PREPARATION AND SPECIFICITY OF ANTIBODIES AGAINST COAT PROTEINS OF BROAD BEAN STAIN VIRUS

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Summary. – Mouse polyclonal antibodies were prepared against broad bean stain virus (BBSV, Comovirus group) and its coat protein subunits, large (L) and small (S) protein. These subunits were less immunogenic than native virus. Antibodies against L protein (Anti-L) and native virus (Anti-BBSV) did not react in immunoblots with S protein, but Anti-BBSV antibody reacted with S protein in plate-trapped antigen ELISA. Anti-S antibodies did not react with the related red clover mottle comovirus (RCMV), but Anti-L and Anti-BBSV antibodies reacted with RCMV similarly to BBSV. We assume that all the linear epitopes of the BBSV S protein are hidden in the native virions and the antigenic similarity between BBSV and RCMV is based mainly on their common linear L-specific epitopes.

Key words: broad bean stain virus; coat protein epitopes; monoclonal antibodies; immunoblot

Introduction

BBSV has an icosahedral capsid composed of equimolar amounts of two polypeptides, L and S (Gibbs and Smith, 1970) of M_r of 36.5 K and 22 K, respectively (Šubr *et al.*, 1993). Structural analysis of comovirus particles revealed an arrangement of 60 L subunits into 20 trimers forming a kind of skeleton filled with 12 S subunit pentamers (Stauffacher *et al.*, 1987). Each L subunit consists of two folding units (8-strand beta-barrels), while each S subunit contains one such domain (Chen *et al.*, 1989). The virion surface thus consists of a total of 180 scale domains in a P=3 (pseudo T=3) arrangement (Chelvanayagam *et al.*, 1992).

Since both proteins participate in the structure of the virion in a ratio of 2:1, a similar ratio of the amounts of epitopes pertaining to the L and S protein can be anticipated.

Monoclonal antibodies (MoAbs) against red clover mottle virus (RCMV) as well as against BBSV have been prepared recently in our laboratory (Šubr *et al.*, 1993). Most of them cross-reacted with both these viruses. All MoAbs were directed against linear detergent-stable epitopes localized on L proteins.

Our idea about comovirus antigen determinants includes all 3 epitope types (Van Regenmortel, 1982; Kalmar and Eastwell, 1989) in accordance with their presence in native virions and isolated viral protein. Neotopes, localized on the surface of virions are detergent-sensitive, and thus discontinuous (conformational) epitopes, while cryptotopes are uncovered only by detergent treatment of virions. The native structures hidden in mature particles are not detectable, because the protein-protein interactions are very strong especially in comoviruses and the coat protein solubilization is possible only under conditions leading to its denaturation (Wu and Bruening, 1971). Thus the cryptotopes are hidden linear (sequence) epitopes.

The proteins may obviously have different space arrangements also in the presence of denaturation agents, however, because of instability of such structures we can not really think of some conformational cryptotopes.

Finally, the metatopes are present in native virions and isolated coat protein as well – they are the surface linear epitopes.

According to this categorization none of MoAbs prepared by us (Šubr *et al.*, 1993) was directed against a neotope. Polyclonal rabbit antisera used as controls in those experiments bound conformational and linear epitopes as well, but none of them bound the S protein in immunoblots. The size of comovirus S protein (about 20 K) in fact excludes the possibility of a complete absence of linear epitopes. Therefore we attempted to prepare antibodies by immunizing mice with isolated coat proteins.

Materials and Methods

Antigens (L and S proteins) were isolated by electroelution from Coomassie Brilliant Blue-stained SDS-polyacrylamide gels following discontinuous electrophoresis (SDS-PAGE) (Laemmli, 1970) of BBSV purified according to Musil *et al.* (1983) with subsequent dialysis against 50 mmol/l Tris-HCl pH 7.0. Balb/c mice were immunized ip with 3 doses each of 0.25 mg antigen (L, S protein or BBSV) mixed 1:1 (v:v) with complete Bacto-Difco adjuvant, given at weekly intervals. One week after the last injection the mice were given ip Sp2/0 mouse myeloma cells. Ascitic fluids from the mice were collected 15 days later and immunoglobulins from them were prepared by DEAE-52 cellulose (Serva) chromatography and stored at +4 °C.

Polyacrylamide gels were silver-stained (Marcinka *et al.*, 1992). Western blot analysis was carried out according to Hirano and Watanabe (1990) and the immunological detection of proteins on nitrocellulose membranes (Schleicher and Schuell) as described by Šubr *et al.* (1993).

The plate-trapped antigen (PTA) and double antibody sandwich arrangements of ELISA (DAS ELISA) were employed using buffers described by Clark and Adams (1977). The concentration of antigens was 2 µg/ml, antibodies were diluted 1:500. In DAS ELISA rabbit anti-BBSV antiserum (VsM) (Musil *et al.*, 1983) was used as the coating layer.

The immunoelectrophoresis was run in 1% agarose C (Pharmacia) buffered with 0.01 mol/l phosphate pH 7.0 (Musil and Gallo, 1982).

Results and Discussion

We obtained antibodies designated Anti-L, Anti-S and Anti-BBSV corresponding to the respective antigen used for immunization. PTA ELISA titers of antibodies against isolated coat proteins were about 100-times lower than those of the antibody against native BBSV (3,200 for Anti-L and 1,600 for Anti-S).

The specificity of obtained antibodies with regard to BBSV, its isolated coat proteins and RCMV was tested in PTA ELISA (Table 1). The cross-reactions of Anti-L and Anti-S with whole virions were probably caused by the antigen deformation after binding onto the plate surface (Van Regenmortel, 1990), which perhaps led to a partial uncovering of some hidden epitopes.

However, this fact cannot explain the ability of Anti-BBSV to recognize the coated S protein. Most probably the isolated coat proteins partialy fold into their natural tertial structure after removal of SDS by dialysis and thus they can react also with antibodies directed against virus neotopes.

RCMV was detected by both Anti-BBSV and Anti-L, and vice versa the control anti-RCMV rabbit antiserum reacted with BBSV and its isolated L protein (Table 1). This confirmed a considerable representation of group-specific antibodies in comovirus antisera described by Gallo and Musil (1988).

Table 1. Specificity of the antibodies in PTA ELISA

Antigen	Antibody			
	Anti-BBSV	Anti-L	Anti-S	Anti-RCMV
BBSV	+++	+	+	+++
BBSV-L	+++	+		+
BBSV-S	+		+	
RCMV	+++	+		+++

Results obtained with anti-RCMV rabbit antiserum are included for comparison. Mouse IgG were detected with commercial (ÚSOL, Prague) swine antibodies labelled with horseradish peroxidase, using o-phenylenediamine as substrate. A_{490} values were scored as follows: 0.06 – 0.30 (+), 0.30 – 0.80 (++), > 0.80 (+++). The sap of healthy pea plants gave no reaction with any antibody and the ascitic fluid from AMV-immunized mice bound none of comovirus antigens.

The binding ability of antibodies with BBSV and RCMV is summarized in Table 2. The PTA ELISA results correspond to those of immunoblots confirming considerable influence of the plate surface binding on the antigen structure. On the contrary the native structure of the viruses was conserved in DAS ELISA and the results were in accordance with those of the immunoprecipitation.

Table 2. Comparison of the reactivity of antibodies against BBSV and its coat proteins with BBSV and RCMV in different assays

Antibody	Reaction with BBSV/RCMV as antigens in different assays			
	PTA ELISA	IB	DAS ELISA	IE
Anti-BBSV	+/+	+L/+L	+/–	+/–
Anti-L	+/+	+L/+L	–/–	–/–
Anti-S	+/–	+S/–	–/–	–/–

Positive (+) or negative (–) reaction; L or S – respective coat protein; IB – immunoblot; IE – immunoelectrophoresis.

Anti-S gave no immunoblot reaction with RCMV. Apparently, S proteins of the two comoviruses do not share common sequential epitopes.

The immunological similarity between both viruses looks like to be caused mainly by common L-specific linear epitopes. The negative reaction of Anti-BBSV with native RCMV could indicate the absence of common surface epitopes. Nevertheless, such epitopes do exist. At least one of MoAbs prepared previously (Šubr *et al.*, 1993) which reacted both with RCMV and BBSV was of anti-metatope

character. Furthermore most of polyclonal rabbit antisera against RCMV or BBSV precipitated both viruśes. This discrepancy could be caused by another immunization protocol. After ip injection of the virus into mice the common surface epitopes could be immunologically presented less efficiently than after iv injection into rabbits. The level of this fraction of antibodies in Anti-BBSV could be undetectable.

The same may be said about the absence of anti-metatopes in Anti-L and Anti-S, taking into account the unability of these antibodies to bind native viruses. After immunization by isolated coat proteins the metatopes were not presented at all, or the anti-metatopes erose at low level only.

In immunoblots, in addition, we tested the cross-reactivity of the antibodies obtained with two BBSV strains, namely pea green mottle virus (PGMV) and pea seed-borne symptomless virus (PSbSV) (Musil *et al.*, 1983). As shown in Fig. 1, Anti-BBSV and Anti-L bound L proteins of all ascitic fluid from alfalfa mosaic virus-immunized mice (*amv10*); Gallo and Matisová, 1993 used as negative control did not bind any comovirus antigen in immunoblots (data not shown). No quantitative difference was observed in immunoblots between reactions of Anti-BBSV and Anti-L. After SDS-PAGE there reacted only the fraction of antibodies directed against sequential (detergent-stable) epitopes occuring in the isolated protein as well as in the native virus.

Joisson and van Regenmortel (1991) presume in another comovirus, bean pod mottle virus (BPMV), an effect of the C-terminus of its S protein subunit on the antigenicity of the native virus. They obtained antisera against synthetic peptides corresponding to 12 or 18 C-terminal amino acids of the S protein which reacted with intact BPMV virions in DAS ELISA. However, more recent results of these authors (Joisson *et al.*, 1993) did not confirm this reaction. This part of the S protein is probably really localized on the virion surface in accordance with the data of Chen *et al.* (1989)

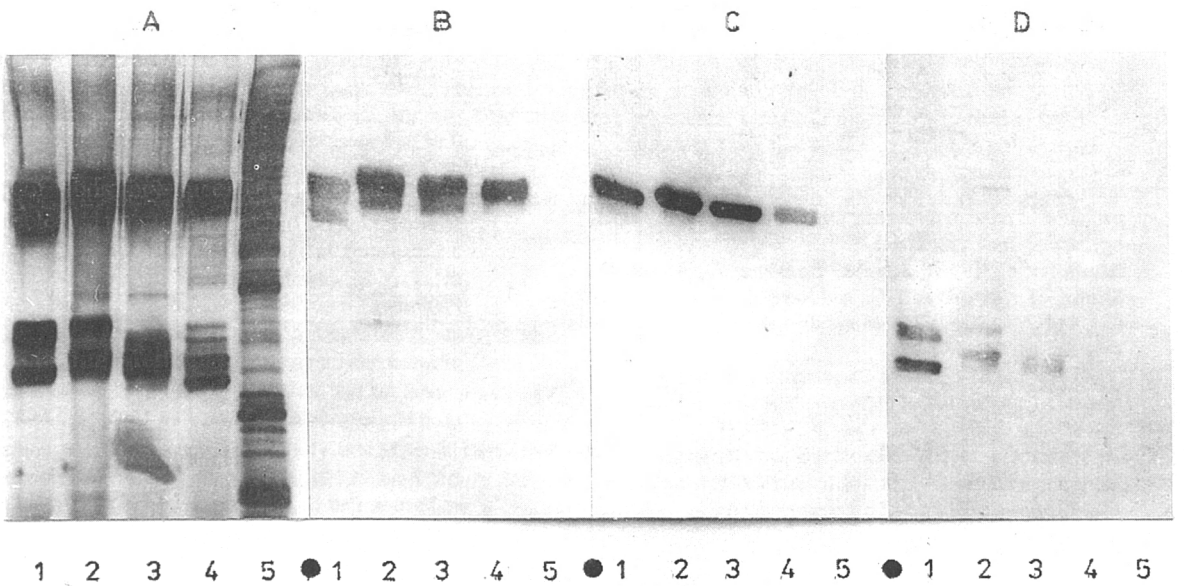

Fig. 1
Immunoblot specifity of antibodies
BBSV (lanes 1), PGMV (lanes 2), PSbSV (lanes 3), RCMV (lanes 4) and chloroform-treated sap of healthy pea plants (lanes 5) were electrophoresed in 12% polyacrylamide gel (A – silver staining) and blotted onto nitrocellulose membranes. Immunological detection of blots was done with antibodies Anti-BBSV (B), Anti-L (C) and Anti-S (D). All antibodies were diluted 1:500.

four isolates (including RCMV), while Anti-S reacted with the BBSV strains only. Also weak and nonreproducible reactions of Anti-S with PGMV and PSbSV L proteins and of Anti-BBSV with PGMV S protein were noted, probably of nonspecific character. A sample of healthy plants gave no reaction with any antibody (Fig. 1, lanes 5) and the who, based on X-ray diffraction analysis, predicted a three-dimensional model of the comovirus capsid in which both coat proteins are exposed on the capsid surface.

Further experiments with synthetic oligopeptides (Joisson *et al.*, 1993) showed that native comovirions were bound in DAS ELISA by antibodies against an oligopeptide

corresponding to S protein amino acids 20 – 27. However, this was true only in the case when a cyclic oligopeptide was used for immunization. In the linear form it did not show any epitope homology with native virus particles demonstrating probable conformational character of this antigenic site. It could be partially imitated by the molecule deformation caused by stable cyclization by means of an S-S bond. Using succinimidyl cyclization product with a lower deformation effect, antibodies with weaker ability to bind virus were obtained. These results are in good agreement with our observations.

The fact that following ip or iv immunization of animals with native comoviruses no antibodies were formed against sequential epitopes of the S protein subunit of virus capsid suggests that all such epitopes located on the S protein are hidden or masked by the larger coat protein. Also the inability of Anti-S to bind native viruses confirms this fact.

S protein epitopes located on the virion surface (including the C-terminus of its proteolytically unprocessed form and the part between amino acids 20 – 27 investigated by Joisson and coworkers (Joisson *et al.*, 1993)) are detergent-sensitive and thus are exclusively of the conformational type.

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