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SORPTION OF BACTERIOPHAGES BY THE CATION-SUBSTITUTED FORMS OF MONTMORILLONITE

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Summary. — The peculiarities of the absorption of phages T2 or MS2 on the Na-, Ca- or Al- forms of cherkassky montmorillonite have been studied. This depended on the size of the virus and the type of the exchanged cation of the mineral. The affinity of the phages to various forms of the montmorillonite has been defined and their maximum phage-sorptive activities determined.

Key words: bacteriophages T2 and MS2; sorption of bacteriophages by montmorillonite; cation-substituted forms of montmorillonite

Introduction

The capability of viruses to be sorbed by clayey minerals can be used for their concentration and purification, in the study of biological properties of viruses (Dunn and Hitchborn, 1965; Shyrobokov, 1972, 1974) and for their isolation and removal from water (Kazantseva *et al.*, 1978; Lesichkov *et al.*, 1978; Turk *et al.*, 1980). The information on the effect of the size of viruses and the cation form of the mineral on the sorption is necessary for the rational use of clayey minerals when solving theoretical and practical problems. In this connection we have studied the sorption of bacteriophages of different size by the cation-substituted forms of montmorillonite.

Materials and Methods

The T2 *Escherichia coli* B and MS2 *Escherichia coli* K-12 phages were obtained by using the synthetic medium M9. The admixtures were removed by the multi-stage low-temperature hypotonic dialysis and by centrifugation (Nykovskaya *et al.*, 1979). After additional purification by ultracentrifugation in CsCl density gradient, the preparation of phage T2 was used in the comparative experiments. The concentration of phage (c) in the samples was defined by Gracia's method of agar layers. The results of the plaque calculation were processed statistically and expressed in the number of plaque forming units per 1 ml (PFU/ml).

The montmorillonite of the Cherkassky deposit (Ukrainian S.S.R.) which is the greatest in Europe was obtained from the natural clay by separation of colloid fractions. Its cation-substituted forms were prepared by treatment of the mineral with 1 mol/l NaCl, $CaCl_2$ and $AlCl_3$ (Tarasevich and Ovcharenko, 1975).

The sorption of phages by the montmorillonite was studied under equilibrium conditions. The mineral (50 mg/ml, experimental specimens) was introduced into distilled water of pH 6.3 – 6.6 containing 0.5×10^9 PFU/ml of phage T2 or 0.8×10^{10} PFU/ml of phage MS2 (control specimens) and mixed at 22 °C until the sorbtion equilibrium was established. The initial concentration of phage (C_0) was defined after the centrifugation at 13,600 g for 15 min in the supernatant of the control specimens. The equilibrium concentration (C_e) was defined in the experimental specimens. The value of the specific sorption of phages by the montmorillonite (a, PFU/mg) was calculated by the equation $a = \frac{C_o - C_e}{m}$ (1) where m is the concentration of the mineral (mg/ml)

In the mathematical description of the phage sorption by the montmorillonite Langmuir's equation was used. To define the ratios of the equation and its applicability, the methods of equilization and average were utilized (Batuner and Posin, 1971).

Results

The dependence of the specific sorption (a) of T2 and MS2 phages by the Na-, Ca- and Al-forms of montmorillonite on the equilibrium concentration of virions (C_e) is shown in Fig. 1. The isotherms of the phages resemble Langmuir's curves. They were described by means of the linearized form of Langmuir's equation $C_e/a = 1/a_{max} . K + C_e/a_{max}$ (2) where a_{max} is the maximal sorption of the phages under condition of saturation of all active centres of the sorbent (PFU/mg). K is the constant of sorption equilibrium which is equal to the relation of the speed of the phage sorption to the speed of its elution and is proportional to the affinity of the phage to the sorbent (ml/PFU).

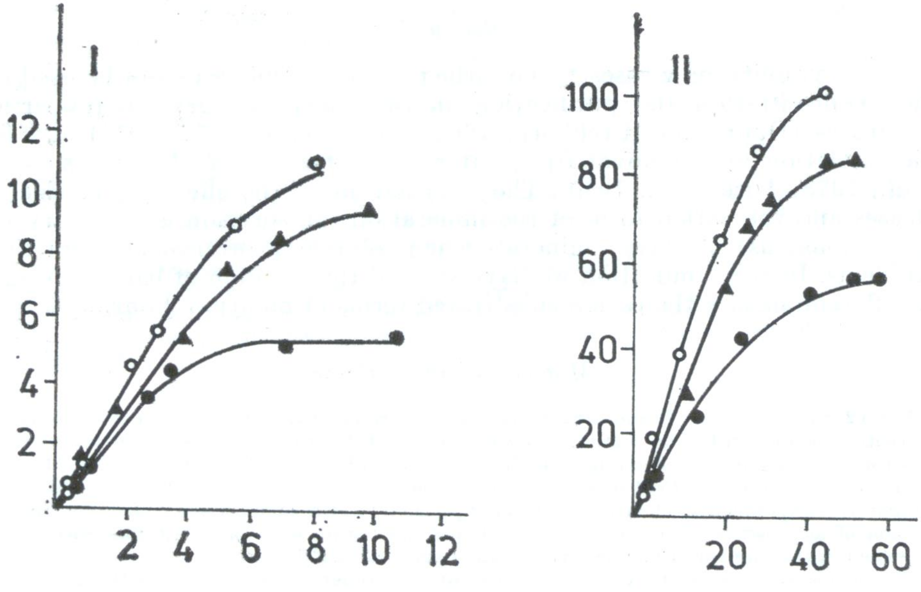

Fig. 1.
Isotherms of sorption of phages T2 (I) and MS2 (II) by the cation-substituted forms of montmorillonite
Abscissa: $C_e . 10^{-9}$ (PFU/ml). The white circles denote the Na-form, the triangles denote the Ca-form, the black circles denote the Al-form; ordinate: $a . 10^{-10}$ (PFU/mg).

Fig. 2. shows the isotherms of the phage sorption in 3 forms of montmorillonite. They were done in the coordinates of equation 2. The experimental points do not deviate much from the calculated paths of the isotherms. (The average error $< 10\%$). Consequently, Langmuir's equations can be applied to the description of the phage interaction with the montmorillonite. This attests to the single-layer sorption of the viruses by the clayey mineral. The values a_{max} and K calculated in equation 2 are given in the Table 1.

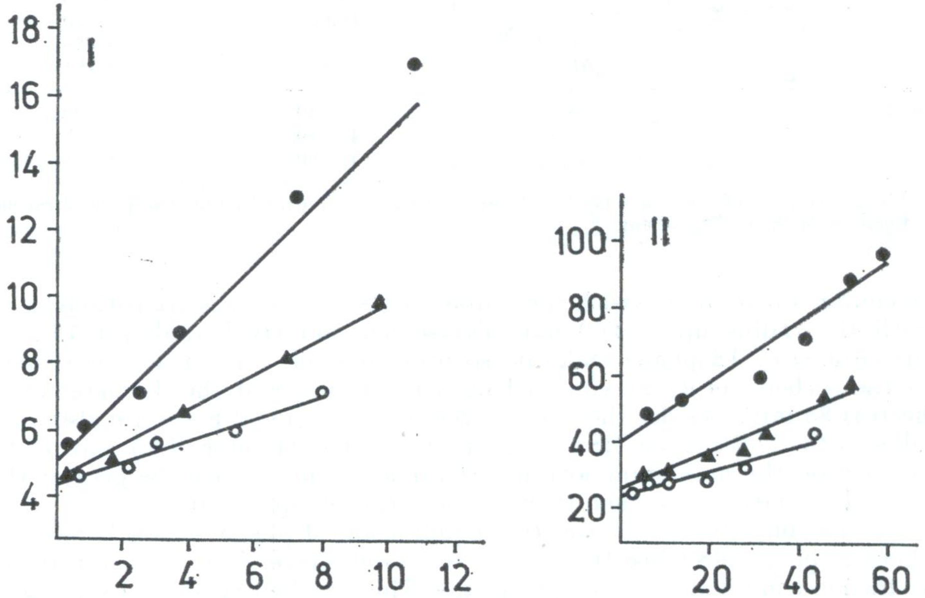

Fig. 2.
Isotherms of the sorption of T2 (I) and MS2 (II) phages by the cation-substituted forms of montmorillonite in the coordinates of Langmuir's equation
Abscissa: see Fig. 1; ordinate $C_e/a.10^1$ (ml/PFU)

Discussion

The a_{max} and K values for the Ca-forms of montmorillonite do not differ much when the highly and partially purified preparations of T2 phage are used. This testifies to the fact that our method of obtaining the lysates of phages and their purification from admixtures with the help of dialysis and centrifugation yields phage preparations pure enough to be used in studies on phage sorption to minerals.

The maximum sorption a_{max} of phage MS2 by the three forms of montmorillonite is one order higher than of phage T2 (see Table 1). It must be connected with the poriferous structure of the mineral. The montmorilloni-

Table 1. The characteristics of sorption of T2 and MS2 phages by the cation-substituted forms of montmorillonite

Phage	Form of the mineral	$a_{max} \cdot 10^{-10}$ (PFU/mg)	$K \cdot 10^{10}$ (ml/PFU)
T2	Na—	34.50	0.620
	Ca—	16.98	1.640
	Ca—*	16.44	1.826
	Al—	8.80	2.350
MS2	Na—	373.40	0.100
	Ca—	191.50	0.189
	Al—	109.60	0.221

* The preparation of the phage used in the experiments was purified additionally by ultracentrifugation in the CsCl gradient.

te contains macropores with the radius > 200 nm and the transitional pores with the radius up to 200 nm (Tarasevich and Ovcharenko, 1975). The dimensions of T2 phage make its sorption possible only in the macropores of the sorbent as its maximum length is 240 nm and the diameter of the head is 85 mm. Besides the macropores the transitional pores are also accessible to the ball-shaped MS2 phage as it has the diameter of 25 mm. In this connection the maximum sorption of the small phages can be greater than that of the large ones when there is the sorption equilibrium.

As the constants of the sorption equilibrium of phage T2 are higher than those of phage MS2 (see the Table), the large phage T2 has greater affinity to the montmorillonite than MS2 phage. This can be explained by using the general principle of amplification of the interaction of large and long sorptives with the sorbent. The sorptive capacity of the Na-form of montmorillonite in relation to the phages is higher than that either of Ca- and Al-forms, although the Al-form has more marked affinity to the phages.

The Ca-form of montmorillonite is widely spread in nature. It ocupies the intermediate position between its Al- and Na-forms by the values of sorptive capacity and activity towards the viruses. That is why it can be recommended for the removal of viruses from natural and sewage waters, and other sources by absorption. Our method of sorption of viruses by the montmorillonite with the help of Langmuir's equation has shown that the viruses differing in size are also different in the level of maximum sorption and the affinity to the cation forms of montmorillonite. The method can be applied to the evaluation of the sorptive properties of minerals in relation to the viruses and to the choice of the most prospective sorbents.

References

Batuner, L. M., and Posin, M. E. (1971): Mathematical methods in chemistry engineering (in Russian), pp. 708—711. *Chemistry*, Leningrad.

Dunn, D. B., and Hitchborn, J. H. (1965): The use of bentonite in the purification of plant viruses. *Virology* **25**, 171—192.

Kasantseva, V. A., Ayzen, M. S., Drozdov, S. G., and Codking, G. H. (1978): Comparison of the effectiveness of the methods of extraction of enteroviruses from water by means of adsorption on diaphragm filters, ion-exchange tar and bentonite (in Russian). *Gig. Sanit.* (3), 1978, 79—83.

Lesychkov, V. S., Globa, L. I., and Kirillova, L. G. (1978): The utilization of mineral sorbents in north-eastern Bulgaria for removing viruses from water, pp. 555—558. In: *7th Int. Congr. Infect. Paras. Dis.*, Reports 111, Oct. 1978, Varna, Bulgaria.

Nykovskaya, G. N., Globa, L. I., and Rotmistrov, M. N. (1979): T2 *E. coli.* B and MS2 *E. coli* K-12 bacteriophages' survival by dialysis and storage (in Russian). *Mikrobiol. Zh. A.N.U.R.S.R.* **41**, 177—179.

Shyrobokov, V. P. (1972): The study of physiological peculiarities of the Coxsacckie viruses by their adsorption on bentonite (in Russian). *Mikrobiol. Zh. A.N.U.R.S.R.* **34**, 122—123.

Shyrobokov, V. P. (1974): The use of bentonite on the purification and concentration of enteroviruses (in Russian). *Vop. Virus.* **2**, 228—233.

Tarasevich, Ju. I., and Ovcharenko, F. D. (1975): Adsorption on clayey minerals (in Russian), pp. 9—14, 84—92. Naukóva Dumka, Kiev.

Turk, C. A., Moore, B. E., Sagic, B. P., and Sorber, C. A. (1980): Recovery of indigenous viruses from waste water sludges using a bentonite concentration procedure. *Appl. Environ. Microbiol.* **40**, 423—425.