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Surface plasmon resonance for monitoring the interaction of *Potato virus Y* with monoclonal antibodies

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ABSTRACT

Surface plasmon resonance (SPR)-based biosensors have been widely utilized for measuring interactions of a variety of molecules. Fewer examples include higher biological entities such as bacteria and viruses, and even fewer deal with plant viruses. Here, we describe the optimization of an SPR sensor chip for evaluation of the interaction of the economically relevant filamentous *Potato virus Y* (PVY) with monoclonal antibodies. Different virus isolates were efficiently and stably bound to a previously immobilized polyclonal antibody surface, which remained stable over subsequent injection regeneration steps. The ability of the biosensor to detect and quantify PVY particles was compared with ELISA and RT-qPCR. Stably captured virus surfaces were successfully used to explore kinetic parameters of the interaction of a panel of monoclonal antibodies with two PVY isolates representing the main viral serotypes N and O. In addition, the optimized biosensor proved to be suitable for evaluating whether two given monoclonal antibodies compete for the same epitope within the viral particle surface. The strategy proposed in this work can help to improve existing serologic diagnostic tools that target PVY and will allow investigation of the inherent serological variability of the virus and exploration for new interactions of PVY particles with other proteins.

Surface plasmon resonance (SPR) is one of the leading techniques for studying molecular interactions [1]. SPR biosensors measure the changes in the refractive index that occur upon binding of an analyte to its specific ligand immobilized on a sensor chip surface. Principal advantages of SPR in comparison to other techniques are direct real-time measurement of the interaction among label-free molecules, determination of the association and dissociation rates of the binding process, and the small amounts of sample used in the assay (usually nanomolar concentrations). Despite SPR biosensors having been typically applied to the study of proteins or peptides (i.e., pharmaceutically active compounds, receptors, and antibodies), an increasing number of studies dealing with higher order biological entities such as viruses and bacteria can also be found in the literature [2,3]. In contrast, only a few examples of studies describing the application of SPR to viruses of plant origin are available. The following plant viruses are among the few that have been involved in SPR studies: *Apple stem pitting virus* [4], *Tomato leaf curl New Delhi virus* [5], *Tobacco mosaic virus* [6–12], *Cowpea mosaic virus* [7], and *Lettuce mosaic virus* [13]. The main focus in these works was one or more among the following: virus detection [4,6], evaluation of the virus–antibody interaction [5,7–12], or assessing epitope interference/overlapping [13]. In none of these works were all these features confronted in the same study using the same virus system.

Potato virus Y (PVY) is a member of the genus *Potyvirus* [14] that infects species from the Solanaceae family, such as potato, tobacco, tomato, and pepper [15], using aphids as vectors [16]. The PVY particle is structured as a flexuous filament of 740 × 11 nm dimensions, consisting of a polymer of coat protein units encapsidating the genomic RNA molecule. The potato tuber necrosis ringspot disease caused by certain PVY variants results in a notable reduction in tuber quality that can lead to effective losses of up to 100% [17]. Recently PVY has been classified into the group of the 10 most

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important viruses from economic and scientific points of view [18]. A variety of molecular methods have been developed to characterize, quantify, and detect the various circulating PVY variants [19,20]; however, antibody-based serologic tests (enzyme-linked immunosorbent assay, or ELISA) are still the most widely used method for fast and high-throughput detection and characterization of the virus. Using proper specific antibodies, the two main PVY serotypes, N and O, can be distinguished [21,22] and this distinction can serve as a preliminary assessment of the virus impact on potato and tobacco crops. The specificity and sensitivity of the assay depends on the quality of the antibody or antibody cocktail used. To overcome the natural virus evolution [23,24], the antibodies intended to form part of a successful cocktail should preferably target independent epitopes. In this way, mutations in one epitope resulting in the impairment of the interaction between the surface of the virus and one antibody, would be compensated for with other antibodies that react to other epitopes. The main features enabled by SPR (detection of viruses, kinetic evaluation of the antibody–virus interactions, and evaluation of epitope interference) could notably help in improving the selection of antibodies used in research and diagnostics of PVY virus. In addition, SPR biosensors may allow the measurement of new interactions among PVY and other molecules rather than just antibodies.

The objective of this study was to optimize an SPR-based biosensor to enable detailed evaluation of the interactions between PVY and monoclonal antibodies (mAbs). For this purpose we first tested if the filamentous PVY particles can be captured onto a previously formed polyclonal antibody (pAb) surface covalently immobilized on a carboxymethyl 5 (CM5) sensor chip. The stability of the capture over time was monitored and the dependence of the SPR response on the applied PVY dose was compared with already established techniques such as ELISA and RT-qPCR. Next, two PVY isolates, representing the two main viral serotypes (N and O), were captured and used to study the specificity and kinetic parameters of a group of mAbs. Finally, the possibility of using the optimized biosensor to explore if two different mAbs compete for the same viral epitope was also explored.

Material and methods

Viral material

Three PVY isolates from a collection at INRA–Le Rheu were used. Two reference isolates were the Irl isolate (PVY^{O} strain) and the NZ isolate (PVY^{NTN} variant), which have been previously described as isolates of serotype O and serotype N, respectively [25,26]. The third isolate, 08Fr29 (PVY^{N} strain), was a field isolate, characterized as serotype N using a commercial PVY^{N}-specific mAb (Bioreba, Reinach, Switzerland). PVY isolates were propagated in *Nicotiana tabacum* cv. Xanthi by mechanical inoculation as described previously [27]. Inoculated plants were maintained in the greenhouse at 20 ± 2 °C for 15–20 days before being sampled for virus purification. PVY isolates were purified from infected tobacco leaves using the method of Leiser and Richter [28] modified as reported by Rupar et al. [29]. The purification reached different yields depending on the PVY isolate. The yield was 7.47 mg/kg of fresh tobacco leaves for Irl isolate, 8.3 mg/kg for NZ, and 6.44 mg/kg for 08Fr29. The concentration (mg/ml) of virus in each purified suspension was calculated spectrophotometrically as described previously [29].

Serological reagents

The bovine serum albumin (BSA)-free universal anti-PVY pAb used as capture molecule was from Bioreba. PVY serotype N-specific mAb, N^{acw}, was kindly supplied by Agroscope-Changins (ACW; C. Balmelli). N SASA and O/C SASA mAbs, specific for the N and O serotype, respectively, were kindly supplied by Craig Douglas from Science and Advice for Scottish Agriculture (Edinburgh, Scotland, UK). The mAbs Y123 and 16E4 were obtained from INRA–Le Rheu Laboratory (INRA/FN3PT) and detect both PVY serotypes O and N ([22] and L. Glais, personal communication). Another mAb was obtained from the commercial Y^{N} assay from Adgen (Neogen Europe, Scotland, UK). This particular mAb, denoted as Y^{all}, is the coating antibody used in the mentioned assay and has confirmed reactivity to both N and O serotypes (Rob Langley, Neogen Europe, personal communication). When necessary, BSA was removed from the mAb solution by affinity chromatography using a HiTrap Protein G HP column (GE Healthcare).

Surface plasmon resonance

Immobilization

SPR analysis was performed using a Biacore T100 (GE Healthcare) and a Series S sensor chip CM5 (GE Healthcare). Virus isolates were immobilized by using a capture approach. The chip was docked and primed twice with SPR running buffer (10 mM Na_2HPO_4, 2 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4, 0.005% P20 detergent). BSA-free polyclonal anti-PVY antibody (Bioreba) was covalently attached to the surface of the chip by amine coupling, after activating the surface with a 7-min pulse of a 1/1 (v/v) mixture of 0.4 M 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide and 0.1 M *N*-hydroxysuccinimide. Such pAb is described by the manufacturer to bind PVY strains belonging to both N and O serotypes. The next step in the immobilization procedure varied depending on the experiments that followed. For the virus titration experiments, the pAb was injected only over the second flow cell while the first flow cell was left empty and served as a control for nonspecific binding of viruses to the dextran matrix of the chip. The limit of detection of the virus binding to the polyclonal surface was defined as the virus concentration that achieved a response value higher than the buffer injection +2× SD. For the titration of mAbs and competition assays, both flow cells were covered with pAb. PVY particles were captured only on one of the flow cells (i.e., flow cell 2), while the other (flow cell 1) was used as a reference to account for nonspecific binding of the mAbs to the pAb surface. Finally, 1 M ethanolamine, pH 8.5, was injected for 7 min over both flow cells to block the remaining surface.

Titration of Irl isolate

Irl isolate was injected in a concentration series (0, 0.15, 0.312, 0.624, 1.25, 2.5, 5 10, 20, and 40 μg/ml) for 300 s and the dissociation was monitored for 420 s. The injection of a virus at 5 μg/ml was repeated at the end of the experiment to contrast the stability of the surface. The surface was regenerated with a 5-s injection of 10 mM glycine, pH 2.0. The flow rate was 30 μl/min.

Titration of mAbs

PAb on the surface of flow cell 2 served as a capture molecule to immobilize virus isolates Irl or NZ. The virus was diluted in the running buffer and injected over flow cell 2 for 300 s to obtain a binding response of around 50–100 response units (RU). The first flow cell was covered only with pAb and served as a reference cell to correct for the refractive index changes and nonspecific binding. The injection of the virus was followed by titrations of various antibodies. MAbs were diluted in the running buffer to various final concentrations (1.56–800 nM). A regeneration step including two 4-s injections of 10 mM glycine, pH 2.0, removed the virus and the mAb from the capturing pAb. The single-cycle experiment was additionally used to evaluate kinetic data for the interaction of antibody Y123 to both virus isolates. The single-cycle titration

consisted of a sequential injection of increasing concentrations of mAb (0.5, 1.5, 4.5, 13.5, 40.5 nM) without regeneration between injections. For the immobilization of the pAb, virus captures, and mAb titrations the flow rate was set to 10 μl/min. The flow rate during regeneration injections was 30 μl/min. All experiments were performed at 25 °C. The obtained sensorgrams were globally fitted to 1:1 and bivalent binding models by using Biacore T100 Evaluation software to obtain association and dissociation rates and affinity constants. We report averages ± SD for each derived parameter from two or three independent titrations.

Competition assays

Virus isolates (08Fr29 or Irl) were captured onto the pAb surface to approximately 40 RU, followed by an 8-min mAb injection. Regeneration was as described above. For each pair of mAbs investigated, three injections were performed: two of each mAb separately at a 50 nM concentration, and an injection of both mAbs together at a 50 nM concentration each. In another experiment the injection of one antibody was followed by a subsequent 8-min injection of a second antibody.

Real-time RT-qPCR

Samples applied to RT-qPCR consisted of Irl virus isolate dilutions in SPR running buffer. Before the RT-qPCR measurements, RNA was extracted from 140 μl of each sample using a QIAamp viral RNA mini kit (Qiagen, USA) according to the manufacturer's instructions. The detection of PVY RNA was done using a specific PVY-Uni RT-qPCR assay developed by Kogovšek et al. [19]. All samples were applied to RT-qPCR in triplicate. AgPath-ID One-Step RT-PCR reagents (Life Technologies, Carlsbad, CA, USA) and an Applied Biosystems 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) were used. Amplification conditions were 10 min at 48 °C, 10 min at 95 °C, and 45 cycles of 15 s at 95 °C and 1 min at 60 °C. Quantification cycles (C_q) for each sample were determined using SDS version 2.3 software (Applied Biosystems). A result was considered positive if at least two reactions within a triplicate gave measurable C_q values.

ELISA

Double antibody sandwich ELISA was performed using polystyrene microtiter plates (Greiner) according to Clark and Adams [30]. Commercial polyclonal coating and conjugate antibodies from a PVY universal detection assay (Bioreba) were selected. Buffers and incubation conditions were adapted from those recommended by the manufacturer of the assay. Briefly, plate wells were incubated with coating antibody for 4 h at 37 °C and then rinsed. Next, each sample was added to the wells in duplicate and incubated at 4 °C overnight. After the wells were rinsed, conjugate antibody was added to the wells and incubated 4 h at 37 °C. All samples consisted of Irl virus isolate dilutions in SPR running buffer. The same buffer was included in the ELISA as negative control. Absorbance was measured at 405 nm using a spectrophotometer (Sunrise; Tecan) after incubation with the substrate (1 mg/ml paranitrophenyl phosphate) for 30 min, 1 h, and 2 h. Data were processed with Megallan version 6.6 software. To estimate the limit of detection, a result was considered positive if the measured OD_{405} value was at least twice that of the negative control.

Results and discussion

Virus capture

In this paper we developed an SPR-based biosensor approach for the detection and characterization of PVY virus particles. We have used Irl and NZ isolates as representatives of N and O serotypes. A third viral isolate, 08Fr29, was used in epitope competition assays. According to electron microscopy all three virus suspensions used in the study consisted of intact PVY particles with their characteristic filamentous shape (740 × 11 nm; Supplementary Fig. S1).

A biosensor approach intended for studying the interactions between mAbs and PVY particles should ideally allow capture of different PVY isolates by polyclonal antibodies immobilized on the surface of the sensor chip. For this purpose a BSA-free universal anti-PVY pAb (Bioreba) able to bind the majority of the described PVY isolates was used as capture molecule. The pAb was covalently immobilized onto a CM5 chip to a final 8000 RU as described under Material and methods (Fig. 1A, top scheme). The pAb surface generated in such a way was used in all subsequent tests for capturing PVY particles (Fig. 1A, middle scheme).

All three virus isolates used can be successfully captured after injection onto the pAb surface (Fig. 1B). Viruses remained stably bound with no evident dissociation observed during the recorded time. To evaluate the stability of the pAb surface over time, independent of the number of cycles, 45 captures and regenerations were performed with the Irl isolate. The reproducible response levels and kinetics confirmed the stability of the pAb surface over time (Fig. 1C). The slope of the sensorgram and the final response observed upon virus capture were dose dependent (Fig. 1D). This dependency can be exploited for using such pAb surface for quantification of PVY particle concentration. In Fig. 2 we compare the dose dependence observed in the SPR approach with two other methods used for PVY detection and quantification, ELISA and RT-qPCR. The same serial dilutions of Irl isolate in SPR running buffer were applied to the three techniques. The RT-qPCR method is the method of choice for quantification of plant viral nucleic acids, since it is characterized by a great sensitivity and large linear quantification range [19,31]. ELISA detection is based on the interaction between antibodies with the coat protein from the surface of the viral particles. For coating the microplate the same pAb as the one used in the SPR approach was used. As expected, RT-qPCR showed the highest sensitivity, detecting PVY RNA in virus dilutions down to 1.22×10^{-6} μg/ml (Fig. 2) and it also showed the broadest linear range for quantification. However, for many plant viruses, including PVY, the number of virus RNA genomes and the actual number of packed virus particles present in the plant can notably differ, depending on many factors [32]. Within the plant tissue, in cells in which the virus is actively replicating, the amount of nonencapsidated viral RNA genomes can notably exceed the number of encapsidated ones. Therefore, methods such as ELISA and SPR, which target the protein structural unit of the virus particles instead of the nucleic acid, are required in certain studies such as those addressing questions linked to epidemiology and aphid transmission. The ELISA and SPR detection ranges were similar. This was expected, since both methods target the same interaction among pAbs and viral coat proteins. The limit of detection of the SPR approach was approximately 1 order of magnitude less sensitive (0.31 μg/ml) than ELISA (0.019 μg/ml) (Fig. 2). Among the scarce existing data, a limit of detection of 16 μg/ml was described for *Cowpea mosaic virus* [33], which is approximately 2 orders of magnitude less sensitive than the one we observed for PVY. In an older study *Tobacco mosaic virus* and *Cowpea mosaic virus* were captured to a CM5 chip using polyclonal antibodies but the authors used only a single concentration of 100 μg/ml [7]. It was proposed that high concentrations are needed to obtain signals in SPR assays for larger biological entities, such as bacteria ($>10^7$ cells/ml), and most likely also viruses [3], which is related to physical limitations that originate from the in-flow nature of the assay and the size of the analyte. In addition to these factors and for the particular case of ELISA, the contact time between antibody and

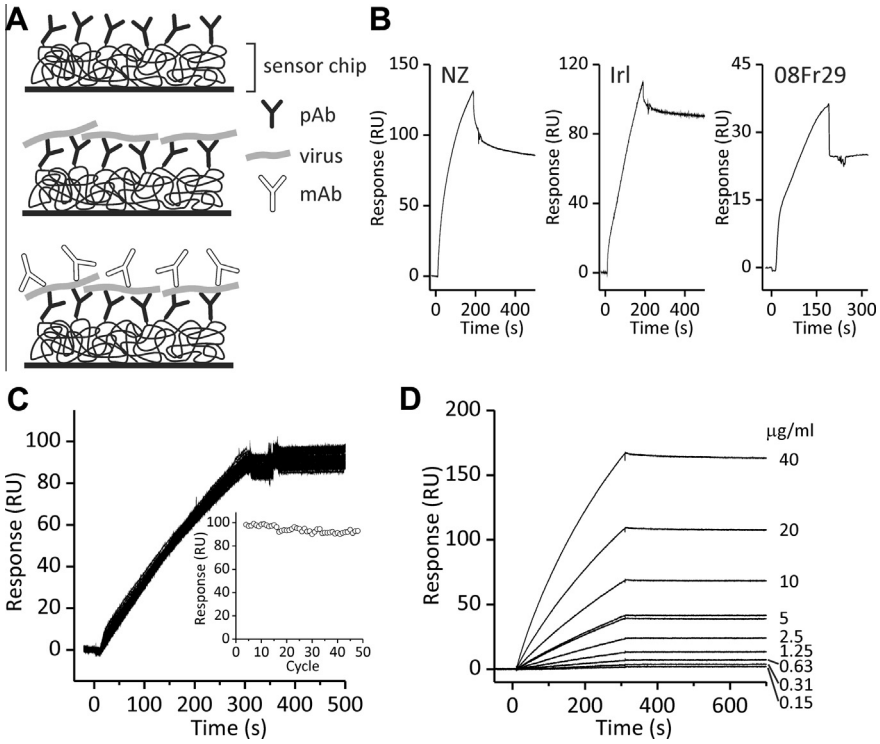

Fig.1. Strategy and virus capture. (A) Scheme depicting the strategy followed in the study. Top: immobilization of the polyclonal anti-PVY antibody to the CM5 chip surface. Middle: capture of the virus particles via the pAb. Bottom: binding of mAb to the generated virus surface. (B) Sensorgrams showing injection of each virus isolate (5 µg/ml NZ, 20 µg/ml Irl, 3 µg/ml 08Fr29) onto the polyclonal surface. (C) Reproducibility of the capture of isolate Irl, 20 µg/ml, over 45 cycles, using the same pAb surface. Final response obtained after each injection is depicted in the inset. (D) Dose dependency of the binding of Irl to the polyclonal anti-PVY antibody surface. The range of concentrations was 0.15–40 µg/ml. Concentration 5 µg/ml was done in duplicate in the middle and at the end of the titration.

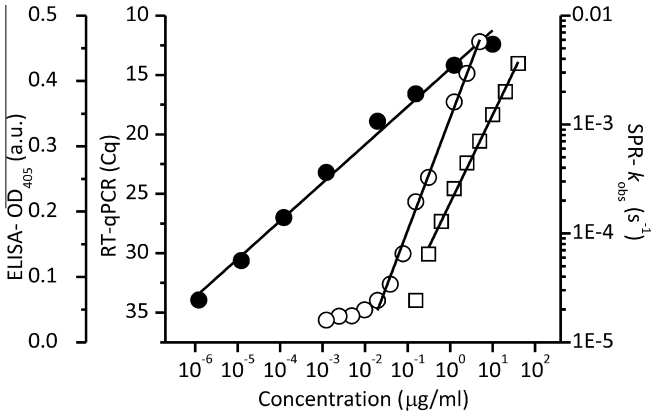

Fig.2. Comparison of the PVY detection and dose dependency using SPR (open squares), ELISA (open circles), and RT-qPCR (solid circles). In each case the virus concentration is plotted against the slope of the sensorgrams (SPR), the optical density at 405 nm after 1 h incubation with the substrate (ELISA), and the quantification cycle (C_q) for RT-qPCR. For each plot the data points that gave a linear regression with $R_2 \geqslant 0.99$ are depicted with a trend line.

epitope in this assay occurred during hours (overnight incubation) instead of a few hundred seconds (SPR), which, together with the presence of a secondary antibody, may explain the better sensitivity observed with ELISA.

Interaction of mAbs with the virus surface

From the above-mentioned results it is clear that SPR does not improve the PVY detection procedures used at present. However, the main objective of this work was not to develop a new diagnostic tool, but to create a stable virus surface for evaluation of the interaction of mAbs with different isolates (Fig. 1A, bottom scheme). In the past decades a variety of mAbs or antibody cocktails have been produced for the detection and identification of different PVY serotypes (N and O) and strain groups (N, O, and NTN) [21,22]. The sensitivity and specificity of such methods depend directly on the affinity and specificity of the interaction between the mAb and its epitope within the viral coat protein. A biosensor that could enable evaluating the affinity and specificity of such interaction would represent a valuable tool for improving serologic tests for PVY diagnostics and characterization. We selected two reference virus isolates, Irl and NZ, representing PVY serotypes O and N, respectively, together with 6 mAbs with known specificities. Fig. 3 shows a typical cycle in which a given concentration of mAb is injected onto the virus surface previously captured onto the CM5 chip via immobilized pAb. Two sequential injections of glycine, pH 2.0, enabled complete regeneration of the pAb surface, allowing another cycle to be performed.

The biosensor efficiently confirmed the predicted specificity of each tested mAb. The Y^{all}, 16E4, and Y123 mAbs were able to interact with both O and N serotype isolates (Fig. 4A). This correlates with the serologic reactivity previously described for these three mAbs ([22]; L. Glais personal communication; Rob Langley, Neogen Europe, personal communication). On the other hand, N-serotype-specific mAbs, N^{acw} and N (SASA), and an O-serotype-specific mAb, O/C (SASA), reacted only to their specific predicted isolate (Fig. 4B). The data also allowed estimating the affinity of each mAb/virus interaction by fitting the data to appropriate binding models using the BIAevaluation software (see below) (Table 1). The highest affinity, below nanomolar level ($K_{D1} = 10^{-10}$ M^{-1}), was observed for the interaction between mAb N^{acw} and NZ isolate, while the lowest

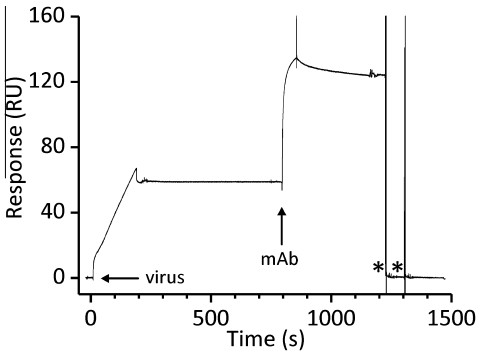

Fig.3. Sensorgram showing a representative cycle of virus capture, stabilization, mAb binding and dissociation, and regeneration. Arrows indicate the moment of injection of virus and mAb, while asterisks indicate the two 10 mM glycine, pH 2.0, injections done to achieve regeneration of the surface.

affinity (K_{D1} = 10^{-7} M^{-1}) was observed for the interaction of the Y^{all} mAb with NZ isolate.

Most of the mAb–virus interactions were best fitted using the bivalent model (Fig. 4, Table 1). This model describes the binding of a bivalent analyte to an immobilized ligand, in which one analyte molecule can bind to one or two ligand molecules [34,35]. This model is relevant to studies using intact antibodies that bind to an immobilized antigen, as is the case of our study. Each of the captured virus particles is built of polymerized coat proteins that are available at high surface density for the binding of mAbs, allowing two binding regions of a mAb to interact simultaneously with the same epitope at the surface of the virus. However, some of the interactions (Y^{all} to Irl, 16E4 to Irl, and N (SASA) to NZ) were satisfactorily fitted to the 1:1 model (based on the χ^2 value, which was for all reported fits 1% of the maximal response (R_{max}) or below; Fig. 4 and Table 1). Interestingly, the data for the interactions of Y^{all} and 16E4 mAbs with the NZ isolate were better fitted with the bivalent model in contrast to the 1:1 model that best fitted their interactions with Irl isolate (Fig. 4 and Table 1). This could be a consequence of a change in the orientation of the epitope among both isolates. The affinity constants obtained in two independent experiments showed high reproducibility, independent of the level of captured virus in each experiment. Titrations repeated on surfaces generated on different CM5 chips also showed good reproducibility (data not shown).

In addition to the multicycle titrations shown in Fig. 4, the single-cycle titration approach was also tested for the interaction of mAb Y123 with both virus isolates (Fig. 5). Single-cycle titration does not involve regeneration steps and is done entirely on the same virus surface, allowing one to save the precious purified virus material. The single-cycle kinetics were best fitted to a bivalent analyte model, similar to their multicycle counterparts (Fig. 5).

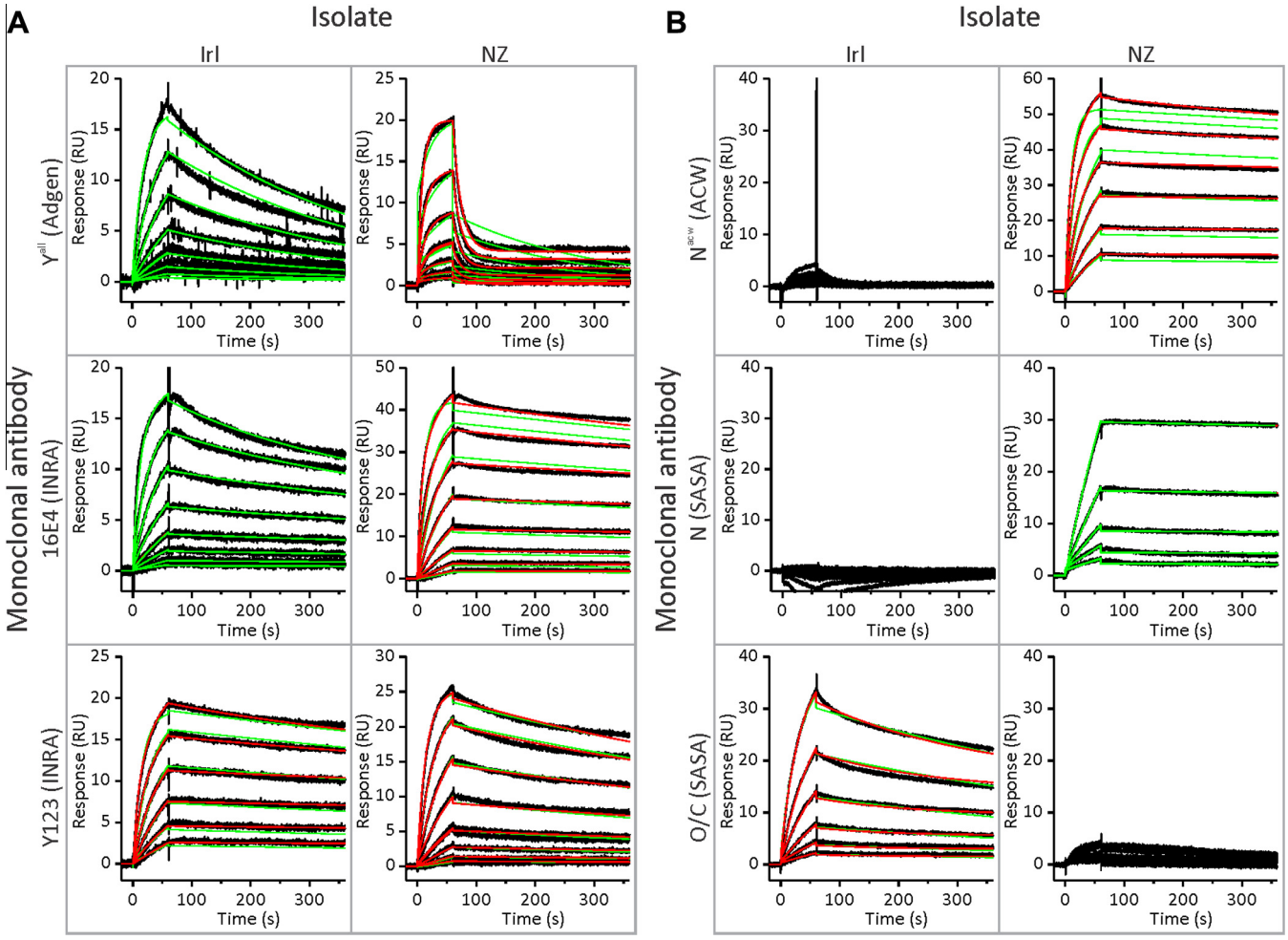

Fig.4. Titrations of each mAb to both Irl and NZ isolates. Experimental data (black lines) are shown together with fits. The concentration range used for the titration was between 1.56 and 800 nM, depending on the antibody. Red traces are fits to a bivalent model, fits to the 1:1 model are shown in green. (A) Titrations with antibodies that recognize both isolates. (B) Titrations with antibodies that recognize particular virus isolates.

Table 1
Kinetic constants derived from the kinetic measurements.

Antibody	Virus	1:1 model			Bivalent model					R_{max}[a] (RU)	χ^2[a] (RU^2)
		k_a (M^{-1} s^{-1}) × 10^4	k_d (s^{-1}) × 10^{-4}	K_D (M^{-1}) × 10^{-9}	k_{a1} (M^{-1} s^{-1}) × 10^4	k_{d1} (s^{-1}) × 10^{-4}	k_{a2} (RU^{-1} s^{-1}) × 10^{-4}	k_{d2} (s^{-1}) × 10^{-3}	K_{D1} (M^{-1}) × 10^{-9}		
Y^{all} (Adgen)	Irl	24 ± 1.7	29.3 ± 0.4	12.1 ± 1						24.8	0.17
Y^{all} (Adgen)	NZ				9.6 ± 2.7	657 ± 27.5	1 ± 0.4	0.14 ± 0.19	744 ± 234	44.4	0.08
16E4 (INRA/FN3PT)	Irl	9.8 ± 1.2	7.8 ± 5.4	8.3 ± 6.5						22.6	0.17
16E4 (INRA/FN3PT)	NZ				4 ± 4.3	6.5 ± 0.9	188 ± 165	113 ± 98.1	35.1 ± 35.2	50.2	0.30
Y123 (INRA/FN3PT)	Irl				39.3 ± 16.4	15 ± 6.1	399 ± 61	106 ± 8.6	3.8 ± 0.04	23.5	0.05
Y123 (INRA/FN3PT)	Irl[b]				14.3 ± 8.7	1.9 ± 1.2	15.2 ± 10.9	3.5 ± 3.3	1.9 ± 2	30.4	0.08
Y123 (INRA/FN3PT)	NZ				8 ± 0.8	12.1 ± 1.4	17.7 ± 12.9	24.2 ± 15.2	15.2 ± 0.3	26.9	0.29
Y123 (INRA/FN3PT)	NZ[b]				6.2 ± 1.7	7 ± 4.9	4.2 ± 1.9	0.01 ± 0.02	12.8 ± 11.9	116.8	1.09
O/C (SASA)	Irl				21.4 ± 5	20 ± 1.2	18.5 ± 23.9	39.1 ± 55.3	9.7 ± 2.8	44.0	0.22
N^{acw} (ACW)	NZ				146 ± 25	5.3 ± 0.2	118 ± 34	56.8 ± 7.7	0.4 ± 0.08	70.2	0.23
N (SASA)	NZ	3.2 ± 0.5	1.3 ± 0.1	4.2 ± 0.3						86.3	0.10

The average ± SD of two independent titrations is shown, except for the binding of Yall to NZ and Y123 to NZ (single cycle), for which three independent titrations were performed.
[a] R_{max} and χ^2 are presented for the example shown in Figs. 4 and 5.
[b] Data from single-cycle SPR experiment.

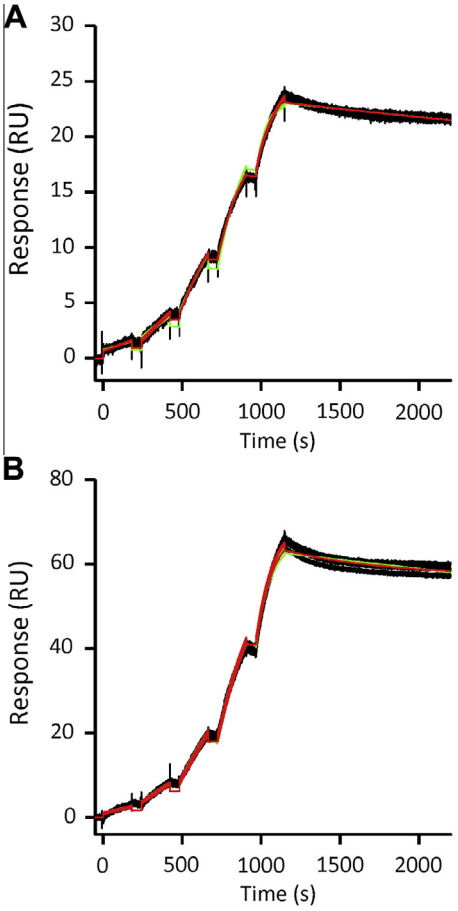

Fig.5. Single-cycle titrations of mAb Y123 (A) to Irl and (B) to NZ. Red and green traces correspond to the fitting of the interaction to a bivalent analyte model and the 1:1 model, respectively.

The obtained K_D values correlated well in both approaches (Table 1), demonstrating that the multicycle approach can also be chosen as an option to perform kinetic studies with a PVY-based biosensor.

In summary, the developed biosensor approach has proved to be an efficient tool to screen the affinity and specificity of the interaction between a given mAb and a chosen PVY isolate.

Competition assays

Viruses, and especially those bearing RNA genomes such as PVY, evolve rapidly because of the high mutation rate inherent to their replication cycle. A mutation in a single nucleotide within an epitope-spanning region can abolish the reactivity of an otherwise previously reactive mAb [23]. Such drawback can be overcome by designing antibody cocktails that include mAbs with the pursued specificity but targeting independent epitopes. For this reason we evaluated if the biosensor can be used to explore whether two mAbs compete for the same epitope. The most illustrative examples of independent and overlapping epitopes were observed when assessing the epitope competition of mAbs Y123 and N^{acw} using isolate 08Fr29 (Fig. 6A) and mAbs Y123 and 16E4 using isolate Irl (Fig. 6B and C). In a typical competition assay, a given concentration of each mAb was injected separately over the virus surface. The interaction of each mAb alone was then compared with an injection of the same concentration of mAbs together (Fig. 6A and B). The interaction time in these experiments was increased

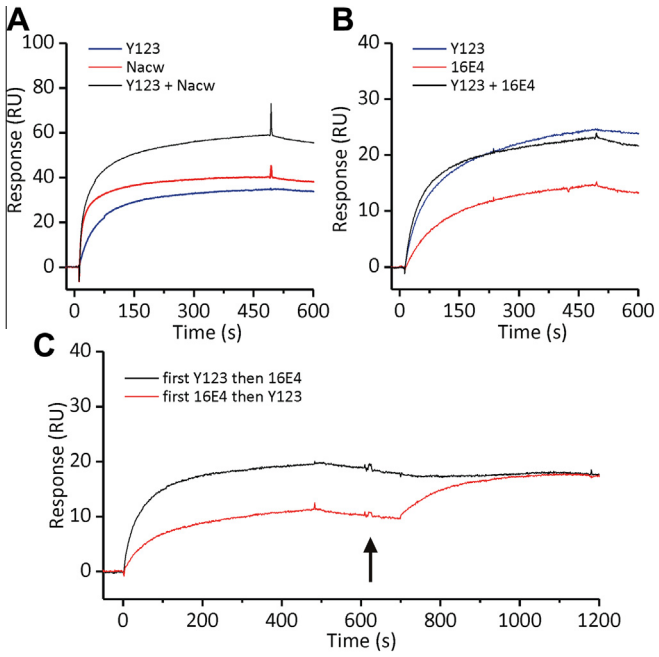

Fig.6. Competition assays. (A) Injections of mAbs Y123 and N^{acw}, each one separately and both simultaneously, to isolate 08Fr29. (B) Injections of mAbs Y123 and 16E4, each one separately and both simultaneously, to isolate Irl. (C) Sequential injection to isolate Irl of first mAb Y123 and second mAb 16E4 and vice versa. The injection of the second antibody is denoted by an arrow.

from the 60 s used in the titration assays to 480 s, to allow for a more complete saturation of the epitopes.

MAbs N^{acw} and Y123 showed similar interactions when injected alone onto a surface of 08Fr29 isolate, producing a response increment of 40 and 34 RU, respectively (Fig. 6A). Ideally, for an independent epitope scenario, the final response obtained when both mAb's are injected together should increase, being equivalent to the sum of the responses reached by each mAb on saturation when injected alone. In the case of N^{acw} and Y123, the final response indeed increased (Fig. 6A), meaning that a higher mass was being bound to the virus surface and pointing to an interaction of these two mAbs with different epitopes. Actually, the response obtained when injecting both mAbs together, 60 RU, was slightly lower than 74 RU, but the slope of the sensorgram observed in the end of the association phase indicates that the surface was not fully saturated yet. A situation in which, albeit independent, the epitopes are still located in close vicinity within the coat protein could explain this behavior. In such case, when injected together, each mAb would still be able to interact with its own epitope, but the proximity among them may contribute to slowing down this interaction, and thus the observed lower-than-predicted maximum and the slower saturation.

The competition assay performed with mAbs Y123 and 16E4 on isolate Irl represents a different scenario. When injected alone, mAb Y123 reached a higher final response (24 RU) than mAb 16E4 (15 RU) (Fig. 6B). When injected together, the obtained sensorgram was nearly identical to the one obtained when injecting Y123 alone. This suggests that both mAbs target the same epitope, or that they target different epitopes that overlap within the tertiary structure of the coat protein. In this scenario, the binding of Y123 would not allow 16E4 to interact. To confirm this, we did an additional experiment, in which we injected sequentially Y123 and 16E4 and vice versa (Fig. 6C). Such strategy [36] was previously used to study the interference of a set of mAbs against *Lettuce mosaic virus* [13]. When mAb 16E4 is injected right after Y123, no mass is bound to the surface, indicating that all the epitopes targeted by 16E4 are already blocked by Y123. On the other hand, when Y123 is injected right after 16E4, an increase in the response is observed, reaching a final response equivalent to the one obtained when Y123 was injected alone. MAb Y123 not only blocks mAb 16E4 from binding to its epitope (Fig. 6C, black trace), but it is also able, because of its higher affinity for Irl isolate (Table 1, Fig. 6B), to displace 16E4 from their shared epitope (Fig. 6C, red trace). This explains why the injection of Y123 after 16E4 results in nearly the same final bound mass (18 RU) as when Y123 is injected alone (19 RU).

The competition assays performed prove that the developed biosensor can be readily used to explore, not only the affinity and specificity of the interaction among PVY isolates and mAbs, but also to estimate if different mAbs compete for the same epitope. The potential of the biosensor for assessing all these features within the mAb/PVY interaction constitutes a powerful tool for accurate screening of the most appropriate mAbs to be included in serological tools.

Conclusion

The SPR was applied for the first time to study the interactions of the economically relevant filamentous PVY virus with antibodies. The developed biosensor can be applied to the detection and quantification of PVY virus particles. The approach can be used to study the affinity and interaction mechanism of monoclonal antibodies and to confirm whether the epitopes targeted by those monoclonal antibodies overlap. This tool can be used to improve the antibody-based virus diagnostics assays and enables new studies on the serological variability of the PVY virus. Moreover, the stable PVY virus surface achieved in the biosensor may be used to explore PVY interactions with other molecules different from mAbs, such as proteins from the host plant.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ab.2013.10.032.

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