Note

Molecular Weight Dependence of the Antitumor Activity of Schizophyllan

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Schizophyllan, a water-soluble beta-1,3-D-glucan with single beta-1,6-linked glucopyranose residues, has host-mediate antitumor activity.¹⁾ An aqueous solution of schizophyllan is too viscous to be injected clinically, so it is partially depolymerized to lower molecular fragments by ultrasonic irradiation²⁾ or hydrodynamic shearing.³⁾

Norisuye *et al.*⁴⁾ found that both native and depolymerized schizophyllan dissolve in water as a rod-like triple helix, while it is dispersed molecularly in dimethyl sulfoxide (Me_2SO) as a random coil. Concerning the antitumor activities of beta-1,3-D-glucans, Chihara⁵⁾ hypothesized that a certain ordered structure of a glucan is necessary for its antitumor activity. As mentioned above, schizophyllan in water has indeed such an ordered structure.

We have investigated both the antitumor activity of schizophyllan against Sarcoma 180 ascites, and its behavior in dilute aqueous solution by bioassay, viscometry,

and gel-filtration chromatography, using low molecular weight samples.⁶⁾ Data on intrinsic viscosity $[\eta]$ combined with chromatographic data showed that below an M_w (weight-average molecular weight) of about 9×10^4 , both rigid triple helices and single chains coexist in aqueous solution, and that the proportion of helices decreases monotonically with decreasing M_w . With this decrease in helices, the tumor-inhibition ratio diminishes. These findings suggested that for beta-1,3-D-glucans, the triple helical conformation is the ordered structure invoked by Chihara.⁵⁾ However, we do not know if the triple helical structure of schizophyllan is essential for its antitumor activity, independent of molecular weight, since the effects of molecular weight and schizophyllan conformation on tumor inhibition cannot be separated.

To answer this question, the schizophyllan samples (U-16, -11, -15, -16, and -18) we used before were separated into triple-helix species (UH-6, -11, -15, -16, and -18) and random-coil species (UR-6, -11, -15, -16, and -18), using a preparative Sephadex G-100 column (5×80 cm). Then we examined the antitumor activity of these species against Sarcoma 180 ascites.

The tumor inhibition ratio was calculated by the following method. Sarcoma 180 ascites (0.1 ml; about 2×10^6 cells) was implanted subcutaneously into the groins of ICR-JCL mice weighing 20~25 g. After 24 hr, a test sample dissolved in physiological saline solution in 0.5 wt% was injected intramuscularly at the dose of 10 mg/kg. Then, 31 days after the tumor implantation, the mice were killed for evaluation of the effect of the treatment on tumor growth. Inhibition ratios were calculated by the following equation

$$\text{Inhibition ratio (\%)} = [(A - B)/(A)] \times 100$$

where A is the average tumor weight of the control group, and B is that of the treated group.

FIG. 1. Molecular Weight Dependence of the Inhibition Ratio for Triple-helix Species (●) and Random-coil Species (△) of Schizophyllan.

The molecular weight of the triple helix species was assumed to be three times that of the original (non-separated) sample in Me_2SO , on the basis of the following findings. The estimated M_w of U-16 in Me_2SO is 1.35×10^4 . The viscosity-average molecular weight M_v of UH-16 was estimated to be 3.9×10^4 from $[\eta]$ in water, 0.133 dl/g, using the theoretical curve of $[\eta]$ vs. M_w for the rigid schizophyllan triple helix in water. This M_v is close to three times the M_w of U-16 in Me_2SO (4.05×10^4). Furthermore, an $[\eta]$ of 0.159 dl/g for UR-16 in water yields 1.15×10^4 for the M_v of this random-coil species, which is not very different from the M_w of U-16 in Me_2SO . The ratio of M_v of UH-16 to that of UR-16 is about 3.4.

Figure 1 shows the molecular weight dependence of the tumor inhibition ratio for the random-coil and the triple-helix species of schizophyllan. With decreases in M_w , the curve for the triple helix species fell sharply at the molecular weight of about 5×10^4 . This indicates that the schizophyllan triple helix lost its antitumor activity when the molecular weight is below 5×10^4 . On the other hand, the inhibition ratios for the random-coil species were

almost zero, which means that these species have virtually no antitumor activity in the region of molecular weight tested. Thus, we conclude that schizophyllan has antitumor activity against Sarcoma 180 only when it has a molecular weight higher than 5×10^4 and a triple helical structure in aqueous solution.

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