Carbohydrate Polymers 17 (1992) 289-297

Thermal denaturation and degradation of schizophyllan

Frédéric Zentz, Jean-François Verchère & Guy Muller*

URA 500 du CNRS 'Polymères, biopolymères, membranes' Faculté des Sciences, BP 118, 76134 Mont-Saint-Aignan, France

(Received 8 April 1991; accepted 15 April 1991)

Size exclusion chromatography and low-angle laser light scattering have been used for studying the evolution of schizophyllan polysaccharide during a thermal treatment ($t > 100°C$) in aerated solution. Thermal denaturation of the native triple helices into single chains is initiated above 135°C and is complete in 10 min at 160°C. Both conformations can coexist in the 130–140°C temperature range. In the presence of oxygen, both forms of the biopolymer undergo severe thermal degradation. The rate of degradation was found to be independent of chain length and conformation. An activation energy of 104 kJ mol^{-1} was determined. The reaction was base-catalyzed. Analysis of chromatographic patterns indicate that the degradation probably occurs through an 'all-or-none' process.

INTRODUCTION

Schizophyllan is a water-soluble extracellular polysaccharide produced by the fungus *Schizophyllum commune* (Kikumoto *et al.*, 1970, 1971). Its primary structure consists of linearly linked β-1,3-D-glucopyranose residues with one β-1,6-D-glucopyranoside side-chain for every three main-chain residues. This is the same repeating unit as that of scleroglucan (Yanaki *et al.*, 1981), another fungal polysaccharide.

Both biopolymers show similar behavior in solution (Yanaki & Norisuye, 1983) and possess very interesting viscosifying properties which could result in many industrial applications including enhanced oil recovery (EOR). In neutral aqueous medium, they adopt a triple helical conformation and behave as semi-rigid rods for $M_w > 5 \times 10^5$ g mol^{-1}, which explains the high viscosity (Yanaki *et al.*, 1980). This ordered secondary structure is stable at room temperature, up to pH 12·8 (Kashiwagi *et al.*, 1981). Denaturation occurs at higher pH ([NaOH] > 0·20 M), at high temperature and/or in DMSO (Rinaudo & Vincendon, 1982; Sato *et al.*, 1983; Kitamura & Kuge, 1989) and results in a dissociation of the triple helix into single coiled chains. The denaturation is due to breaking of intermolecular hydrogen bonds responsible for the stability of the native ordered form and results in a large viscosity loss and optical rotation changes (Norisuye *et al.*, 1980; Bo *et al.*, 1987).

The efficiency of schizophyllan for oilfield applications including EOR closely depends on its ability to give aqueous solutions with high viscosity over a large pH and temperature range. However, schizophyllan samples from different commercial batches often display variable properties and heterogeneities which could originate from unsuitable extraction processes. Post-biosynthesis operations generally involve alkalinization of the growth medium followed by a thermal treatment, that either denature or degrade the biopolymer.

The objective of this work was to evaluate the effects of pH and temperature on the solution properties of schizophyllan with special emphasis on thermal degradation in the presence of dissolved oxygen.

EXPERIMENTAL

Two purified schizophyllan samples were used. One (sample S: $\overline{M}_w = 5 \times 10^6$, $[\eta] = 8000$ ml g^{-1}) was obtained as a stock solution (concentration = 1·25 g liter^{-1} = 1250 ppm) and had not been precipitated from the broth (Sanofi, France). The second (sample L: $\overline{M}_w = 4{\cdot}5 \times 10^5$, $[\eta] = 400$ ml g^{-1}) was a lyophilized sample (Taito, Japan) kindly donated by Dr Yanaki.

Solutions of both samples were prepared in NaCl

---

*To whom correspondence should be addressed.

Carbohydrate Polymers 0144-8617/92/$03.50

(20 g liter^{-1}) and were protected from bacterial attack by NaN_3 (0·4 g liter^{-1}). They were stored at 6°C before use.

Low angle laser light scattering (LALLS) measurements were conducted at 25°C with a KMX 6 low angle ($\theta \simeq 5°$) photometer (Chromatix LDC/Milton Roy). A Contraves low shear LS 30 viscometer was used for viscosity measurements in the Newtonian regime.

High performance size exclusion chromatography (HPSEC) experiments were made by using a TSK G 6000 PW column (Toyo Soda). Schizophyllan samples were detected with a refractive index (RI) detector (Model 6A, Shimadzu). The results are expressed as the partition coefficient, defined as: $K'_d = (V_e - V_0)/(V_e^T - V_0)$ where V_e is the elution volume, V_0 is the total exclusion volume and V_0^T is the total permeation volume (salt peak).

Alkaline denaturation was studied according to the following procedure: the pH was adjusted by adding 1 M NaOH under vigorous stirring of the solutions (final volume: 20 cm^3, final polymer concentration: 10^{-2} g liter^{-1}). Measurements were performed on filtered solutions (0·22 μm) 3 days after NaOH addition. The proportions of native and denatured forms were estimated from the integration of chromatographic area obtained with the RI detector (dn/dc had the same value, 0·145 ± 0·003 ml g^{-1}, for both forms).

Thermal denaturation and degradation were studied in the temperature range 102–161°C, using a thermostated oil bath ($t \pm 1$°C). Typical conditions for denaturation were: solution volume 2–5 cm^3, polymer concentration: 0·1–0·2–0·3 g liter^{-1}.

Thermal degradation was monitored by size exclusion chromatography only. Light scattering was found inappropriate because of the parallel denaturation and chain aggregation processes. The percentage of degradation was evaluated by comparing the amount of polymer eluted at ambient temperature before and after the solution had been subjected to heat treatment. All experiments were performed in sealed bottles in which air was not excluded. The solutions (2–5 cm^3) contained about 5 ppm of dissolved oxygen (CHEM metric) at room temperature.

RESULTS

pH- and temperature-induced denaturation

Alkaline treatment of schizophyllan at room temperature shows the main following features:

- Denaturation of triple helices into single coils is very fast in 0·2 M NaOH, since chromatographic data reveal that intact native triple helices do not exist any more after 10 min (Fig. 1). The denatured solution is stable when stored at room temperature in the presence of sodium azide. A similar observation was reported for scleroglucan under the same conditions (Bo *et al.*, 1987).

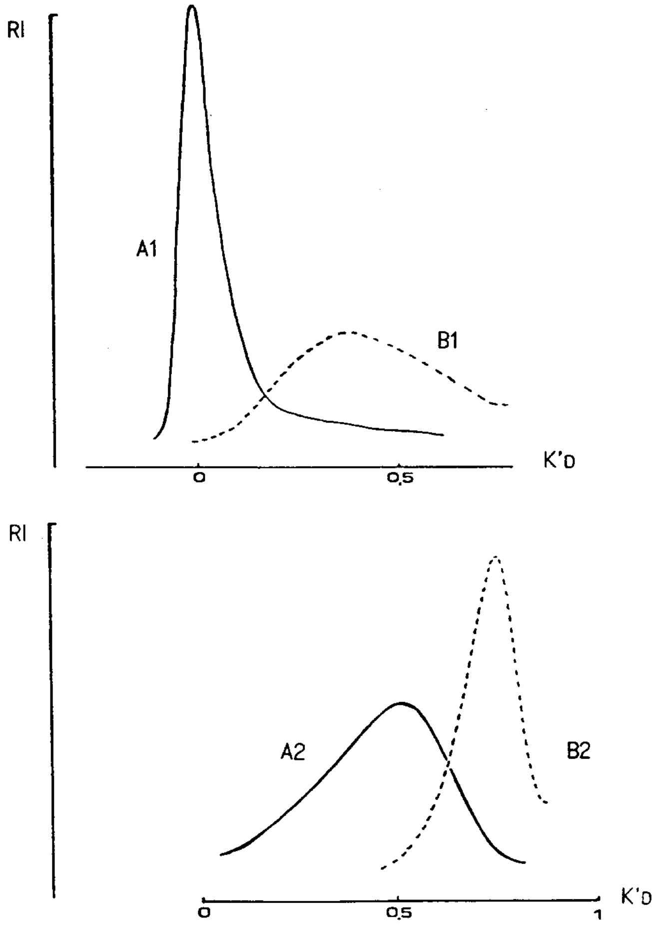

Fig. 1. Alkaline denaturation of schizophyllan (A: sample S; B: sample L) in aqueous solution (NaCl 20 g liter^{-1}). Chromatographic profiles. A_1: —— initial solution ($M_w = 5 \times 10^6$ g mol^{-1}, pH = 7). A_2: —— solution treated 10 min in 0·23 M NaOH and neutralized at pH = 7 ($M_w = 1{\cdot}6 \times 10^6$ g mol^{-1}). B_1: ---- initial solution ($M_w = 4{\cdot}45 \times 10^5$ g mol^{-1}, pH = 7). B_2: - - solution treated 10 min in 0·23 M NaOH and neutralized at pH = 7 ($M_w = 1{\cdot}5 \times 10^5$ g mol^{-1}).

- No denaturation occurs upon alkalinization by 0·1 M NaOH as long as the final NaOH concentration does not exceed 0·05 M.
- SEC data reveal that, even in 0·05 M NaOH, triple helices and single chains could coexist (Fig. 2) when this final concentration was obtained by adding concentrated NaOH (1 M) and diluting under vigorous stirring (Vortex). The ratio of both forms was found to be time independent at room temperature.
- Evidence is found from SEC that no restoration of triple helices could occur upon neutralization to pH = 7 of a denatured solution. This suggests that the denaturation process is irreversible. Moreover it indicates that in the 0·05–0·2 M NaOH range, where both schizophyllan species

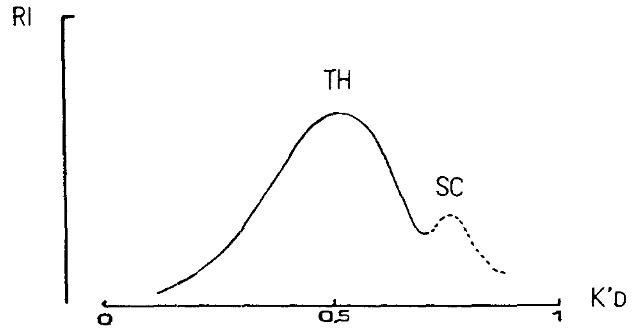

Fig. 2. Chromatographic profile showing coexistence of triple helix (TH) and single chain (SC) forms of schizophyllan (sample L) in neutral aqueous solution, after treatment in 0·05 M NaOH (obtained by 1/20 dilution of 1 M NaOH).

coexist, no chemical equilibrium exists. This behavior is the same as solvent (DMSO)-induced denaturation (Kitamura & Kuge, 1989).

- Denatured solutions which were neutralized (pH = 7) and stored at 6°C showed changes in SEC profiles, suggesting the formation of aggregated single chains upon aging. It is probably associated with the poor filterability and the plugging tendency observed with such solutions.

As monitored by SEC, thermal denaturation into single chains only occurs above a temperature threshold near 130–140°C and is complete after 10 min at 160°C (Fig. 3). It agrees with previous differential thermal analysis data (Kitamura & Kuge, 1989). At temperatures between 130 and 140°C, the reaction was slower and incomplete. The presence of both conformations could be observed for several hours. Because, a degradation reaction occurred during thermal treatment at all temperatures and its rate increased with temperature, the study of the kinetics of denaturation became impossible above 140°C.

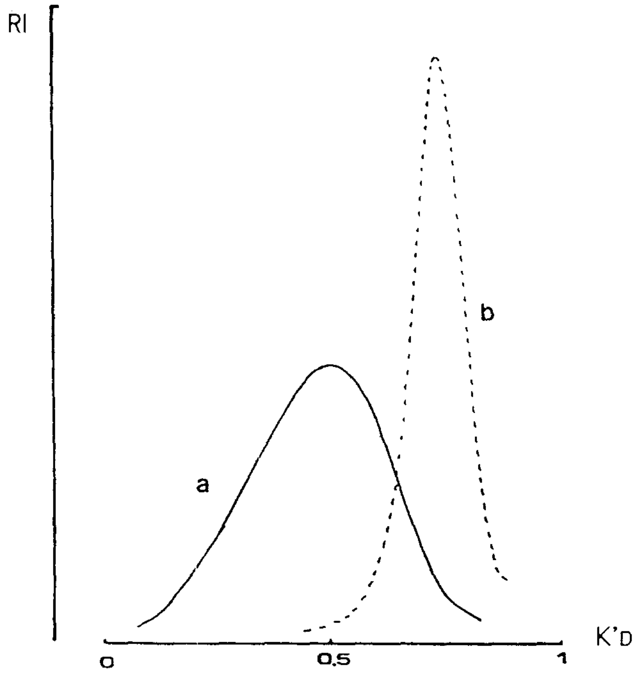

Fig. 3. Thermal denaturation of schizophyllan (sample L) at 161°C in neutral aqueous solution (NaCl 20 g liter^{-1}). Chromatographic profiles. (a) $t = 0$; (b) $t = 10$ min.

Thermal degradation

Solutions of schizophyllan in the pure single chain conformation could be prepared by heating the native polymer for a short time, but a comparison of the chromatographic area obtained before and after complete thermal denaturation (10 min, 161°C), indicated a loss of polymer ($\simeq$15%). That it involved a degradation reaction was indicated by a parallel decrease in apparent molecular weights values determined by LALLS for solutions of denatured schizophyllan from different batches. The single chains resulting from thermal denaturation had a lower molecular weight than those formed by alkaline denaturation at room temperature, showing that only the thermal treatment could induce degradation (Table 1).

The same degradation was found to occur within the whole temperature range explored (102–161°C), whatever the conformation adopted by the polymer.

The chromatographic analysis of polymer samples in which both conformations were present (corresponding to temperatures 131 and 137°C) showed that while degradation took place, the proportion of the single chain did not increase as expected (the triple helix was simultaneously denatured at a higher rate), but slowly decreased. At 131°C, we even found that the single chain form completely disappeared after 6 h (Fig. 4).

Our first hypothesis was that single chains were destroyed faster than triple helices. However, a kinetic study (see below) demonstrated that the degradation rates of both forms were almost identical. Then we assumed that the single chains were able to form aggregates that had an elution volume close to that of the triple helix form. We have shown above that such aggregation occurred at lower temperatures.

This hypothesis was confirmed by a kinetic study

Table 1. Molecular weights of schizophyllan denatured by alkaline or thermal treatment

Sample	Molecular weights $\bar{M}_w$ (g mol^{-1})		
	Initial	Denatured	
		[NaOH] = 0·23 N	161°C/10 min
S	5×10^6	$1{\cdot}6 \times 10^6$	$1{\cdot}25 \times 10^6$
L	$4{\cdot}45 \times 10^5$	$1{\cdot}5 \times 10^5$	$1{\cdot}3 \times 10^5$

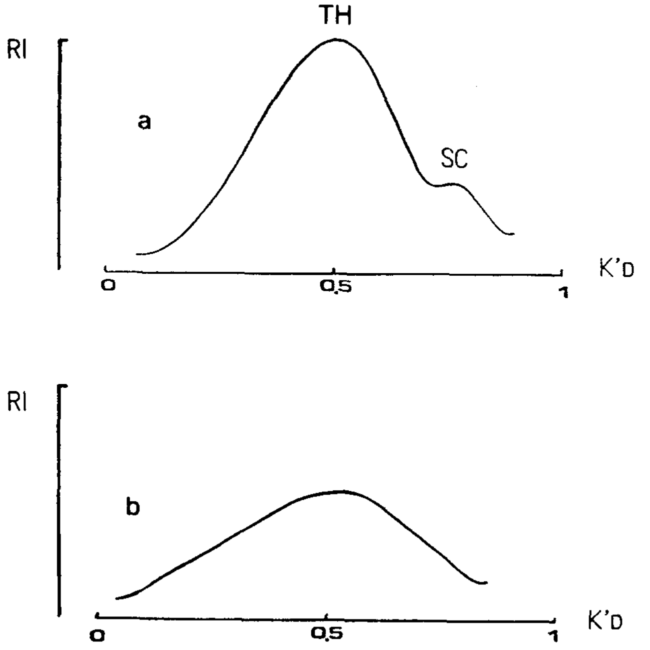

Fig. 4. Thermal denaturation of schizophyllan (sample L) at 131°C in neutral aqueous solution (NaCl 20 g liter^{-1}). Chromatographic profiles. (a) $t = 31$ min; (b) $t = 6$ h. Peaks are marked for the triple helix (TH) and single chain (SC) forms.

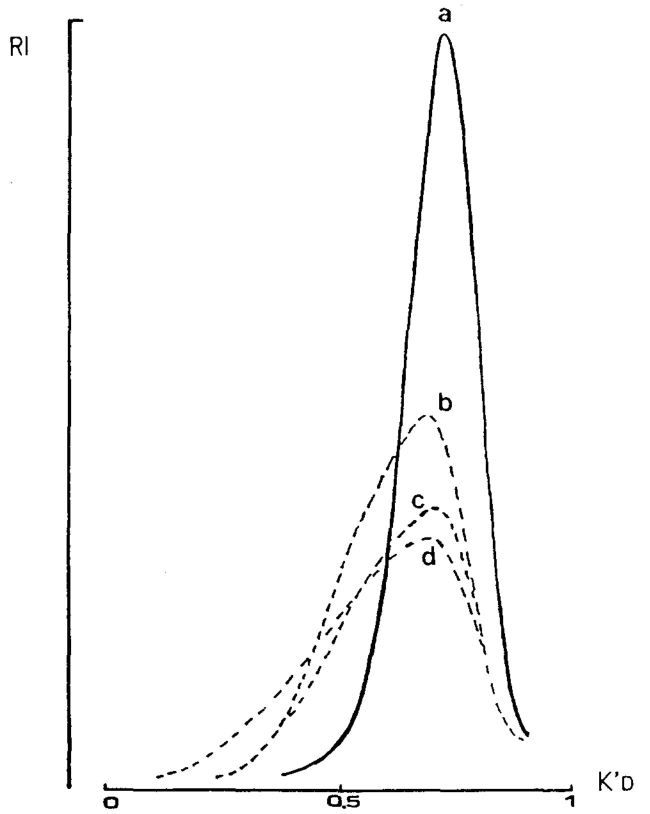

Fig. 5. Aggregation of denatured schizophyllan (sample L) at 102°C in neutral aqueous solution (NaCl 20 g liter^{-1}). Chromatographic profiles. (a) $t = 0$; (b) $t = 31$ min; (c) $t = 118$ min; (d) $t = 157$ min.

performed on a sample of schizophyllan in single chain conformation (obtained by thermal denaturation, 161°C/10 min, $\bar{M}_w = 1{\cdot}3 \times 10^5$). Its degradation at 102°C was monitored using various techniques. The evolution of SEC profiles (Fig. 5) showed that the species with hydrodynamic volumes higher than that of isolated single chains were generated during the heat treatment. The LALLS behavior of the same sample showed that, after heating at 102°C during 140 min, the intensity of scattered light nearly doubled (experimental ratio: 1·7). It confirmed the aggregation of the single chains, which can probably be held responsible for the poor filterability of heated polymer solutions.

Finally, it should be stated that the single chains do not form aggregates at temperatures above 140°C, contrary to what happens at 102°C. Thus the formation of aggregates is characterized by a temperature threshold identical to that for the denaturation reaction. It suggests that similar hydrogen bonds are involved in both processes.

Kinetics of thermal degradation

Several parameters can influence polymer degradation: chain length, conformation, presence of dissolved oxygen, temperature and pH.

Influence of chain length

The degradation of several samples of schizophyllan differing in molecular weight was studied at 102°C. We verified that the study was not complicated by the much slower denaturation of the polymer. The results (Fig. 6(a)) show that the reaction rate did not depend on chain length. Thus all following kinetic runs were carried out using only the low-molecular weight sample L.

Influence of conformation

The degradation of a sample previously denaturated to the single chain conformation by a short thermal treatment (10 min, 160°C) was studied at the same temperature as above, 102°C (Fig. 6(b)). A comparison of Figs 6(a) and 6(b) shows that the rate of degradation was almost independent of the polymer conformation. The calculated $t_{1/2}$ values were in close agreement: triple helix, 5·5 ± 0·5 days and single chain, 4·5 ± 0·5 days.

Our finding that the rate of degradation does not vary with the chain stiffness agrees with the hypothesis of an initial attack of reaction sites that are not involved in hydrogen bonds responsible for the triple helix stability.

Kinetic parameters

The kinetics of the degradation of schizophyllan were carried out at various temperatures (102–161°C). Although the reaction probably involves dissolved

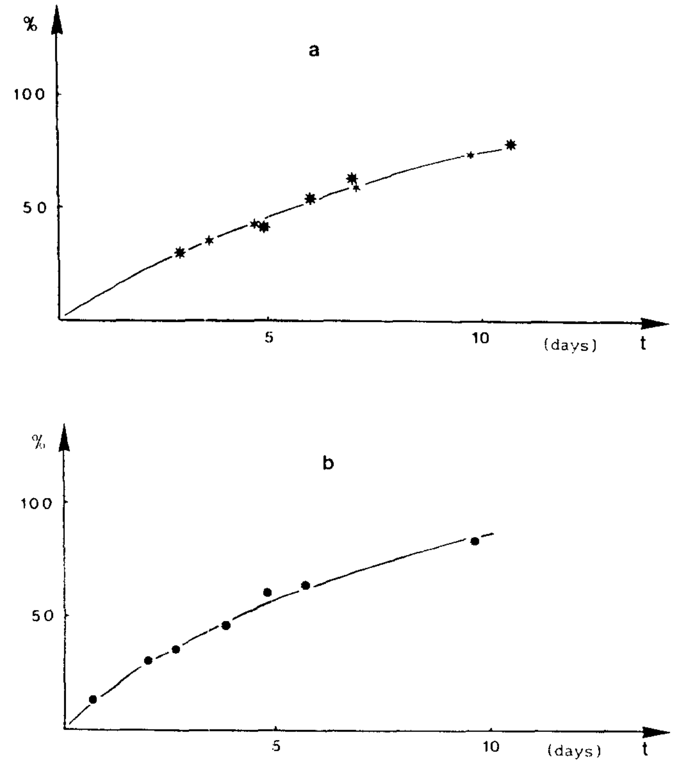

Fig. 6. Thermal degradation of schizophyllan (sample L) in neutral aqueous solution (NaCl 20 g liter^{-1}). Kinetic study at 102°C (% degradation vs time t). (a) Samples in triple helix conformation: *, sample S, $M_w = 5 \times 10^6$ g mol^{-1}; *, sample L, $M_w = 4{\cdot}45 \times 10^5$ g mol^{-1}. (b) Sample L in single chain conformation ($M_w = 1{\cdot}3 \times 10^5$ g mol^{-1}).

oxygen (see below), this reagent was present in excess in all experiments. Thus its concentration remained constant in the reaction medium, in agreement with the experimental pseudo-first-order (Table 2).

The variations of the rate constant k with temperature T, according to the Arrhenius relationship: $\ln k = \ln A - E/RT$(R is the universal gas constant) gave a single linear (correlation coefficient: 0·992) plot (Fig. 7). It is a fundamental result, because if the mechanism had been dependent on the conformation (triple helix below 130°C, single chain above 140°C), a break would have been observed around 135°C. This distinctly differs from data for the degradation of xanthan (Lambert & Rinaudo, 1985), which was reported to be conformation-dependent. The temperature threshold was much lower (60°C) in the case of xanthan.

Table 2. Kinetics of the thermal degradation of schizophyllan. Method of time ratio

Theoretical ratio^a		$t_{1/2}/t_{1/4}$	$t_{1/2}/t_{1/3}$	$t_{1/3}/t_{1/4}$
t(°C)	Conformation^b	2·41	1·71	1·41
		Experimental ratio		
102	TH	2·31	1·68	1·36
102	SC	2·51	1·77	1·42
120	TH	2·66	1·88	1·41
131	TH + SC	2·93	2·00	1·41
137	TH + SC	2·70	1·90	1·41
140	SC	2·55	1·85	1·39
161	SC	2·45	1·81	1·35

^{a}For a first-order reaction.
^{b}TH, triple helix; SC, single chain.
$t_{1/n}$ is the time corresponding to $1/n$ completion.

The pre-exponential factor A and activation energy E for the thermal degradation of schizophyllan were calculated: $A = 9 \times 10^9$ s^{-1}, $E = 27{\cdot}2$ kcal mol^{-1} or 104 kJ mol^{-1}.

The high stability of schizophyllan at lower temperatures could be evaluated by extrapolation of the kinetic data. For example, at 25°C, the half-life time would approach 150 years.

The value of the activation energy is in the same range as those found (Bradley & Mitchell, 1988) for the thermal degradation of carboxymethylcellulose: 80 kJ mol^{-1} and κ-carrageenan: 105 kJ mol^{-1}.

The value of the activation entropy was calculated using Eyring's equation, $\Delta S^{\ddagger} = -60$ J mol^{-1} K^{-1}. Monomolecular reactions are generally characterized by values near zero, whereas bimolecular reactions possess negative activation entropies (Schaleger & Long, 1963). Thus the negative value found supports a bimolecular mechanism involving an oxygen molecule, in agreement with the pseudo-first-order kinetics observed experimentally.

Catalysis by hydroxide ions

The kinetics of the degradation of schizophyllan (sample L) at 102°C were studied at two concentrations of NaOH. It is worth mentioning that the use of 0·01 M NaOH at room temperature was recommended by some authors (Kashiwagi *et al.*, 1981) for obtaining solutions free of microgel aggregates.

The experimental values for $t_{1/2}$ were: 150 ± 15 min in 0·005 M NaOH and 42 ± 4 min in 0·01 M NaOH. In these conditions, we did not observe any denaturation of the triple helix conformation (Fig. 8), as was the case at room temperature. The half-life time values were much smaller than those for the same sample in neutral medium, ($t_{1/2} \simeq 5$ days), indicating an effective catalysis by the alkali.

Effect of dissolved oxygen

The influence of dissolved oxygen was investigated from the variations of viscosity and molecular weight induced by a thermal treatment at 102°C of a sample of schizophyllan in native form.

The macromolecular parameters (M_w and $[\eta]_o$) of the polymer decreased with time (0–2–8 days) whereas the Huggins constants increased (k': 0·5–1–1·7) indicating an increasing trend towards the creation of intermolecular interactions (Fig. 9).

However, viscosity data (Fig. 10) revealed that the unreacted polymer remained in the triple helix conform-

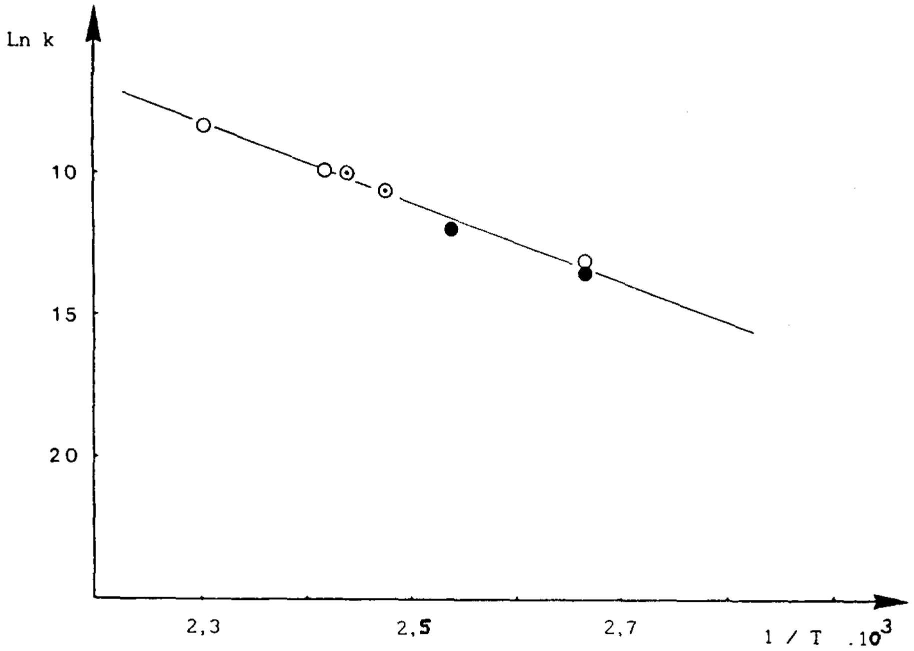

Fig. 7. Arrhenius plot for the thermal degradation of schizophyllan. k is the pseudo-first-order rate constant and T is temperature. Samples were respectively in: ●, triple helix form; ⊙, mixture of both conformations; ○, single chain form.

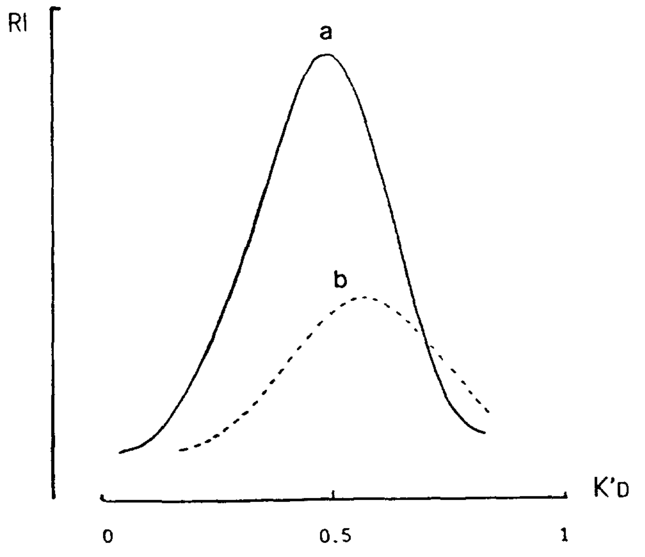

Fig. 8. Thermal degradation of schizophyllan (sample L) at 102°C in aqueous 0·01 M NaOH (NaCl 20 g liter^{-1}). Chromatographic profiles. (a) $t = 0$; (b) $t = 70$ min.

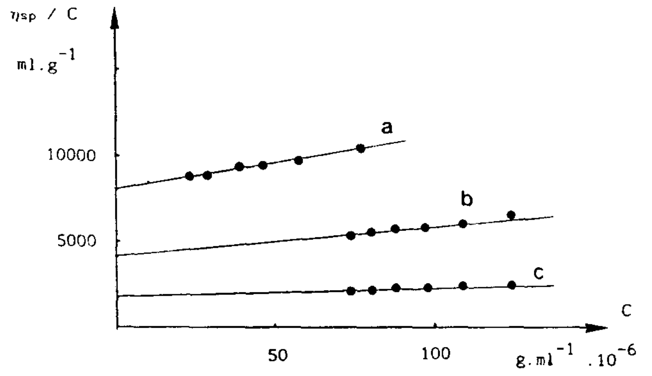

Fig. 9. Variations of specific viscosity of neutral aqueous solutions of schizophyllan vs polymer concentration during thermal degradation at 102°C (sample S, in triple helix form). k' are Huggins constants. (a) $t = 0$; $M_w = 5 \times 10^6$ g mol^{-1}; $[\eta)]_0 = 8000$ ml g^{-1}, k' = 0·5. (b) $t = 2$ days; $M_w = 3{\cdot}6 \times 10^6$ g mol^{-1}; $[\eta]_0 = 4100$ ml g^{-1}, k' = 1·0. (c) $t = 8$ days; $M_w = 1{\cdot}6 \times 10^6$ g mol^{-1}; $[\eta]_0 = 1700$ ml g^{-1}, k' = 1·7.

ation, as evidenced by the characteristic value, 1·24, of the exponent in the Mark–Houwink relationship (Zentz, 1991). It meant that the denaturation reaction could be neglected during the degradation at 102°C, in the presence of oxygen. This result was not unexpected, as the temperature was below the denaturation threshold. Thus the enhancement of intermolecular interactions probably indicates the aggregation of degradation residues, as it cannot be attributed to the aggregation of the polymer in the denatured form.

For comparison, it was reported that treatment of scleroglucan for more than one year at 90°C in synthetic sea water, *under a nitrogen atmosphere* (oxygen concentration lower than 5 ppb), allowed good molecular dispersion of the polymer and gave solutions having a satisfactory filterability (Rivenq *et al.*, 1989; Kalpakci *et al.*, 1990). No viscosity change was observed, indicating that a thermal treatment at ≃100°C in anaerobic conditions was not associated with both denaturation and degradation processes.

On the contrary, the present results conclusively show that a thermal treatment *in the presence of oxygen* results in considerable degradation of the polymer. Lower stability of biopolymers in the presence of

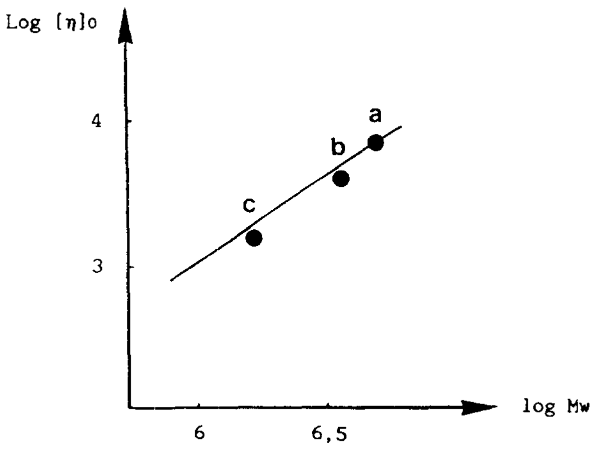

Fig. 10. Variations of intrinsic viscosity of neutral aqueous solutions of schizophyllan (sample S) vs molecular weights, during thermal degradation at 102°C. Solid line was drawn according to the Mark-Houwink type relationship: $[\eta]_0 = 4{\cdot}14 \times 10^{-5} M_w^{1{\cdot}24}$ (see text). (a) $t = 0$; (b) $t = 2$ days; (c) $t = 8$ days.

oxygen is well documented (Wellington, 1983). This oxidative reaction is catalyzed by various free radical species. Moreover, polysaccharides like xanthan, scleroglucan and schizophyllan are particularly sensitive (Kalpakci *et al.*, 1990) as the acetal carbon can stabilize a free radical species through resonance. Accordingly, stabilizing agents active against autoxidation include combinations of a sacrificial alcohol and free radical scavengers (Wellington, 1983).

It follows that dissolved oxygen is a driving factor for thermal degradation of schizophyllan. This result has practical implications. For use in enhanced oil recovery, the effect of oxygen is not of primary importance, since oil reservoirs are essentially oxygen-free. However, industrial or laboratory processes are generally performed in aerobic conditions, particularly the post-fermentation treatment that involves heating the polymer in the aerated solution. Moreover, many impurities are present in the fermentation broth, that can increase the pH value and catalyze the degradation process. It is probably the origin of the differences between commercial samples of schizophyllan.

DISCUSSION

Mechanism of the degradation reaction

Oxidations of polysaccharides by oxygen proceed via a free-radical mechanism (El Khadem, 1988). In the initiation steps, free-radicals $R^{\cdot}$ are generated by abstraction of hydrogen from an activated molecule of substrate $R{*}H$ to form activated radicals $R{*}^{\cdot}$, which are then converted by oxygen into peroxidate radicals $R{*}{-}O{-}O^{\cdot}$. Ultimately, reaction with another substrate molecule forms the radicals $R^{\cdot}$. The rate-limiting step is generally assumed to be the first reaction in the overall sequence:

$$\begin{aligned} R{*}H + O_2 &\longrightarrow R{*}^{\cdot} + HOO^{\cdot} \\ R{*}^{\cdot} + O_2 &\longrightarrow R{*}{-}O{-}O^{\cdot} \\ RH + R{*}{-}O{-}O^{\cdot} &\longrightarrow R^{\cdot} + R{*}{-}O{-}OH \end{aligned}$$

The initial degradation proceeds by a random scission of the main chain. Then the chain is rapidly destroyed by hydrolytic cleavage of the 1→3 linkages.

Base catalysis is common in the reactions of carbohydrates, since it is well known that mono- and poly-saccharides ionize in alkaline solution, forming enediol anions which are the starting intermediates for isomerization or degradation reactions. In the case of schizophyllan, a mechanism can be considered (Gersmann *et al.*, 1963) in which the first step is the deprotonation of a glucose monomer RH to give a carbanion R^-, which then reacts with an oxygen molecule to form a radical $R^{\cdot}$.

$$\begin{aligned} RH + OH^- &\rightleftharpoons R^- + H_2O \\ R^- + O_2 &\longrightarrow R^{\cdot} + O{-}O^- \end{aligned}$$

Taking into account the fast pre-equilibrium, the reaction rate can be written

$$v = k[RH]\,[OH^-]\,[O_2] = k'[RH],$$

$$\text{with } k' = k\,[OH^-]\,[O_2]$$

Because oxygen and hydroxide ions are present in excess, the reaction is of pseudo-first-order. The half-reaction time is: $t_{1/2} = \ln 2/k' = C/[OH^-]$, where C is a constant term.

The experimental dependence of the rate constant on NaOH concentration did not agree with this model, which required an inverse relationship between the half-reaction time and $[OH^-]$.

We considered two possible reasons for this result. First, our $t_{1/2}$ values could have been inaccurate, because the reaction was very slow. However, the difference seems too large to arise exclusively from experimental error. Second, the experimental order in OH^- could be higher than one. A similar situation was reported for the alkaline degradation of monosaccharides (de Bruijn *et al.*, 1987). For example, the degradation of D-fructose deviated from simple first-order kinetics with respect to the hydroxide ion, probably because it involved a second ionization step of the carbohydrate. By analogy, it can be assumed that the mechanism of the degradation of schizophyllan presents similar characteristics.

Structurally, two possible mechanisms can be considered for the chain degradation of schizophyllan:

(1) An 'all-or-none' process, according to a mechanism beginning by a slow, rate-determining step of chain attack, followed by faster steps in which the chain is rapidly and completely destroyed. A consequence would be that the

molecular weight of the remaining chains should not vary.

(2) A stepwise degradation, in which all the chains shorten at the same rate. It would be characterized by the progressive decrease of the molecular weight of the sample.

Careful examination of elution patterns of heated schizophyllan samples under both single coiled and triple helix conformation suggests that degradation more probably occurs through the 'all-or-none' process. It is observed that the decrease in the chromatographic area (which is proportional to the degradation of the biopolymer into small sugar molecules) is not accompanied by a significant shift of the peak towards higher elution volumes (Figs 11 and 12). The value of the effective partition coefficient, K'_d, was found to be constant or nearly constant, thus indicating that the reaction medium during the degradation was constituted by a mixture of only two species: undegraded polymer eluted in the initial solute retention volume, V_e, and small molecules eluted in the total permeation volume, V_e^T, of the column. The fact that SEC did not detect the presence of shortened triple helices strongly supports an 'all-or-none' degradation process.

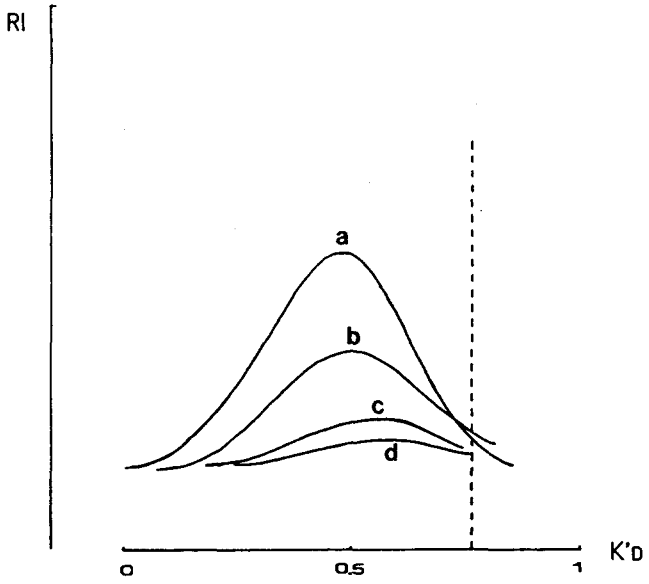

Fig. 12. Thermal degradation of schizophyllan (sample L, in native form) at 121°C in neutral aqueous solution (NaCl 20 g liter^{-1}). Chromatographic profiles. The dashed line shows the location of the peak corresponding to the denatured form (from Fig. 11). (a) $t = 0$; (b) $t = 28$ h; (c) $t = 72$ h; (d) $t = 125$ h.

CONCLUSION

Denaturation of the native triple helical conformation of schizophyllan upon alkaline treatment above 0·2 M NaOH and/or heating above 135°C results in an irreversible dissociation into single coils, followed by aggregation of the denatured molecules if the temperature is lower than 140°C. In some cases, the coexistence of triple helices and single chains was observed in 0·05 M NaOH, which could be ascribed to local pH inhomogeneities generated at the time of adding concentrated base.

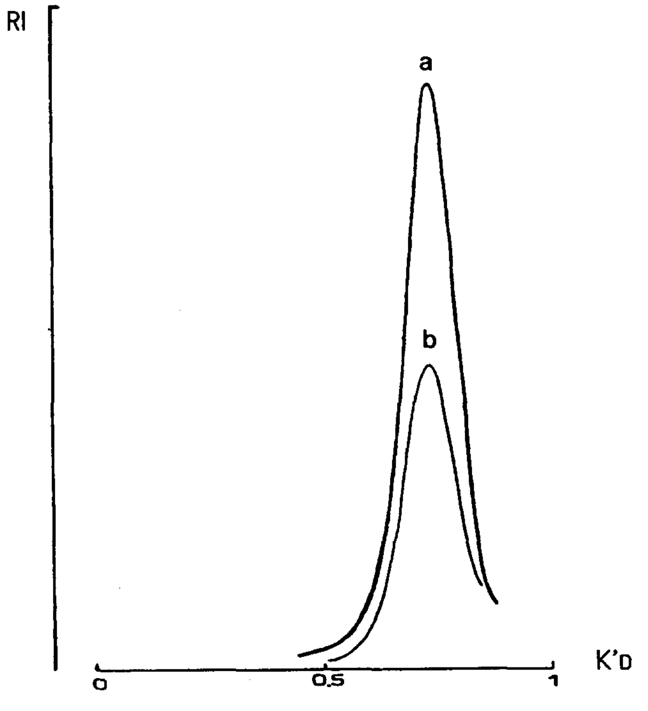

Fig. 11. Thermal degradation of schizophyllan (sample L, in native form) at 161°C in neutral aqueous solution (NaCl 20 g liter^{-1}). Chromatographic profiles. (a) $t = 10$ min, showing complete denaturation; (b) $t = 70$ min.

The rate of thermal degradation of schizophyllan in the presence of dissolved oxygen (activation energy $E = 104$ kJ mol^{-1}) does not depend on polymer conformation and molecular weight. Base catalysis was demonstrated.

Experimental evidence suggests that degradation proceeds in an 'all-or-none' mechanism, e.g. the initial attack of the macromolecule is rate-determining and is followed by a fast destruction of the polymeric chains. Chromatographic analyses made before completion could only detect the presence of nearly undegraded polymer molecules and small sugar residues eluted in the total permeation volume of the column.

ACKNOWLEDGEMENTS

The authors thank M. Brigand (Sanofi Bio-Industries, Baupte) for the generous gift of several samples of schizophyllan and Institut Français du Pétrole for financial support.

REFERENCES

Bo, S., Milas, M. & Rinaudo, M. (1987). *Int. J. Biol. Macromol.*, **9**, 153-7.

Bradley, T. D. & Mitchell, J. R. (1988). *Carbohydr. Polym.*, **9**, 257-67.

de Bruijn, J. M., Kieboom, A. P. G. & van Bekkum, H. (1987). *Recl. Trav. Chim. Pays-Bas*, **106**, 35-43.

El Khadem, H. S. (1988). *Carbohydrate Chemistry: Monosaccharides and their Oligomers*, Academic Press, San Diego, USA, p. 152.

Enomoto, H., Einaga, Y. & Teramoto, A. (1984). *Macromolecules*, **17**, 1573.

Gersmann, H. R., Nieuwenhuis, H. J. W. & Bickel, A. F. (1963). *Tetrahedron Lett.*, 1383-5.

Kalpakci, B., Jeans, Y. T., Magri, N. F. & Padolewski, J. P. (1990). *Soc. Pet. Eng. J.*, 603-13.

Kashiwagi, Y., Norisuye, T. & Fujita, H. (1981). *Macromolecules*, **14**, 1220-5.

Kikumoto, S., Miyajima, T., Yoshizumi, S., Fujimoto, S. & Kimura, K. (1970). *J. Agric. Chem. Soc. Jpn*, **44**, 337-42.

Kikumoto, S., Miyajima, T., Kimura, K., Okubo, S. & Komatsu, N. (1971). *J. Agric. Chem. Soc. Jpn*, **45**, 162-8.

Kitamura, S. & Kuge, T. (1989). *Biopolymers*, **28**, 639-54.

Lambert, F. & Rinaudo, M. (1985). *Polymer*, **26**, 1549-53.

Norisuye, T., Yanaki, T. & Fujita, H. (1980). *J. Polym. Sci.*, **18**, 547-58.

Rinaudo, M. & Vincendon, M. (1982). *Carbohydr. Polym.*, **2**, 135-44.

Rivenq, R. C., Donche, A. & Noik, C. (1989). *Soc. Pet. Eng. J.*, 775-84.

Sato, T., Norisuye, T. & Fujita, H. (1983). *Macromolecules*, **16**, 185-9.

Schaleger, L. L. & Long, F. A. (1963). *Advan. Phys. Org. Chem.*, **1**, 1-33.

Wellington, S. L. (1983). *Soc. Pet. Eng. J.*, 901-12.

Yanaki, T. & Norisuye, T. (1983). *Polym. J.*, **15**, 389-96.

Yanaki, T., Norisuye, T. & Fujita, H. (1980). *Macromolecules*, **13**, 1462-6.

Yanaki, T., Kojima, T. & Norisuye, T. (1981). *Polym. J.*, **13**, 1135-43.

Zentz, F. (1991). Thesis, Université de Paris 6.