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Simple method for plant virus detection: effect of antibody immobilization technique

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Abstract

The possibility has been demonstrated for applying a surface plasmon resonance for detecting plant viruses in real samples. An optimal mode for antiviral immunoglobulin immobilization on sensor surfaces is described. Out of three proposed techniques for sensor surface treatment, namely, unmodified gold surface, gold surface treated with (a) thiocyanate and (b) thiocyanate and protein A (*Staphylococcus aureus*), the latter was chosen as most suited for retention of the formed native immunoglobulin layer.

Keywords: Oriented protein immobilization; Surface plasmon resonance; TMV

Studies of routes used by viruses for propagation and circulation among their natural hosts in planta, as well as in artificial model systems, are of considerable importance (Boyko, 2001). Such investigations make it possible to predict virus behavior in natural conditions and inspect molecular details of interaction between a virus and host cell. One of the most commonly used models is the tobacco mosaic virus (TMV) (Van Regenmortel et al., 1993).

The interaction between TMV and specific antibodies was studied using the surface plasmon resonance (SPR) technique. This technique is based on light beam interaction with a thin metal (Au, Ag, etc.) film deposited onto an insulator surface (e.g. glass) (De Bruijn et al., 1992; Liedberg et al., 1995). The SPR technique provides additional information that cannot be obtained with traditional methods at least, without additional sophistications. In particular, the SPR technique makes it possible to study kinetics of processes that result in the host–guest interaction (Altschug et al., 1992; Glaser and Hausdorf, 1996). The use of this method also makes it possible to exclude the application of labeled reagents (that are sometimes very expensive), as well as to reduce time required for testing (Davies, 1994).

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There are, however, some limitations on the application of this technique to virology. The ability of the device to detect the presence of molecules is determined by the depth of penetration into the dielectric medium for the wave that appears when the light beam is incident on the boundary of the gold layer. This penetration depth is about 200 nm, so that the size of the objects to be studied should not exceed 100 nm (Rengevych et al., 1999). Most human and animal viruses do not meet this requirement. However, the SPR technique could be easily applied for studies of plant viruses because many are of small sizes (Sanual and Van Regenmortel, 1995; Van Regenmortel, 1999). TMV whose diameter is below 18 nm seems to be a very convenient model.

One of the main conditions for an adequate analysis is retention of the native state of specific receptor sites immobilized on the physical transducer surface. To this end additional treatment of the gold chip surface is required when the SPR technique is applied, because an unmodified metal film could have a destructive effect on the receptor molecules namely, an immediate contact with a metal surface may lead to denaturation of molecules, the receptor sites are arbitrarily oriented, or by immobilization to give a high level of nonspecific binding (Ramsden, 1997). These problems can be solved by forming a buffer interlayer between the metal surface and protein (Karlsson and Fält, 1997). Various functional thiols are used most often as such interlayers. However, in many cases the problems related to lending the required chemical function to thiol monolayers either do not permit the formation of an oriented bimolecular layer on their surfaces, or do not provide strong immobilization (Löfås et al., 1995).

Several different approaches have been suggested to solve this problem. In particular, one of the possible ways is to modify the sensor surface by treatment with KNCS (Boltovets et al., 2001a). It is known that behavior of composite protein molecules in the native conditions depends essentially on their charge. Therefore the position of the molecule under consideration can be changed relative to the charged sensor surface by altering the charge of definite amino acid groups via changing pH of the ambiance, which in turn affects its activity at the surface (Mattews et al., 2000). In this context, preliminary treatment of the gold surface with NCS^- may be of interest because (i) the presence of the sulfur atom in NCS^- favours strong coupling with metal surface; (ii) the formed monolayer provides reliable protection of the metal surface, being at the same time sufficiently thin; (iii) the surface modified in this way has an extra effective charge, and this enables oriented immobilization of molecules. This approach was tested earlier (Boltovets et al., 2001b) with the soybean trypsin inhibitor model. However, such an approach presupposes the presence of positively charged centers (particularly, histidine) in the area of the contact with the chip surface. In the case of immunoglobulins there are many such sites located in different areas at the molecular surface so that oriented character of immobilization becomes doubtful.

On the other hand, protein A *Staphylococcus aureus* which is known to have pseudoimmune interaction with the Fc fragment of immunoglobulins by five homologous IgG-binding domains (Cedergreen et al., 1993; Jenderberg et al., 1996) seemed to be a useful agent for antiviral immunoglobulin immobilization and orientation at the gold surface. But since it is a protein itself and can be damaged by immediate contact with the gold layer, previous treatment of the gold surface was necessary. It was shown (Boltovets et al., 2000) that previous treatment of the gold layer by NCS^- promotes the adsorption of the protein ? at the surface.

An SPR spectrometer 'PLASMON' with a GaAs laser ($\lambda = 670$ nm) was used as source of excitation. The spectrometer was designed at the Institute of Semiconductor Physics of the National Academy of Sciences of Ukraine (Beketov et al., 1998; Chegel et al., 1998; Snopok et al., 2000). The glass plates (refractive index $n = 1.61$) with an as-sputtered (through an adhesive Cr interlayer 1–1.5 nm thick) gold layer 50 nm thick (Snopok et al., 2001) have been treated previously with 'piranha' (mixture of 30% H_2O_2 and 98% H_2SO_4 in the ratio of 1:3) to remove organic contaminations. After treatment the glass plates were fixed on a supporting glass prism (refractive index $n = 1.61$). Optical contact was provided us-

ing an immersion liquid (polyphenyl ether, refractive index $n = 1.6$). A 6×10^{-2} M solution of sodium thiocyanate (analytical grade) was prepared immediately before the experiment.

The glycine and carbonate buffers were prepared by the standard procedure (Dawson et al., 1986). For the glycine buffer (pH 2.2), 25 ml of 0.2 M glycine was mixed with 22 ml of 0.2 M HCl and then diluted by distilled water to 100 ml. For preparation of the carbonate buffer (pH 9.6) 30 ml of 0.1 M Na_2CO_3 was mixed with 70 ml of 0.1 M $NaHCO_3$.

In this study the green alga *Bracteacoccus minor* cells that were infected by TMV at the zoospore stage, served as the virus source. The homogenate of cells was prepared by pounding the cell suspension in carbonate buffer (pH 9.6). The mixture was centrifuged (at 85 g) to remove undamaged cells and larger organelles. The pellet free supernatant was used for further analysis.

Four types of samples were investigated: (i) purified type strain of TMV whose concentration was known; (ii) supernatant, obtained from infected cells after one passage (two months of growth); (iii) supernatant, obtained from infected cells after two passages (six months of growth); (iv) supernatant, obtained from virus free cells.

At the first stage of the investigation the binding efficiency of known concentrations of purified TMV were tested. The efficiency of virus binding to the antibodies that were adsorbed on the sensor surfaces of the following three types were compared: (i) unmodified gold; (ii) gold treated with KNCS; (iii) gold treated in series with KNCS and with immobilized protein A *S. aureus*. It was shown that the antibodies to TMV are adsorbed efficiently onto all the above surfaces. However, they retained their ability for TMV binding in one case only, namely, when the surface was treated in series with KNCS and protein A *S. aureus*.

The principal course of the experiment is shown in Fig. 1. The rabbit antiviral serum was applied to the sensor surface treated previously. The unbound antibody excess was washed off with carbonate buffer (pH 9.6). The viral preparation was then applied to the surface washed with buffer. The preparation excess was washed off with carbonate buffer (pH 9.6). The test for binding specificity was then carried out by treating the surface with glycine buffer (pH 2.2) that brakes the protein–protein bonds. The cell was then washed with carbonate buffer (pH 9.6) once more.

At the second stage of the test, the above procedure was used to check the efficiency of the green alga *B. minor* cells infection with TMV depending on the number of culture passages as shown in Fig. 1. The viral antigens from the alga *B. minor* cells that were infected previously with TMV could be unambiguously detected only by the sensor on whose surface protein A *S. aureus* had been immobilized. It was shown also that the viral concentration in the algal suspension diminished appreciably after the second passage in comparison with the first one.

The results obtained with the SPR technique correlate with those obtained by ELISA as shown in Fig. 2 demonstrating, that the concentration of the viral antigen is much higher after one passage than after two passages.

The light microscopy investigation results shown in Fig. 3 indicate that after one passage algal cells change appreciably their shape and size, whereas, after two passages no considerable difference from the control is observed.

It was shown that binding between the virus and antibodies is specific but is broken by buffer at pH 2.2. This feature makes it possible to use repeatedly the sensor surface after it has been washed in this way. Thus the SPR technique is recommended for the detection of plant viruses in samples and can be used for rapid diagnosis of virus diseases of plants.

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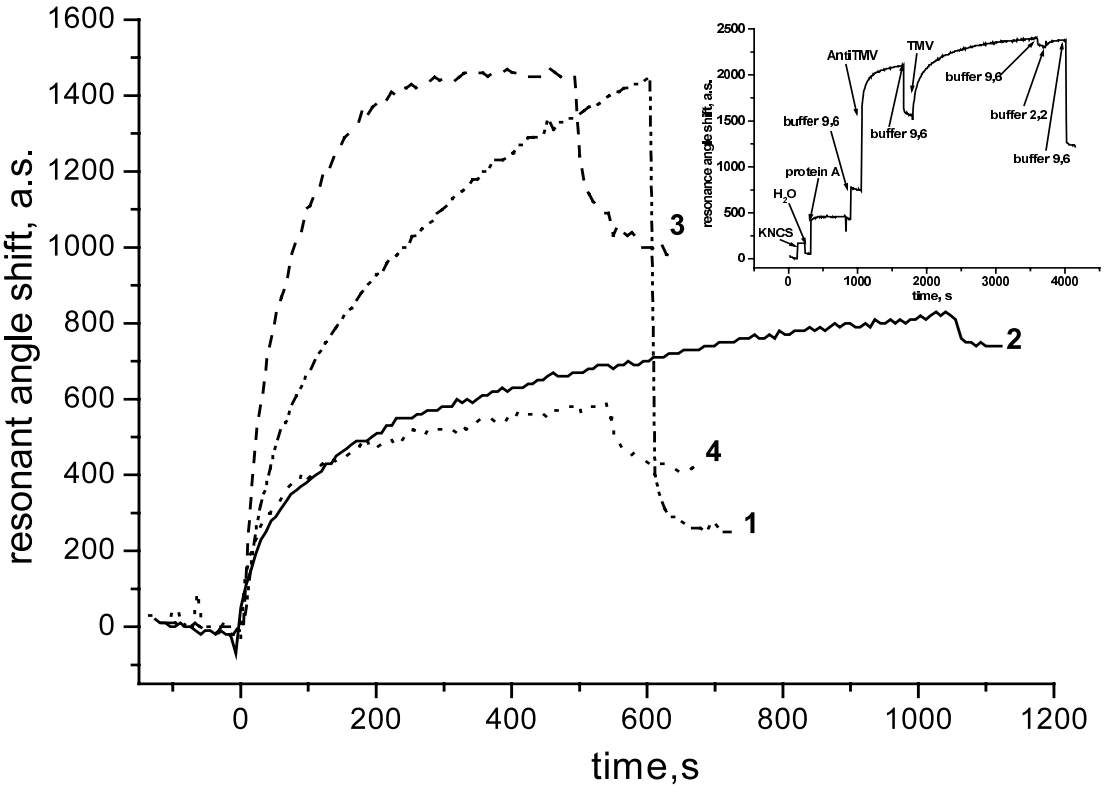

Fig. 1. Binding of antigens derived from *Bracteococcus minor* cells infected with TMV to the antiTMV IgG immobilized at the KNCS-protein A modified surface. Insert: the experimental sequence for TMV control samples. The extent of binding for different test samples. 1, *Br. minor* control; 2, TMV control; 3, *Br. minor* + TMV after one passage; 4, *Br. minor* + TMV after two passages.

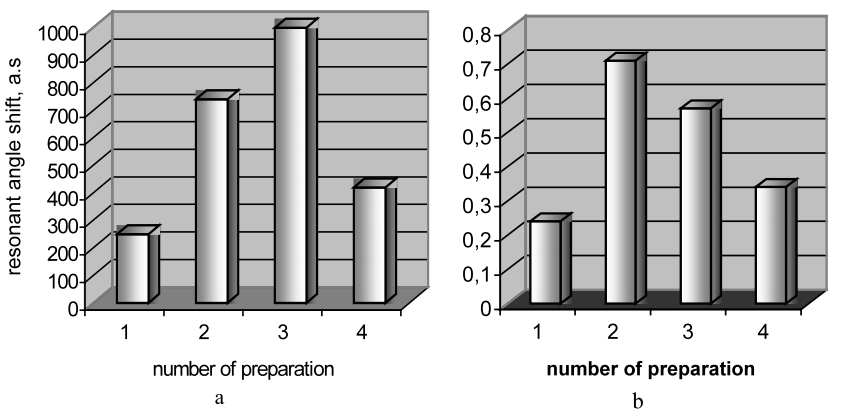

Fig. 2. Effectiveness of the *Bracteococcus minor* cells infection by TMV demonstrated by SPR (a) and ELISA (b). -···-···, 1, *Br. minor* control; —, 2, TMV control;-- -, 3, *Br. minor* + TMV after one passage; —, 4, *Br. minor* + TMV after two passages.

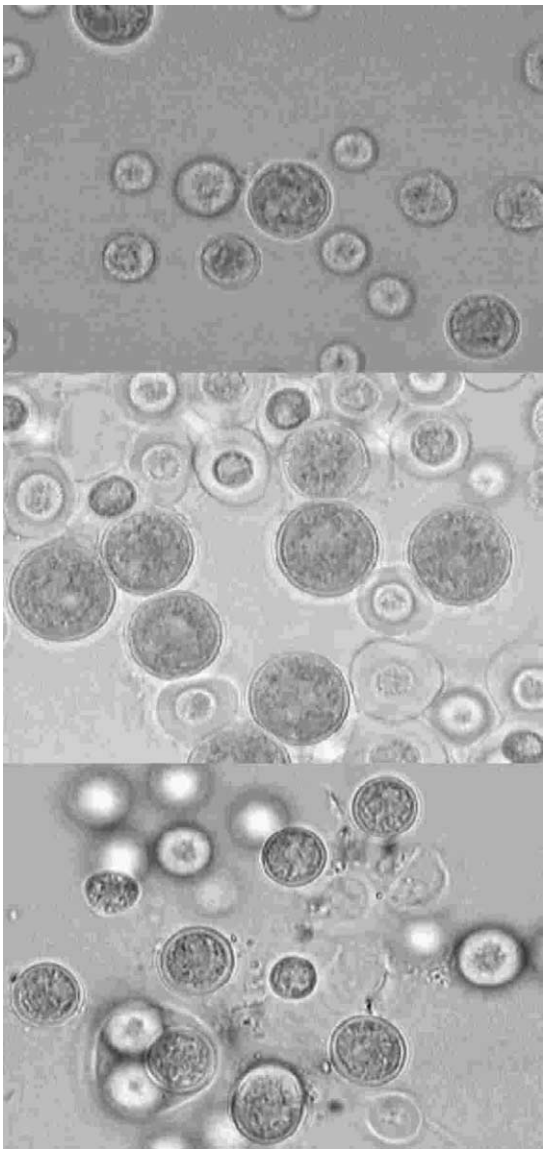

Fig. 3. Cell size and shape changes due by TMV infection untreated. (a) *Bracteococcus minor* cells. (b) *Bracteococcus minor* cells infected by TMV after 1 passage. (c) *Bracteococcus minor* cells infected by TMV after 2 passages.

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