THE SPR ANALYSIS OF THE INTERACTION OF INACTIVATED POLIOVIRUS VACCINE ATTENUATED STRAINS WITH ANTIBODIES

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The interaction of inactivated poliovirus vaccine strains with oriented antibodies immobilized to protein A via Fc fragments has been investigated. Using an SPR biosensor, the kinetic and equilibrium parameters of the interaction of vaccine attenuated polioviruses of the Sabin strains type 1 and type 2, inactivated by various methods were determined. The strongest interaction was observed between polyclonal antibodies to Sabin strain type 2 poliovirus and Sabin strain type 2 poliovirus inactivated with β-propiolactone, $K_D = 1.04 \cdot 10^{-11}$ M, as well as the interaction of monoclonal antibodies to Sabin strain type 1 poliovirus and Sabin strain type 1 poliovirus inactivated with formaldehyde, $K_D = 1.39 \cdot 10^{-11}$ M. The high-affinity interaction of inactivated vaccine polioviruses of the Sabin strains type 1 and type 2 with immobilized antibodies indicates that the D-antigen retained its structure after virus inactivation with β-propiolactone or formaldehyde.

Keywords: antigen-antibody interaction; surface plasmon resonance; vaccine attenuated poliovirus strains

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INTRODUCTION

Surface plasmon resonance (SPR) is a powerful analytical technique commonly used in the analysis of a wide range of intermolecular interactions. In contrast to various biophysical methods such as nuclear magnetic resonance [1, 2], X-ray crystallography [3], mass spectroscopy [4], isothermal titration calorimetry [5], affinity capillary electrophoresis [6], affinity chromatography [7], using SPR it is possible to perform real time monitoring of molecular interactions. Such SPR-based analysis usually requires small amounts (micrograms) of samples for analysis and it generally does not require any modifications or labeling of immobilized ligands.

Biosensor analysis serves as one of the most important tools for process monitoring or batch integrity testing in vaccine development and production. Biacore biosensors have been used in the development of the following COVID-19 vaccines: mRNA vaccines (Moderna/NIAD, Curevac), DNA vaccine (Inovio), vector non-replicating (J&J), recombinant vaccine (Sanofi/Barda), vaccine based on virus-like particles (Medicago), adjuvant systems (GSK), recombinant peptide vaccine (Epivax).

Biacore was also used to evaluate the antigenicity of hepatitis E vaccines [8, 9] and to measure hemagglutinin concentrations in multivalent influenza vaccines [10]. It should be noted that the implementation of such testing method can accelerate availability of the influenza vaccine to the population by at least 6 weeks. The results have shown that the SPR-based method developed using Biacore is robust and independent of the type of reference antigens (inactivated whole virus, split or vaccine-derived subunit material) and regardless of their use as monovalent or multivalent preparations.

Biacore plays a significant role in the development of HIV vaccines [11]. SPR was also used to assess the presence of conformation-dependent neutralizing epitopes on VLP L1 (virus-like particles derived from the HPV-16 and HPV-18 L1 protein) included in the human papillomavirus (HPV) prophylactic vaccine [12].

Poliomyelitis is a highly contagious viral disease. The poliovirus affects the human nervous system and can cause complete paralysis within hours. For many years, two types of vaccines have been used to prevent poliomyelitis: live oral vaccine (LOV) and inactivated vaccine (IPV). Attenuated Sabin strains (Sabin strain type 1 LSc 2ab, Sabin strain type 2 P712 Ch 2ab, Sabin strain type 3 Leon 12a1b) are used to produce LOV. For a long time, only wild strains (type 1 — Mahoney, type 2 — MEF-1, type 3 — Saukett) were used to produce inactivated vaccine [13].

Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products of Russian Academy of Sciences has developed an inactivated poliovaccine based on the Sabin PoliovacSin strains [14], which is currently used to prevent poliomyelitis in Russia. In the production of IPV based on both wild strains and attenuated strains, formaldehyde is used to inactivate the polio virus [14, 15]. Inactivation of the virus with formaldehyde is a long and labor-intensive stage of the technological process. However, the poliovirus can be also inactivated by β-propiolactone [16], which can potentially reduce the vaccine production time [17]. Pseudovirus particles based on viruses inactivated by various methods can be the basis of a promising platform for the development of new types of vaccines [18].

The antigenicity of IPV is expressed in D-antigen units. Manufacturers and official drug control laboratories use their own antibodies and methods. Studies have shown that there is a problem of standardization of parameters when it is necessary to characterize quantitatively the IPV samples. This is true both for the assessment of D-antigen concentration [19] and for the assessment of the immunogenic properties of vaccines [20]. Kersten et al. [21] have demonstrated that Biacore can be used to determine the D-antigen content of IPV. More recent studies [22, 23] described a Biacore biosensor-based method for assessing D-antigen concentration. The results were comparable with those of enzyme-linked immunosorbent assay (ELISA). This indicates that the SPR biosensor is an alternative means for assessing the efficacy of IPV. Unlike ELISA, the capture antibodies are monoclonal and detection antibodies or conjugates are not required since the biosensor signal is mass-dependent. Inter-laboratory variations can be reduced due to the automated nature of the biosensors. Using biosensors it is possible to perform both rapid characterization of antibodies and quick screening and selection of optimal antibodies.

The aim of this work was to perform a SPR-biosensor based-study of the affinity interaction between attenuated polioviruses of the Sabin strains type 1 and type 2 inactivated in various ways and antibodies.

MATERIALS AND METHODS

The analysis of the interaction between attenuated polioviruses of the Sabin strains type 1 and type 2 and antibodies was performed using a Biacore X-100 optical biosensor (Cytiva, USA), operating on the surface plasmon resonance (SPR) effect. The biosensor signal has been recorded in resonance units RU (1 RU corresponds to the binding of 1 pg of protein on the surface of the optical chip). Chips with shortened dextran CM3 (Cytiva), recommended for working with viral particles, were used [23].

Reagents

The following reagents were obtained from Cytiva: HBS-N (150 mM NaCl, 10 mM HEPES, pH 7.4); 10 mM acetate buffer, pH 4.5; a reagent kit for covalent immobilization of proteins via primary amino groups (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide-HCl, N-hydroxysuccinimide, 1 M ethanolamine-HCl, pH 8.5), 10 mM glycine buffer, pH 1.5. Protein A was obtained from IMTEK (Russia). The monoclonal antibodies α-Polio1 Mab234 (Mab1), α-Polio2 Mab1050 (Mab2), and α-Polio3 Mab520 (Mab3) to the Sabin vaccine strains of poliovirus types 1, 2 and 3, respectively, were obtained from the National Institute for Biological Standards and Control (UK). The D-antigen concentration in the Sabin vaccine strains of poliovirus types 1 and 2 inactivated with β-propiolactone (SI bpl, SII bpl) or formaldehyde (SI form, SII form), as well as inactivated with formaldehyde followed by dialysis (SI form (d), SII form (d)) was determined by ELISA at the Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products of Russian Academy of Sciences (Chumakov FSC R&D IBP RAS) [24].

Production of Polyclonal Antibodies

Polyclonal antibodies to the Sabin strain of poliovirus type 2 (Pab2) were prepared using the serum of immunized Soviet Chinchilla rabbits. The animals were immunized three times with an interval of 21 days. For immunization, CsCl gradient-purified poliovirus type 2 antigens were used at a concentration of 300–1260 μg/ml. The presence of antibodies to the D-antigen was tested in neutralization assays performed using Vero cells. The IgG fraction of antibodies was obtained from sera with a titer of at least 1:2000. Purification was performed using affinity chromatography on HiTrap, Protein G HP columns (Cytiva). The target fraction was eluted with 0.1 M glycine-HCl buffer (pH 2.7) and pH value was immediately adjusted to 7.0 by adding 1 M Tris-HCl (pH 9.0). For further use, purified antibodies were transferred to 0.1 M carbonate-bicarbonate buffer (Sigma, USA) or phosphate buffer solution, pH 7.2 (Chumakov FSC R&D IBP RAS) by dialysis.

Inactivated Virus Preparation

Polioviruses of the Sabin strains type 1 and type 2 were obtained by infecting a monolayer culture of Vero cells. The viral suspension was filtered, concentrated [14, 25], and chromatographically purified [13, 26]. Inactivation with formaldehyde was carried out as described previously [24]. Additionally, the inactivated virus was dialyzed against phosphate-buffered saline (pH 7.2) using centrifugal concentrators with a 30 kDa membrane (Jet Biofill, China), according to the manufacturer's recommendations. For inactivation with β-propiolactone (Molecula, USA), the viral suspension was incubated

under stirring for 48 h at 2–8°C with the inactivating agent used at a concentration of 0.2% (v/v). Purification was carried out by sequential procedures of filtration, concentration, and chromatography as described previously [13, 14, 25, 26].

Protein A Immobilization on the CM3 Chip

Protein A immobilization in both channels of the optical biosensor chip was carried out by forming covalent bonds between carboxyl groups on the CM3 optical chip surface and amino groups of the protein. The carboxyl groups of the chip were activated by injecting a mixture of 0.2 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide-HCl/0.05 M N-hydroxysuccinimide for 7 min at a flow rate of 5 μl/min. Injection of protein A solution (100 μg/ml) in 10 mM acetate buffer (pH 4.5) was performed at a flow rate of 5 μl/min for 7 min. Unreacted activated groups were blocked with 1 M ethanolamine hydrochloride solution (pH 8.5) for 3 min at a flow rate of 5 μl/min. HBS-N was used as a buffer.

Antibody Immobilization on the CM3 Chip

Monoclonal antibodies α-Polio1 Mab234 (Mab1) or α-Polio2 Mab1050 (Mab2), or α-Polio3 Mab520 (Mab3), or polyclonal antibodies (Pab2) in a 1:20 dilution were injected into the working channel of the biosensor at a flow rate of 5 μl/min for 3 min. This was followed by injection of the working buffer (HBS-N).

Interaction of the Sabin Type 1 and Type 2 Polioviruses, Inactivated with β-Propiolactone or Formaldehyde, with Antibodies

SI bpl, SII bpl, SI form, SII form, SI form (d), SII form (d) were injected 5 min after injection of Mab1 or Mab2, or Pab2 antibodies, respectively, at a rate of 5 μl/min for 3 min. The interaction of inactivated poliovirus vaccine strains with antibodies was studied in the range of D-antigen concentrations from 0.05 nM to 5 nM. Registration of interactions of inactivated particles of Sabin poliovirus vaccine strains types 1 and 2 with immobilized antibodies was performed in real time by injecting samples through the control (without antibodies) and working channels of the optical biosensor. The resulting sensorgrams represented the difference in signals in the working and control channels. The chip surface was regenerated with glycine buffer, pH 1.5, at a flow rate of 30 μl/min for 30 s after each cycle of interaction of antibodies with immobilized protein A and subsequent interaction of inactivated strains with antibodies. The experimental curves were processed using the Biaevaluation 4.1.1 program.

RESULTS AND DISCUSSION

In the SPR system, one of the interacting biomolecules (ligand) is immobilized on the surface of the sensor chip. Two approaches are used to immobilize ligands: covalent immobilization and non-covalent capture. In the first case, functional groups on the chip surface react with the corresponding functional group of the ligand (amino, carboxyl, sulfhydryl groups, etc.) to form stable bonds [27, 28]. In the latter case, capture involves the use of ligands with a high-affinity tag [29, 30] or antibodies [23, 31], as well as proteins A, G [32]. We have chosen the method of oriented immobilization of antibodies to protein A via Fc fragments [32]. Using this method it is possible to remove immobilized antibodies and the antigen bound to them and thus to regenerate protein A for the next interaction. Protein A was immobilized in the working and control channels to exclude non-specific interaction of inactivated poliovirus vaccine strains with this protein. 1530 RU and 1120 RU were immobilized in the working and the control channels, respectively. Then, Mab1 or Mab2, or Pab2 antibodies were immobilized through protein A in the working channel of the biosensor. In contrast to these antibodies, we did not observe Mab3 interaction with protein A. It appears that they could not be immobilized using this method.

The SPR-based analysis included the study of: (a) interaction of SI bpl, SI form, SI form(d) samples with immobilized Mab1 (Fig. 1); (b) interaction of SII bpl, SII form, SII form(d) with immobilized Mab2 (Fig. 2); (c) interaction of SII bpl, SII form with immobilized Pab2. The interaction of the analyte (i.e. D-antigen) with antibodies was monitored in real time, and the specific binding was reflected by the change in the magnitude of the biosensor response before and after antigen injection (Figs. 1, 2). In addition, we did not observe the interaction of the inactivated attenuated poliovirus of Sabin strains type 2 with Mab1. As a result of processing the experimental curves, the association rate constant (k_a), dissociation rate constant (k_d), equilibrium dissociation constant K_D, and affinity K_A constant were obtained (Table 1).

The K_A values of antigen-antibody complexes decreased in the following order: (Pab2/SII bpl) ≈ (Mab1/SI form) > (Pab2/SII form) > (Mab2/SII bpl) > (Mab1/SI form(d)) > (Mab1/SI bpl) > (Mab2/SII form) ≈ (Mab2/SII form(d)). The interaction of polyclonal antibodies to the Sabin strain of poliovirus type 2 with this strain of poliovirus inactivated with β-propiolactone (SII bpl), as well as the interaction of monoclonal antibodies to the Sabin strain of poliovirus type 1 and this strain of poliovirus inactivated with formaldehyde (SI form) demonstrated the highest affinity.

CONCLUSIONS

SPR-based analysis of interaction of inactivated attenuated poliovirus of Sabin strains type 1 and type 2 with the corresponding antibodies was performed

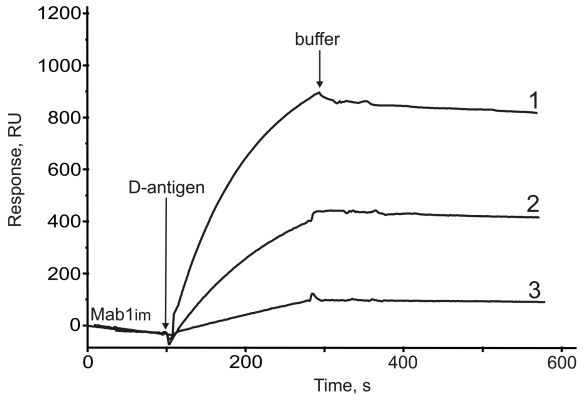

Figure 1. Sensograms of the interaction of inactivated particles of the Sabin vaccine strain of poliovirus type 1 with Mab1. 1 – SI form, D-antigen concentration 1.42 nM; 2 – SI form(d), D-antigen concentration 4.2 nM; 3 – SI bpl, D-antigen concentration 0.12 nM.

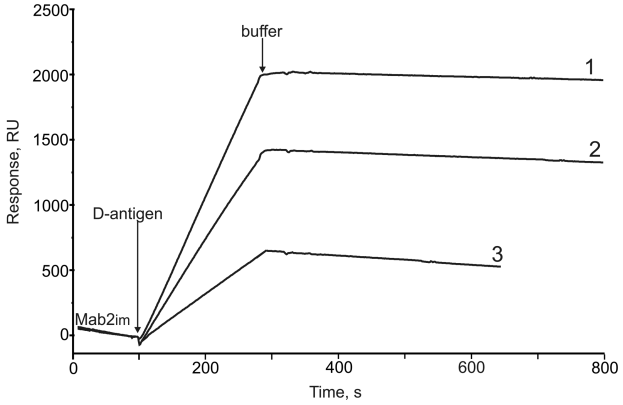

Figure 2. Sensograms of the interaction of inactivated particles of the Sabin vaccine strain of poliovirus type 2 with Mab2. 1 – SII bpl, D-antigen concentration 0.08 nM; 2 – SII form(d), D-antigen concentration 0.46 nM; 3 – SII form, D-antigen concentration 0.08 nM.

Table 1. Kinetic (k_a, k_d) and equilibrium parameters (K_D, K_A) of the antigen-antibody interaction

Complex	k_a, $M^{-1}\cdot s^{-1}$	k_d, s^{-1}	K_D, M	K_A, M^{-1}
Mab1/SI bpl	$5.37\cdot10^{5}$	$2.15\cdot10^{-4}$	$4.01\cdot10^{-10}$	$2.49\cdot10^{9}$
Mab1/SI form	$6.10\cdot10^{6}$	$8.52\cdot10^{-5}$	$1.39\cdot10^{-11}$	$7.19\cdot10^{10}$
Mab1/SI form (d)	$1.58\cdot10^{6}$	$2.46\cdot10^{-4}$	$1.56\cdot10^{-10}$	$6.41\cdot10^{9}$
Mab2/SII bpl	$5.22\cdot10^{5}$	$2.68\cdot10^{-5}$	$5.13\cdot10^{-11}$	$1.95\cdot10^{10}$
Mab2/SII form	$2.70\cdot10^{5}$	$1.59\cdot10^{-4}$	$5.91\cdot10^{-10}$	$1.69\cdot10^{9}$
Mab2/SII form (d)	$2.27\cdot10^{5}$	$1.75\cdot10^{-4}$	$7.71\cdot10^{-10}$	$1.30\cdot10^{9}$
Pab2/SII bpl	$3.39\cdot10^{7}$	$3.52\cdot10^{-4}$	$1.04\cdot10^{-11}$	$9.61\cdot10^{10}$
Pab2/SII form	$1.15\cdot10^{7}$	$2.37\cdot10^{-4}$	$2.06\cdot10^{-11}$	$4.85\cdot10^{10}$

using oriented antibodies immobilized to protein A via Fc fragments. Comparable affinity of the interaction of vaccine strains of poliomyelitis virus inactivated by different methods (β-propiolactone or formaldehyde) with antibodies was revealed. The high-affinity interaction of inactivated attenuated poliovirus of Sabin strains type 1 and type 2 with immobilized antibodies indicates that the D-antigen retained its structure after virus inactivation.

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COMPLIANCE WITH ETHICAL STANDARDS

The experiments were conducted in compliance with generally accepted norms of humane treatment of laboratory animals. The study was performed in accordance with the Order of the Ministry of Health of the Russian Federation No. 199n of April 1, 2016 “On Approval of the Rules of Good Laboratory Practice” and Directive 2010/63/EU of the European Parliament and the Council of the European Union of September 22, 2010 on the protection of animals used for scientific purposes.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SPR-АНАЛИЗ ВЗАИМОДЕЙСТВИЯ ИНАКТИВИРОВАННЫХ ВАКЦИННЫХ АТТЕНУИРОВАННЫХ ШТАММОВ ВИРУСА ПОЛИОМИЕЛИТА С АНТИТЕЛАМИ

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Исследовали взаимодействие инактивированных вакцинных аттенуированных штаммов вируса полиомиелита с антителами, ориентированно иммобилизованными за Fc-фрагмент через протеин А. С помощью SPR-биосенсора определены кинетические и равновесные параметры взаимодействия вакцинных аттенуированных штаммов вируса полиомиелита типа 1 и 2 (штаммов Сэбина), инактивированных различными способами. Наиболее прочным было взаимодействие поликлональных антител к вирусу полиомиелита типа 2 (штамм Сэбина), со штаммом Сэбина вируса полиомиелита типа 2, инактивированным β-пропиолактоном, K_D = $1{,}04 \cdot 10^{-11}$ М, а также взаимодействие моноклональных антител к вирусу полиомиелита типа 1 (штамм Сэбина) со штаммом Сэбина вируса полиомиелита типа 1, инактивированным формальдегидом, K_D = $1{,}39 \cdot 10^{-11}$ М. Высокоаффинное взаимодействие инактивированных вакцинных штаммов Сэбина вируса полиомиелита типов 1 и 2 с иммобилизованными антителами свидетельствует о том, что D-антиген сохранил свою структуру после инактивации вируса β-пропиолактоном или формальдегидом.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: взаимодействие антиген-антитело; поверхностный плазмонный резонанс; вакцинные аттенуированные штаммы вируса полиомиелита

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