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ScienceDirect
Journal of Virological Methods 137 (2006) 1–6
Journal of Virological Methods
www.elsevier.com/locate/jviromet

Cloning and expression of functional single-chain Fv antibodies directed against NIa and coat proteins of *potato virus Y*

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Received 12 January 2006; received in revised form 7 April 2006; accepted 3 May 2006

Abstract

Three single-chain variable fragment (scFv) antibodies recognizing the nuclear inclusion a (NIa) and capsid proteins of *potato virus Y* were obtained from two mouse derived hybridoma clones secreting, respectively, an anti-NIa (22-1) and an anti-coat protein (136-13) monoclonal antibodies. The first monoclonal antibody was able to inhibit in vitro the PVY polyprotein cleavage by blocking the NIa protease activity. The amplified scFv cDNAs were first inserted into the TOPO vector and then sequenced. Several recombinant *E. coli* clones carrying the accurate scFv sequences were selected and the corresponding cDNAs were subcloned in pHEN phagemid and transferred in *E. coli* strain. The expressed scFv fragments showed an antibody activity that recognized the viral target proteins in infected tissues. Their activity was comparable to the parental monoclonal antibodies.

Keywords: *Potato virus Y*; ScFv; NIa; Expression; Proteolytic cleavage inhibition

1. Introduction

Potato virus Y is one of the most important pathogens causing damage of potato and other solanaceous plant cultures. Different strategies were developed to overcome plant infection by this virus (Wilson and Watkins, 1986). Transgenic plants expressing genes encoding structural (De Zoeten and Fluton, 1975) and non-structural (Golemboski et al., 1990; Vardi et al., 1993) proteins have been described. They showed efficient resistance against the virus. However, risks due to the expression of viral proteins such as complementation or heterologous encapsidation may limit wide application of these strategies. Indeed, these phenomena may lead to worse symptoms or to plant sensitivity to new virus strains (Tepfer, 2002). Heteroencapsidation may occur in transgenic plants between the expressed viral segment and the genome of related viruses (White and Morris, 1994). The recombination may induce the emergence of new virulent virus strains (Jakab et al., 1997).

The expression of monoclonal antibodies directed against viral proteins was suggested as an alternative approach (Hiatt et al., 1989). Antibodies were expressed as a single-chain variable fragment (scFv) directed essentially against the viral capsid protein (Voss et al., 1995; Fecker et al., 1996; Tavladoraki et al., 1993). The antibodies were active in plants and have led to 60% protection rate against the virus infection. The production of scFv directed against potyviral proteins has been described (Boonham and Barker, 1998; Xiao et al., 2000; Hust et al., 2002). These proteins were produced for the detection of specific virus strains or as a broad range method for detection of potyviruses.

Two monoclonal antibodies (mAb) directed against PVY NIa and coat (CP) proteins have been produced and characterized previously (Rouis et al., 2001). The NIa viral protease is responsible for the cleavage of 2/3 of the viral polyprotein into functional proteins. It is also associated with the NIb protein to form the nuclear inclusion body. These viral proteins were chosen as antigens because of their important role in viral infec-

Abbreviations: PVY, *potato virus Y*; NIa, nuclear inclusion body A; scFv, single-chain variable fragment

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doi:10.1016/j.jviromet.2006.05.011

tion and also for their relative sequence conservation among potyvirus isolates. When used for in situ immunofluorescence detection of the virus, both mAbs recognized specifically the target proteins (Rouis et al., 2002). In vitro, the anti-NIa mAb was able to recognize the different NIa protein species including precursors and cleavage products. It was also able to inhibit the cleavage of the polyprotein detected in the semi purified NI fraction.

The cloning and expression in *E. coli* of the scFv directed against PVY NIa and capsid proteins are described in this paper. To our knowledge this is the first report on the expression of anti-NIa and anti-coat protein scFv from anti-PVY parental antibodies. In contrast, the mAbs described by Hust et al. (2002) were selected from an scFv human library. Immunochemical properties of the different scFv expressed in *E. coli* were determined and compared to the parental monoclonal antibodies.

2. Materials and methods

2.1. Hybridoma cell lines used

Murine hybridoma cells secreting IgG antibodies recognizing the PVY NIa and coat proteins were selected and cloned previously as anti-NIa (22-1) and anti-coat protein (136-13), respectively (Rouis et al., 2001).

2.2. scFv cDNA synthesis and cloning

Total RNAs were isolated from 5×10^6 cells of mouse hybridoma cells secreting anti-NIa and coat protein mAbs (RNAXEL^R, Eurobio). The synthesis of the first strand cDNA was carried out using random hexanucleotides (Pharmacia) and AMV reverse transcriptase (Gibco-BRL). The cDNAs encoding the heavy (VH) or light (VL) chain variable regions were then amplified by PCR using *Taq* DNA polymerase (Promega) according to the manufacturer's protocol. Two primers (H4 For and H4 back) were used for the amplification of the heavy chain cDNA. Six primers (VK, CK, VLA1, VLA′1, VK2 For and VK2 back) were used for the light chain cDNA. Their sequences were previously described by Clackson et al. (1991) and Evans et al. (1995). The heavy and light fragments were assembled into the scFv form using four primers (Linkback, LinkFor, H4ForMut and H4backSfi). They were then cloned into TOPO plasmid (Invitrogen) according to the manufacturer's instructions. The resulting plasmids were introduced into TG1^{TR} cells provided by the kit and transformed clones were screened by PCR using *Taq* DNA polymerase (Promega) and the H4Back/H4For primers, according to the manufacturer's protocol.

Automated sequencing of the cloned cDNA fragments was carried out by Genome Express S.A. (Grenoble, France), using the pUC19 reverse and M13-40 sequencing primers.

The scFv sequences were submitted to GenBank (Bankit). Their accession numbers are the following: AY196321 (VH1), AY196322 (VL1), AY196323 (VL′1), AY196324 (VH2) and AY196325 (VL2).

2.3. Production of scFv constructs

The selected cDNAs cloned into TOPO vector were digested by *Sfi*I and *Not*I, since the corresponding sites were present in the primers used for scFv assembling. They were then inserted into pHEN phagemid (Invitrogen) treated with the same enzymes. The pHEN scFv phagemids were transferred in the TG1^{TR} *E. coli* strains.

Soluble scFvs were produced as follows: the recombinant TG1^{TR} strains containing the pHEN-scFv phagemid were grown at 30 °C, induced by addition of 1 mM isopropyl-β-D-thiogalactoside (IPTG, Sigma) and incubated for 4 h. Bacteria were then pelleted at 4000 rpm at 4 °C for 15 min and resuspended in phosphate buffer (PBS) containing 0.1 mM phenylmethylsulphonylfluoride (PMSF, Sigma). Sonicated cells were centrifuged at 20,000 × *g* at 4 °C for 15 min and the supernatant was used for further analysis.

Phage-scFv were produced as follows: recombinant TG1^{TR} strains containing the pHEN-scFv vectors were grown at 37 °C and infected with 10^{10} pfu of M13 KO7 helper phage according to manufacturer's instructions (Pharmacia).

2.4. ELISA tests

ELISA tests were performed as reported previously by Thullier et al. (1999) using crude extract from infected plant leaves as antigen. For inhibition assays, ELISA was carried out as described by Choumet et al. (1998). Briefly, various concentrations of crude plant extracts were incubated in solution overnight at 4 °C with defined quantities of scFv or Mab until equilibrium was reached. The antibody concentration used was deduced from preliminary ELISA calibration and was chosen in order to ensure a quantitative formation of the complex. Each mixture (100 μl) was transferred to microtiter plates coated previously with antigen and incubated for an hour at 20 °C.

2.5. Western blot analysis

The different samples containing scFv fragments were separated by electrophoresis on 10% polyacrylamide slab gel (SDS-PAGE) and blotted onto a nitrocellulose membrane by electrotransfer. The membranes were reacted with anti *C-myc* monoclonal antibody (Invitrogen) and visualized using the immunoperoxidase method (ECL kit, Amersham) as described previously (Rouis et al., 2001).

2.6. Indirect fluorescent antibody (IFA) assay

IFA assays were carried out as described previously by Rouis et al. (2002).

2.7. Virus strain and multiplication

The Tunisian isolate of PVY^O strain was replicated in potato plants (*Solanum tuberosum* L. cv Spunta) in a greenhouse after mechanical inoculation by dusting using carborundum (Gargouri-Bouzid et al., 2005).