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PHYSICOCHEMICAL PROPERTIES OF FOUR TYMOVIRUSES

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Summary. - Isoelectric point (pI), free mobility and retardation coefficient (K_R) of turnip yellow mosaic virus (TYMV), erysimum latent virus (ELV), belladonna mottle virus (BelMV) and scrophullaria mottle virus (ScrMV) have been studied. The use of Ferguson plots for the physical identification of these four viruses and for confirmation of their identity is discussed. For TYMV, ELV, BelMV and ScrMV the pI values of 3.64, 4.73, 6.25 and 3.95, respectively, were found. Using K_R the differences between the diameter of particles of the four tymoviruses were estimated. The particle diameter decreases as follows: ELV, ScrMV, TYMV, BelMV.

Key words: tymoviruses; Ferguson plot; isoelectric point; free mobility; retardation coefficient; electrophoresis; exclusion chromatography

Introduction

The tymovirus group includes plant viruses with one molecule of single stranded (+)RNA as a genome, that is packed into icosahedral capsid (Walkey, 1991). In sucrose density gradient the viruses sediment as two components: the bottom (B) component contains a nucleic acid, and the top (T) is formed by empty shells. The best characterized tymovirus is TYMV. On the other hand, there is only a limited knowledge about the electrophoretic properties of the other tymoviruses. The pI of ELV, TYMV, kennedya yellow mosaic virus and poinsettia mosaic virus were published by Shukla and Gough (1980), Matthews (1980), Gibbs (1978) and Koenig *et al.* (1986). The direction of movement of fourteen tymoviruses and some of their isolates in the electric field was investigated by Koenig (1976). The free mobility is known for the cacao yellow mosaic tymovirus only (Brunt, 1970).

In this paper we investigated the isoelectric point, the free mobility and the retardation coefficients of TYMV, ELV, BelMV and ScrMV.

Materials and Methods

Viruses. The TYMV isolate Dobříš (Špak and Polák, 1985), BelMV (Pelikánová *et al.*, 1992) and ELV (Špak *et al.*, 1993) were used. The Czech isolate of ScrMV was found in the *Scrophularia nodosa*

L. plants and was serologically identical with the Italian isolate of the virus (Rana *et al.*, 1988). All the viruses were propagated in systemic host plants which were harvested 14 days after inoculation.

The purification of viruses were conducted as described by Pelikánová *et al.* (1992) with small differences: The viruses were extracted in 0.01 mol/l phosphate buffer pH 7.0, and then precipitated with 4 % polyethylene glycol 6000 (Serva) and 4 % NaCl. After centrifugation at 12 000 rpm for 10 min in Janetzki K 24 centrifuge the size exclusion chromatography as a more gentle method was used for the isolation of viral particles from the crude isolate. About 2 mg of each virus was loaded on Sephadex G-50 (Pharmacia) column (25 × 0.9 cm) equilibrated with 0.01 mol/l phosphate buffer pH 7.0 and eluted with the same buffer. The first fraction highly absorbing at 260 nm was collected and used for further studies.

The estimation of pI of viral particles was performed by nonequilibrium pH gradient electrophoresis in agarose gel. The viruses were loaded with 10 % glycerol on 1 % agarose gel (Loeve Biochemica) 90×60×3 mm with 12 % w/v of sorbitol and 3 % v/v 3-10 Pharmalyte (Sigma). The electrolytes were 0.5 N NaOH and 0.02 N H_2SO_4. The field strength was 3 V/cm and duration of electrophoresis was 800 V.hr. The gels were stained with Coomassie Brilliant blue and the pH gradients were measured after elution of 0.5 cm pieces of one gel strip in distilled water.

The Ferguson plots were conducted according Orbán and Chrambach (1988) on a series of 0.4 % – 2.0 % agarose gels 30×120×3 mm in 0.01 mol/l phosphate buffer pH 7.0 and the same buffer as electrolyte. The electrophoresis was carried out at 4 °C using an electric field strength of 5 V/cm for 48 hr. Gels were stained with ethidium bromide (50 μg/ml).

We used the semilogarithmic plots of mobility versus gel concentration (Ferguson plots) for the derivation of free mobility and retardation coefficient. The approximation of the plot to zero concentration of agarose is logarithm of free mobility ($\log\mu_0$). This parameter is a measure of the surface charge. The slope of the plot is the retardation coefficient (K_R), physical meaning of which is a measure of size of separated particles. Both the values ($\log\mu_0$ and K_R) were calculated according Gombocz and Chrambach (1989) from the linear part of slopes from data of three independent experiments. The identity test was based on plotting the joint 95 % confidence envelopes of K_R and the free mobility of separate viruses and was done as described (Chrambach and Rodbard, 1988).

Results and Discussion

The results are summarized in Table 1. The pI of the four tymoviruses were found in the acid region. The pI of TYMV 3.64 is very close to previously published value 3.75 (Matthews, 1980), but the pI 4.73 of the Czech isolate of ELV is more distant from the value 5.40 obtained for the German isolate of ELV by Shukla and Schmelzer (1972). According to our knowledge the pI of BelMV and ScrMV were not published previously.

The TYMV and ELV moved toward anode, whereas BelMV and ScrMV moved to cathode. The differences in the free mobility of viral particles reflect their different net charge.

The Ferguson plots of log of absolute mobility versus gel concentration of the four tymoviruses are shown in Fig. 1, 2. The plots are linear between 1–2 % agarose with the exception of ELV. Nonlinear Ferguson plot was described for hibiscus chlorotic ringspot and turnip crinkle carmoviruses, too (Gombocz *et al.*, 1987). The nonlinearity occurred under different electrophoretic conditions between different agarose concentrations. It seems, that this phenomenon depends on the quality of agarose used for the experiment.

Table 1. Isoelectric point (pI), free mobility and retardation coefficient (K_R) of TYMV, ELV, BelMV and ScrMV

Virus	pI	Free mobility [$cm^2/V/s \times 10^5$]	K_R	
			$\bar{x}$	S. D.
TYMV	3.64	-9.39	0.30	0.03
ELV	4.73	-6.15	0.55	0.11
BelMV	6.25	6.89	0.16	0.05
ScrMV	3.95	5.71	0.44	0.01

The mobility of viral particles toward the anode is marked with (-). $\bar{x}$ - average value, S. D. - standard deviation.

Under the conditions used in our experiments (0.01 mol/l phosphate buffer pH 7.0, 4 °C and agarose separating gel) we did not found statistically significant difference between the K_R of ELV and ScrMV at the 95 % coincidence level. Therefore we suppose that the diameter of particles of ELV and ScrMV is identical.

The K_R of turnip yellow mosaic virus and of belladonna mottle virus is significantly smaller when compared with those of ELV and ScrMV. The diameter of particles is proportional to a square root of the K_R value. We can therefore estimate the differences between the diameter of the four viruses more preciselly, than by measuring the particles from electron micrograph. ELV and ScrMV have approximately the same diameter of particles, TYMV has smaller

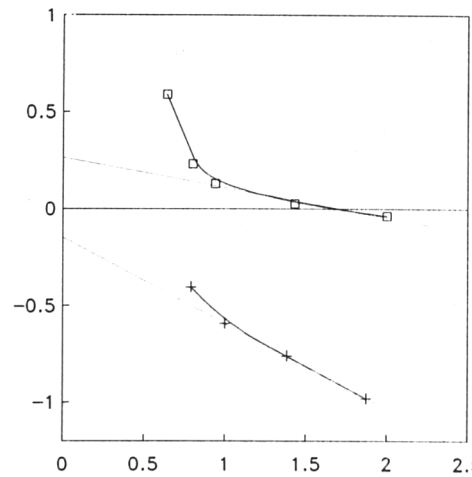

Fig. 1
Ferguson plots for BelMV and ScrMV
Abscissa: agarose concentration (%); ordinate: absolute mobility (log μ + 5). The approximation of the linear part of the plot is illustrated by dotted line. BelMV (□), ScrMV (+).

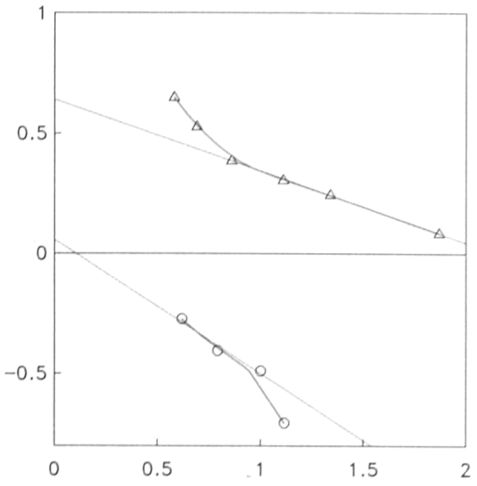

Fig. 2
Ferguson plots for TYMV and ELV
For legend see Fig. 1. TYMV (△), ELV (○).

particles and the smallest ones was found by BelMV. As far as we know retardation coefficients of tymovirus particles were not yet published. A comparison of our data with those of Gombocz *et al.* (1987) is not possible, because they used different ways of diameter estimation.

The tymovirus group consists of at least two clusters of viruses, when compared their biological and biophysical properties and sequence data (Jellison, 1987; Jacob *et al.*, 1992). One cluster is formed the BelMV, ScrMV together with physalis mottle, eggplant mosaic and ononis yellow mosaic viruses, whereas TYMV with kennedya yellow mosaic and cacao yellow mosaic viruses form the second one. The erysimum latent virus is more distant to both the clusters.

The viruses studied in the present report are from all the clusters. The differences found for the four viruses could support the classification, as the pI of all the viruses is in acid region, BelMV, ScrMV and most of viruses of this cluster move slowly to the cathode, TYMV and other viruses of that cluster move quickly and ELV slowly to the anode. It seems, that the direction of movement expressed as free mobility is more important feature for the characterization of tymoviruses (as it correlates with other viral characteristics) than the retardation coefficient (where is no correlation for BelMV and ScrMV with their position in one cluster).

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