100°, were each determined by the phenol-sulfuric acid procedure (Dubois et al., 1956). In the case of ovomucoid the sugars were separated by chromatography on Whatman No. 1 with n-butanol: ethanol: water (4: 1: 5 V/V, upper layer).

D-Glucosamine was determined by the method of Boas (1953).

D-Glucuronic acid was determined by both the carbazole method (Dische, 1955) and the phenol-sulfuric acid procedure (Montgomery, 1961).

Results and Discussion

Some of the results for sodium heparinate (Wolfrom et al., 1943), ovalbumin (Kekwick and Cannan, 1936), and ovomucoid are summarized below. The ovomucoid was prepared by the method of Lineweaver and Murray (1947), followed by chromatography on diethylaminoethyl-cellulose.

Few sugar residues in heparin are oxidized by periodate although the consumption of periodate continues. That this is "over-oxidation" and not due to the slow cleavage of sterically hindered residues is demonstrated by the constant level of D-glucosamine and D-glucuronic acid in the oxidation products. No inorganic sulfate is liberated until the oxidation exceeds about three moles of periodate per 4736g. The carbohydrate analyses correspond to one D-glucuronic acid residue and one D-glucosamine residue oxidized per sulfated hexadecasaccharide unit (4736 g-mol) in which the mole ratio of glucosamine: glucuronic acid: sulfate is 2: 2: 5. The present results are at variance with a previously proposed structure (Wolfrom et al., 1950) which was based on the consumption of one mole of periodate per sulfated tetrasaccharide.

Oxidation of the sugar residues in ovomucoid is complete in 2 to 3 days, after which time approximately two thirds of the D-glucosamine and half of the D-mannose residues are oxidized. The fate of the D-galactose residues cannot be accurately stated because chromatographic analyses of the oxidation products have failed to separate the unoxidized D-galactose from a slightly

Periodate Oxidation of Ovomucoid, Heparin and Ovalbumin

Time (days)	Periodate consumption	Carbohydrate Content, %			
		Ovomucoid			
	moles/mole[b]	Glucosamine	Total Hexose[a]	Mannose	"Galactose"
0	4	14.5	5.8	4.6	1.0
6(hrs)	22	8.0	3.5	2.4	1.0
2	38	5.4	3.6	2.5	0.9
21	111	5.4	3.7	2.4	0.8
		Sodium Heparinate			
	moles/4736g	Glucosamine	Glucuronic Acid		
			Phenol-H_2SO_4	Carbazole[d]	
0	0	21.0	24.9	42.6	
4(hrs)	-	19.7	23.0	41.5	
1	0.9	18.4	22.2	38.6	
2.5	1.3	18.3	21.6	38.6	
29.5	2.0	18.1	21.3	37.4	
64.5	2.4	-	-	-	
		Ovalbumin			
	moles/mole[c]	Glucosamine	Mannose		
0	12	1.2	2.2		
6(hrs)	27	0.8	1.1		
18(hrs)	51	0.7	1.0		
43(hrs)	55	0.7	1.0		
93	169	-	-		

a. Total hexose expressed as mannose.
b. Molecular weight of ovomucoid taken as 28,000.
c. Molecular weight of ovalbumin taken as 45,000.
d. The carbazole procedure is known to give abnormally high values for glucuronic acid in heparin (Dische, 1955) and is included here to demonstrate the same relative change as the phenol-H_2SO_4 procedure.

faster moving component produced during the oxidation. All of the D-galactose is not oxidized however (cf Bragg and Hough, 1961) and maybe part is linked to the N-acetyl neuraminic acid which acts as a blocking group. In contrast to the carbohydrate residues, the protein is continuously oxidized, thus permitting little structural deduction from the moles of periodate consumed.

About 1 hour after the periodate oxidation of ovalbumin is commenced the product begins to precipitate. Nevertheless the oxidation of the carbohydrate is complete in 18 hours, at which point one of the three D-glucosamine and three of the six D-mannose residues in the glycoprotein are cleaved. An analysis of the amino acid composition of the periodate-oxidized glycoproteins shows that the cysteine-cystine residues are oxidized rapidly to cysteic acid, methionine is converted to the sulfoxide and sulfone, and the tryptophan and tyrosine are eventually destroyed. No other amino acids are significantly changed.

The results with ovalbumin and ovomucoid indicate that the carbohydrate structures are either highly branched or else have a number of different glycosidic linkages (see, for example, Smith and Montgomery, 1959). No such conclusion is possible from the periodate consumption data.

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