Short Communication

Synthesis and conformational properties of cyanoethyl–Scleroglucan

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Abstract

Cyanoethyl derivatives were obtained as intermediates in the chemical modification of the neutral polysaccharide Scleroglucan, aimed at attaining new ionic carbohydrate polymers. The chemical and conformational properties of the cyanoethylated polymer were investigated and compared with those exhibited by the native one. In particular, capillary viscosity and small angle X-ray scattering experiments demonstrated that in aqueous solutions cyanoethyl derivatives were not able to assume the triple helix structure typical of the parent sclecroglucan. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Cyanoethyl derivatives of Scleroglucan were obtained aiming at possible modifications of cyanogroups to get a variety of different products. This way, any type of final product exhibits an identical distribution of functional groups with these either being randomly present on the saccharidic units or located on specific positions.

Scleroglucan is a β-(1 → 3) linked glucan exhibiting a β-(1 → 6) glucose residue, as a lateral chain, on each third monomer. In aqueous solutions, Scleroglucan assumes a triple helix structure, which exhibits a rigid rod-like behaviour of the polymer chains (Bluhm, Deslandes, Marchessault, Perez & Rinaudo, 1982). In dimethylsulfoxide, as well as in aqueous solutions at pH higher than 12, Scleroglucan exists as single chains exhibiting a more flexible conformation (Yanaki, Kojima & Norisuye, 1981).

This communication deals with the synthesis and the chemical characterisation of cyanoethyl–Scleroglucan, together with clarification on its conformational properties.

2. Experimental

Scleroglucan (Actigum CS11™, hereafter referred as SCL) was kindly donated by SKW Biosystems (Baupte,

France). All chemicals were analytical grade commercial products. Aqueous solutions were prepared using double distilled water. Solution concentrations (w/v) were determined by weighing the solute. The water contents of the freeze-dried polymers were detected by thermogravimetry.

The cyanoethyl derivatives were obtained by the addition of acrylonitrile to a strongly basic SCL aqueous solution at room temperature. An appropriate amount of acrylonitrile was added either as a single addition or in separate portions. Different acrylonitrile-to-polymer ratios were used (Table 1). After neutralisation with HCl, the resulting solution was exhaustively dialysed against distilled water and then lyophilised. The degree of substitution obtained was determined by both elemental analysis (using a mod. 1106 Carlo Erba Elemental Analyser, Italy) and NMR (Table 1). ^{13}C NMR spectra were recorded on a Bruker AC 200 instrument operating at 50.32 MHz. Proton decoupled spectra were obtained at 80°C. Peak assignment was carried out according to the data from the literature (Crescenzi, Gamini, Paradossi & Torri, 1983).

Weight average molecular weights and molecular weight distribution curves of both native and derivatised Scleroglucan were determined in DMSO solutions by means of size exclusion chromatography (SEC) resorting to Zorbax PSM bimodal kit (Du Pont Inst.) columns combined with low angle laser light scattering detection (LALLS, Chromatix CMX-100). The dn/dc refractive index increment was set in agreement with Yanaki et al. (1981). Experiments were carried out at 40°C.

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Table 1
Acrylonitrile-to-polymer ratios, used in different cyanoethyl derivatives preparations, and degree of substitutions (DS) obtained. Polymer concentration: 2 g/l in 0.5 M NaOH

[CH$_2$CH$_2$CN]/[Glc][a]	DS
2.0[b]	0.03
4.9[b]	0.06
9.8[b]	0.10
14.7[b]	0.15
19.6[b]	0.26
19.6[b]	0.11
24.5[b]	0.30
23.0	0.42
24.5	0.55

[a] Number of cyanoethyl groups per glucose residue.
[b] Addition of reactive as a single injection.

Reduced viscosity measurements were carried out using 0.5 g/l solutions of either Scleroglucan or cyanoethyl–Scleroglucan. Prior to measurement, the solutions of Scleroglucan and cyanothyl derivatives were filtered on 3.0 and 0.45 μm membranes (Millipore), respectively. A Schott-Geräte AVS/G apparatus equipped with both a CT 1150 Schott-Geräte thermostatic bath and a Ubbelohde capillary (i.d. = 0.53 mm) was used. Measurements were carried out at 25°C.
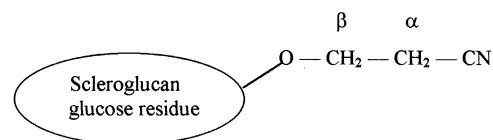
Small angle X-ray scattering (SAXS) experiments were carried out at the Austrian SAXS beamline (Amenitsch et al., 1997) available at the ELETTRA synchrotron radiation facility (Trieste, Italy), using 8 keV radiation and point collimation. The sample-to-detector distance was approximately 2.5 m. An one-dimensional Gabriel-type gas detector was used. Polymer solution samples were introduced in a quartz capillary with 1 mm diameter. Scattering angle calibration was carried out using rat tail tendon.

3. Results and discussion

3.1. Synthesis and chemical characterisation

Cyanoethyl derivatives of Scleroglucan (hereafter referred as CN-SCL) were obtained in very basic conditions (pH near 14), where the polymer exists as a single chain. Therefore, it may be assumed that the accessibility to hydroxyl groups by cyanoethyl groups was complete because of the absence of triple helix structures. The rather severe basic conditions suggested a possible reduction in the polymer molecular weight during the reaction. Therefore, the duration of the reaction was limited to 90 min. A sketch of the chemistry of cyanoethyl-derivatives is shown in Scheme 1.

The polymer molecular weights were investigated in dimethylsulfoxide (DMSO). A low substituted CN-SCL (0.03 CN groups per Glc residue) was used in order to use the same refractive index increment as for the native SCL. The values obtained are shown in Table 2 and indicated that

Scheme 1.

minor, or no, depolymerization had occurred. The slight increase in the molecular weight was because of both the addition of pendant groups onto the polymeric chain and, possibly, the low precision of the refractive index increment used. In any case, the $\langle M_w \rangle$ values obtained excluded a major molecular weight decrease.

The degrees of substitution (DS) obtained were not particularly high (Table 1). The highest DS obtained indicated that, on an average, only one hydroxyl residue for every 1.8 glucose units was cyanoethylated. This was partly because of the limited reaction time, and partly because of the degradation of the acrylonitrile reagent, which is unstable in strong basic conditions used for the derivatization. As a matter of fact, the higher substitution degrees were obtained when the reagent was freshly added in four portions during the reaction time (see Table 1). The ^{13}C NMR spectrum obtained for a sample exhibiting a degree of substitution of 0.38 is shown in Fig. 1. Resonance peaks pertaining to unsubstituted carbon atoms are clearly detected and assigned according to the results outlined in the literature (Crescenzi et al., 1983); they are also those with the highest intensity. In addition to these, a number of new peaks indicated the effect of cyanoethylation. The peak at 18.4 ppm was assigned to the α-methylene group of the cyanoethyl substituent (see Scheme 1). The integration of the peak due to unsubstituted C6 carbon atoms (61 ppm) with respect to that of the anomeric carbon atoms (103.2 ppm) indicated that one primary hydroxyl group for every 7.5 was substituted; i.e. the degree of substitution of the primary hydroxyl groups was 0.13 per glucose residue. Therefore, the remaining substitution occurred on the available C2, C3 and C4 hydroxyl groups with an average degree of substitution equal to 0.08 per hydroxyl group.

3.2. Conformational properties

The conformational properties of CN-SCL were investigated by both viscosity measurements and small angle X-ray scattering (SAXS). The experiments were aimed at

Table 2
Weight averaged molecular weights of Scleroglucan before and after cyanoethylation

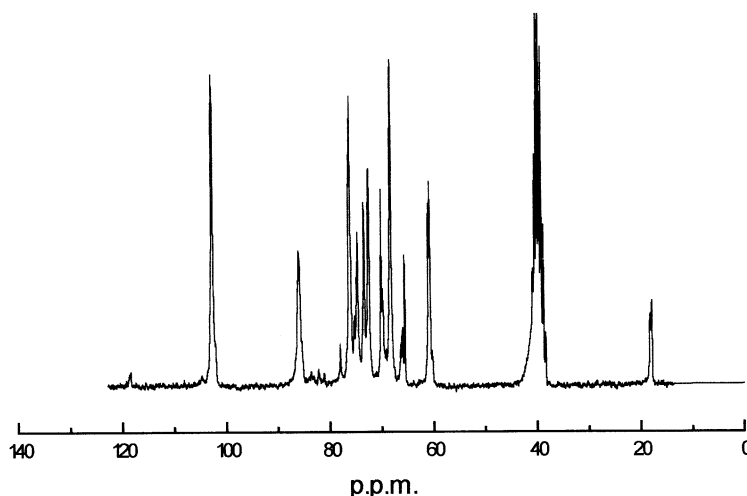| Sample	$\langle M_w \rangle$ (g/mol)
Native Scleroglucan	670 000
Cyanoethyl–Scleroglucan (0.03 CN groups /Glc residue)	800 000

Fig. 1. ^{13}C NMR spectrum of cyanoethyl–Scleroglucan (DS = 0.38) in DMSO-d$_6$.

determining whether CN-SCL could assume a triple helix conformation similar to that of native SCL. It is known from the literature that the viscosity of aqueous Scleroglucan solutions strongly depends on the pH (Bluhm et al., 1982). In the pH range of 12–13 the viscosity dramatically decreases due to the disruption of the triple helix conformation caused by partial ionisation of hydroxyl groups. According to data available in the literature (Kashiwagi, Norisuye & Fujita, 1981), a SCL sample having $\langle M_w \rangle = 700,000$ g/mol exhibits an intrinsic viscosity of about 30 dl/g in 0.01 M NaOH where it assumes a triple helix conformation. The reduced viscosity, far from the limit of zero polymer concentration, must have larger values.

The reduced viscosity of CN-SCL solutions as a function of pH is shown in Fig. 2. Two samples with the degrees of substitution equal to 0.11 and 0.42, respectively, were analysed. Insignificant changes in either polymer conformation or chain aggregation could be recognised on decreasing

the pH from 12.5 to less than 12. In fact, the small increase in the reduced viscosity indicated that some chain aggregation with a possible formation of short triple helical stretches was present in both samples. However, the reduced viscosity increase (from 4 to 6 dl/g) was still low with respect to that expected for native SCL (up to about 30 dl/g; Bluhm et al., 1982). In addition to this, the reduced viscosity increase was lower for the sample exhibiting a higher degree of substitution (Fig. 2). As a matter of fact, the reduced viscosity measured for a native sample Scleroglucan in 0.01 N NaOH at the same polymer concentration as CN-SCL gave a value of 31 dl/g.

In conclusion, the capillary viscosity experiments suggested that random cyanoethylation of SCL samples led to a new polymer which failed to retain the ability to form triple helix structures. Apparently, the rather bulky substituents prevent favourable chain–chain contacts, and perturb the local conformation, preventing extended secondary structure stretches. However, the polysaccharide backbone was still able to produce chain aggregation as, at pH values lower than 10, micro-gel formation was clearly detected.

The SAXS studies gave quantitative estimates of the structural parameters pertaining to the polysaccharidic chains. Measurements were carried out on both native and cyanoethylated SCL samples. In addition, SAXS experiments were performed on an SCL sample suspended for 90 min in the same alkaline medium as for cyanoethylation (blank sample, hereafter referred as B-SCL).

A linear behaviour of the angular dependence of the scattering curves was obtained when $\log(I \times h)$ was plotted as a function of h^2 (here I represents the scattered intensity and h is the scattering vector, $h = 4\pi \sin \theta/\lambda$). In the limit of long rods ($L \gg R$, where L is the length of the cylinder and R is its radius), the scattered intensity is proportional to $(1/h) \exp(-h^2 R_c^2/2)$ where R_c is the cross-sectional radius. Therefore, a linear plot of $\log(I(h) \cdot h)$ vs h^2 is expected. The
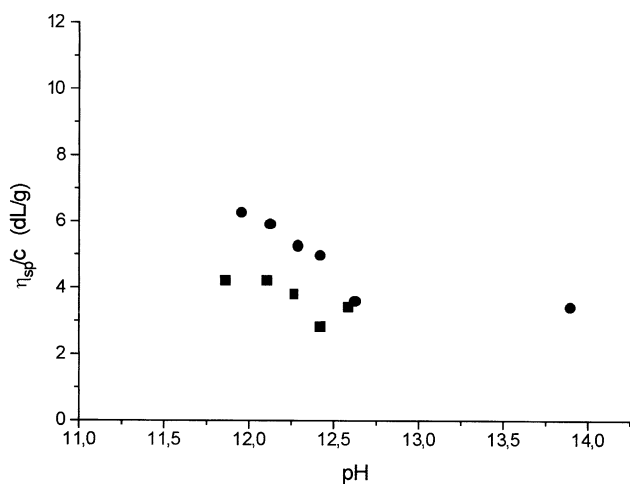

Fig. 2. Reduced viscosity of cyanoethyl–Scleroglucan (●, DS = 0.11; ■, DS = 0.42) as a function of pH.
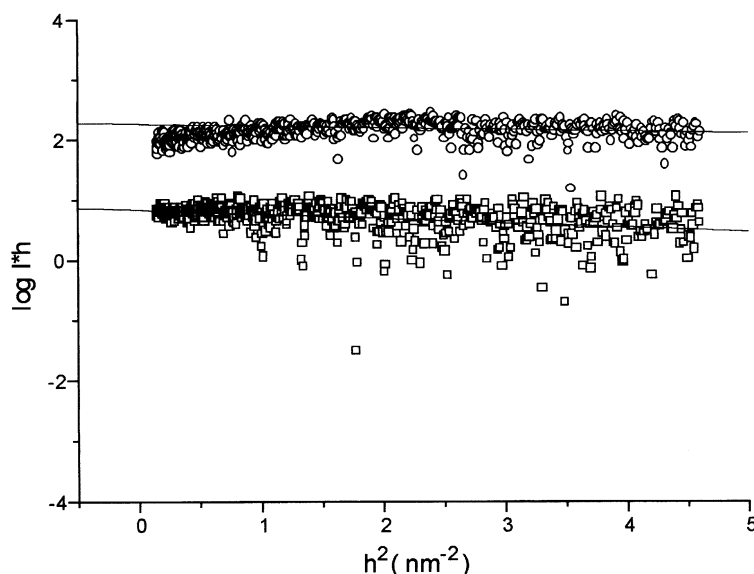
Fig. 3. Guinier plot for native Scleroglucan in 0.01 M NaOH (□) and in DMSO (○) ($C_p = 0.25$ w/v).

slope of the straight-line gives the value of R_c. For a uniform rod of radius R, R_c is given by $R_c = R/\sqrt{2}$. The data reported in Figs. 3 and 4 as cross-sectional Guinier plots for SCL and CN-SCL show that the polymers investigated could be considered as rigid rods. The diameters of the rods ($D = 2R$) are reported in Table 3.

SAXS measurements carried out as a function of polymer concentration (C_p) exhibited a dependence of R_c on C_p for concentrations greater than 0.3% (w/w). Therefore, structural data were derived from the experiments carried out on solutions whose polymer concentrations were as lower as possible.

X-ray diffraction data obtained on Scleroglucan fibres (Bluhm et al., 1982) showed that the dimensions of the hexagonal cell containing the triple helix structure were 17.3 Å for both a and b axes located in a plane perpendicular to the fibre axis. This number represents the distance between the two triple-helix axes in adjacent cells and can be considered, in good approximation, as the diameter of the triple-helix rod. The figures obtained for SCL by SAXS measurements both in water and in 0.01 M NaOH, where SCL is present as a triple helix, are in very good agreement with the crystallographic findings (samples 1 and 2 in Table 3). It is worth mentioning that Gawronski, Park, Magee and Conrad (1999) published a D value relative to the native Scleroglucan in water obtained by SAXS measurements. They found $D = 16.6$ Å which is in good agreement with our findings. Other diameter values, obtained by the same
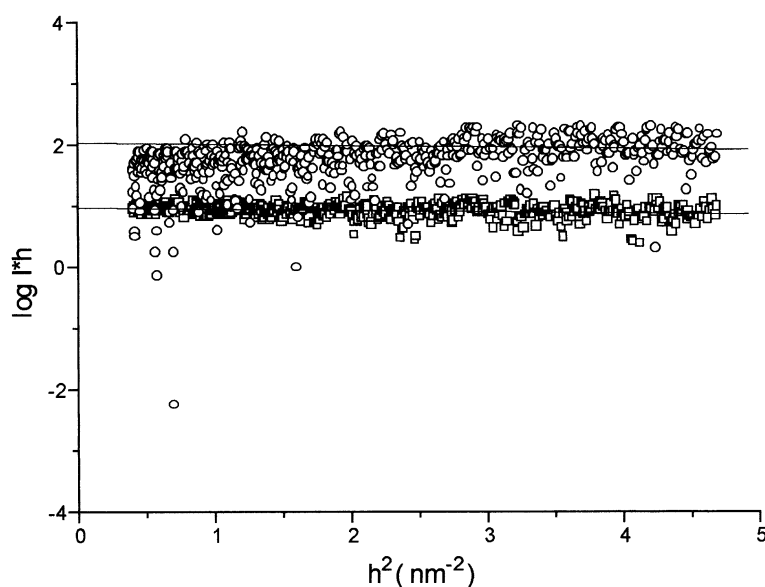

Fig. 4. Guinier plot for CN-SCL in 0.01 M NaOH (□) and in DMSO (○) (DS = 0.55, $C_p = 0.3$ w/v).

Table 3
Diameters of polymer chain cylinders as determined by SAXS (D $\pm$ 0.7 Å)

Sample	Polymer	C_p (w/w%)	Solvent	D (Å)
1	SCL	0.15	Water	18.4
2	SCL	0.25	M NaOH (pH 11.5)	16.4
3	SCL	0.25	DMSO	7.0
4	B-SCL	0.25	M NaOH (pH 11.5)	20.7
5	B-SCL	0.30	DMSO	7.2
6	CN-SCL DS = 0.55	0.30	M NaOH (pH 11.5)	9.6
7	CN-SCL DS = 0.55	0.30	DMSO	7.3

technique, were published for triple stranded polysaccharidic chains (Gawronski, Conrad, Springer & Stahman, 1996). These values ranged from 15.2 to 18.2 Å, again in very good agreement with our data.

The D value obtained for native SCL in DMSO (7.0 Å, Table 3) could consequently be considered as the diameter of the SCL single chain. The high R values found for B-SCL indicated that the reaction conditions did not prevent Scleroglucan from resuming the triple helix structure in appropriate solvent conditions.

Contrary to this, CN-SCL exhibited low D values: 9.6 Å at pH 11.5 (aqueous NaOH) and 7.3 in DMSO (Table 2, samples 6 and 7, respectively). Therefore, even in 0.01 M NaOH, where native Scleroglucan is able to give a triple-helix structure, the cyanoethylated sample exhibited a rod diameter only slightly higher than that found in DMSO. In agreement with capillary viscosity data, this increase of the polymer chain diameter very likely indicates the presence of some molecular aggregation which, at pH values lower than 10, is responsible for micro-gel formation.

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