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Method for the simultaneous assay of the different poliovirus types using surface plasmon resonance technology

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ABSTRACT

The inactivated polio vaccine (IPV) contains viral samples that belong to serotypes 1, 2 and 3. We report here a surface plasmon resonance (SPR)-based technique that permits the simultaneous assay of the individual viral types in the IPV as well as in different bulk intermediates from the industrial vaccine production process. Monoclonal antibodies specific to each of the 3 viral types along with a negative control antibody are captured via an anti-IgG antibody on the surface of the 4 flow cells of the SPR instrument. The viral samples are then injected over these flow cells and the increase in resonance units as a result of virus binding is measured. The method was calibrated by an analysis of the European Working Standard (EWS) for poliovirus vaccines. We show that the antibodies used recognize viruses with functional affinities in the picomolar range permitting an effective capture of the antigen. In addition we demonstrate that the antibodies are highly specific to a given virus type and that the heat induced destruction of the D-antigen abolishes antibody recognition entirely. The technique was found to be reproducible and robust and its response was linear to the antigen concentration. Due to the rapidity of analysis this technique permits an almost real-time follow-up of the industrial production process and may present an alternative to the established ELISA assay for the analysis of the intermediates and the final product.

1. Introduction

Polioviruses (PVs) are the causative agents of poliomyelitis. The virus is transmitted through contaminated food and water, and multiplies in the intestine, from where it can invade the nervous system. PVs cause the destruction of motor neurons in the central nervous system. Due to an extensive vaccination campaign the incidence of this disease has been reduced drastically [1]. However, the objective to eradicate this disease by the year 2000 has not been met [2,3].

PVs belong to the genus enterovirus which forms part of the picornaviruses family. PVs are small, non-enveloped particles. The surface of the virion is composed of 60 copies of each of the four capsid proteins. PVs comprise three serotypes [4] that are all able to cause poliomyelitis. The 3D structure of viruses belonging to these serotypes has been solved [5–7] and their comparison revealed that structural differences among the three PV serotypes occur primarily in the loop regions of the viral capsid proteins [7].

The successful fight against poliomyelitis is due to the use of two different vaccines. The formalin-inactivated PV vaccine (IPV), developed by Salk et al. [8], was the first vaccine to be licensed followed by the oral PV vaccine (OPV) developed by Sabin [9].

The last decade saw an increase in the use of IPV as compared to OPV which was due to the excellent safety and efficacy record of IPV as well as cost-effective protocols for its production. Research at Sanofi Pasteur has contributed significantly to this gain of importance of the IPV vaccine. The initial version of the IPV was based on viral cell cultures on primary monkey kidney cells and Montagnon et al. [10] developed a procedure for viral culture on Vero cells.

The IPV is formulated as a trivalent product containing a representative virus isolate of each serotype. All three viral serotypes contain the D-antigen, which induces protective antibodies. After mild heating and other treatments the antigens are rapidly converted to the H or C forms that are not protective [11]. The antigenic structure of PVs consists of at least four different antigenic sites [4,12,13] and the D-antigen content represents the combined activity of multiple epitopes [14]. The potency of IPV vaccines is

Abbreviations: EDC, 1-ethyl-3-(3-dimethylpropyl)-carboiimide; DU, D-antigen unit; ELISA, enzyme-linked immunosorbent assay; EWS, European Working Standard; HBS-EP, hepes-buffered saline containing EDTA and the P20 detergent; Ig, immunoglobulin; IPV, inactivated polio vaccine; Mab, monoclonal antibody; MCK, multi-cycle kinetic; PV, poliovirus; NHS, N-hydroxysuccinimide; OPV, oral polio vaccine; SCK, single-cycle kinetic; SPR, surface plasmon resonance; PV, poliovirus; RU, resonance unit.

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determined by measuring the D-antigen content. Several ELISA methods have been developed using polyclonal or monoclonal antibodies [14–17].

The last two decades were marked by major technological advances in the study of molecular interaction, exemplified by appearance of the surface plasmon resonance technology (SPR), high sensitivity microcalorimetry and atomic force spectroscopy. The SPR technology is based on the measurement of changes in the refractive index caused by ligand binding to an immobilised molecule [18]. Advantages of SPR include the facts that only a small amount of sample is necessary, that analysis occurs in real time and that there is no need to label the interactants. SPR has been successfully used to characterize many different vaccines [19–22]. In addition, some SPR instruments allow to define 4 flow cells on the sensor chip surface which permit simultaneous binding studies to different immobilised molecules. We describe here a SPR-based method for the simultaneous quantification of the three different PV serotypes from vaccine samples.

2. Materials and methods

2.1. Materials

Mabs: The anti-mouse IgG (BR-1008-38, GE Healthcare) was covalently immobilised on the sensor chip as the capture antibody. A murine monoclonal anti-hapten antibody (NeoMarkers Inc., Fremont, CA, reference NC-748-P) was captured on one flow cell which served as a negative control. The following purified Mabs were obtained from the National Institute for Biological Standards and Control (NIBSC): anti-type 1 PV Mabs 234, 236, 237 and 423 as well as the anti-type 2 PV Mabs 1121, 1037 and 1050. The generation and characterization of these Mabs have been reported by Minor et al. [4]. The hybridomes of the 3 anti-type 3 PV Mabs were generated by Sanofi Pasteur and the corresponding antibodies were produced and purified by Biotem (Le Rivier d'Apprieu, France): 1E3-3G4, 4B7-1H8-2E10 and 4H8-3A12-2D3 using protein A chromatography.*Viral samples*: Samples were provided by the Production Department of Sanofi Pasteur. They included viral type 1 (Mahoney strain), type 2 (MEF-1 strain) and type 3 (Saukett strain) samples and the trivalent vaccine. In addition the European Working Standard (EWS, reference P2160000, European Council, Strasbourg, France) for the trivalent polio vaccine was analysed, of which batch 2,1 was used, containing 320, 67 and 282 units of D-antigen/ml for viral types 1, 2 and 3, respectively.

2.2. Surface plasmon resonance measurements

For kinetic experiments a Biacore T200™ instrument (GE Healthcare) was used. CM5 sensor chips (BR-1005-30) were activated (NHS/EDC) following the instructions provided by the manufacturer. Anti-mouse IgG capture antibodies at a concentration of 35 μg/ml in 10 mM acetate buffer pH 4.5 (BR-1003-50) were injected on the sensor chip. This process leads to covalent antibody immobilisation (around 8000 RU) by an amine coupling mechanism. Subsequently, virus-type specific antibodies were diluted between 0.2 and 1 μg/ml in HBS-EP buffer and addressed on the different flow cells at a flow rate of 10 μl/min during 15 s causing an increase by 10–30 RU. After a washing step with HBS-EP, 5 concentrations of each virus type were injected at a flow rate of 70 μl/min. The complexes formed on the surface of the sensor chip were dissociated by the injection of 2.7 μl of 10 mM glycine, pH 1.5 (BR-1003-54) at a flow rate of 10 μl/min either between viral sample injections (MCK) or at the end of the cycle (SCK). Kinetic parameters analysis was performed using both multi-cycle kinetic (MCK) and single-cycle kinetic (SCK) methods.

For assay experiments a Biacore 3000™ instrument (GE Healthcare) was used. The assay was developed in mass transport limitation conditions. CM5 sensor chip (BR-1000-14) was activated (NHS/EDC) following the instructions provided by the manufacturer. Seventy microliters of anti-mouse IgG capture antibodies at 35 μg/ml in 10 mM acetate buffer pH 4.5 (BR-1003-50) were injected on the sensor chip at a flow rate of 5 μl/min, which gave a signal increase by around 13,000 RU. Subsequently, virus-type specific antibodies were diluted to 50 μg/ml in HBS-EP buffer and addressed on the different flow cells at a flow rate of 5 μl/min which resulted in a signal increase by 1100 to 1600 RU depending on the Mabs (for details see Supplementary Table 3). Antibodies specific to the three viral types were found to be captured with similar efficiencies. After a washing step with HBS-EP, diluted viral samples were injected on the chip at a flow rate of 30 μl/min and the increase in RU was measured over 50 s. From these data the increase in RU/time was calculated. The complexes formed on the surface of the sensor chip were dissociated as detailed above.*Calibration of the assay*: Five serial dilutions of the EWS were prepared which cover a concentration range of 6.25–100 D-antigen units/ml (DU/ml) for type 1, 1.3–21 DU/ml for type 2 and 5.5–88 DU/ml for type 3. These dilutions of EWS were then injected on the assay surface. Calibration curves were obtained by plotting the slope of RU (following correction using binding data to negative control antibody and buffer alone) caused by the binding of individual PV types to their cognate antibodies against the D-antigen concentration of the EWS standard. Data were fitted by linear regression and the resulting calibration curves were then used to calculate the D-antigen titers.

3. Results

3.1. Mabs recognize with picomolar functional affinity and high specificity the different types of PV

A prerequisite for the use of the Mabs for viral assay purposes resides in their capacity to effectively capture the antigen. To address this issue the functional affinities of ten different antibodies for their target antigen were determined. The PV is a multi-epitope antigen and therefore affinity measurements reflect more avidity than true affinity. We therefore use the term functional affinity. The different Mabs were captured on the sensor chip through an anti-IgG Mab. Different concentrations of viral samples were then injected (direct immobilisation of the anti-poliovirus Mabs on the sensor chip was not possible due regeneration issues). Sensorgrams presented in Fig. 1 and Supplementary Fig. 1 were obtained and their analysis resulted in the calculation of the association and dissociation rate constants, which are shown in Table 1 and Supplementary Table 1. From these constants the equilibrium constant of binding was determined. Even though we were not in a classical 1–1 interaction model, the Langmuir model gave the best fit. The K_D values for these 10 antibodies ranged from 17 pM to 1.1 nM, indicative of a very tight binding. The K_D values in the lower pM range correspond to the lower affinity limit of the SPR instrument used. These results show that antigens are captured effectively, permitting a use for analytical quantification purposes.

Another prerequisite is to assess the specificity of the Mabs for the individual viral types. To this end binding experiments of the 10 Mabs with any of the three virus types were conducted (Fig. 2, Supplementary Fig. 2). Efficient viral binding was observed when experiments were conducted with the cognate antibodies listed in Table 1. However, in all cases the exposure of any of the Mabs to the remaining two viral types did not result in any binding interaction (Fig. 2, Supplementary. Fig. 2). It can be concluded that the Mabs used are highly specific for their cognate viral type.