Carbohydrate Research, 109 (1982) 295–298
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

Hydrodynamic behavior of lentinan molecules as studied by quasielastic light-scattering*

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(Received January 20th, 1982; accepted for publication, February 12th, 1982)

Lentinan is an antitumor homoglucan[1] isolated from *Lentinus edodes*, an edible mushroom popular in Japan, and it has a backbone constituted mainly of a chain of β-D-(1→3)-linked D-glucopyranosyl residues[2,3]. Its molecular weight was recently reported[4] to be 3×10^5 to 8×10^5. Its hydrodynamic characteristics are, however, not yet known precisely.

We have now performed quasielastic light-scattering measurements on lentinan solutions, in order to study their hydrodynamic behavior, and to establish the relationship between the molecular weight (M) and the diffusion coefficient (D) of lentinan molecules. Because lentinan samples are highly polydisperse, and quasielastic light-scattering experiments on such samples are difficult to interpret, we employed polydispersity information provided by gel-permeation chromatographic data for the samples in our analysis[5].

Lentinan samples were isolated and purified by the method reported by Chihara *et al.*[6]. Gel-permeation chromatographic study was conducted by one of the authors (K.S.) and his colleagues[4]. Some of the gel-permeation chromatograms of lentinan samples used in the present study are shown in Fig. 1.

After being clarified by centrifuging at 10,000*g* for 1 h at room temperature, lentinan solutions (0.1%) in 0.12M borate buffer, pH 10, were carefully placed in a light-scattering cell. The fluctuation in the intensity of the scattered light was measured at various scattering-angles by means of single-photon counting, all measurements being conducted at 20°.

The light-scattering profiles of the lentinan solutions did not change for over 48 h, which shows that lentinan molecules are stable, and have little tendency to form aggregates. The measured autocorrelation functions of the scattered light were

*Dedicated to Professor Sumio Umezawa on the occasion of his 73rd birthday and the 25th anniversary of the Microbial Chemistry Research Foundation.

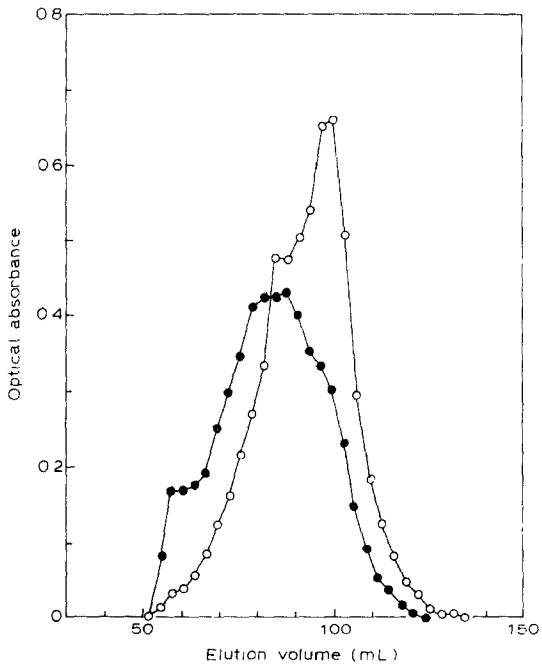

Fig. 1. Chromatograms of lentinan. [Open circles: sample No. 3; filled circles: sample No. 7. Lentinan concentration 0.1%, 0.12M borate buffer, pH 10.]

TABLE I

SUMMARY OF EXPERIMENTAL RESULTS

Sample No.	*Gel permeation*		*L.S.*	*Calculation*[a]	
	M_W (10^4)	M_W/M_N	$\langle D \rangle_z$ (10^{-8} $cm^2.s^{-1}$)	M_D (10^4)	M_D/M_W
1	36.8	4.30	9.25 ±0.23	52.1	1.41
2	64.8	4.13	8.04 ±0.42	85.6	1.36
3	31.2	2.67	12.3 ±1.3	38.9	1.25
4	73.4	3.40	6.47 ±0.31	92.5	1.26
5	65.5	3.88	7.28 ±0.50	86.3	1.32
6	54.2	3.06	9.53 ±0.57	68.0	1.26
7	66.2	3.21	8.09 ±0.25	83.7	1.26
8	55.8	3.96	8.65 ±0.62	73.6	1.32

[a] $M_D/M_W = P_i \cdot (\Sigma P_i M_i)^{(1-\alpha)/\alpha}/(\Sigma P_i M_i^{1-\alpha})^{1/\alpha}$ is calculated for $\alpha = 0.593$.

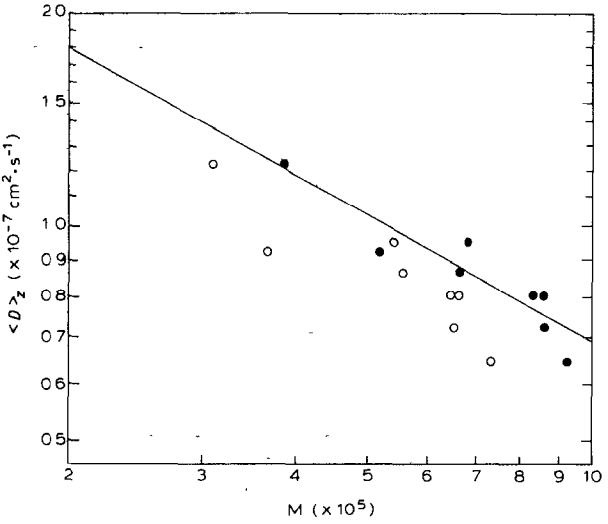

Fig. 2. Double-logarithmic plot of observed z-average diffusion coefficient *versus* molecular weight for lentinan samples. [Open circles correspond to the weight-average molecular weight (M_W), and filled circles to the diffusion-average molecular weight (M_D). The solid line indicates $\langle D \rangle_z = 2.50 \times 10^{-4}\ M_D^{-0.593}\ cm^2 \cdot s^{-1}$.]

analyzed by the method of cumulant expansion[5,7], and the "z-average" diffusion-coefficient $\langle D \rangle_z$ of lentinan samples was obtained from the average relaxation-frequency $\bar{\Gamma}$ of the autocorrelation functions. If the relationship $D = K_D M^{-\alpha}$ holds for each lentinan molecule, with K_D being a proportionality constant, $\langle D \rangle_z$ corresponds to the "diffusion-average" molecular weight, M_D, calculated by[5]:

$$M_D = M_W \frac{\Sigma P_i \cdot (\Sigma P_i M_i)^{(1-\alpha)/\alpha}}{(\Sigma P_i M_i^{1-\alpha})^{1/\alpha}},$$

where M_W is the weight-average molecular weight; P_i, the optical absorbance of the i-th elution volume v_i; and M_i is the molecular weight of the species included in v_i. Computation procedure for the estimation of α, K_D, and M_D from $\langle D \rangle_z$ and gel-permeation data has been described[5].

The best-fit relationship between $\langle D \rangle_z$ and M_D was found to be $\langle D \rangle_z = 2.50 \times 10^{-4}\ M_D^{-0.593}\ cm^2 \cdot s^{-1}$ (see Fig. 2), and the experimental results are summarized in Table I. Molecular-weight dependence of the diffusion coefficient $D = K_D M^{-\alpha}$ can be combined with that of intrinsic viscosity $[\eta] = K_\eta M^\beta$: the "equivalent hydrodynamic-sphere" model[8] predicts $3\alpha = 1 + \beta$. Nakamura *et al.*[4] measured the intrinsic viscosity of lentinan samples, and obtained $[\eta] \propto M_W^{0.877}$; $\beta = 0.877$ yields $\alpha = 0.626$, which is in fairly good agreement with our light-scattering results, considering that our α-value (0.593) was obtained for M_D, and intrinsic viscosity data for M_W.

The α-value obtained indicates that the main chain of lentinan molecules in 0.12M borate buffer, pH 10, is a random coil having some long-range interactions[9]; attraction between polymer segments and solvent molecules is greater than that

between polymer segments themselves. This conclusion is consistent with the stability of lentinan molecules in 0.12M borate buffer solution as described in this note. Besides, when the values of $\langle D \rangle_z$ for lentinan are compared with those for samples of dextrans[5], which are also homoglucans, of almost the same molecular weight, it is obvious that, in 0.12M borate buffer, the lentinan molecules spread more widely than the dextran molecules. This result may be ascribed to the difference in the D-glucopyranosyl linkage, *viz.*, β-D-(1→3) and α-D-(1→6).

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