ELECTROCHEMICAL PROFILING OF POLIOVIRUS PARTICLES INACTIVATED BY CHEMICAL METHOD AND IONIZING RADIATION

L.E. Agafonova[1]*, V.V. Shumyantseva[1,2], Yu.Yu. Ivin[1,3], A.N. Piniaeva[1,3], A.A. Kovpak[3], A.A. Ishmukhametov[3], S.V. Budnik[4], R.S. Churyukin[4], D.D. Zhdanov[1], A.I. Archakov[1,2]

[1]Institute of Biomedical Chemistry,
10 Pogodinskaya str., Moscow, 119121 Russia; *e-mail: agafonovaluba@mail.ru
[2]Pirogov Russian National Research Medical University,
Ostrovityanova str. 1, Moscow, 117997 Russia
[3]Chumakov Federal Scientific Center for Research and Development
of Immune-and-Biological Products of Russian Academy of Sciences,
8/1 Moskovsky settlement, Polio Institute settlement, Moscow, 108819 Russia
[4]CEO Teocortex LLC, 34/6 Pervomaysky settlement, Moscow, 108808 Russia

Electrochemical profiling of formaldehyde-inactivated poliovirus particles demonstrated a relationship between the D-antigen concentration and the intensity of the maximum amplitude currents of the poliovirus samples. The resultant signal was therefore identified as electrochemical oxidation of the surface proteins of the poliovirus. Using registration of electrooxidation of amino acid residues of the capsid proteins, a comparative electrochemical analysis of poliovirus particles inactivated by electrons accelerated with doses of 5 kGy, 10 kGy, 15 kGy, 25 kGy, 30 kGy at room temperature was carried out. An increase in the radiation dose was accompanied by an increase in electrooxidation signals. A significant increase in the signals of electrooxidation of poliovirus capsid proteins was detected upon irradiation at doses of 15–30 kGy. The data obtained suggest that the change in the profile and increase in the electrooxidation signals of poliovirus capsid proteins are associated with an increase in the degree of structural reorganization of surface proteins and insufficient preservation of the D-antigen under these conditions of poliovirus inactivation.

Key words: electrochemical profiling; poliovirus; pseudovirus, poliomyelitis; inactivation; functional characteristics

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INTRODUCTION

Importance of preparation and investigation of pseudoviral constructs is determined by their use in fundamental biology and biomedicine. Pseudoviral constructs are widely used as the main component for the creation of vaccines [1–4] and gene therapy vectors in biotechnology [5, 6]. The pseudoviral model is the preferred object for accurate analysis of the phenotype of viral envelope proteins subjected to rapid accumulation of mutations *in vivo* [6]. Using pseudoviruses, the molecular determinants of the pathogenicity of viruses are studied [6]. Nanoreactors based on the enzyme encapsulation inside virus-like particles represent an alternative for enzyme replacement therapy [7]. Virus-like particles are biodegradable, uniformly organized and porous nanostructures/nanocontainers that transport and protect the biocatalyst (enzyme) from the external environment without significant loss of its biological activity [7]. For this purpose the enzyme is encapsulated within a capsid, and the surface of the nanoreactor can then be functionalized with specific ligands to target specific receptors, cells, or tissues [7].

Pseudoviral (inactivated) particles are prepared by inactivating viruses using various chemical (treatment with formaldegin, β-propiolactone) and physical (irradiation with waves and particles of various natures) methods. Inactivation is aimed at destroying the genetic material (DNA or RNA) of the virus in order to completely suppress its ability to reproduce, but preserving the protein part for the production of antibodies to the resulting pseudovirus [3, 8–11].

The electrochemical analysis of various viruses based on genosensory and immunosensory approaches has already been described in the literature [3, 12–16]. Electrochemical strategies for detecting viruses, either from RNA or from viral proteins or antibodies, rely on measuring signals using electron transfer mediators, electroactive and enzymatic tags, and using various modifications of working electrodes to increase the electroactive area and enhance the signal. Direct electroanalysis of viral particles based on electrochemical profiling of biomolecules has not been described yet. Previously, we used comparative electrochemical profiling of transfected cells and DNA to study transfection processes and analyze DNA fragmentation induced by restriction enzymes and apoptosis [17, 18].

The goal of this work was to perform direct electrochemical profiling of poliovirus particles inactivated by various doses of ionizing radiation (accelerated electrons) and treated with formaldehyde. In connection with this goal, the following tasks have been formulated:

- to conduct a comparative analysis of the electrochemical profiles of polioviral particles inactivated by the chemical method and ionizing radiation;

- to characterize the nature of the registered signal based on electrical analysis data;

- to calculate electrochemical parameters (potential, maximum current amplitude and peak area) from electrochemical profiles;

- to analyze changes in electrochemical parameters depending on inactivation conditions.

MATERIALS AND METHODS

Cells and Viruses

The Vero RCB 10-87 cell line was used as a cellular system for production of the viral material. Samples of the producer culture were prepared from the cell pool of a certified working Vero cell bank (RCBVero, Chumakov Federal Scientific Center for Research and Development of Immunobiological Preparations RAS (Polio Institute), Russia).

Attenuated Sabin strains of poliovirus type 1 (strain LSc 2ab, hereinafter referred to as SI) and type 3 (strain Leon 12a1b, hereinafter referred to as SIII) were used to obtain samples and to perform the neutralization reaction. The viral material used in this work was obtained from the original strains by passaging in Vero cells. The cells were cultivated in a bioreactor using Cytodex 1 microcarriers (Cytiva, USA) in the Eagle MEM medium (Polio Institute) supplemented with 5% fetal bovine serum (LTBiotech, Lithuania). Next, the Eagle MEM medium was replaced with a support medium (medium 199 without added serum) and the cells were infected with the required strain of the poliovirus. The virus was cultivated at 34°C until the monolayer of Vero cells on microcarriers was completely degraded, and the viral suspension was collected. The suspension was filtered using filter cascades with a rating (with pore sizes) of 70 μm — 0.65/0.45 μm — 0.22 μm. The cleared viral suspension was concentrated 200–500-fold using a tangential flow ultrafiltration procedure using a 100 kDa cut-off membrane [19, 20].

Virus Inactivation

Pseudoviral particles, the polioviral particles subjected to inactivation with formaldehyde and various doses of ionizing radiation (accelerated electrons) have been investigated in this work.

To inactivate with formaldehyde, poliovirus concentrates were purified using chromatographic procedures (gel filtration and ion exchange chromatography) [21, 22] and incubated with 0.025% formaldehyde for 13 days at 37°C. After inactivation, the samples were subjected to dialysis and transferred to a phosphate-buffered saline solution (PanEco, Russia) [20].

To inactivate poliovirus by ionizing radiation, samples with a virus titer of 10^{10} TCD_{50}/ml were used. The samples (3–4 ml) were dispensed into 5 ml cryovials and placed in sealed containers. The samples were irradiated with doses of 5–30 kGy at room temperature on a pulsed linear electron accelerator with a power of 15 kW and electron energy of 10 MeV. Irradiation was performed on one side. The absorbed dose was accumulated in one irradiation cycle (not fractionally). The samples arrived at the irradiation site via a roller conveyor in sealed transport containers; time spent "under the beam" varied from 0.2 s to 2 s. Dosimetric studies were carried out using an absorbed dose detector of an approved type: a standard sample of the absorbed dose of photon and electron radiation (copolymer with a phenazine dye) SO PD (F) R-5/50, passport of a standard sample of an approved type GSO 7865-2000, batch no. 22.57. To determine the absorbed dose, a PE-5400UF spectrophotometer (Ekros, Russia) was used. Actual absorbed doses were: 5.2±0.5 kGy, 11.4±1.4 kGy, 15.0±1.8 kGy, 23.3±2.8 kGy, 30.4±3.7 kGy.

Analysis of Residual Infectivity of Virus Samples

The inactivation effectiveness was analyzed by titrating samples on a sensitive Hep-2 cell culture (Cincinnati) obtained from NIBSC (National Institute of Biological Standards and Control, UK) according to a 50% cytopathic effect (TCD_{50}) [23], as well as by a series of passages on a sensitive Vero cell culture and assessment of the state of the cell monolayer [24]. Hep-2 cells were grown in the Eagle MEM medium with a double set of amino acids and vitamins, Vero cells were grown in the DMEM medium. In both cases 5% fetal calf serum (Biolot, Russia), streptomycin (0.1 mg/ml) and penicillin (100 units/ml) (PanEco, Russia) were added to the culture medium.

Analysis of the D-Antigen Content

Analysis of the D-antigen content of the poliovirus was carried out using an ELISA test system based on purified rabbit polyclonal antibodies specific to the D-antigen. In a 96-well panel (Corning, Germany), affinity purified Ig G to the poliovirus (obtained by immunizing rabbits with the purified antigen and further purification using affinity chromatography) was adsorbed for 20 h at 2–8°C. After the sorption procedure, the wells were washed twice with a washing solution (0.01 M phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20). After removal of the washing solution, a blocking procedure was performed using a blocking solution (0.01 M phosphate buffered saline, pH 7.4, with 1% fetal bovine serum) for 1 h at 37°C. The test samples, diluted in ELISA buffer (0.01 M phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20, and 1% fetal calf serum), were added to the plate wells and incubated for 2 h at 37°C. The samples were washed

twice with the washing buffer. The D-antigen was detected using Ig G, conjugated with biotin; Ig G specific to the poliovirus of the corresponding type was obtained by immunizing rabbits with the purified antigen, and after purification by affinity chromatography it was biotinylated. The samples in the wells were incubated with it for 1 h at 37°C, and then washed with the washing buffer. Streptavidin conjugated peroxidase (Sigma, USA) was added to the wells and the samples were incubated for 1 h at 37°C, and then were washed twice with the washing buffer. For detection, we used a tetramethylbenzidine solution (TMB solution, cat. no. T0440, Sigma), which was incubated for 15 min. The results were registered using an iMark microplate spectrophotometer (Bio-Rad, USA). A sample with a known D-antigen content, measured using the international standard NIBSC (National Institute for Biological Standards and Control, UK), was used as a standard sample.

Electrochemical Analysis

Electrochemical measurements were carried out using a PalmSens potentiostat (PalmSens BV, the Netherlands) with the PSTrace software (version 5.8). In the work, we used three-contact electrodes (PGE), obtained by screen printing (ColorElectronics, Russia), with graphite working and auxiliary electrodes, and a silver chloride reference electrode. The diameter of the working electrode was 0.2 cm (area 0.0314 cm^2). All potentials are given relative to the silver chloride reference electrode (ref. Ag/AgCl).

Monopotassium phosphate and sodium chloride (Reakhim, Russia) were used to prepare 0.1 M potassium phosphate buffer, pH 7.4, containing 0.05 M NaCl (PPB). The working electrode was modified with 2 μl of an aqueous dispersion (0.75±0.05) mg/ml of single-walled carbon nanotubes (CNTs) stabilized by carboxymethylcellulose (PGE/CNTs, OCSiAl, Russia). The electrodes were dried for 30 min at room temperature and then pretreated (4 scans in the working potential range). After that 2 μl of an analyte was applied to the modified CNT electrode and incubated for 24 h at 4°C. Then 60 μl of electrolyte PPB buffer was applied to the electrode and measurements were carried out in horizontal mode under aerobic conditions at room temperature. To analyze the electrochemical profiles, the method of square wave voltammetry (SWVA) was used in the potential range (0.0 ∩ 1.2) V with a potential step of 5 mV, an amplitude of 20 mV and a frequency of 10 Hz.

The signal intensity values were processed using the PSTrace program and baseline correction. The electrochemical parameters were the numerical values of the maximum amplitude of the oxidation peak current (in microamperes, μA) and the peak area (in μA×V) at the corresponding potentials (in Volts, V). All electrochemical data presented represent the results of three parallel experiments.

RESULTS AND DISCUSSION

Using electrochemical analysis, protein molecular profiles (signatures) and nucleotide molecular profiles (after DNA and RNA isolation) of cells are recorded [17, 18]. Using comparative electroanalysis of pseudoviral particles it is possible to assess the impact of chemical reagents or inactivation parameters of electron irradiation on polioviruses. The obtained comparative electrochemical profiles of polioviruses reflect altered conformation and, as a consequence, a change in the exposure of viral capsid proteins to the process of electrochemical oxidation of protein amino acid residues.

Electroanalysis of Samples Subjected to Chemical Inactivation

Electrochemical profiles of Sabin strain of poliovirus type 1 (strain LSc 2ab, SI) and type 3 (strain Leon 12a1b, SIII), inactivated by formaldehyde, were studied by the SWVA method (Fig. 1) based on the electrooxidation of amino acid residues of the viral capsid proteins.

The data obtained have been used for calculation of quantitative electrochemical parameters (maximum current amplitude and peak area at corresponding potentials) shown in Table 1.

The electrochemical profile of the SI poliovirus contained two electrooxidation peaks with a maximum at potentials of 0.53±0.01 V and 0.81±0.01 V (Fig. 1, Table 1). Electroanalysis of protein molecular fingerprints of the SIII poliovirus showed a more complex electrooxidation profile as compared to the SI strain (Fig. 1, Table 1). For the SIII, two peaks were recorded with a maximum at a potential of the first peak shifted to the cathode region of 0.42±0.01 V. This indicates a thermodynamically more favorable process of electrooxidation on the electrode, with a wide "shoulder" at 0.53±0.01 V and almost unchanged potential of the second peak at 0.83±0.01 V (Fig. 1, Table 1). There was a relationship between the D-antigen concentration and the intensity of the maximum amplitude of electrochemical oxidation currents of poliovirus strains. The obtained comparative electrochemical parameters of SI and SIII samples (Fig. 1) with different contents of D-antigen at the same concentration of total protein suggest that the recorded signal may be identified as the electrooxidation of the surface capsid proteins in the studied poliovirus samples.

Electroanalysis of Samples Irradiated with Accelerated Electrons

Pilot samples of poliovirus type 1 (strain LSc 2ab, SI) at an initial concentration of 10^{10} particles/ml (10^{10} TCD_{50}/ml) inactivated with accelerated electrons in doses of 5 kGy, 10 kGy, 15 kGy, 25 kGy, 30 kGy at room temperature were analyzed using

Table 1. Comparative electrochemical parameters of Sabin strains of poliovirus type 1 (strain LSc 2ab, SI) and type 3 (strain Leon 12a1b, SIII) inactivated with formaldehyde

**[total protein in samples]/915 μg/ml	Electrochemical parameters (maximum current amplitude, I_{ox}, μA, and oxidation peak area, S, μA×V at corresponding potentials (E_{ox}, V) and *concentration of D-antigen, [D], DU/ml		
		SI	SIII
1 peak	E_{ox}	0.53±0.01	0.42±0.01
	I_{ox}	4.52±0.40	9.51±0.85
	S	0.66±0.17	1.88±0.23
	[D]	88	5401
2 peak	E_{ox}	0.81±0.01	0.83±0.01
	I_{ox}	0.530±0.064	0.526±0.090
	S	0.039±0.006	0.040±0.009

* D-antigen concentration was determined by ELISA; ** total protein in poliovirus samples was determined by the Lowry method.

Table 2. Electrochemical parameters of Sabin strains of poliovirus type 1 (strain LSc 2ab, SI) irradiated at room temperature with doses 5–30 kGy

Sample SI/ quantitative electrochemical parameters	Oxidation potential, E_{ox}, V	Maximum current amplitude, I_{ox}, μA	Peak area, S, μA×V
1	0.51±0.01	6.69±0.55	1.460±0.150
2	0.50±0.01	6.74±0.33	1.450±0.083
3	0.50±0.01	10.74±0.26	2.300±0.004
4	0.49±0.01	13.46±0.69	2.790±0.110
5	0.50±0.01	13.29±1.23	2.820±0.230

1,2,3,4,5 – samples of poliomyelitis virus type 1 (strain LSc 2ab, SI) at concentrations of 2×10^7 particles per electrode inactivated by ionizing radiation at doses of (5, 10, 15, 25, 30) kGy, respectively. The increase in the irradiation dose and change in the conformation of the virus protein shell (structural reorganization of surface proteins) are accompanied by an increase in the electrooxidation signals (I_{ox}, S).

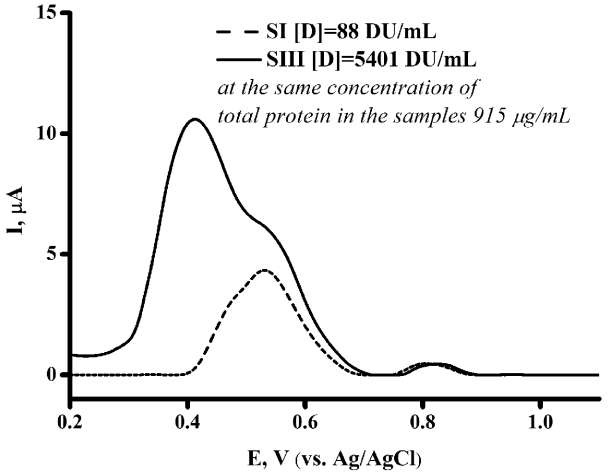

Figure 1. Square-wave voltammograms of Sabin strains of poliovirus type 3, strain Leon 12a1b, (PGE/CNT/SIII, solid curve) and type 1, strain LSc 2ab, (PGE/CNT/SI, dashed curve) with different D-antigen contents at the same concentration of total protein. Square wave voltammograms were baseline corrected.

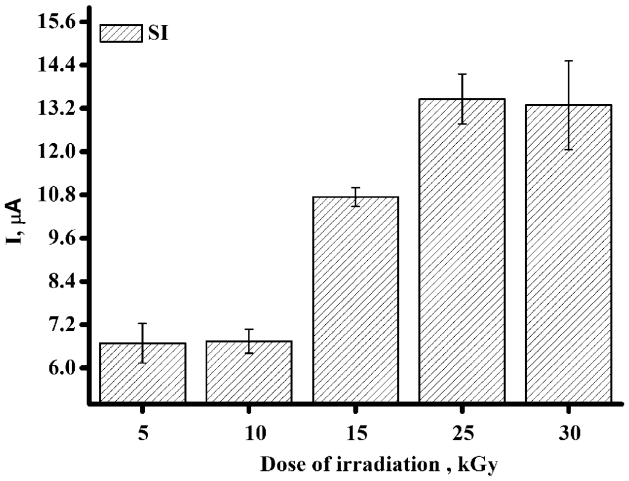

Figure 2. Diagram of the maximum current amplitudes of the SI poliovirus (strain LSc 2ab), inactivated by irradiation at room temperature, depending on the radiation dose of 5 kGy, 10 kGy, 15 kGy, 25 kGy, 30 kGy.

the SWVA method. Electroanalysis of poliovirus samples is based on recording of electrooxidized residues of the capsid surface proteins [25].

Electrochemical profiling of SI virus samples has shown the presence of a broad electrooxidation peak in the potential range (0.3 ∩ 0.8) V with a maximum at 0.50±0.01 V, which is typical for the electrochemical oxidation of protein molecules [17, 25]. Based on the obtained data on the maximum amplitudes of oxidation currents of amino acid residues of the SI capsid surface proteins (Table 2) a diagram was constructed (Fig. 2). According to this diagram,

the increase in current intensity with increasing radiation dose occurs due to changes/disturbances in the conformation of the poliovirus protein shell, which probably became more accessible for electrooxidation at the electrode.

From Table 2 and the diagram in Figure 2 it follows that irradiation with doses of 5-30 kGy leads to the registration of currents in the range of 6.7–13.5 μA at potentials E = 0.50±0.01 V. A significant increase in signals (I_{ox}, S) electrooxidation of poliovirus capsid proteins was detected upon irradiation at doses of 15–30 kGy. This may indicate an increase in the degree of structural reorganization of surface proteins and is confirmed by ELISA data on poor preservation of the D-antigen under these conditions of poliovirus inactivation.

According to ELISA tests, the initial concentration of D-antigen in samples of poliovirus type 1 (strain LSc 2ab) was 740 DU/ml. In samples irradiated with doses of 15–30 kGy, D-antigen was not detected (<1 DU/ml). After irradiation with the dose 5 kGy, the D-antigen content was 50 DU/ml, and after irradiation with the dose 10 kGy, the D-antigen content was 4 DU/ml. Thus, the loss of D-antigen after irradiation with the doses of 5 kGy and 10 kGy without cooling was 93.2% and 99.5%, respectively.

It is known that poliovirus samples can contain not only the immunogenic D-antigen, but also the H(C) antigen, which does not have immunogenic properties. The transformation of the D-antigen into the H(C)-antigen can be provoked by various factors (irradiation under suboptimal conditions, heating at 56°C, 1 h) [4].

Based on the experiments performed, it can be concluded that the conditions for inactivation of poliovirus type 1 (strain LSc 2ab, SI) by accelerated electrons without cooling the samples are not optimal for obtaining pseudoviral constructs suitable for creating vaccines, since the D-antigen is destroyed.

CONCLUSIONS

Comparative electrochemical profiling of poliovirus particles inactivated by formaldehyde and various doses of accelerated electron irradiation was carried out. Based on the data obtained, the recorded signal was identified as the electrooxidation of the surface capsid proteins in the studied poliovirus samples.

Samples of SI poliovirus (strain LSc 2ab) were analyzed at a concentration of 2×10^7 particles on the electrode, inactivated by irradiation with accelerated electrons in doses of 5 kGy, 10 kGy, 15 kGy, 25 kGy, 30 kGy at room temperature. An increase in the irradiation dose to 15–30 kGy apparently causes a change in the conformation of the viral capsid proteins and is accompanied by an increase in the maximum amplitudes of currents and peak areas of electrooxidation of amino acids of the poliovirus capsid proteins (Table 2, Fig. 2) due to their larger accessibility to electrochemical reactions on the electrode. According to the ELISA data, in samples irradiated with doses of 15–30 kGy, D-antigen was not detected (<1 DU/ml). The latter suggests the ineffectiveness of inactivation conditions for samples treated with accelerated electrons to obtain pseudoviral particles suitable for creating vaccines.

Based on the experiments performed in this study, it is reasonable to propose that inactivation by accelerated electrons should be carried under conditions of sample cooling in order to avoid structural rearrangement of the D-antigen and preservation of the immunogenicity of pseudoviral particles for the production of vaccines.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans or the use animals as objects.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ЭЛЕКТРОХИМИЧЕСКОЕ ПРОФИЛИРОВАНИЕ ВИРУСНЫХ ЧАСТИЦ ПОЛИОМИЕЛИТА, ИНАКТИВИРОВАННЫХ ХИМИЧЕСКИМ СПОСОБОМ И ИОНИЗИРУЮЩИМ ИЗЛУЧЕНИЕМ

Л.Е. Агафонова[1]*, В.В. Шумянцева[1,2], Ю.Ю. Ивин[1,3], А.Н. Пиняева[1,3], А.А. Ковпак[3], А.А. Ишмухаметов[3], С.В. Будник[4], Р.С. Чурюкин[4], Д.Д. Жданов[1], А.И. Арчаков[1,2]

[1]Научно-исследовательский институт биомедицинской химии имени В.Н. Ореховича, 119121, Москва, ул. Погодинская, 10; *эл. почта: agafonovaluba@mail.ru
[2]Российский национальный исследовательский медицинский университет имени Н.И. Пирогова, 117997, Москва, ул. Островитянова, 1
[3]Федеральный научный центр исследований и разработки иммунобиологических препаратов имени М.П. Чумакова РАН (Институт полиомиелита), 108819, Москва, поселение Московский, посёлок Института полиомиелита, домовладение 8, корп. 1
[4]"Теокортекс", 108808, Москва, поселение Первомайское, кв-л 328, 34, стр. 6

Электрохимическое профилирование вирусных частиц полиомиелита, инактивированных формальдегидом, продемонстрировало взаимосвязь между концентрацией белков D-антигена и интенсивностью максимальной амплитуды токов образцов полиовирусов. Это позволило идентифицировать сигнал как электрохимическое окисление поверхностных белков полиовируса. Используя регистрацию процессов электроокисления аминокислот, входящих в состав капсидных белков, проведён сравнительный электрохимический анализ вирусных частиц полиомиелита, инактивированных ускоренными электронами в дозах 5 кГр, 10 кГр, 15 кГр, 25 кГр, 30 кГр при комнатной температуре. Увеличение дозы облучения сопровождалось увеличением сигналов электроокисления. Существенный рост сигналов электроокисления белков капсида полиовируса был выявлен при облучении в дозах 15–30 кГр. Полученные данные позволяют предположить, что изменение профиля и рост сигналов электроокисления белков капсида полиовируса связан с увеличением степени структурной реорганизации поверхностных белков и недостаточной сохранностью D-антигена при данных условиях инактивации полиовируса.

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Ключевые слова: электрохимическое профилирование; полиовирус; псевдовирус; полиомиелит; инактивация; функциональная характеристика

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