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Polysaccharides

Functional Group Modification in a Non-ionic Extracellular Schizophyllan Polysaccharide

G. Muller[1]*, G. Chiron[2], and G. Levesque[2]

[1] UA 500 du CNRS "Polymères, Biopolymères, Membranes", Faculté des Sciences de Rouen, F-76130 Mont-Saint-Aignan, France

[2] Laboratoire de Chimie des Composés Thio-Organiques, UA 480 du CNRS, Université de Caen, Institut des Sciences de la Matière et du Rayonnement, 5 Avenue d'Edinbourg, F-14032 Caen Cedex, France

Summary

Chemical modification of schizophyllan,a neutral fungal polysaccharide which assumes a triple helical structure in aqueous solution,was performed at room temperature in DMSO in the presence of triethylamine and dimethyl-4 aminopyridine.No molecular weight degradation results from the modification.The modified polysaccharide which contains 2.3 phtalic acid groups per repeat unit behaves as a polyelectrolyte in aqueous solution.Due to the presence of charged acidic groups a single chain conformation is evidenced in both DMSO and aqueous solution.

Introduction

Schizophyllan (Figure 1),a neutral water soluble polysaccharide coming from fungal fermentation,dissolves as a rigid triple helix in aqueous solution and as a single random coil in dimethylsulfoxide (DMSO) (NORISUYE et al. 1980;KASHIWAGI et al.1981).

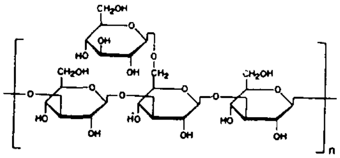

Figure 1.Primary structure of schizophyllan.

Interchain hydrogen bonds are responsible for the stabilization of the triple helix (T.NORISUYE et al.1980) disruption of which occurs upon heating or in the presence of 0.1M NaOH.The same behaviour is observed with scleroglucan which has the same primary structure as that of schizophyllan (T.YANAKI et al.1983).
A recent work described the solution behaviour of a polycarboxylic acid derived from scleroglucan by periodate oxidation (CRESCENZI et al.1983;A.GAMINI et al.1984).Starting from a freeze-dried schizophyllan sample kindly supplied by Dr.T.YANAKI (Tato Co.,Japan)

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* To whom offprint requests should be sent

we have prepared a modified schizophyllan containing phtalic acid groups by using the system DMSO/triethylamine/dimethyl-4 aminopyridine which has been reported successful for esterification of polyols and polysaccharides at room temperature (G.CHIRON,1985).
Here we report the effect of such a functional group modification as concerns the conformational behaviour in solution.

Experimental

To a solution of 0.33g($5x10^{-4}$mole) of the initial schizophyllan (Sample I) in 20 ml dimethylsulfoxide (DMSO) was added 0.5ml triethylamine and 0.015g($1.23x10^{-4}$mole) dimethyl-4 aminopyridine then 0.247g($1.5x10^{-3}$ mole) phtalic anhydride.The mixture is stirred at room temperature during 3h30'.The modified polysaccharide (Sample II) was isolated by precipitation in acidified water (1% HCl) and centrifugation.It was then purified by dissolving it in a mixture methoxy-2 ethanol/methylene chloride from which it was precipitated in acetone. The precipitate was dried to constant weight.
Potentiometric titration gave a ionic content of $2.3x10^{-3}$eq.acid/g.
The light scattering measurements were performed on a low angle laser light scattering photometer KMX-6. The weight average molecular weight $\bar{M}_w$ was calculated according to $KC/\Delta R_\theta=1/\bar{M}_w+2A_2c$ where A_2 is the second virial coefficient, K the optical constant which includes the refractive index increment dn/dc and ΔR_θ ($\theta=4.8°$) is the excess Rayleigh ratio due to the polymer.
Viscosities were measured with a Ubbelhode-type viscometer (Fica viscomatic).Optical rotation $|\alpha|$ was measured at 365nm with a Perkin Elmer 241 polarimeter.

Results and discussion

The molecular characteristics of the schizophyllan samples I (initial) and II (modified) are given in Table I.
The drop in both $|\eta|$ and $\bar{M}_w$ of sample I observed by changing the solvent composition is due to the melting of triple helix (stable in water) to disordered single coiled chains (stable in DMSO and/or in 0.2M NaOH) which is otherwise evidenced by a marked change in the optical rotation from +40° to -25°.
By comparing viscosity and molecular weight data in DMSO wherein coil conformation exists for both samples I and II it is obvious that no molecular weight degradation resulted from the modification reaction.
The acidic schizophyllan (sample II) as well assumes a coil conformation in aqueous solution with a $\bar{M}_w$ about three times as small as that of sample I as revealed from the light scattering measurements (in 0.1M NaCl) shown in Figure 2.

TABLE I

Characteristics of schizophyllan samples

Sample	Solvent	$\bar{M}_w$	$\lvert\eta\rvert$ ml/g	$\lvert\alpha\rvert^{25°C}_{365nm}$	Conformation
I	DMSO	$1.2x10^5$	100	-	Single coil
	Water	$3.5x10^5$	250	+40°	triple helix
	0.2 NaOH			-25°	Single coil
II	DMSO	$1.4x10^5$	100	-	Single coil
	Water +0.1 NaCl (pH=7)	$1x10^5$	100	-35°	Single coil

$|\alpha|$ is the specific rotation

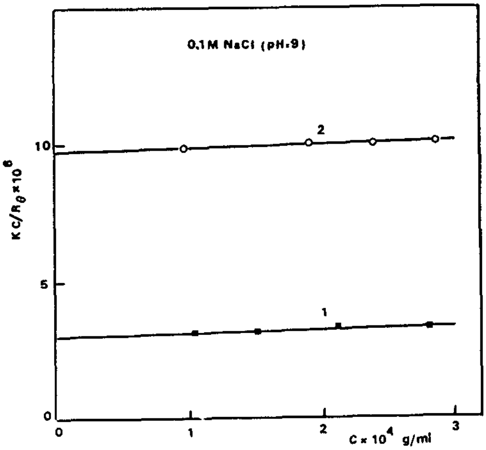

Figure 2. Plot of $KC/\Delta R_\theta$ (θ=4.88°) versus concentration for samples I and II in 0.1M NaCl.

1. Sample I $A_2=5x10^{-4}$ dn/dc=0.142 ml/g
2. Sample II $A_2=7x10^{-4}$ dn/dc=0.145 ml/g

Data reported indicate that conditions for interchain association (H bonding) are no longer existing due to the presence of charged acidic groups in Sample II.

As shown in Figure 3 samples I and II exhibit different viscosity behaviour in aqueous solution. Contrary to what is observed for sample I, which assumes the same reduced viscosity in the absence and in the presence of salt as expected from the absence of ionic groups in its chain,the viscosity behaviour of sample II is typical of a polyelectrolyte.In pure water the polyion assumes extended conformation with large reduced viscosity owing to electrostatic interaction between the charged acidic groups located along the chain. In the presence of added salt the chain expansion is suppressed due to shielding of ionic charges and the viscosity is only dependent on the size of the polymer molecule and no longer on its shape.

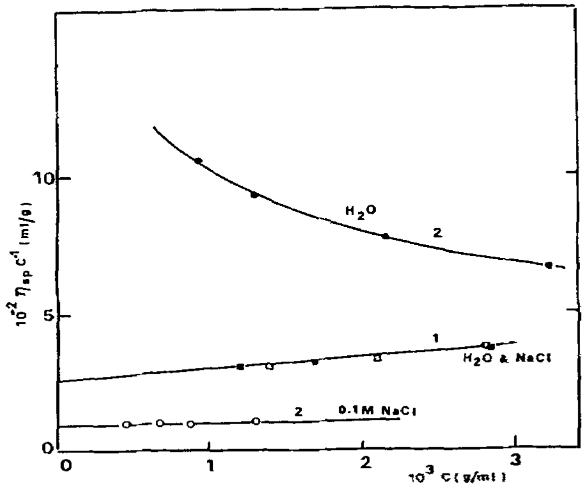

Figure 3. Plot of the reduced viscosity η_{sp}/C vs. concentration for sample I both in pure water and 0.1M NaCl (1) and for sample II in pure water (●) and in the presence of salt (○).

Conclusion

The above reported data clearly indicate that the modified schizophyllan in its ionized form (Na-salt at pH=7-8) assumes a disordered random coil conformation more or less expanded according to the extent of added salt. In 0.1M NaCl the conformation is similar to that observed in DMSO. It is to be expected that different conformations exist in aqueous solution as a function of the degree of dissociation of acidic groups. Preliminary results (G.MULLER,1985) show a clear-cut change in chiro-optical data in a narrow ionization range suggesting the existence of quite different secondary structures the stability of which may be attributed to the effect of intramolecular hydrogen bonding. Further work is in progress concerning the pH and salt dependence of the physicochemical behaviour of this acidic polysaccharide.

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