Carbohydrate Polymers **6** (1986) 177–191

Charge-Induced Order–Disorder Transition in an Ionic Polysaccharide Derived from Schizophyllan

Guy Muller

UA 500 du CNRS 'Polymères, biopolymères, membranes', Faculté des Sciences de l'Université de Rouen, 76130 Mont Saint Aignan, France

(Received: 12 November 1985)

SUMMARY

The conformational behaviour of a schizophyllan polysaccharide containing phthalic acid groups (2·3 acid groups per repeating unit) has been examined in organic (DMSO) and in aqueous media. The acidic polysaccharide adopts a single chain conformation in both media and behaves as a weak polyacid in aqueous solution ($\mathrm{p}K_0 \simeq 3$)*. Potentiometric titration data reveal the existence of a conformational transition occurring over a small ionization range. Viscosity and optical rotation measurements suggest that the transition can be interpreted as a change from an ordered compact conformation at low degrees of ionization, probably stabilized through intramolecular H-bonds, to a disordered more extended one at higher degrees of ionization.*

INTRODUCTION

Schizophyllan is a neutral extracellular polysaccharide of microbial origin (*Schizophyllum commune*) (Kikumoto *et al.*, 1971). Its primary structure which consists of a linear (1 → 3)-β-D-glucan to which single β-D-glucopyranosyl groups are linked (1 → 6) at every third unit of the main chain is exactly the same as that of scleroglucan (Tabata *et al.*, 1981) (Fig. 1(a)). Both of these polysaccharides can adopt different secondary structures according to the solvent conditions, namely a rigid triple helical ordered conformation in water and a single coiled

Carbohydrate Polymers 0144-8617/86/$03.50 — © Elsevier Applied Science Publishers Ltd, England, 1986. Printed in Great Britain

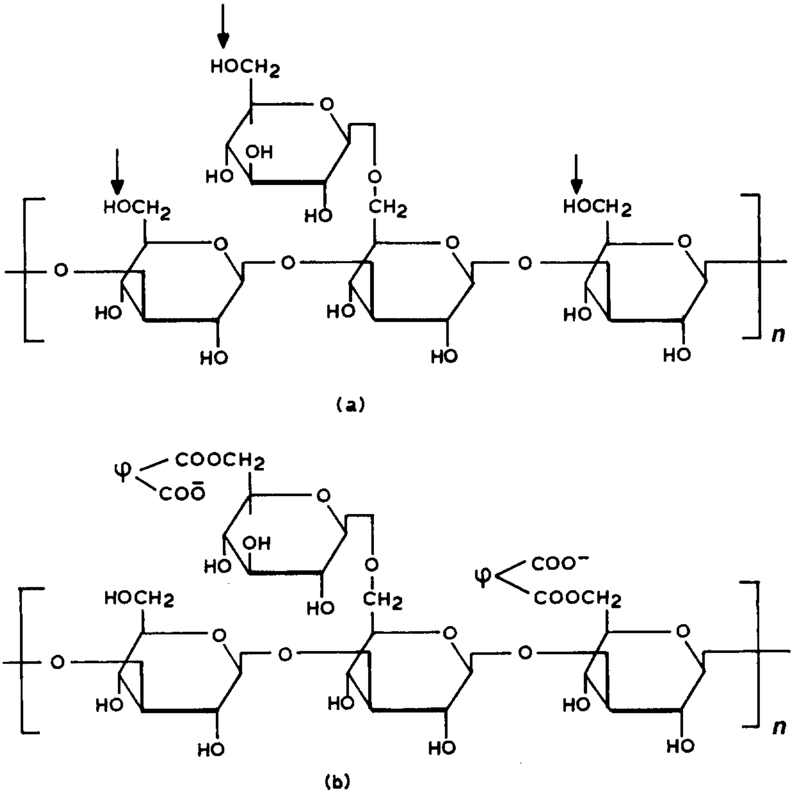

Fig. 1. Primary structures of schizophyllan polysaccharides: (a) Unmodified (the arrows indicate the position of primary OH); (b) modified, with the more probable position of attachment of the substituent phthalic acid groups.

disordered conformation in dimethylsulphoxide (DMSO) (Norisuye *et al.*, 1980; Kashiwagi *et al.*, 1981; Yanaki *et al.*, 1981; Yanaki & Norisuye, 1983). Experimental evidence clearly indicates that the order–disorder transition is not a simple reorganization of the secondary structure but is accompanied by chain dissociation as a result of breaking of intermolecular H-bonds responsible for the stabilization of the triple helical structure in water. Recently, it has been reported that polycarboxylated polymers obtained by periodate oxidation (in water) of scleroglucan glucopyranosic side chains exhibited unusual conformational behaviour in aqueous solution, which was particularly evidenced in their interactions with Ca^{2+} ions (Crescenzi *et al.*, 1983; Gamini *et al.*, 1984). On the other hand, we have recently described the effect of introducing acidic groups (phthalic acid) on the conformation of schizophyllan in aqueous solution and shown that a single chain conformation prevailed for the modified schizophyllan (Fig. 1(b)) in both DMSO and aqueous solution (Muller *et al.*, 1985). It is well recognized that polysaccharides (xanthan, schizophyllan or scleroglucan) can adopt ordered double (or triple) helical conformations in their native state favouring the stacking of chains in solution and giving rise to technologically important applications. Melting of such structures to single chains can result from changes in temperature and/or solvent conditions. It has been shown (Norisuye *et al.*, 1980) that the triple helix of schizophyllan which exists in water cannot reform once it is broken in DMSO; instead the addition of water may result in the formation of larger aggregates (interchain association) as for the renaturation of collagen.

It is of interest to determine the influence of charge on the conformational behaviour of schizophyllan in aqueous media, particularly on interchain association.

In the present work we report the effect on chain conformation of increasing the degree of dissociation of the acidic groups located on the polysaccharide chain. A clear-cut change in potentiometric, viscosity and chiro-optical data takes place over a narrow ionization range where an order–disorder conformational transition is initiated.

EXPERIMENTAL

Polysaccharide samples

The neutral schizophyllan (Sample 1) was a freeze dried sample kindly supplied by Dr T. Yanaki (Tato Co., Japan) and prepared from a native schizophyllan by sonication followed by fractionation.

A modified schizophyllan (Sample 2) which contains 2·3 phthalic acid groups per repeating unit (as determined by potentiometric titration) was prepared from Sample 1 as previously reported (Muller, 1985; Muller *et al.*, 1985), i.e. at room temperature in DMSO in the presence of dimethyl-4-aminopyridine/triethylamine.

Potentiometry

For potentiometric titrations, a weighed amount of Sample 2 was first dissolved in dilute aqueous sodium hydroxide (0·01 M) then percolated through a cationic exchange resin column (Dowex 50W X8, acid form). The pH titrations were performed at 25°C using a Radiometer PHM 64 pH-meter, titrant being delivered with a Gilmont S 3200A syringe microburette.

Static laser light scattering

Measurements were performed at 25°C on solutions which were optically clarified by passage through 0·22 μm Millipore filters. A low angle laser light scattering photometer KMX6, operating at very low angle (4·88°) which circumvents the extrapolation to zero angle, was used. 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 is the excess Rayleigh ratio due to the polymer (both measured at the same wavelength of 633 nm).

The refractive index increments were determined by using a Brice Phoenix differential refractometer.

Viscosity measurements were made at 25°C using a Ubbelhode capillary viscometer (Fica viscomatic).

Optical rotation $|\alpha|$ was measured at 365 nm and 25°C with a Perkin-Elmer 241 polarimeter.

RESULTS AND DISCUSSION

Chain conformation of schizophyllan polysaccharides

Figure 2 shows the specific optical rotation change for aqueous solutions of both schizophyllan samples with increasing NaOH concentration. Whereas Sample 1 assumes a triple helical ordered conformation so long as the NaOH concentration does not exceed 0·1 M, a disordered coil conformation prevails for Sample 2 in the same NaOH concentration range.

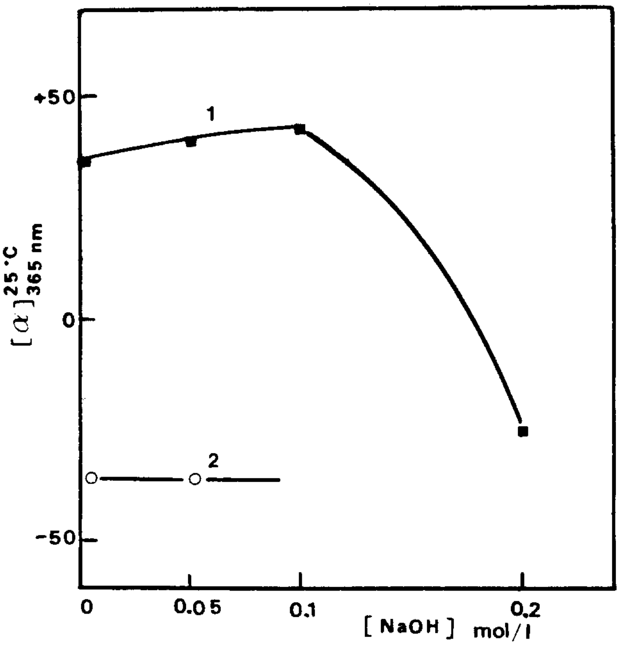

Fig. 2. Dependence of the specific optical rotation $|\alpha|$ measured at 365 nm on the NaOH concentration: 1, Unmodified schizophyllan (Sample 1); 2, acidic schizophyllan (Sample 2). (In DMSO, $|\alpha| \simeq -50°$ for both samples.)

In DMSO both samples assume the same $|\alpha|$ value ($|\alpha| \simeq -50°$) characteristic of the random coil conformation as reported for scleroglucan (Rinaudo & Vincendon, 1982).

Figure 3 illustrates the concentration dependence of KC/R_θ ($\theta = 4{\cdot}88°$) for Samples 1 and 2 in DMSO and aqueous solution (pH = 8).

In agreement with data reported in the literature, the molecular weight $\bar{M}_w$ of Sample 1 in 0·1 M NaCl (curve 1) is about three times as large as that measured in DMSO (curve 1*). This confirms that the polysaccharide has a trimer structure in aqueous solution and is a single random coil in DMSO. From the intercept of curve 2 it appears that the molecular weight of Sample 2 in 0·1 M NaCl is about three times as low as that of Sample 1 in the same salt solution. Furthermore, identical $\bar{M}_w$ are measured for Sample 2 both in DMSO and 0·1 M NaCl. The value found is near to that observed for Sample 1 in

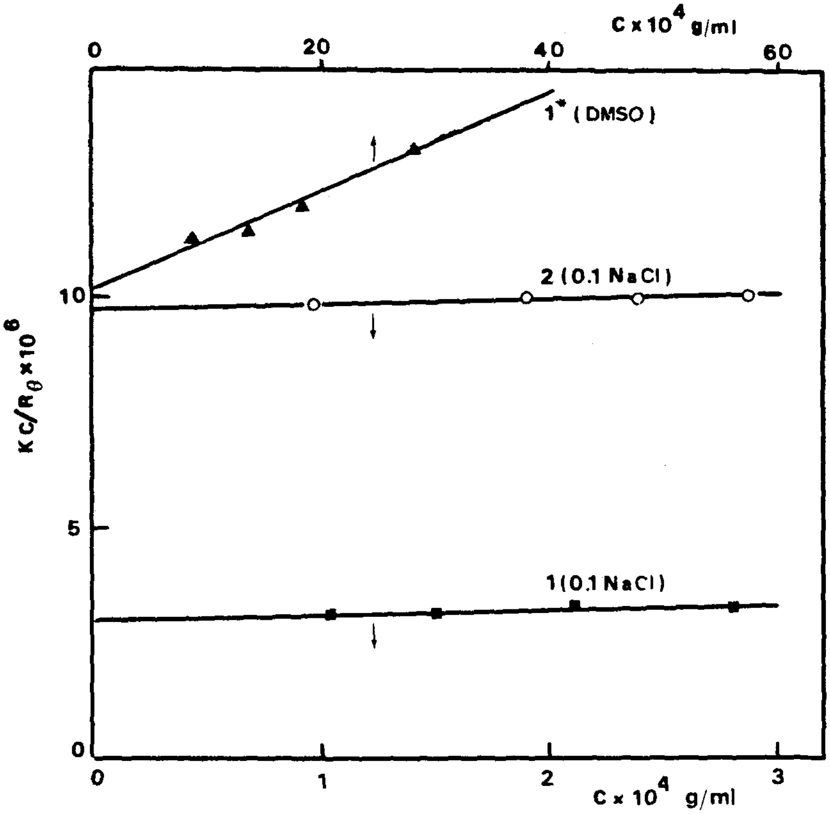

Fig. 3. Plots of KC/R_θ ($\theta=4{\cdot}88°$) versus concentration for schizophyllan samples: 1, 1*, Sample 1 in DMSO (▲) and in 0·1 M NaCl (■); 2, Sample 2 in 0·1 M NaCl (○) (a nearly identical plot is observed for this sample in DMSO). In 0·1 M NaCl, $\bar{M}_w(1)/\bar{M}_w(2)\approx 3{\cdot}2$.

DMSO. From these data it can be deduced that a single chain conformation prevails for Sample 2 whatever the solvent may be. As the modification was performed in DMSO when the initial triple helix will be dissociated, this means that the initial conformation is no longer restored in aqueous solution, which agrees with reported data (Yanaki *et al.*, 1985). On the other hand, no evidence for aggregate formation is observed, contrary to what happens with the unmodified schizophyllan (Yanaki *et al.*, 1985), which can be attributed to charged acid groups located on the chain preventing formation of intermolecular hydrogen bonds.

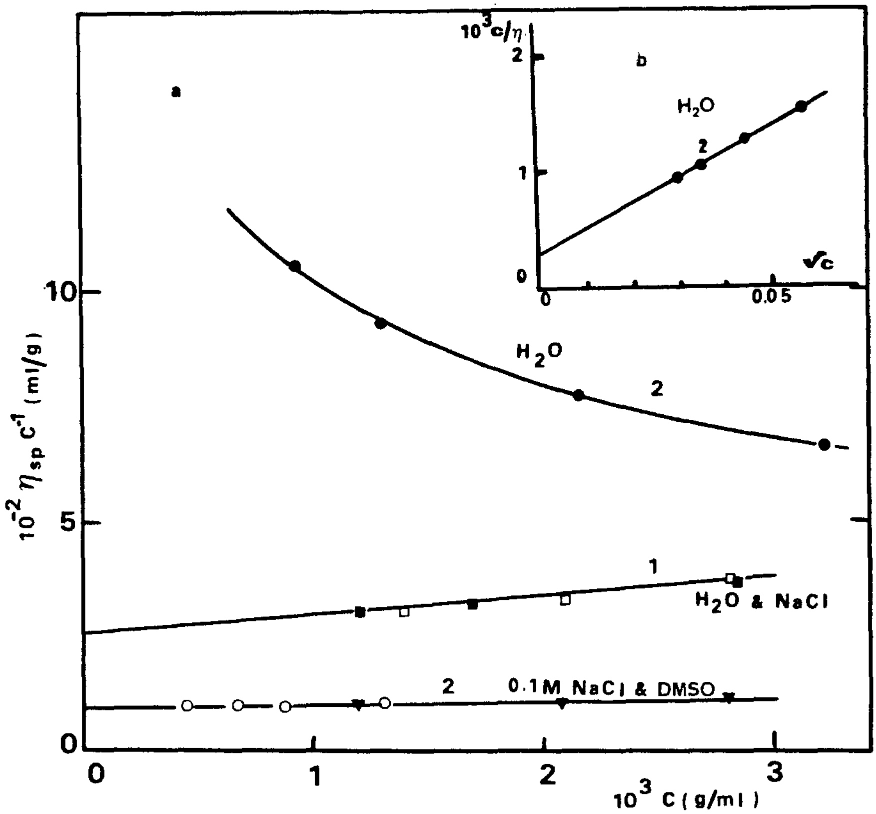

Fig. 4. (a) Dependence of the reduced viscosity η_{sp}/C on polymer concentration for Sample 1 in aqueous solution (curve 1) and Sample 2 in pure water (●), in 0·1 M NaCl (○) and in DMSO (▼) (the viscosity plot for Sample 1 in DMSO, not shown here, is the same as that for Sample 2 in DMSO). (b) Fuoss plot for Sample 2 in pure water.

Figure 4 shows the plots of the reduced viscosity η_{sp}/C as a function of polymer concentration for Samples 1 and 2. The viscosity behaviour of Sample 1 can be described by a Huggins-type equation both in aqueous solution (with or without added salt) and in DMSO, as would be expected for an uncharged polymer. The intrinsic viscosity values found in both solvent conditions, i.e. a larger viscosity in aqueous solution, are consistent with the light scattering data.

On the other hand, distinct polyelectrolyte behaviour, i.e. a concave upward curvature in the plot of η_{sp}/C vs. C, is observed for Sample 2 in pure water due to increasing charge repulsion within the polymer chain at low concentrations, resulting in a more extended conformation. The intrinsic viscosity $|\eta| \simeq 1000$ ml g^{-1} when determined from the empirical Fuoss equation (Fuoss, 1951). This behaviour disappears upon addition of salt due to shielding of ionic charges and the intrinsic viscosity measured in 0·1 M NaCl ($|\eta| \simeq 100$ ml g^{-1}) is the same as that in DMSO, showing that a single coil conformation exists in both solvent conditions similar to that prevailing for Sample 1 in DMSO.

Charge-induced conformational transition

The ionization process of a poly(monoprotonic acid) can be described as (Arnold & Overbeek, 1950):

$$\mathrm{pH} - \frac{0{\cdot}434}{RT}\frac{\mathrm{d}G}{\mathrm{d}\beta} = \mathrm{p}K_0 + \log\frac{\beta}{1-\beta} \tag{1}$$

or

$$\mathrm{p}K_\mathrm{a} = \mathrm{pH} + \log\frac{1-\beta}{\beta} \tag{2}$$

where K_0 is the dissociation constant for an isolated acid group, K_a is the apparent dissociation constant of the polyacid, β is the dissociation degree of the polyacid, and dG is the differential change in the electrostatic and conformational free energy per monomole which accompanies a differential change in the dissociation degree dβ.

As usual β is defined by

$$\beta = \bar{\beta} + C_{\mathrm{H}^+}/C_\mathrm{p} \tag{3}$$

with $C_{\mathrm{H}^+} \simeq 10^{-\mathrm{pH}}$ (if activity coefficients can be put equal to unity), $\bar{\beta}$ the degree of neutralization being equal to $\bar{\beta} = C_\mathrm{B}/C_\mathrm{p}$ (C_B is the molar concentration of added base and C_p the monomolar polyelectrolyte molar concentration). Figure 5 shows the plot of pK_a, as defined by eqn (2), against the degree of dissociation β for Sample 2 at ionic strengths of 0 and 5×10^{-2} M NaCl. The titration curve reveals a sharp rise at low β, followed by a maximum or plateau region, and rises again at higher β. The abnormal shape of this curve is consistent with the occurrence of a conformational transition within the molecule during ionization as

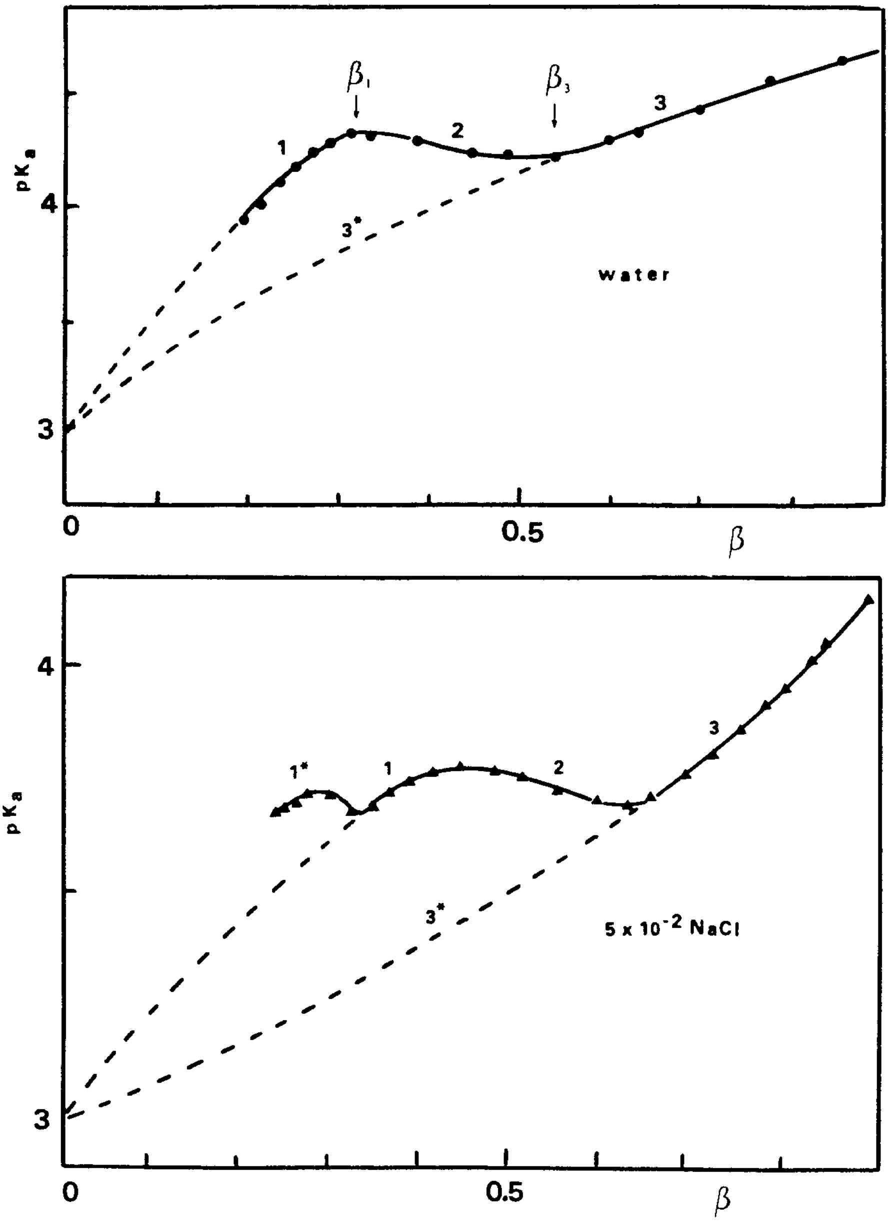

Fig. 5. Potentiometric titration curves of Sample 2 in pure water and in 5×10^{-2} M NaCl. β_1 and β_3 represent the beginning and the end of the transition. Extrapolation of curves at $\beta > \beta_3$ to the region of low β shows the behaviour of a hypothetical disordered coil over the whole ionization range (broken line 3*).

is well documented for some other polyelectrolytes (Wada, 1960; Leyte & Mandel, 1964; Barskaya & Ptitsyn, 1971; Muller *et al.*, 1975). The titration curves can also be represented by the Henderson–Hasselbach plot in which pH is plotted as a function of log $(1-\beta)/\beta$. An example of such a plot is given in Fig. 6. The linear portions of these plots correspond to the titration of two different conformations labelled 1 (stable at low β) and 3 (stable at higher β), and the middle part labelled 2 is the transition region between them.

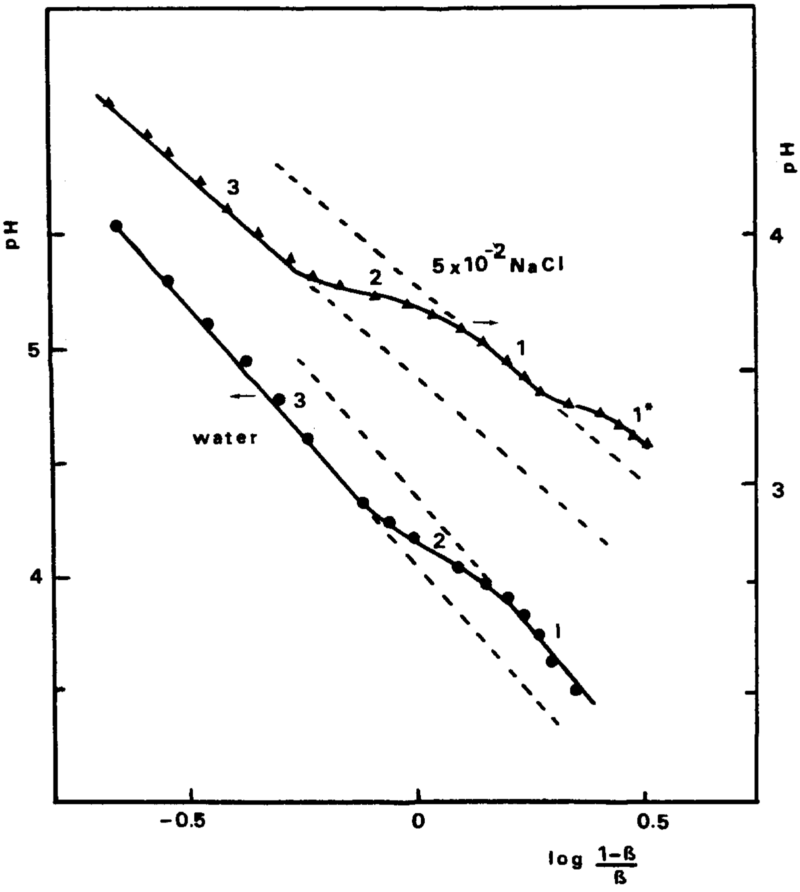

Fig. 6. Henderson–Hasselbach plots for the titration of Sample 2 in pure water and in 5×10^{-2} M NaCl.

Extrapolation of pK_a against β to $\beta \to 0$ yields the intrinsic dissociation constant for the acidic group's pK_0 which is near p$K_0 = 3$ irrespective of the ionic strength in the range investigated ($0\text{–}5 \times 10^{-2}$ M NaCl). For salt concentrations above 5×10^{-2} M NaCl, precipitation of polymer is observed at low β values; therefore the region labelled 1* can be attributed to intermolecular aggregation. The β value at which the transition starts is shifted to higher values as the ionic strength increases. It is interesting to note that the slope of the rise of pK_a with

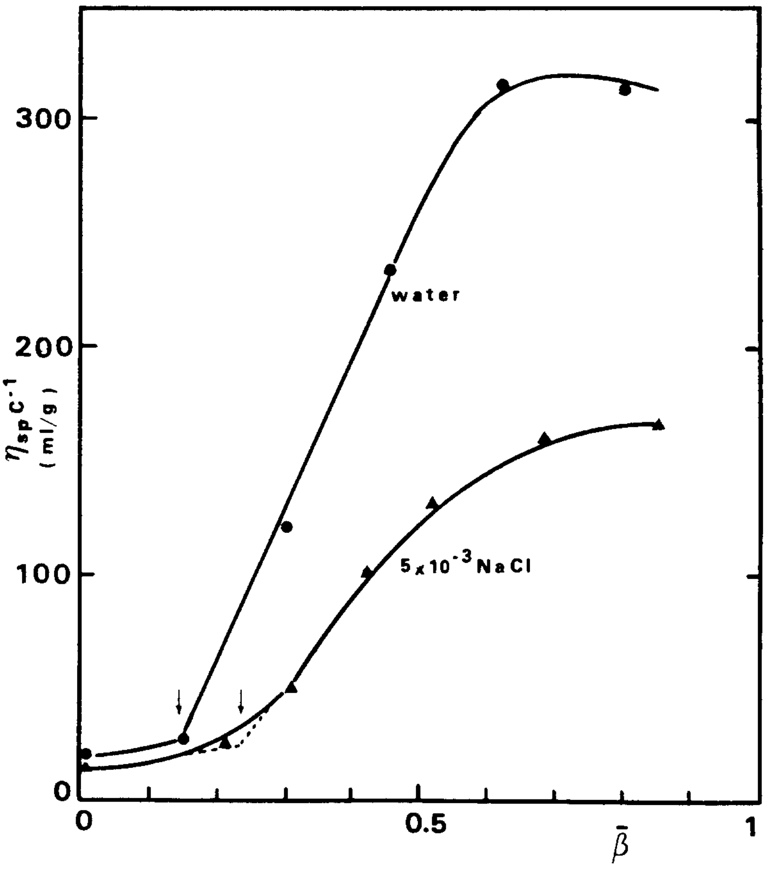

Fig 7. Reduced viscosity as a function of the degree of neutralization $\bar{\beta}$ for Sample 2 in pure water and in the presence of 5×10^{-3} M NaCl (polymer concentration $C = 8 \times 10^{-4}$ g ml^{-1}).

$\bar{\beta}$ after the completion of the transition ($\bar{\beta} > 0{\cdot}55$ in pure water) is smaller than before the transition. This is also observed for poly(methacrylic acid) (Bartels & Arends, 1981) and is an indication that the transition occurs between a rather compact conformation existing at low $\bar{\beta}$ and a more extended one at higher $\bar{\beta}$. This conclusion is supported by viscosity measurements. The dependence of the reduced viscosity η_{sp}/C on the degree of neutralization $\bar{\beta}$ presented in Fig. 7 shows a breakdown in the viscosity plots occurring within a small $\bar{\beta}$ interval. In the $\bar{\beta}$ range corresponding to the regions labelled 1 in Figs 5 and 6, the viscosity is low and only slightly dependent on salt concentration which is consistent with the existence of a rather com-

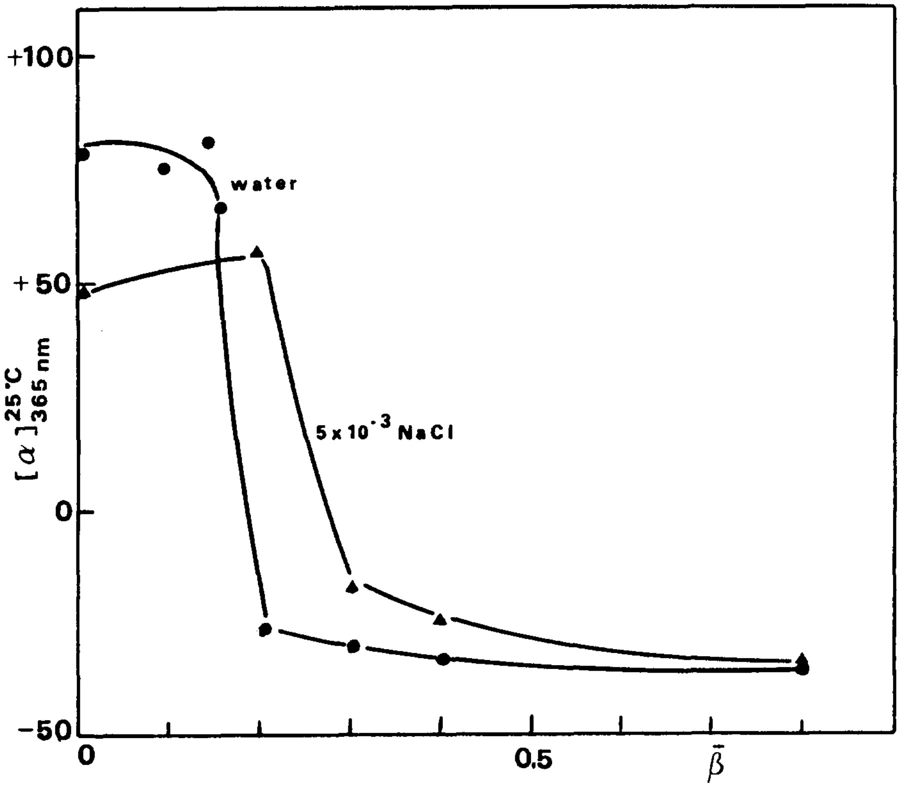

Fig. 8. Dependence of the specific optical rotation of Sample 2 on the degree of neutralization $\bar{\beta}$.

pact conformation. A large increase in reduced viscosity is observed over the ionization range corresponding to the transition regions. This is due to the uncoiling of the chains due to increasing charge repulsion between charged groups and is typical of weak polyacids such as for example polyacrylic acid. In this domain it is well recognized that the viscosity is sensitive to an increase of the ionic strength. These viscosity results clearly show that Sample 2 undergoes a change in conformation from a compact to an extended form as $\bar{\beta}$ increases.

As shown in Fig. 8, the specific optical rotation $|\alpha|$ goes through a sharp sigmoidal change in the ionization range corresponding to the conformational transition. The inflections in the S-shaped $|\alpha|$ vs. $\bar{\beta}$ curves occur at $\bar{\beta}$ values located in the middle part of the neutralization range, covered by the plateau in the potentiometric plots (region labelled 2 in Fig. 5).

The sharp change in $|\alpha|$ observed over a rather small ionization range is indicative of a co-operative change in conformation and is consistent with the pK_a and viscosity results. The sharp decrease in $|\alpha|$

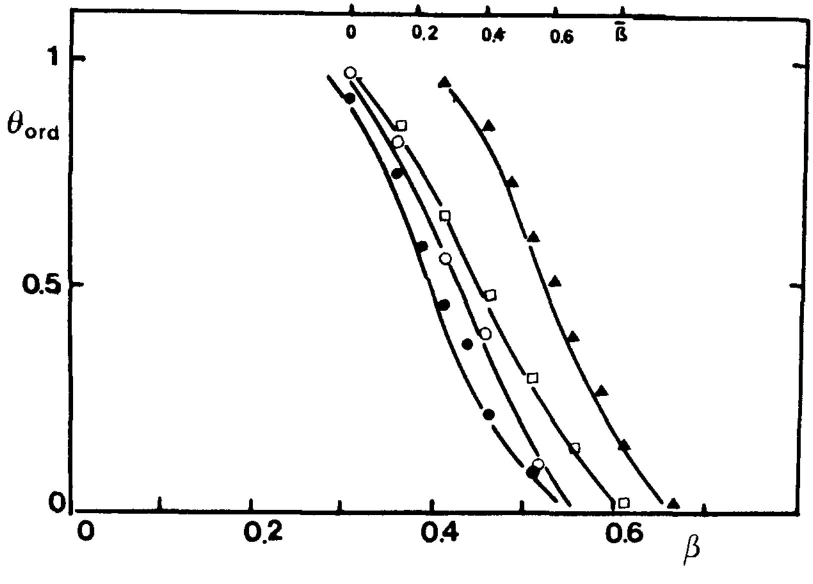

Fig. 9. Normalized titration curve of Sample 2 determined from the pH-titration data at NaCl concentration: ●, 0; ○, 5×10^{-3} M; □, 10^{-2} M; ▲, 5×10^{-2} M. θ_{ord} is the fraction of residues in the ordered state vs. β (lower scale) and $\bar{\beta}$ (upper scale).

together with the large $|\eta|$ increase observed as the transition between the two conformations is initiated suggests a transition from a contracted ordered structure, stable at low $\bar{\beta}$, to a more disordered extended random coiled form. It is clear that changes in ionization alter the balance between non-covalent attractions and repulsions because the electrostatic contribution changes. In our opinion the adoption of a compact molecular structure is primarily due to intramolecular H-bonding, although other types of non-covalent interaction, e.g. van der Waals, dipolar, can also play a role.

Figure 9 shows the normalized transition curve, i.e. the fraction θ_{ord} of the ordered form in the molecule vs. the degree of dissociation β, calculated from the pH–titration data by assuming a two-state model (Nitta *et al.*, 1975). From these plots it clearly appears that the compact ordered form becomes more stable with increasing salt concentration.

CONCLUSIONS

The above light scattering, potentiometric, viscosity and optical rotation data indicate that the acidic polysaccharide derived from schizophyllan exists in a single chain conformation whatever the solvent may be, in contrast to the neutral starting material. In aqueous solution it undergoes a clear-cut conformational transition from a rather compact ordered form to an extended disordered coiled one upon ionization of acidic groups located on the polysaccharide chain. Intramolecular H-bonds are probably responsible for the stabilization of the compact secondary structure prevailing in the low ionization range. This conformation is more stable at higher salt concentrations.

REFERENCES

Arnold, A. & Overbeek, J. Th. (1950). *Rec. Trav. Chim.* **69**, 192.

Barskaya, T. V. & Ptitsyn, O. B. (1971). *Biopolymers* **10**, 2181.

Bartels, T. & Arends, J. (1981). *J. Polym. Sci., Polym. Chem. Ed.* **19**, 127.

Crescenzi, V., Gamini, A., Paradossi, G. & Torri, G. (1983). *Carbohydr. Polym.* **3**, 273.

Fuoss, P. M. (1951). *Disc. Faraday Soc.* **11**, 125.

Gamini, A., Crescenzi, V. & Abruzzese, R. (1984). *Carbohydr. Polym.* **4**, 461.

Kashiwagi, Y., Norisuye, T. & Fujita, H. (1981). *Macromolecules* **14**, 1220.

Kikumoto, S., Miyajima, T., Kimura, K., Okabo, S. & Komatsu, N. (1971). *J. Agr. Chem. Soc. Japan* **45**, 162.

Leyte, J. C. & Mandel, M. (1964). *J. Polym. Sci.* Part A **2**, 1879.

Muller, G. (1986). *7th European Symposium on Polymer Spectroscopy*, Dresden (*Teubner-Texte zur Physik*, in press).

Muller, G., Fenyo, J. C., Braud, C. & Sélégny, E. (1975). In *Polyelectrolytes and their applications*, eds A. Rembaum and E. Selegny, Dordrecht, Holland, Reidel Publishing Co., p. 15.

Muller, G., Chiron, G. & Levesque, G. (1985). *Polym. Bull.* **15**, 125.

Nitta, K., Yoneyama, M. & Ohno, N. (1975). *Biophys. Chem.* **3**, 323.

Norisuye, T., Yanaki, T. & Fujita, H. (1980). *J. Polym. Sci., Polym. Phys. Ed.* **18**, 1135.

Rinaudo, M. & Vincendon, M. (1982). *Carbohydr. Polym.* **2**, 135.

Tabata, K., Ito, W., Kojima, T., Kawabata, S. & Misaki, A. (1981). *Carbohydr. Res.* **89**, 121.

Wada, A. (1960). *Mol. Phys.* **3**, 409.

Yanaki, T. & Norisuye, T. (1983). *Polymer J.* **15**, 389.

Yanaki, T., Kojima, T. & Norisuye, T. (1981). *Polymer J.* **13**, 1135.

Yanaki, T., Tabata, K. & Kojima, T. (1985). *Carbohydr. Polym.* **5**, 275.