Chemistry of Natural Compounds, Vol. 34, No. 1, 1998

MOLECULAR HETEROGENEITY AND ANTIVIRAL AND INTERFERON-INDUCING ACTIVITIES OF THE DRUG SAVRATS

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UDC 576.858.095.393

The molecular-mass distribution of the drug Savrats has been studied by gel filtration on HW-55 f. It has been shown that the drug is heterogeneous and that the fraction with a molecular mass of 25-25 kDa exhibits a high biological activity.

In the creation of biologically active substances from high-molecular-mass compounds the molecular mass is of great importance. It determines the solubility of the compound, its penetrability through a cell membrane, its degree of conversion into other compounds in the organism, and its degree of excretion from the organism, and other properties. A drug based on oxidized water-soluble acetylcellulose — Savrats — has a broad spectrum of biological action [1, 2].

We have studied the molecular-mass distribution of the drug Savrats and the biological activities of the fractions obtained. To determine the molecular heterogeneity of the drug we used gel filtration on TSK gels of the HW-55 f type. The markers used were blue dextran and polyethyleneglycols having MMs of 20 and 40 kDa covalently stained with Cibacron F3GA.

As can be seen from Fig. 1, the drug was heterogeneous and was eluted in several peaks: the first maximum corresponded to a molecular mass of 40-35 kDa (32%); the second, unsymmetrical, maximum to 35-25 kDa (56%); the third to 10 kDa (10%); and the last to 1 kDa (2%).

Antiviral activities were determined in cultures of L-929 murine fibroblast cells in relation to the virus of murine encephalomyocarditis (EMC) in a dilution of 10^{-2} (Table 1).

At a concentration of 250 μg/ml the unfractionated drug Savrats showed an interferon-inducing activity of 256 AU/ml, and its antiviral effect in relation to EMC in cultures of L-929 murine fibroblasts amounted to 42-48%.

As can be seen from Table 1 and Fig. 1b, all the fractions exhibited biological activity, the highest antiviral effect at a concentration of 250 μg/ml — for fraction 2 — being 60-80%, and the interferon titers being 320-640 AU/ml.

EXPERIMENTAL

To determine the ratios of the various fractions, they were precipitated with acetone, dried in the air, and weighed. The results are given in Table 1.

For gel filtration we used Toyo Soda HW-55 f gel (Japan) with exclusion limits of from 1000 to 200,000 Da (for polysaccharides). Detection was effected with the aid of a LKB Uvicord instrument (Sweden).

The fractions were separated in the following way: 1) fraction with MM from 40 to 35 kDa; 2) from 35 to 25 kDa; 3) 10 kDa; 4) about 1 kDa. The fractions were precipitated with acetone, dialyzed against distilled water, and freeze-dried.

Synthesis of Cibacronized PEG. Separate 50-mg samples of Ferak PEG (Germany) with MMs of 40,000 and 20,000 were dissolved in 2 ml of water. After complete dissolution, 10 mg of Cibacron dissolved in 1 ml of water was added to each, Da and the pH of the reaction mixtures was brought to 12 with sodium carbonate. The mixtures were left in a thermostat at

Mirzo Ulugbek Tashkent State University, fax (3712) 46 24 72. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 112-114, January-February, 1998. Original article submitted October 7, 1996; revision submitted September 16, 1997.

 0009-3130/98/3401-0086$20.00

TABLE 1. Interferon-Inducing and Antiviral Activities of Fractions of the Drug Savrats

Dose, mg/ml	IFN, Au/ml fractions			
	1	2	3	4
125	20*	160**	<10*	<10*
250	40–80**	320–640***	40–80**	40–80**
500	10–20**	80–160**	160**	80–160**

*CPD_{50}/ml 25%;
**CPD_{50}/ml 50%;
***CPD_{50}/ml 75%.

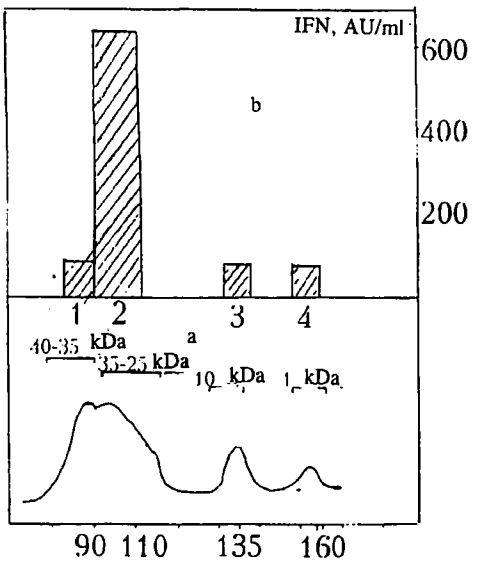

Fig. 1. Molecular-mass distribution of fractions of the drug Savrats (*a*) and biological activities in a cell culture of the fractions 1-4 obtained (*b*).

55°C for 12 h and were then desalted with G-25 resin. After evaporation of the water, each of the residues was redissolved in the minimum volume of water. Each solution was centrifuged and was then deposited on a column of G-25 resin (0.75 × 30 cm) and eluted with distilled water. The fractions were collected on the basis of their color and were dried in the air. The yield for the PEG with MM 40,000 Da was 40 mg (78%), and that for the 20,000-Da PEG was 35 mg (70%).

Determination of the Molecular Mass of the Drug Savrats. A 1 mg/ml solution of blue dextran (2 ml) was deposited on a column of HW-55 f resin (0.8 × 70 cm). The eluent was 0.2 M KH_2PO_4 (pH 4.6) at a rate of 30 ml/h, V_0 being 47 ml. Separate 5 mg/ml samples of each Cibacronized PEG were deposited on the column. The eluent was 0.2 M KH_2PO_4 (pH 4.6) at a rate of 30 ml/h. The maximum for the elution of the PEG with MM 40 kDa was 90 ml, and that for the 20-kDa PEG 115 ml. A residue-free solution of 10 mg of Savrats in 2 ml of water was centrifuged and then the sample was deposited on a column and was eluted with 0.2 M KH_2PO_4 (pH 4.6) at a rate of 30 ml/h.

Determination of the Biological Activities of the Fractions. The interferon-inducing activities of fractions 1-4 were studied in cultures of L-929 murine fibroblast cells at various concentrations. To determine antiviral activities, cultures of murine fibroblast cells were treated with solutions of the fractions obtained, in various concentrations, and after 4 h the solutions were evaporated, the cells were washed twice with Hanks' solution and were then infected with EMC virus having an activity of 10, and after 24 h the cytopathic actions of the drug fractions were determined. To determine interferon-inducing activities, the fractions obtained were added in various concentrations to monolayers of cell cultures. After contact for 4 h, the cells were washed twice with Hanks' solution, and 100 μg of a nutrient medium containing 2% of bovine serum was added. Interferon titers were measured after 24 h by the micromethod in 96-well plates.

REFERENCES

1. Kh. A. Aslanov, A. M. Saiitkulov, and S. A. Maulyanov, "Clinical prospects of an interferon inductor of natural origin," in: Proceedings of the Xth Regional Symposium [in Russian], Riga (1989), p. 11.
2. S. A. Maulyanov, S. A. Auelbekov, A. M. Saiitkulov, et al., "The use of aromatic hydroxyamines for the synthesis of antiviral drugs," in: Proceedings of the IIIrd Regional Conference on Chemical Reagents [in Russian], Tashkent (1990), p. 329.