

Study of the effect of inactivating agents on the structure of adenovirus particles using small-angle X-ray scattering and transmission electron microscopy

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In this work, the effect of inactivating agents (formaldehyde and beta-propiolactone) on the structure of viral particles was investigated using small-angle X-ray scattering and transmission electron microscopy. Data were obtained indicating the destruction of the natural structure of the adenovirus due to the effect of β -propiolactone. The results of this study may be useful in the development of vaccines

Keywords: viruses, inactivation, small-angle X-ray scattering, synchrotron research.

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The structure of inactivated viruses may be reconstructed with a resolution up to a few angstroms using cryogenic electron microscopy [1,2]. However, it is an expensive and complex method that remains inaccessible to a wide range of researchers [3].

The relatively simple and accessible method of small-angle X-ray scattering (SAXS) is currently being used actively in the study of complex biological molecules and viruses [4]. Specifically, SAXS was used to analyze the structure of various viruses: hepatitis B virus and SV40 at different stages of their life cycle [5], potato virus X and alternanthera mosaic virus [6], and ethanol-inactivated bacteriophage Phi6, which often serves as a substitute for human pathogenic viruses [7]. The SAXS method combined with transmission electron microscopy (TEM) was applied in the examination of structure of tobacco mosaic virus [8] and intact and partially trypsinized samples of potato virus A [9].

We found no published SAXS studies focused on the comparison of structural changes of viruses under the influence of various inactivators. The heterogeneity of viruses necessitates the use of an additional method that allows one to compare the structure of virions exposed to different chemical agents.

In the present study, a combination of SAXS and TEM methods revealed that β -propiolactone has the capacity to destroy adenovirus particles, while the mild influence of formaldehyde does not induce any changes in the overall structure of virions. The discovery of this effect expands our knowledge of the virucidal activity of β -propiolactone and formaldehyde and may facilitate vaccine development.

Two adenovirus strains were studied: adenovirus type 6 (strain Tonsil-99) and adenovirus type 26 (strain 415). These adenoviruses were accumulated in the A-549 cell culture, and virus suspensions were obtained via ultracentrifugation. The resulting suspension of each strain was

divided into six parts for treatment with β -propiolactone and formaldehyde. These inactivators were chosen as the most widely available and frequently used in the production of vaccines. All virus-containing liquid suspensions were pre-clarified and, following inactivation procedures, concentrated in a sucrose gradient. Concentrated adenovirus suspensions were resuspended in STE buffer (Sodium Chloride-Tris-EDTA, 1X Solution, pH = 8.0), and the protein concentration was determined using a PierceTM BCA Protein Assay Kit. To ensure data accuracy and reproducibility, the protein content of all samples was kept at 1 μ g/ml. Inactivation testing of the samples was carried out in accordance with MU 3.5.2431-08 (Study and Evaluation of the Virucidal Activity of Disinfectants, para. 3 and 5). All 12 samples were tested for infectious activity in the A-549 cell culture. The infectious titer of the virus from the sample is zero for all samples. In concentrated preparations of purified adenoviruses, the obtained samples were examined using a JEM 1011 transmission electron microscope (JEOL, Japan) at an accelerating voltage of 80 kV within the instrumental magnification range of 50 000–250 000.

The characteristics of adenovirus samples are presented in the table.

Electron micrographs reveal virtually no change in the structure of virions inactivated by the same inactivator with different concentrations. In view of this, we present two electron micrographs: a sample inactivated with formaldehyde and a sample inactivated with β -propiolactone. It will be shown below that the SAXS data for samples inactivated by the same inactivator with different concentrations are also virtually indistinguishable. The electron micrograph of sample No. 1 inactivated with formaldehyde is shown in Fig. 1.

Characteristics of adenovirus samples

Sample number	Virus	Inactivation (volume:volume)	Results of measurement of the protein concentration, $\mu\text{g/ml}$	Infectious titer of the virus from the sample
1	Adenovirus 6	Formaldehyde (1:40)	1.01	0
2	Adenovirus 6	Formaldehyde (1:400)	1.07	0
3	Adenovirus 6	Formaldehyde (1:4000)	1.08	0
4	Adenovirus 26	Formaldehyde (1:40)	1.05	0
5	Adenovirus 26	Formaldehyde (1:400)	1.05	0
6	Adenovirus 26	Formaldehyde (1:4000)	1.04	0
7	Adenovirus 6	β -propiolactone (1:1000)	1.08	0
8	Adenovirus 6	β -propiolactone (1:10000)	1.04	0
9	Adenovirus 6	β -propiolactone (1:100000)	1.07	0
10	Adenovirus 26	β -propiolactone (1:1000)	1.08	0
11	Adenovirus 26	β -propiolactone (1:10000)	1.04	0
12	Adenovirus 26	β -propiolactone (1:100000)	1.04	0

The electron micrograph of sample No. 11 inactivated with β -propiolactone is shown in Fig. 2.

A linear accelerator, the „Siberia-2“ large storage ring, and the „BioMUR“ experimental facility of the „KISI-Kurchatov“ research complex [10] were used to measure the small-angle X-ray scattering curves of inactivated viral particles. Samples of the virus-containing suspension were introduced into thin-walled quartz capillaries with a length of 80 mm, a diameter of 1.5 mm, and a wall thickness of 0.01 mm (Quarzkapillaren, Germany). A DECTRIS Pilatus3 1M two-coordinate detector with a resolution of 981×1043 pixels and a pixel size of $172 \mu\text{m}$ mounted at a distance of 2500 mm from the sample was used to record X-ray patterns. Three intensity curve measurements with an exposure of 300 s were performed for each of the 12 samples. The experiment was conducted at a temperature of 27°C .

Having integrated experimental two-dimensional scattering patterns, we obtained scattering curves (Fig. 3) that represent the dependence of scattered radiation intensity $I(s)$ on scattering vector magnitude $s = 4\pi \sin \theta / \lambda$ squared, where θ is the scattering angle and λ is the radiation wavelength. Large-angle scattering provides data on small-scale scattering objects (nominal resolution d of the SAXS method is specified by ratio $d = 2\pi/s$) [4]. Accordingly, a

larger value of scattering vector magnitude s is associated with smaller objects.

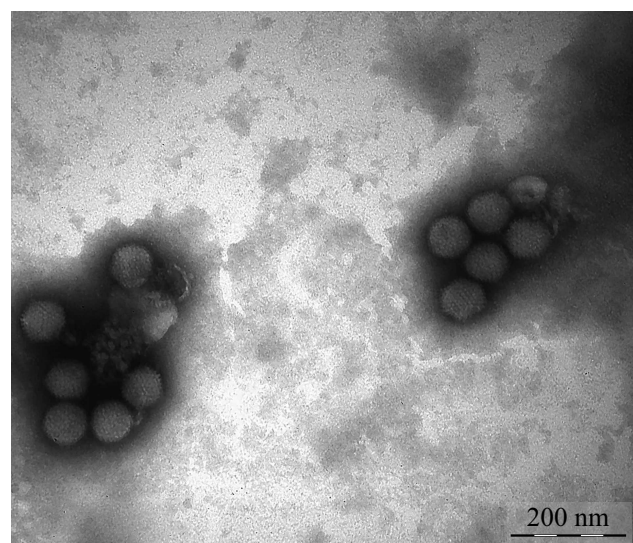


Figure 1. Electron micrograph of adenoviruses from sample No. 1 inactivated with formaldehyde. The suspension contains both the adenoviruses themselves and the remains of A-549 cells in which these adenoviruses were grown. It is evident that the integrity of capsids was not compromised.

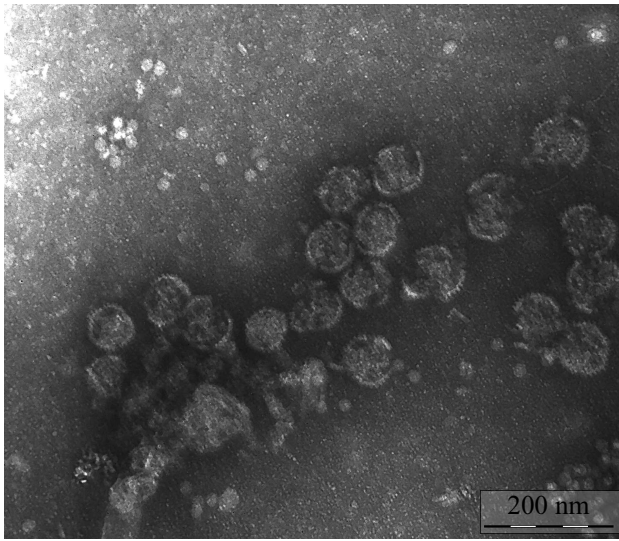


Figure 2. Electron micrograph of adenoviruses from sample No. 11 inactivated with β -propiolactone. The fragmentation of capsids is clearly visible.

A comparative analysis of $I(s)$ curves from all samples of adenovirus type 6 (Ad6) and type 26 (Ad26) inactivated with formaldehyde and β -propiolactone was carried out.

Figure 3 presents the scattering curves for all 12 samples, since, despite the variation of concentration, scattering curves from samples inactivated by the same inactivator differ little from each other.

Highlighted regions *A*, *B*, and *C* correspond to low ($s^2 < 0.01 \text{ nm}^{-2}$) (*A*), medium ($0.01 < s^2 < 0.06 \text{ nm}^{-2}$) (*B*), and high resolutions ($s^2 > 0.06 \text{ nm}^{-2}$) (*C*). The $\{\ln I(s), s^2\}$ dependence is near-linear within region *A* with

the lowest resolution, and the curves deviate only slightly. Within region *B*, the curves marked with triangles shift upward, which is attributable to fragmentation of the capsid under the influence of β -propiolactone (see the electron micrographs of viruses in Fig. 2). The destruction of large particles in suspensions and the formation of smaller particles enhance the relative contribution of the latter to the overall scattering pattern; thus, the aggressive inactivating effect of β -propiolactone is seen most clearly in the *B* region. The *C* region with the highest resolution reveals the presence of small fragments of A-549 cells in the suspension where the virus was grown. The contribution of these fragments to the overall scattering pattern becomes so substantial that it overshadows the contribution of larger adenovirus particles of interest to us, making it impossible to analyze the scattering curves.

It follows from the results of analysis of SAXS curves and electron micrographs that inactivation with β -propiolactone leads to destruction of the viral particle, while formaldehyde does not affect the overall virion structure. This clarifies our knowledge of the virucidal activity of formaldehyde and β -propiolactone and may be used in vaccine development. The capacity of β -propiolactone to alter the overall structure and surface properties of viral particles was also noted in [11].

It should be stressed that the amount of data on the structure of inactivated viruses obtained by TEM only is insufficient to substantiate the results of the present study. This requires analyzing thousands of electron micrographs, which is impossible without the use of machine learning algorithms. The examination of structure of inactivated viruses via TEM and machine learning is of the utmost interest and may be performed in the future. In the present study, the relatively simple and accessible SAXS method

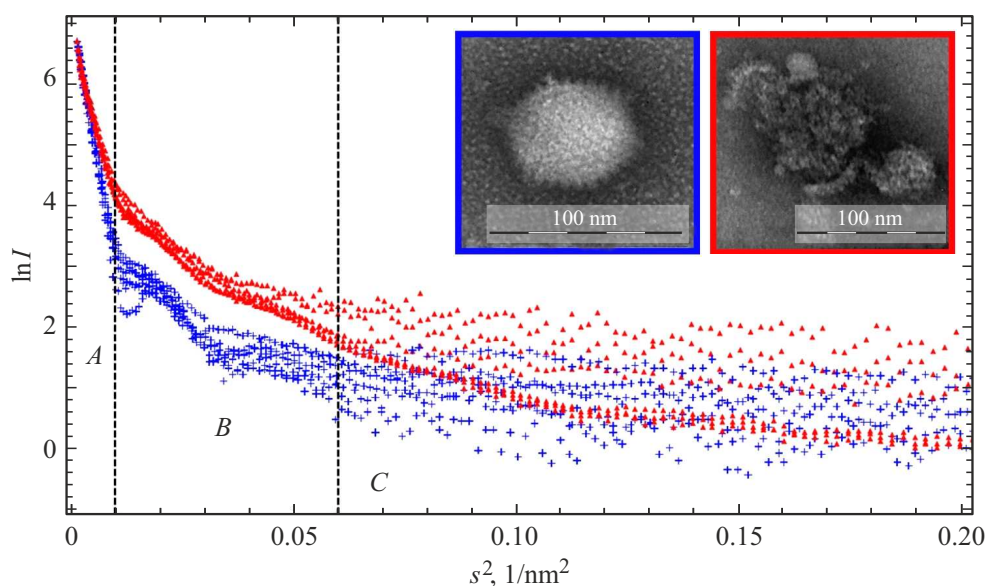


Figure 3. Comparison of scattering curves from samples inactivated with formaldehyde (crosses) and β -propiolactone (triangles). The left and right insets present photographic images of samples inactivated with formaldehyde and β -propiolactone, respectively. The inactivator/suspension ratios for each sample are given in the table.

was used to gather data on the structure of viruses from a large ensemble of randomly oriented particles. The studied virus-containing suspension was a polydisperse system (i. e., consisted of objects of different shapes and sizes). In addition, the presence of destroyed viruses in samples inactivated with formaldehyde could not be ruled out. This necessitated the use of the complementary SAXS method, which provides additional information about the structure of inactivated viral particles. Thus, the reliability of the reported data is ensured by a combination of SAXS and TEM and cannot be guaranteed by either method applied separately.

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Conflict of interest

The authors declare that they have no conflict of interest.

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