ALFALFA MOSAIC VIRUS STRAINS T6 AND 425 DIFFER IN THEIR CAPSID PROTEIN

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Summary. – Capsid proteins (CPs) of two strains of alfalfa mosaic virus (AlMV) – T6 and 425 – were compared using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) peptide mapping and two-dimensional PAGE (2D-PAGE). The CPs had identical molecular mass but differed in their peptide pattern and charge. The AlMV strain T6 was isolated from lucerne in Czech Republic (Gallo, 1977). Previously, its morhology, symptomatology and RNA electrophoretic mobility were characterized and compared with those of the strain 425 (Hagedorn and Handson, 1963; Kúdela and Gallo, 1995). In this study we show that the CP composition of these two AlMV strains differs, too. AlMV, the unique member of the genus *Alfamovirus*, family *Bromoviridae*, is of economic significance in many countries in the world, particularly for its seed transmissibility and relative broad host range. Plenty of strains with different biological properties have been isolated (Van Regenmortel and Pinck, 1981). Some of them have been shown to differ in their RNA non-coding regions or in CP composition (Kraal, 1975; Kraal *et al.*, 1976; Collot *et al.*, 1976; Dore *et al.*, 1989; Neeleman *et al.*, 1991).

Key words: alfalfa mosaic virus; capsid protein; peptide mapping; isoeletric focusing

For this purpose we electrophoresed CPs of strains T6 and 425 in one run of SDS-PAGE (Laemmli, 1970). Virus suspensions were mixed with the Laemmli sample buffer, heated at 100°C for 5 mins and loaded onto a 14% gel. After the run, the gel was silver-stained according to Marcinka *et al.* (1994). MW-SDS-70 Kit (Sigma) was used as standard of M_r of proteins. No difference in the mobility profile of CPs of strains T6 and 425 was observed. Both CPs migrated as two major bands (the faster was probably proteolytically shortened) of M_r of 34 K and 30 K, respectively. However, after cleavage with proteolytic enzymes (Šubr *et al.*, 1993), CPs of the two strains gave different digestion patterns, particularly after V8-protease (Sigma), chymotrypsin (Sigma) and thermolysin (Serva) cleavage, demonstrating amino acid (aa) sequence diversity of CPs of the two strains (Fig. 1).

Investigation of electrophoretic mobility of AlMV virions (Bol and Lak-Kaashoek, 1974) revealed 4 major nucleoprotein components (containing genomic and subgenomic RNAs 1-4) and a set of minor ones reflecting probably slight irregularities in the virion assembly.

We have observed that virions of the strain T6 had in 2D-immunoelectrophoresis a higher content of the faster electrophoretic forms than those of the strain 425 (data not shown). Since there are only small differences between these strains in the genomic RNA mobility (Kúdela and Gallo, 1995), the abovementioned effect was probably caused by differences in CPs.

Abbreviations: aa = amino acid; AlMV = alfalfa mosaic virus; CPs = capsid proteins; IEF = isoelectric focusing; PAGE = polyacrylamide gel electrophoresis; SDS = sodium dodecyl sulphate; 2D-PAGE = two-dimensional PAGE

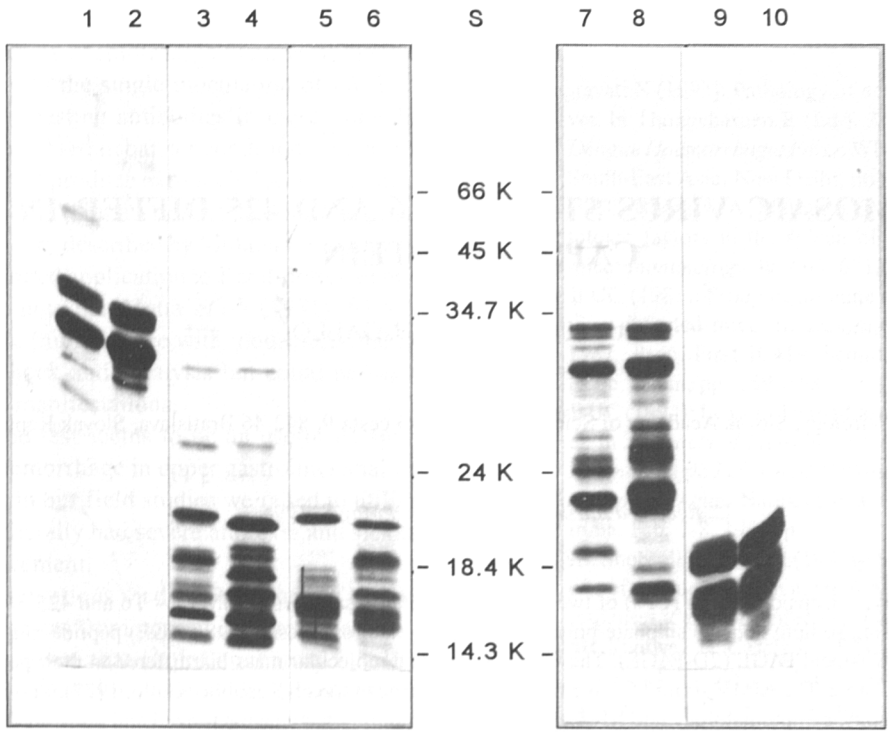

Fig. 1
SDS-PAGE of AlMV CPs
Strain 425: uncleaved (lane 1), cleaved with thermolysin (lane 3), chymotrypsin (lane 5), V8-protease (lane 7) and trypsin (lane 9). Strain T6: uncleaved (lane 2), cleaved with thermolysin (lane 4), chymotrypsin (lane 6), V8-protease (lane 8) and trypsin (lane 10). Protein M_r standards (lane S).

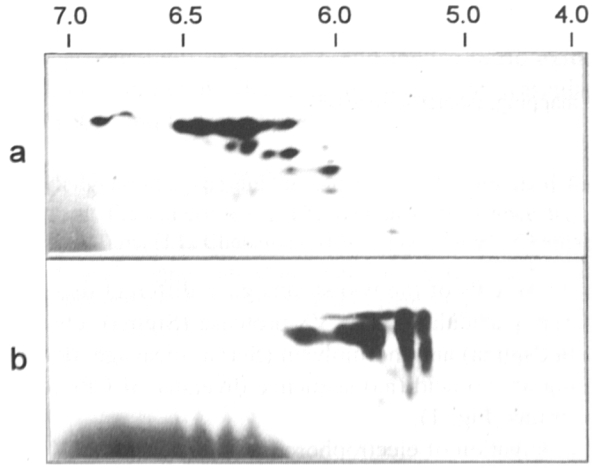

Fig. 2
2D-PAGE of AlMV CPs
Strains 425 (a) and T6 (b). First dimension: IEF in pH range 3 – 10 (the actual pH scale is given on the top); second dimension: SDS-PAGE.

We compared the charges of CPs of the two strains by the isoelectric focusing (IEF) and 2D-PAGE. Virions were disrupted in the presence of 1% SDS, thoroughly dialysed against 0.1 mol/l phosphate buffer pH 7.0 and loaded on the top of polyacrylamide IEF tube gel) with pH range 3 – 10 (Pharmalyt, Pharmacia. After the run the tubes were either stained with Coomassie Brilliant Blue R 250 (results not shown) or subjected to the second dimension run, the SDS-PAGE in 14% gel according to Jäckle (1978). As shown in Fig. 2, the 2D-PAGE patterns of CPs of the two strains were not identical. Moreover, the forms of T6 CP were apparently more acidic (pI 5.5 – 6.2) than those of 425 CP (pI 6.0 – 6.8). These pI values cannot be regarded as precise ones because traces of SDS could have remained bound to the CPs even after thorough dialysis. Nevertheless, the patterns obtained were reproducible and so this sensitive method can be also used for differentiation of the two AlMV strains.

In AlMV infection process CPs have not only the function in forming virions but are also essential for initiation of viral replication (Smit *et al.*, 1981; Zuidema *et al.*, 1983). Thus the symptom differences between strains T6 and 425 observed by Kúdela and Gallo (1995) – "growing" versus "non-growing" small local necrotic lesions on French bean cv. Bountiful – could be causally connected with different aa composition of their CPs. The influence of aa variation in AlMV CPs on the symptoms has been reported with presumption that the charge of CPs can affect their interaction with an induced host response factor (Neeleman *et al.*, 1991). Our results presented here fit this hypothesis.

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