Isotopic Exchange Study of the Scleroglucan Chain in Solution

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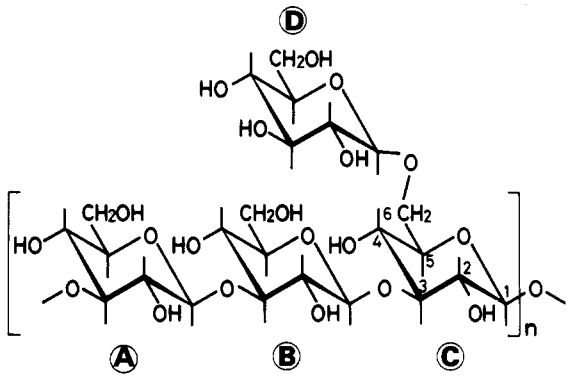
ABSTRACT: OH/OD isotopic exchanges have been achieved on the hydroxyl groups of scleroglucan, a microbial polysaccharide. The isotopic exchange amount has been determined by using ^1H NMR measurements on the remaining hydroxyl proton signals observed in aprotic solvents (DMSO or DMSO–LiCl). In a D_2O solution, scleroglucan adopts an ordered triple-helical structure, which leads to an exchange amount of only 18% of all the hydroxyl protons. When varying the isotopic exchange experimental conditions, i.e., temperature, solvent medium, and lithium chloride concentration, it is observed that at a temperature of 90 °C a maximum of 50% of all the hydroxyl groups can be exchanged. This indicates a remaining ordered structure for the scleroglucan chains in solution under such experimental conditions. Optical rotation measurements on different solutions used in the isotopic exchange experiments lead to the observation of a continuous disorder effect when increasing the lithium chloride concentration as well as temperature, which thus facilitates the hydroxyl group accessibility to D_2O molecules. The observed reversible transition at T_M = 7.5 °C in H_2O and T_M = 18 °C in D_2O indicates a strong solvent effect, which can be related to the existence of an ordered solvation shell around the triple-helix core.

Scleroglucan is a microbial polysaccharide produced by the fungus Sclerotium rolfsii. Its chemical structure corresponds to a regular polysaccharide, with four β-D-glucopyranosyl residues as the repeating unit. One of the D-glucopyranosyl residues is linked as a pendant group through a β(1–6) glycosidic bond, to the linear chain. Both the chemical structure determination and the ^{13}C NMR study agree with the proposed formula.[1]



Scleroglucan has some interesting applications as a thickener used in the food and pharmaceutical industries. The scleroglucan is soluble in water or basic solutions and in dimethyl sulfoxide (DMSO). The conformation of the scleroglucan chain has been extensively studied in solution. In aqueous or slightly basic solutions, the macromolecule is associated in a triple helix structure, whereas in solutions with pH higher than 12.5 or in DMSO the macromolecule is dispersed to take up a single-chain random-coil conformation.[2–4]

Scleroglucan is a highly hydroxylated polymer, which possesses 12 hydroxyl groups per repeating unit. It is well-known that labile protons of hydroxyl or amino groups could be exchanged in deuterated water. The exchange amount depends on the accessibility of the corresponding -OH proton to the D_2O molecules.

The accessibility of the scleroglucan hydroxyl protons in water solution could be expected to depend on the degree of order or association of the macromolecules. For example, cellulose crystallinity can be determined through OH/OD exchange experiments, in the solid state, via the exchange amount determined by means of infrared spectrophotometry. It is found that amorphous regions are more easily accessible to D_2O than the crystalline ones.[5]

Since scleroglucan is soluble in DMSO, an aprotic solvent, it is possible to observe individual hydroxyl proton signals by means of ^1H NMR spectroscopy. However, due to its complex structure, the scleroglucan ^1H NMR spectrum is expected to be unresolved, as 40 signals will resonate between 3 and 5 ppm.

We have already shown that this drawback can be overcome by adding lithium chloride in aprotic solvents, which does not alter their aprotic properties and acts as a shift reagent that spreads out the hydroxyl proton signals as do europium salts and furthermore breaks the intermolecular hydrogen bonds in the polymer chains.[6,7]

In this study we have used ^1H NMR spectroscopy to determine the OH/OD exchange amount of scleroglucan in D_2O solutions and observe the influence of lithium chloride as a dissociating agent.

Experimental Section

Sample. The scleroglucan sample, Polytran CS II, was supplied by CECA (Velizy, France).

OH/OD Exchange Experiments. The standard conditions (otherwise mentioned) used were as followed: Scleroglucan (25 mg) was disolved in 2.5 mL of D_2O; various amounts of lithium chloride were then added to give concentrations of aqueous LiCl solution variyng from 0 to 15% (wt/vol of D_2O).

The solution was then heated 2 h at 90 °C and freeze-dried. The freeze-dried solid was then dissolved into 2.5 mL of D_2O, heated at 90 °C for 2 h, and freeze-dried. Four cycles were always used to reach the maximum exchange amount.

NMR Spectra. Proton NMR spectra were obtained on a Brücker AM400 spectrometer operating at 400 MHz.

Scleroglucan (25 mg) previously exchanged and freeze-dried was dissolved in 1 mL of DMSO-d_6 (kept on molecular siewes) under anhydrous conditions to give a 2.5% (wt/vol) polymer solution. All the chemical shift are referenced to external TMS.

Homonuclear ^1H 2D COSY spectra were obtained under the following experimental conditions: sweep width, SW1 = SW2 = 2000 Hz. The delays were D_1 = 2 s for relaxation and D_0 variable with 256 increment values of 500 μs. The resulting data matrix of 256 × 2K points was multiplied by a "pseudoecho" function and zero filled in each dimension, to build up a 1K × 2K symmetrized matrix with a digital resolution of 1.95 Hz/point. The 90° observe pulse was 8 μs.

Optical rotation measurements were made on a BECKMAN spectropolarimeter at 365 nm with a glass cell of 10-cm optical path length and polymer concentration solutions of 0.72 g/L.

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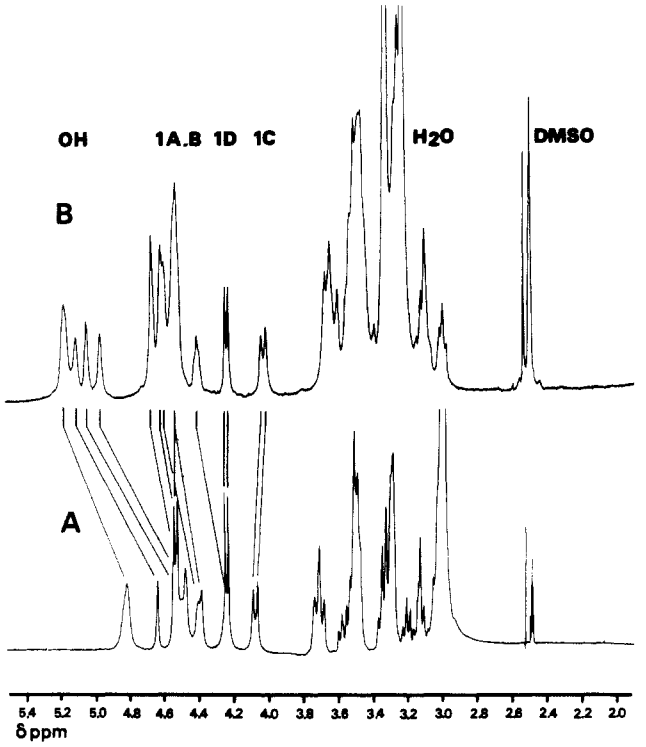

Figure 1. 400-MHz ^{1}H NMR spectrum of scleroglucan at 80 °C: (A) in DMSO-d_6 solution; (B) in DMSO-d_6–LiCl solution.

Results and Discussion

NMR Spectra. We have shown that it is not possible to obtain ^{1}H or ^{13}C NMR signals for scleroglucan in aqueous solutions, due to the existence of a rigid ordered chain conformation.[1] Both proton and ^{13}C NMR spectra can be obtained as far as the scleroglucan chain adopts a random coil conformation in DMSO or in concentrated sodium hydroxyde solutions.

Figure 1A shows the ^{1}H NMR spectrum of scleroglucan in DMSO-d_6 solution at 80 °C. It is difficult to draw any conclusion from this spectrum, except the fact that hydroxyl protons yield individual signals, downfield 4.2 ppm.

Figure 1B shows the spectrum obtained in the same conditions in DMSO–LiCl solvent. The low-field shift induced by lithium chloride on hydroxyl proton signals allows observation of 11 different signals, downfield 4 ppm, corresponding to the 12 hydroxyl groups and the four anomeric H-1 protons of the constitutive unit. The four anomeric proton signals are easily identified as the CH proton signals, insensitive to temperature variation.

The use of the homonuclear ^{1}H 2D COSY technique provides new information, as indicated in Figure 2. COSY[8] gives correlation maps, showing the connectivity between spin–spin scalar coupled protons. In the case of polysaccharides, each sugar unit is independent of the neighboring ones, as no ^{1}H/^{1}H coupling is observed through the interunit glycosidic linkage. The connectivity between protons, inside one β-D-glucopyranosyl ring unit, along the C–C bonds ranging from C-1 to C-6, identifies all the proton signals, as far as one of them can be assigned. Two anomeric H-1 proton signals can be easily identified: the doublet at 4.0 ppm corresponding to the slow-relaxing 1C anomeric proton and that resonating at 4.2 ppm corresponds to the fast-relaxing 1D anomeric proton. All the connectivities of ring protons in C and D units can be observed on Figure 2. More proton signal identification of A and B units can tentatively be achieved; however, due to numerous superimposed signals, a higher field magnet spectrometer would provide better results. Exepting the three primary hydroxyl protons (OH-6A, OH-6B, and OH-6D), OH-2D is the only identified hydroxyl proton signal.

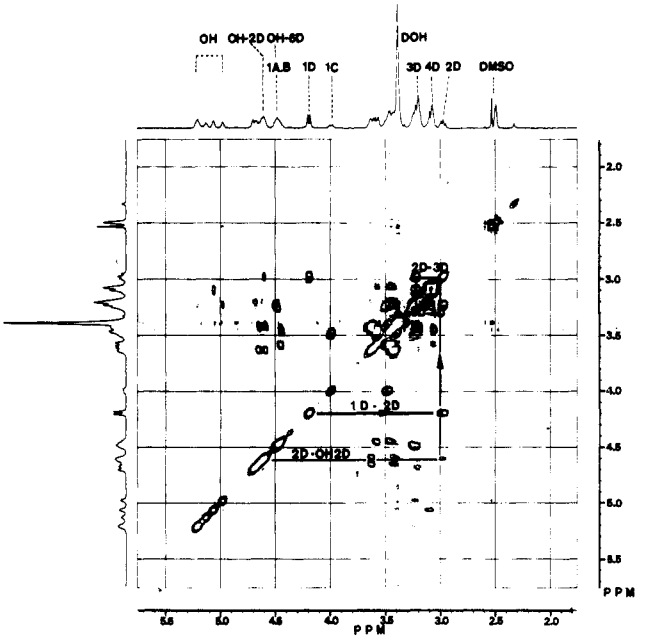

Figure 2. 400-MHz ^{1}H 2D COSY experiment on scleroglucan in DMSO-d_6–LiCl solution at 70 °C.

The average isotopic exchange amount can be determined via the low-field part of the scleroglucan ^{1}H NMR spectrum, integrating hydroxyl signals and using the unexchangeable 1C and 1D C-H anomeric protons as internal reference.

Isotopic OH/OD Exchange Experiments. Since the exchange experiments are conducted in D_2O solutions, scleroglucan will adopt the ordered triple-helix structure, which will prevent the accessibility of all the polymer hydroxyl groups to D_2O molecules.

The standart exchange conditions, described in the Experimental Section (four cycles of dissolving scleroglucan in pure D_2O, keeping the solution at 90 °C for 2 h and then freeze-drying it) lead to 18% of OH groups replaced by OD group. This is a low value considering the rather drastic experimental conditions used. It clearly indicates that in pure D_2O solution, scleroglucan presents a low accessibility to water molecules, due to its rigid and packed triple-helix structure, even at 90 °C.

It is to be remarked that the average OH/OD exchange value obtained by NMR is the result from 12 different values (corresponding to the 12 hydroxyl groups per repeating unit). Since NMR acquisition is conducted in DMSO–LiCl solution, the scleroglucan chain takes a random coil conformation. On the same time scale as NMR, a slow proton exchange occurs and results in the averaging of the exchange values. Thus all the hydroxyl signals on Figure 1B keep individually the same integral value.

In Table I are given the results of the isotopic exchange experiments for scleroglucan in different exchange media. One can observe that the lithium chloride added to D_2O favors the accessibility of the polymer hydroxyl groups. There is a direct relationship between the lithium chloride concentration and the exchange amount of hydroxyl groups. Over the whole lithium chloride concentration range used for these experiments (0–150 g/L) the average OH exchange amount varies from 18 to 48%. This result confirms the fact that lithium chloride presents a high potentiality of dissociation for strongly associated polymers.[10,11]

Table I
Isotopic OH/OD Exchange Experiments of Scleroglucan in D_2O[a]

expt	C polym, g/L	exchange medium	LiCl concn, g/L	av exchange amount, % OD
1	scleroglucan, 20 g/L	D_2O	0	18
2	scleroglucan, 20 g/L	D_2O	25	24
3	scleroglucan, 20 g/L	D_2O	80	35
4[b]	scleroglucan, 20 g/L	D_2O	80	52
5	scleroglucan, 20 g/L	D_2O	150	48
6	scleroglucan, 20 g/L	DMSO–D_2O (50/50)	25	11
7[c]	laminaran, 20 g/L	D_2O	25	70

[a] Four exchange experiments at 90 °C, 2 h (see the Experimental Section). [b] Two exchange experiment at 90 °C, 48 and 2 h. [c] Low molecular weight poly(1–3)-D-glucopyranosyl polysaccharide; one exchange experiment, 2 h at room temperature.

The influence of exchange time was studied in experiment 3 and 4, at the constant lithium chloride concentration of 80 g/L. The substitution of three 2-h exchange cycles by a 48-h one increases the exchange amount from 35 to 52%.

The influence of the degree of order, triple-helix versus random-coil conformation in the scleroglucan chain in solution, was studied in experiment 6. Scleroglucan was first dissolved in a DMSO–LiCl solvent to obtain the random-coil conformation, then D_2O was added to obtain a DMSO–D_2O mixed solvent (50/50) which should increase the accessibility of the polymer. Surprisingly, the exchange amount (11%) was significantly lower than in the corresponding D_2O solution (24%). One can conclude that in this mixed solvent, with a content of 25 g/L of lithium chloride, the triple-helix association remains stable. This experiment confirms the viscosity measurements obtained by Yanaki[2] when increasing the DMSO content in an initial scleroglucan water solution. The solution viscosity decreases sharply with a 87% DMSO content, corresponding to the dissociation of the triple helix into a single-chain state for this composition.

The low accessibility of scleroglucan hydroxyl groups to D_2O molecules in a 50/50 DMSO–D_2O solution could be due not only to the ordered conformation of the polymer chain but also to a solvatation shell of DMSO molecules, which present a high affinity toward the polymer hydroxyl groups. This solvatation shell hinders the accessibility of the OH groups to D_2O molecules.

Optical Rotation Measurements on Scleroglucan Solutions in D_2O/LiCl. From exchange experiments we can see that lithium chloride has an important influence on the scleroglucan chain arrangement in D_2O solutions. Therefore we have studied the optical rotation variation for scleroglucan solutions containing various amounts of lithium chloride within the concentration range used in exchange experiments.

The optical rotation has been shown to be highly sensitive to the degree of order of scleroglucan solutions and particularly to the irreversible triple helix–coil transition.[1,9] The specific optical rotation values $|\alpha|^{365}$ have been plotted as a function of lithium chloride concentration over the 0–300 g/L (0–7 M) concentration range (Figure 3).

$|\alpha|^{365}$ decreases in a constant manner, particularly in the 0.6–2.5 M concentration range. Beyond this point, the salt concentration becomes too important to allow a significant interpretation of the scleroglucan modification. This curve indicates that there is no transition but a continuous disorder process when increasing the lithium chloride concentration. This is in direct relation with the isotopic

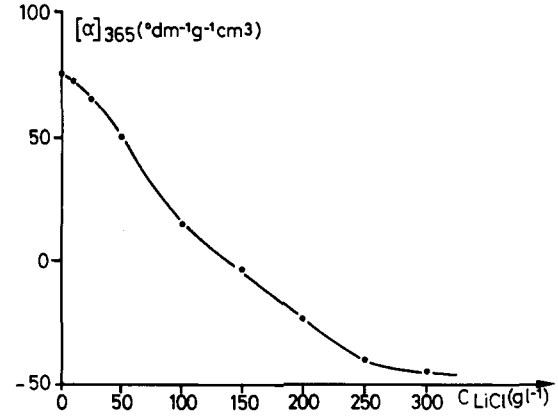

Figure 3. Specific rotation $|\alpha|^{365}$ versus LiCl concentration at 25 °C.

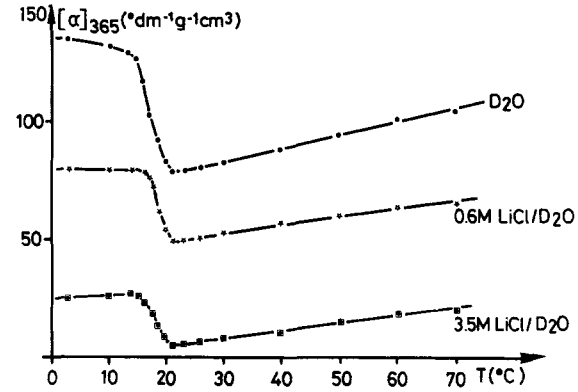

Figure 4. Specific rotation $|\alpha|^{365}$ variation with temperature of a scleroglucan solution (0.72 g/L) in D_2O and D_2O–LiCl solvents.

exchange amount which increases with increasing lithium chloride concentration of the D_2O solutions. Lithium chloride seems to have an intermediary role between sodium hydroxide, which can induce the triple helix–coil transition, and sodium chloride, which plays a negligeable role as a disrupting agent.

As it is well-known that increasing the temperature of a scleroglucan solution can induce a change in the conformation and/or the degree of order of the chains, we have also studied the influence of this parameter on the lithium chloride/D_2O solutions, at different LiCl concentrations. The transformation observed on Figure 4 is found to be reversible as already noticed and can be characterized by the midpoint transition temperature T_M. It has been shown that for both scleroglucan[9] and schizophylan[12]—a polysaccharide from a different origin having the same chemical constitutive unit as scleroglucan—there is a strong isotopic effect on the temperature transitiion T_M, when substituting D_2O to H_2O: T_M = 7.5 °C for H2O and T_M = 18 °C for D_2O solutions. It has also been shown that adding sodium chloride has little effect on the T_M value.

The addition of 0.6 M lithium chloride to scleroglucan in D_2O solution does not affect the transition temperature (T_M = 19 °C) (Figure 4). However, it can be observed that the optical rotation value is strongly lowered, this phenomenon being emphasized when increasing the LiCl concentration up to 3.5 M. The decrease of the $|\alpha|$ values, and at the same time the transition amplitude when increasing the lithium chloride concentration, can be assigned to its potentiality to a partial disruption of the ordered triple-helix structure: deseggregation of several triple helix association; partial mobility of the β(1–6)-D-glucopyranosyl pendant group; disruption of a water shell structure around the triple helix.

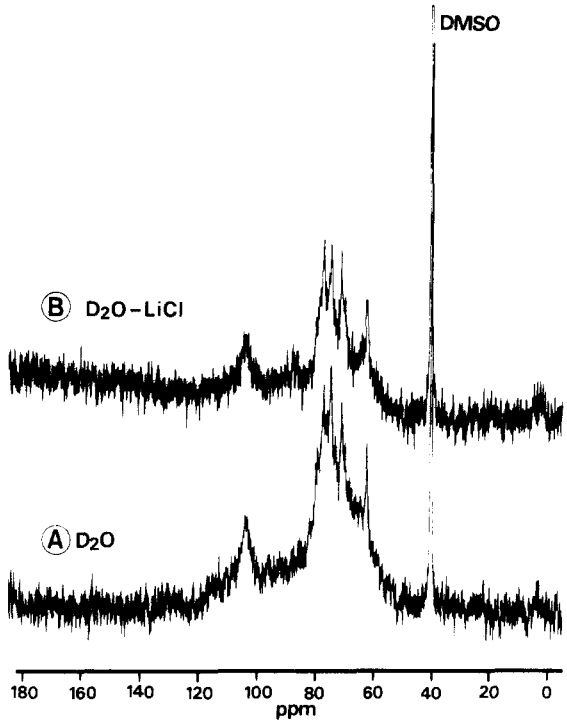

Figure 5. 100-MHz ^{13}C NMR spectra of scleroglucan at 80 °C: (A) in D_2O; (B) in D_2O–LiCl (5%).

The isotopic effect observed on T_M when passing from H_2O to D_2O solutions, for scleroglucan and schizophyllan as well, suggests the influence of water molecules associated to the triple-helix structure. The partial mobility of the side-chain β-D-glucopyranosyl unit has also been proposed as an explanation of this transition in the case of schizophyllan. This was mainly based on the presence of ^{13}C NMR signals of the side-chain unit at a high temperature.

In DMSO solution, scleroglucan adopts a single-chain random-coil conformation and thus yields a well-defined ^{13}C NMR spectrum, the resolution of which is slightly increased when raising the temperature. In that case, we have noticed[1] that the ^{13}C nuclei of the D side-chain unit have higher relaxation times than the main-chain carbon nuclei, which indicates a higher mobility of that unit.

Figure 5 shows the scleroglucan ^{13}C NMR spectra obtained in D_2O (A) and D_2O/LiCl solutions (B) at 80 °C, after one night of accumulation. The broad-band spectrum observed in each case clearly indicates a low-mobility macromolecular systems, showing, however, four more precisely resolved signals, which are assigned to C-5D/C-3D, C-2D, and C-6D carbon atoms with respect to the DMSO spectrum. These ^{13}C NMR spectra indicate that under the experimental conditions used, a higher mobility of the macromolecular triple helix system is observed at this temperature, since no signal is obtained at room temperature.

Lithium chloride has only a small effect on the ^{13}C NMR spectra and then on the interchain interactions. The effect observed on the isotopic exchange amount comes from a better accessibility of the polymer hydroxyl groups to D_2O molecules and must be related either to a dissociation effect of triple-helix aggregation or more probably to the disruption of an ordered hydration water shell around the triple-helix core as proposed for schizophyllan.[13]

Registry No. Scleroglucan, 39464-87-4.

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