Polymer Bulletin 34, 337–343 (1995)

Polymer Bulletin

Conformation and thermal stability of oxidized scleroglucan chains in aqueous NaOH

Tommasina Coviello [2], Mariella Dentini [1], Vittorio Crescenzi [1]

[1] Department of Chemistry, and [2] Department of Chemistry and Technology of Biologically Active Compounds, La Sapienza University, I-00185 Rome, Italy

Received: 17 November 1994/Revised version: 10 December 1994/Accepted: 19 December 1994

Summary

The solution behaviour of the polycarboxylic acid derived from scleroglucan (sclerox) has been investigated as a function of NaOH concentration and temperature by means of polarimetry and viscosimetry techniques. In comparison to the native polymer, sclerox is less sensitive to degradation at high NaOH concentration (up to NaOH equal to 0.4 M) and thermally more stable. The viscosity data show a "normal" polyelectrolyte effect and the polymer chains seem to be not stiff . We conclude that at high pH sclerox chains are in a flexible, probably single stranded conformation.

Introduction.

Scleroglucan is a microbial non-ionic polysaccharide which has been extensively studied for its peculiar solution properties (1-5). By combined periodate-chlorite oxidation of the β-D-glucosyl side residues along scleroglucan main chains, carboxylate groups bearing derivatives can be easily prepared (6,7) .

Both scleroglucan and its oxidized derivatives have been found suitable for the formulation of sustained drug release forms (8,9) .

These polysaccharide polyelectrolytes (sclerox) display interesting solution properties markedly influenced by ionic strength and/or temperature (7,10,11). In particular, the influence of NaCl concentration (up to 5.0 M) on the conformational propensities of sclerox chains in dilute aqueous solution have been recently investigated (12).

We wish to report here the results of a study on the dependence of the dilute solution properties of sclerox, sodium salt (Fig.1), on NaOH concentration and on temperature carried out in order to determine the influence of a strong base on polymer chains conformational states and stability in solution.

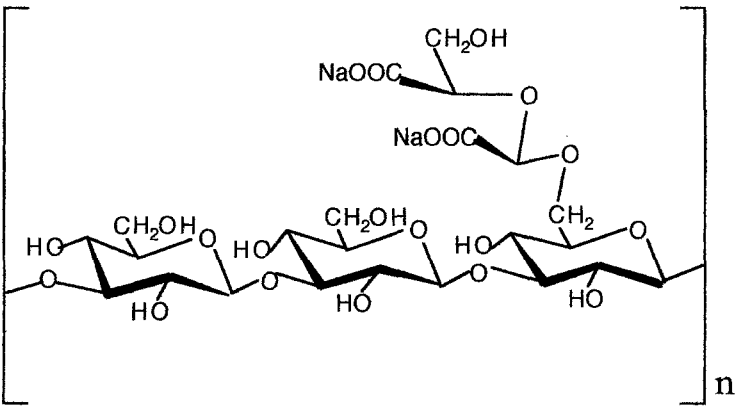

Fig. 1 Repeating unit of oxidized scleroglucan (sclerox).

Experimental

Oxidized scleroglucan (sclerox) samples were prepared according to the procedures described elsewhere (7,8).

Optical activity measurements were performed with a Perkin-Elmer 241 polarimeter using a 10 cm path-length cell, the temperature being controlled by means of a Lauda circulating-water bath.

Viscosity measurements for the Na^+ salt of sclerox were performed at 25°C using a Shott-Geraete automatic viscosimeter equipped with a water thermostat. A range of ionic strengths was investigated; these were controlled by varying the level of NaOH added. The sclerox samples, fresh prepared, and the NaOH solutions were previously filtered four times with Millipore filters with cut-off of 8.0 μm and 0.22 μm, respectively.

Results and discussion

The intrinsic viscosity data for sclerox (25°C) as a function of NaOH concentration are given in Fig.2. The viscosity behaviour is typical of a polyelectrolyte (Fig. 2b) when the charged acidic groups located along the chain are shielded by adding ionic strength and the expansion of the chains is reduced. The linearity of the plot (13) of Fig. 2c indicates that no "anomaly" (14) takes place at 25°C in the range of NaOH concentration considered (up to 0.4 M). Interpolation of such plot yields for the "viscosity parameter" B a value of 0.26.

This may be considered in favour of a flexible character of sclerox chains. An analogous study of the same sclerox sample in a range of NaCl concentrations comprised between 0.01 and 0.4 M (25°C, neutral pH) has shown that B=0.14 (12).

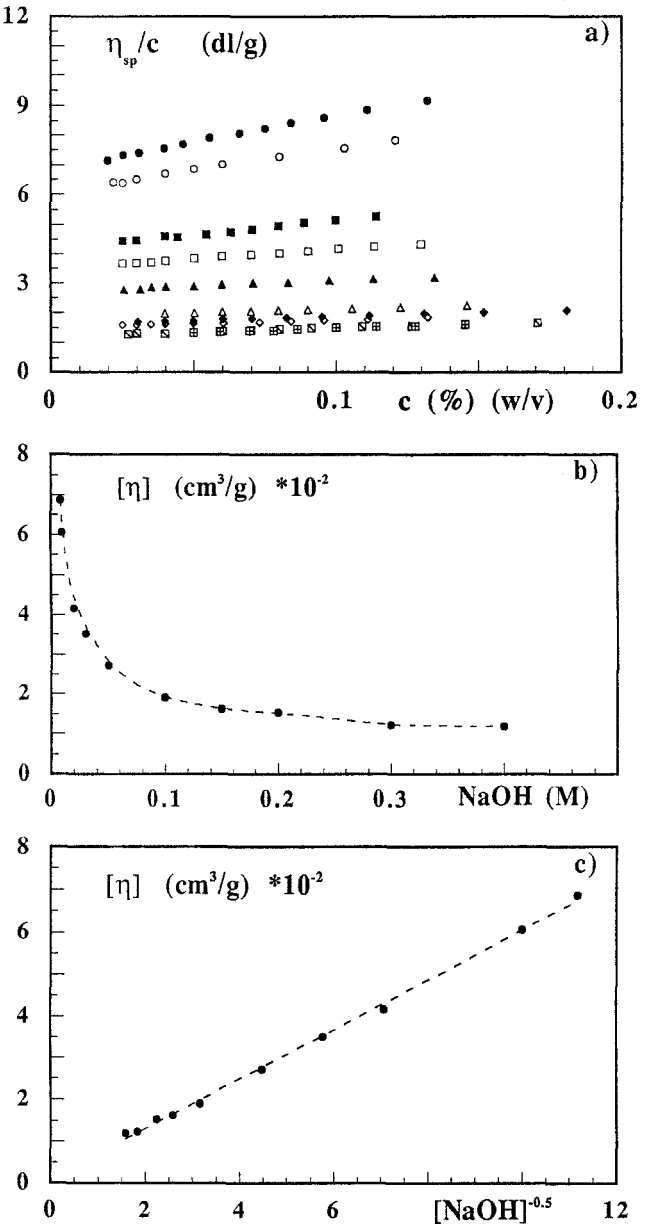

Fig .2 a) Reduced specific viscosity of aqueous sclerox solutions at different NaOH concentrations at 25°C. The NaOH values (M) are:

(●) 0.008; (○) 0.010; (■) 0.020; (❑) 0.030; (▲) 0.050;

(△) 0.100; (◆) 0.150; (◇) 0.200; (⧅) 0.300; (⧄) 0.400.

b) Dependence of sclerox intrinsic viscosity on NaOH at 25°C.

c) Variation of sclerox intrinsic viscosity at 25°C with $[NaOH]^{-1/2}$.

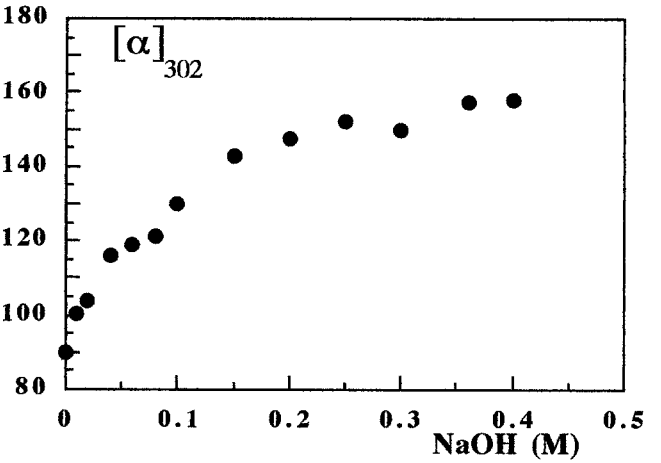

Fig.3 Optical activity (λ= 302 nm, 25°C) dependence on NaOH concentration of sclerox aqueous solutions. Polymer concentration c_p=0.10% (w/v).

It appears therefore that at high pH (aqueous NaOH, up to 0.4 M) sclerox chains may be in a (probably single stranded) more flexible state than in aqueous NaCl in wich at high ionic strengths elongated (likely multistranded), stiff chains shapes would prevail.

It has to be underlined that all 25°C viscosity readings were very stable and exactly reproducible (during almost 24 hrs), with no evidence of polymer aggregation or degradation.

Similar considerations apply to the optical actvity data reported in Fig.3 which also exhibit a regular variation with increasing NaOH concentration (25°C).

In the context, it is worth mention that, different from sclerox solutions, solutions of scleroglucan in 0.10-0.15 M NaOH at 25°C (15,16) already show a time dependent, decreasing viscosity to be traced to polysaccharide chain scission (17).

Evidence of a similar temperature-induced phenomenon in the case of sclerox in 0.15 M and in 0.30 M NaOH is afforded by the optical activity data illustrated in Fig.4.

In fact, succcessive heating-cooling cycles (NaOH 0.15 M: Fig.4a) lead to progressively and irreversibly decreasing $[\alpha]_{302}$ readings. In 0.30 M NaOH, a strong decrease in optical activity is evident for temperatures higher than 55-60°C.

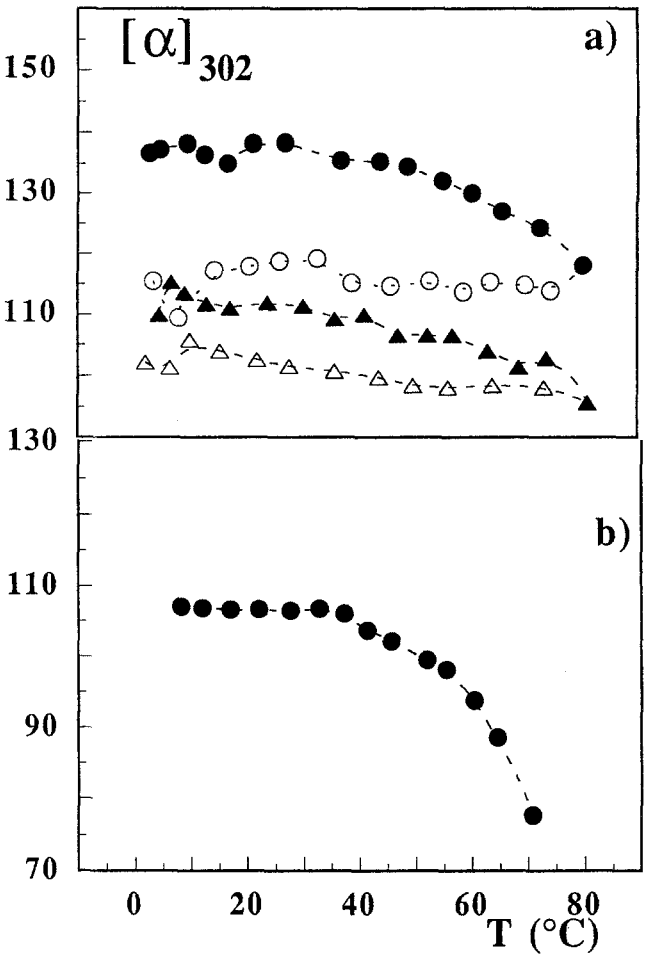

Fig.4 a) Optical activity (λ= 302 nm) dependence on temperature of sclerox solution in 0.15 M NaOH .
First cycle (8 hrs) : (●) heating; (○) cooling.
Second cycle (8 hrs) : (▲) heating; (△) cooling.
Polymer concentration c_p=0.10% (w/v).
b) Optical activity (λ= 365 nm) dependence on temperature of sclerox solution in 0.30 M NaOH and NaCl 1.00 M
Polymer concentration c_p=0.10% (w/v).

Interestingly, the phenomenon is more marked in the case of scleroglucan according to data of Fig.5.: in fact, in 0.3 M NaOH even at 25°C the $[\alpha]_{302}$ values are close to -10, a demonstration of severe, if not total, chains degradation.

Sclerox appears therefore as thermally more stable to alkali induced degradation with respect to its parent uncharged polymer, scleroglucan. Partial exclusion of OH^- ions from the negatively charged sclerox macroions domains might explain this unexpected result.

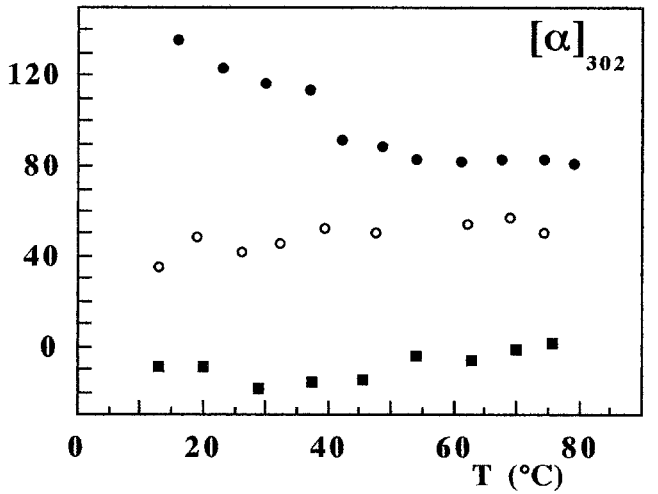

Fig. 5 Optical activity (λ= 302 nm) dependence on temperature of native scleroglucan solutions in (●) 0.11 M , (○) 0.15 M and (□) 0.30 M NaOH . Polymer concentration c_p=0.10% (w/v).

It is also worthwhile to note that the optical activity of sclerox sample in the TBA^+ form (TBA^+= $(CH_3CH_2CH_2CH_2)_4N^+$) which easily dissolves either in water or in methylsulphoxide (DMSO), exhibits almost the same optical activity in the two solvents ($[\alpha]_{302}$ in water = 68±1, $[\alpha]_{302}$ in DMSO = 61 ± 1). Although the similarity of optical activity may be only a poor indicator of polysaccharide conformation it appears possible to presume that in our case the polymer conformation should be the same in both solvents. This is an other indication which supports our idea of a system formed essentially of singularly dispersed sclerox chains.

Similar behaviour is also shown by another polycarboxylated derivative of scleroglucan, containing 2.3 phthalic acid residues per repeating unit, for which the experimental data reported in the literature (18,19) support the existence of a random coil conformation in water and DMSO solution.

Acknowledgements

We wish to acknowledge the financial support of Italian Ministry for University and Scientific and Technological Research (MURST, Rome), "40%" funds.

References

1. Norisuye T., Yanaki T. & Fujita H., (1980) J. Polym. Sci. Polym. Phys. Ed.18:547
2. Norisuye T., (1985) Makromol. Chem., Suppl. 14:105
3. Yanaki T., Norisuye T. & Fujita H., (1980) Macromolecules 13:1462
4. Yanaki T. & Norisuye T. , (1983) Polymer J. 15:389
5. Enomoto H., Einaga Y. & Teramoto A., (1984) Macromolecules 17:1573
6. Hofreiter B.T. , Wolff I.A. & Mehltretter C.L., (1957) J. Am. Chem. Soc. 79:6457
7. Crescenzi V., Gamini A., Paradossi G. & Torri G., (1983) Carbohydr. Polym. 3: 273
8. Alhaique F., Riccieri F.M., Santucci E., Crescenzi V. & Gamini A.(1985) J. Pharm. Pharmacol. 37:310
9. Romanelli L., Alhaique F., Riccieri F.M., Santucci E. & P.Valeri, (1993) Pharmacol. Res. 27 (Suppl.1):127
10. Gamini A., Crescenzi V., Abruzzese R., (1984) Carbohydr. Polym.4:461
11. Crescenzi V., Gamini A., Rizzo R. & Meille S.V., (1988) Carbohydr. Polym. 9: 169
12. Coviello T., Dentini M., Crescenzi V.,Vincenti A.(1994) Carbohydr. Polym. (accepted)
13. Smidsrod O. & Haug H., (1971) Biopolym. 10:1213
14. Smidsrod O., Andresen I.L., Grasdalen H. , Larsen B. & Painter T. , (1980) Carbohydr. Res. 80:C11
15. Bo S., Milas M., Rinaudo M. (1987) Int. J. Biol. Macromol. 9:153
16. Yanaki T., Kojima T. & Norisuye T. , (1981) Polymer J. 13:1135
17. Kashiwagi Y., Norisuye T., Fujita H. (1981) Macromolecules 14:1220
18. Muller G., Chiron G., Levesque G. (1985) Polym. Bull. 15:1
19. Muller G. (1986) Carbohydr. Polym. 6:177