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PRODUCTION OF HERPES SIMPLEX MUTANTS RESISTANT TO DEXTRAN SULFATE

[Following is the translation of an article by M. I. Sokolov, A. B. Germanov, and L. S. Ratushkina, Institute of Virology imeni D. I. Ivanovskiy, AMN USSR, Moscow, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology) 12: 363-64, 1967. It was submitted on 6 Oct 1966.]

Synthetic sulfitization-treated polysaccharide of dextran sulfate is an inhibitor of adsorption and multiplication of many DNA- and RNA-containing viruses [1, 2], including the virus of herpes simplex [3, 4]. Resistance to dextran sulfate is a genetically stable characteristic, used extensively for the genetic study of viruses.

We studied the influence of dextran sulfate on the capacity of the herpes simplex virus to form plaques and the possibility of obtaining mutants which are resistant to this inhibitor.

The L2 strain used in the work was subjected to threefold cloning. Out of individual plaques during incubation in a monolayer culture of fibroblasts of chick embryo under an agar covering in the method of Porterfield on the 4-5th day of incubation at 37° it forms plaques with a diameter of 1-2 mm. The addition of dextran sulfate (preparation from the Swedish firm Pharmacia upsala, molecular weight 2×10^6) to the covering influences both the size and the number of plaques forming. In contrast to the findings of Takemoto and Fabisch [5], dextran sulfate in a concentration of 0.1 mg/ml, based on our findings, did not suppress the cytopathic effect of the virus on a cell culture and did not influence its capacity to form plaques. A decrease in the number and size of plaques was noted only at a concentration of 0.5 mg/ml of dextran sulfate. In a concentration of 1 mg/ml dextran sulfate reduced the effectiveness of seeding by 45-70%; here the diameter of plaques on the 5th day was 0.5 ml. In a concentration of 2 mg/ml the preparation completely suppressed the formation of plaques. The inhibiting action of dextran sulfate was completely removed when into the covering DEAE-dextran was introduced in a concentration equal to the concentration of the inhibitor.

For obtaining mutants resistant to dextran sulfate the culture of chick fibroblasts was infected with 1 ml of an undiluted suspension of virus and sealed with an agar covering containing

dextran sulfate in a dose of 2.5 mg/ml. For determination of the titer of the initial virus suspension the cell culture was infected with a 10-fold dilution of virus and sealed with a covering which did not contain dextran sulfate. Resistant mutants were isolated from plaques, rarely forming under the covering containing dextran sulfate. The frequency of mutations was determined as the ratio of average number of mutants per 1 flask to the average number of PFU in the inoculum.

All told 5 mutants were isolated which were resistant to dextran sulfate. They were designated with the symbol DSR. The frequency of mutations comprised 8.8×10^{-6} . The isolated mutants turned out to be genetically stable and did not lose their resistance during subsequent passages under a covering which did not contain dextran sulfate. Serological typing of the mutants obtained in the reaction of neutralization of cytopathic action with specific serum showed their identity with the initial strain.

In contrast to the initial virus the plaque-forming capacity of DSR-mutants was not suppressed when dextran sulfate was added to the covering in a dose of 1 mg/ml. Findings from the comparative study of the influence of dextran sulfate on the plaque-forming capacity of the initial virus and the resulting mutants are given in the table.

Literature

1. Liebhaber, H., Takemoto, K. K., Virology, 1963, v 20, p 559.
2. Takemoto, K. K., Liebhaber, H., Ibid., 1962, v 17, p 499.
3. Takemoto, K. K., Fabisch, P., Proc. Soc. exp. Biol. (N.Y.), 1964, v 116, p 140.
4. Takemoto, K. K., Spicer, S. S., Ann. N.Y. Acad. Sci., 1965, v 130, p 365.

Influence of dextran sulfate on the plaque-forming capacity of an initial strain of the herpes simplex virus and mutants which are resistant to this inhibitor

(a) Штамм	(b) Концентрация дextrан-сульфата (в мг/мл)	(c) Титр (в БОЕ/мл)	(d) Эффективность посева (в %)	(e) Размер бляшек на 5-е сутки (в мм)
(f) Л2	0	$7,6 \times 10^8$	100	1,5-2
	0,1	$7,6 \times 10^8$	100	1,5-2
	0,5	$4,8 \times 10^8$	63,1	0,5-1,5
	1,0	$4,2 \times 10^8$	55,2	<0,5
	2,0	0	0	Нет
(g) (исходный)	0	$6,0 \times 10^8$	100	1,5-2
	1,0	$2,5 \times 10^8$	41,6	<0,5
	0	$5,5 \times 10^8$	100	1,5-2
DS ₁ ^r	0	$4,5 \times 10^8$	100	1,5-2
	1,0	$4,5 \times 10^8$	100	1,5-2
DS ₂ ^r	0	$4,0 \times 10^8$	100	1,5-2
	1,0	$3,9 \times 10^8$	97,5	1,5-2
DS ₃ ^r	0	$3,6 \times 10^8$	100	1,5-2
	1,0	$3,7 \times 10^8$	100	1,5-2
DS ₄ ^r	0	$5,1 \times 10^8$	100	1,5-2
	1,0	$5,0 \times 10^8$	98,2	1,5-2
DS ₅ ^r	0	$5,2 \times 10^8$	100	1,5-2
	1,0	$5,3 \times 10^8$	100	1,5-2

Key: (a) Strain; (b) Concentration of dextran sulfate (in mg/ml); (c) Titer (in PFU/ml); (d) Effectiveness of seeding (in %); (e) Size of plaques on 5th day (in mm); (f) L2; (g) (initial).