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Carbohydrate Research 309 (1998) 11–16

CARBOHYDRATE RESEARCH

Periodate oxidation of cyclosophoraoses: a quantitative analysis of the reaction products by ionspray mass spectrometry

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Received 28 November 1997; accepted in revised form 17 April 1998

Abstract

The reaction products from the periodate oxidation of cyclosophoraoses, having glucose residues ranging from 17 to 21, were studied by means of ionspray mass spectrometry. Three different samples exhibiting different degrees of oxidation (~30, ~65 and ~85%) were investigated. In order to avoid both insolubility and complex spectra due to water adduct formation, the oxidised cyclosophoraoses were exhaustively reduced in order to convert the aldehydic functions into hydroxyl groups. The individual ions comprising the mass spectral envelope were assigned by taking into account the coexistence of different degrees of oxidation for each cyclosophoraose member. The distribution of variable degrees of oxidation was studied in terms of the statistical Poisson distribution and the experimental data were fitted with the expected theoretical distributions. Detection of unfragmented ions coupled with both high m/z resolution and mathematical modelling allowed the quantitative definition of the distribution of the degree of oxidation for each cyclosophoraose ring size. This information makes possible the use of specific oxidised derivatives as building blocks for complex molecular systems.

Keywords: Cyclosophoraoses; Periodate oxidation; Ionspray mass spectrometry

1. Introduction

The development of soft ionisation techniques, such as electrospray, ionspray and matrix assisted laser desorption, has been a key step for the investigation of large and polar compounds by means of mass spectrometry (MS). These ionisation methods are very effective in producing intact ions from labile or even non-volatile molecules and can be coupled to procedures that allow accurate mass determination with sensitivity down to pico- or even femto-mole levels. As a consequence, large biological molecules are now routinely investigated by mass spectrometry [1–3].

Although organic MS has been primarily applied for elucidation of the covalent structure of organic

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Abbreviations: high performance liquid chromatography, HPLC; mass spectrometry, MS; ionspray mass spectrometry, ISMS; matrix assisted laser desorption ionisation, MALDI.

This work was partly presented at the workshop: Application of MALDI-TOF and ESI-TOF mass spectrometry in the characterisation of synthetic and biological polymers. 30–31 October 1997, Catania, Italy.

0008-6215/98/$19.00
PII S0008-6215(98)00113-X

molecules, the availability of the above mentioned soft ionisation procedures extended the use of MS and MS–MS to different structural problems such as the characterisation of non-covalent interactions, protein and DNA sequencing and drug investigations [4,5]. In this context, a thorough study of the complexes of either cyclodextrins or methylated cyclodextrins with aromatic molecules carried out by Cescutti et al. [6,7] led to the possibility of discriminating between inclusion complexes and non-specific electrostatic adducts by means of ionspray mass spectrometry (ISMS).

Cyclosophoraoses are a family of cyclic β-(1→2)-D-glucans produced by many strains of *Rhizobium* and *Agrobacterium* as a mixture of ring molecules exhibiting a variable number of residues per ring [8–11]. The cyclic nature of such compounds was demonstrated by means of FAB-MS and ^{13}C NMR spectroscopy [12]. In our laboratories, different cyclic glucans, modified by periodate oxidation, are being examined as cross-linking agents in the synthesis of hydrophilic polymer networks (for data concerning oxidised cyclomaltoheptaose, see ref [13].).

In this paper, a quantitative analysis of cyclosophoraoses derived oxidation products is reported. A detailed characterisation of the latter as a function of the stoichiometric degrees of oxidation is relevant also in better defining their potential as the building blocks of hydrogels.

2. Experimental

Periodate oxidation reaction.—The periodate oxidation reaction was performed according to Hay et al. [14]. Three different degrees of oxidation were obtained using a Glc-to-periodate ratio of 0.2, 0.5 and 0.8, respectively.

Reduction and purification of the oxidised cyclosophoraoses.—The oxidised cyclosophoraose samples were reduced by treatment with $NaBH_4$ at room temperature overnight, neutralised with aqueous 50% acetic acid and evaporated to dryness. The samples were re-evaporated with 10% acetic acid in methanol and pure methanol three times in order to remove borate complexes, and desalted on a Biogel P2 column using water acidified with formic acid (pH 3.5) as eluent, yielding the reduced forms RED1, RED2, and RED3.

Ionspray mass spectrometry.—The mass spectra were recorded on an API-I PE SCIEX quadrupole mass spectrometer equipped with an articulated ion spray source and connected to a syringe pump for sample introduction. The instrument was calibrated using a polypropylene glycol (PPG) mixture (3.3×10^{-5} M PPG 425, 1×10^{-4} M PPG1000 and 2×10^{-4} M PPG 2000), 0.1% (v/v) acetonitrile and 2 mM ammonium formate in 50% (v/v) aqueous methanol. The samples were dissolved in 50% (v/v) aqueous acetonitrile/0.63×10^{-4} M ammonium acetate at a final concentration of about 0.5 mg/mL. The spectra were recorded in the positive mode with the ionspray voltage set at 5200 V and the orifice potential at 50 V, using a scan step size of 0.1 or 0.2 amu. The sample flow rate was 5 μL/min.

3. Results and discussion

The ionspray mass spectrum of the least oxidised cyclosophoraoses showed numerous variably hydrated adducts for each ring caused by the presence of aldehydic groups and leading to a very complicated and poorly resolved spectrum. The other two samples, exhibiting higher degrees of oxidation, were almost insoluble in the solvent used. For this reason the three oxidised mixtures were exhaustively reduced to their alcoholic derivatives resulting in better spectra due both to the loss of the hydrated species and to their improved solubility. The reduced forms still reflected the original distribution of the variably oxidised species. By comparison to the reduced compounds, the mass spectrum of the native cyclosophoraoses showed (Fig. 1) a distribution and a relative abundance of the mixture components very similar to those obtained by means of both high performance

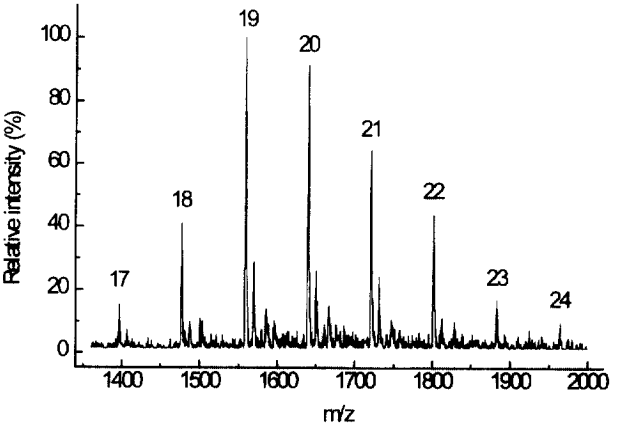

Fig. 1. Positive mode ionspray mass spectrum of the native cyclosophoraoses family.

liquid chromatography (HPLC) [15] and MALDI mass spectrometry [16]. However, in the ionspray mass spectrum only the rings from DP 17 to 24 were detected, while in the MALDI mass spectrum and in the HPLC chromatogram rings up to DP 27 were present. The most abundant ions of the cyclosophoraoses were doubly charged ammonium adducts, whilst the mixed adducts formed by one ammonium and one potassium ion were present to a lesser extent. From this spectrum, the same conclusions as for the MALDI mass spectrum [16] may be drawn: no fragmentation of the rings was observed and this technique was suitable to be used for quantitative determination of the ring content and for recording their molecular weight distribution. Therefore, the quantitative study of the extent of oxidation of the rings could be undertaken.

The ionspray mass spectra of the RED1, RED2 and RED3 (Fig. 2) show members of the cyclosophoraose family ranging from 17 to 24 or 23 glucose monomers per ring, as doubly charged species. In addition, the formation of adducts containing two cyclic molecules was ascertained by the presence of the peaks at high m/z (data not shown). A given degree of oxidation resulted in a slight shift to higher m/z values, as expected. In fact, for every oxidised-reduced Glc residue there was an increase of the molecular weight of 2 amu, and therefore, for doubly charged species, a difference of $+1$ m/z. The experimental values shown in Fig. 2 were compared with calculated values obtained by subsequent addition of 2 amu to the monoisotopic mass of each native ring. The results are reported in Table 1. Although ammonium acetate was used as the ionising agent, the RED3 sample formed adducts preferentially with two sodium ions (as discussed later in more detail). This finding was confirmed by the mass spectra in sodium acetate, used in place of ammonium acetate, where the highest peak in each DP of the RED1 and RED2 mixtures underwent a shift of about + 2.5 amu for mixed adducts, while no significant change in the m/z values of each peak was observed in the RED3 spectrum.

Examination of these spectra showed that each peak was an envelope constituted by individual ions reflecting varying degrees of oxidation. As an example, the relevant enlargements of the mass spectral envelopes for the RED1, RED2 and RED3 samples are reported in Fig. 3. By restraining the detailed description to the RED1 case (Fig. 3(a)), the envelope contained individual ions from 1557.8 to 1569.7 m/z differing within each other for 1 amu. This was in agreement with a collection of molecules exhibiting degrees of oxidation from 0 to 63% which correspond to a number of oxidised diols from 0 to 12. Similar considerations were made for the mass spectral envelopes of all the other rings. The interpretation of the mass spectra permitted the statistical analysis of the oxidation reaction within each member of the oxidised and reduced cyclosophoraose family.

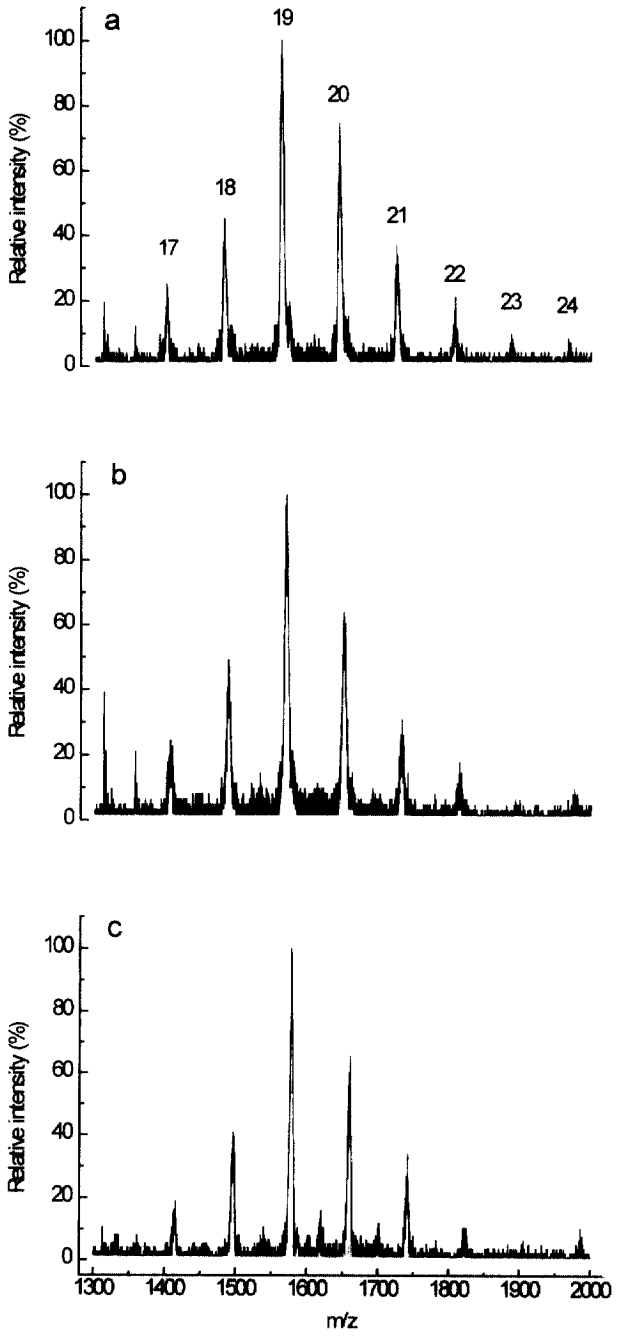

Fig. 2. Mass spectra of the oxidised-reduced cyclosophoraoses mixtures. (a) RED1; (b) RED2; (c) RED3 (see Experimental).

Table 1
Calculated and experimental values for the ionspray mass spectra of both native and oxidised-reduced cyclosophoraoses

DP	Native mixture		RED1		RED2		RED3	
	Calcul.	Exper.	Calcul.	Exper.	Calcul.	Exper.	Calcul.	Exper.
17	1395.5	1396.2	1401.7	1401.2	1407.2	1407.5	1413.5	1413.4
18	1476.5	1476.8	1482.5	1482.6	1488.2	1487.4	1496.5	1496.4
19	1557.5	1558.2	1563.7	1563.3	1569.2	1569.6	1578.5	1578.3
20	1638.5	1639.4	1644.8	1644.7	1650.3	1649.9	1660.5	1660.4
21	1719.6	1720.4	1725.8	1725.4	1731.3	1732.1	1741.5	1741.9
22	1800.6	1801.4	1806.8	1806.8	1812.3	1812.0	1822.6	1822.5
23	1881.6	1882.4	1887.8	1887.8	1893.3	1894.2	1905.6	1905.1
24	1962.6	1964.0	1968.4	1968.2	—	—	—	—

The calculated values refer to $2NH_4^+$ ions adducts for the native rings, RED1 and RED2 samples. RED3 data were analysed in terms of $2Na^+$ adducts.

We assume that the statistical distribution of the oxidation degrees follows the so-called Poisson probability distribution whose probability function is given by:

$$P(i) = \frac{a^i}{e^a i!} \tag{1}$$

where i is a discrete random variable which can take the values $i = 0, 1, 2, 3, \ldots$ and a is the mean value of the Poisson-distributed i variable. In our case, the variable i represented the number of oxidised glucose residues in each molecule.

The experimental distributions were best fitted with a Poisson distribution using the least-squares approach. The fit was obtained simultaneously solving the following two equations which represent minimisation with respect to the fitting parameters a and N:

$$\sum_i \frac{d}{da}\left[\frac{a^i}{e^a i!} - NP_{\exp}(i)\right]^2 = 0 \tag{2}$$

and

$$\sum_i \frac{d}{dN}\left[\frac{a^i}{e^a i!} - NP_{\exp}(i)\right]^2 = 0 \tag{3}$$

where N is a normalisation factor needed to scale the obtained experimental observable (detector counts) to the mathematical probability distribution. The value of a was obtained numerically solving the above equation's system, the N value was then directly attained.

Solution of eqs (2) and (3) leads to the definition of the best Poisson distribution for each oxidised ring and, in addition, the value of a provides the relevant mean degree of oxidation. The best fit of the Poisson distribution with the experimental distribution of the 19-residues-per-ring in the RED1, RED2 and RED3 samples are shown in Fig. 4 and the mean degrees of oxidation are reported in Table 2. The same mathematical approach was used for the other rings. The interpretation of the mass spectral envelopes of the RED3 sample was troublesome due to the simultaneous presence of adducts formed by sodium, ammonium and both ions. As reported in Fig. 3(c), the highest m/z value in this envelope, which corresponds to a completely oxidised ring, could only be assigned to a sodium adduct, the other adducts leading to a degree of oxidation higher than 100%, and therefore not plausible. The concurrent presence of the three types of adducts caused some uncertainty in the assignment of the individual ions, which was reflected in the fitting (Fig. 4(c)) showing a rather high discrepancy between the calculated and experimental values. The summary of all the mean oxidation degrees for the RED1, RED2 and RED3 rings is reported in Table 2 and in Fig. 5.

In conclusion, ISMS proved to be an invaluable tool for the investigation of the statistical distribu-

Table 2
Mean degree of oxidation for the oxidised-reduced cyclosophoraoses samples

Residues per cycle	Mean percentage of oxidation		
	RED1	RED2	RED3
17	36.4	64.6	86.5
18	33.1	65.5	85.9
19	32.4	63.7	86.7
20	31.9	66.1	85.9
21	31.9	63.9	85.0

tion of the periodate oxidation for a mixture of cyclosophoraoses exhibiting a number of glucose residues ranging from 17 to 21. The resolving power of the instrumentation was sufficient for the analysis of the ions comprised in wide ion-envelopes, providing information regarding the pattern of the periodate oxidation on each single molecular species. The quality of the experimental data allowed for fitting the observed oxidation distribution with a Poisson statistical analysis which resulted in the quantitative determination of the distribution of the glucose residues oxidised for the different cyclosophoraose rings thus permitting a better definition of their potential as building blocks for complex molecular systems such as hydrogels.

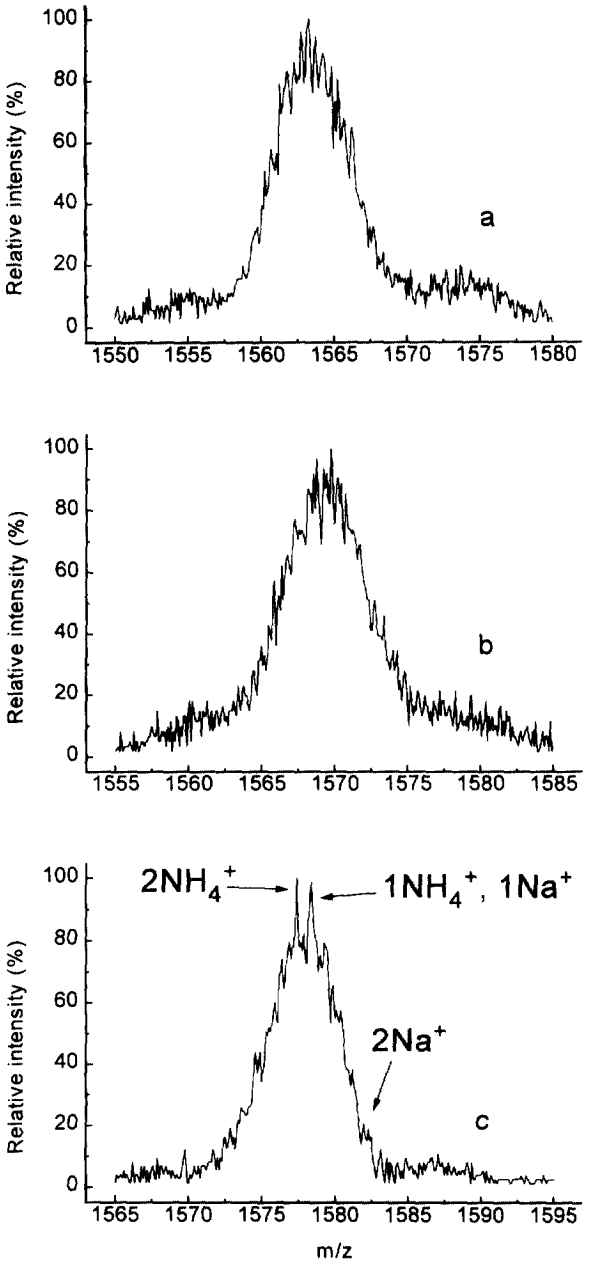

Fig. 3. Ion envelope of the 19-glucose residues cyclosophoraose at the three different degrees of oxidation; (a) 19-glucose residues cyclosophoraose in RED1; (b) 19-glucose residues cyclosophoraose in RED2; and (c) 19-glucose residues cyclosophoraose in RED3. In (c), arrows indicate the upper limit of 100% oxidation for the three different ionisation species reported.

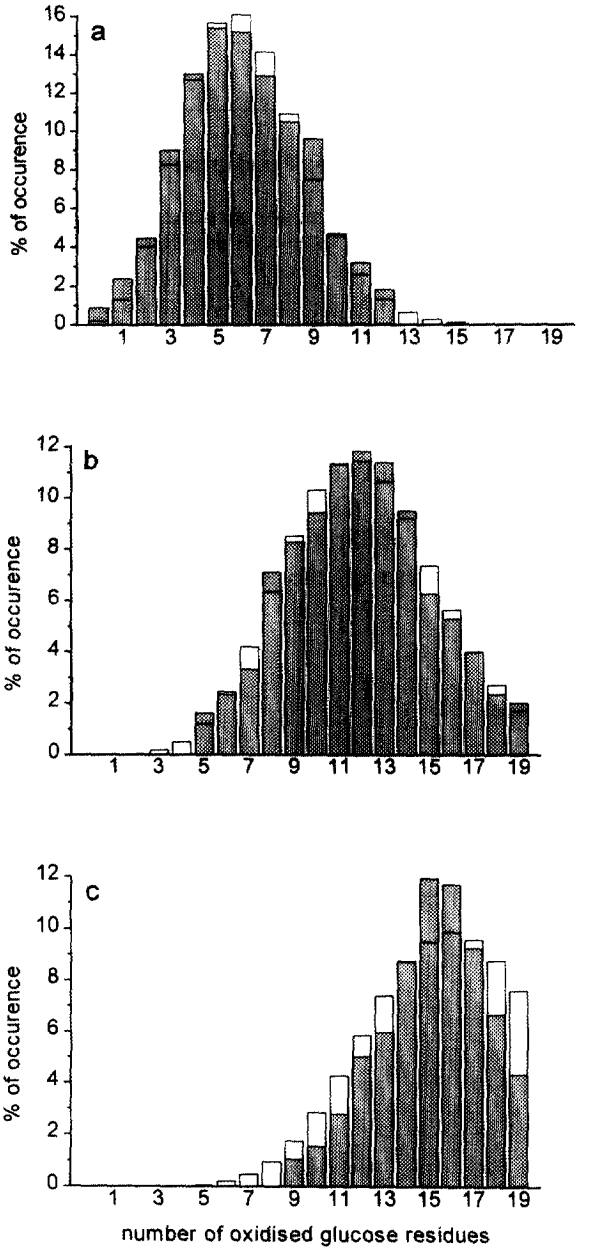

Fig. 4. Fitting of the experimental data with the theoretical Poisson distribution for the 19-glucose residues cyclosophoraose at the three different oxidation degrees investigated: (a) 19-glucose residues cyclosophoraose in RED1; (b) 19-glucose residues cyclosophoraose in RED2; and (c) 19-glucose residues cyclosophoraose in RED3. Hollow bars represent theoretical Poisson distributions; gray bars indicate the experimental data.

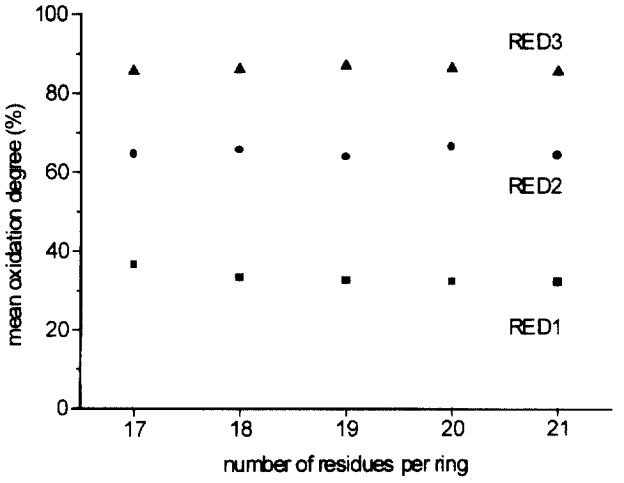

Fig. 5. Mean oxidation degree for each member of the cyclosophoraose family in the three samples: RED1, RED2, and RED3.

Acknowledgements

This work was sponsored by the Italian Ministero dell'Universita' e della Ricerca Scientifica e Tecnologica. The "Centro Grandi Strumenti" of the University of Trieste is gratefully acknowledged for the use of the API-I PE SCIEX mass spectrometer.

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