Ion exchange membrane chromatography and gel filtration as tools to investigate plant RNA viruses

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INTRODUCTION

The net charge of viral particles is one of the important factors determining their interaction with host plant proteins. It also defines the binding of viral particles to positively or negatively charged surfaces as ion exchange membranes. Such membranes can capture viral particles and in turn facilitate fast determination of their net charge in plant extract. Gel filtration can be next used for fast separation of complexes of viral particles with interacting host plant proteins from the rest of host plant proteome. To test these concepts, in this work we have investigated the ability of positively (Q) or negatively (S) charged membranes to bind plant RNA viruses using as models potato virus Y (PVY), potato virus M (PVM) and potato leafroll virus (PLRV). These viruses represent three typical groups of plant viruses differing in genome organization, the shape of particles and mechanisms of replication and translation. Particles of all investigated viruses expressed negative charge in studied conditions. However, the strength of their binding to membrane Q was different. Using PVY as a model virus, we have also developed a gel filtration on high pore beads for fast separation of host plant proteins interacting with viral particles. Both, ion exchange membrane chromatography and gel filtration were proved to be useful tools for plant virus investigation, purification as well as a mean of viruses concentration to increase the sensitivity of virus detection.

Virus concentration by filtration through Ion-Exchange Membranes

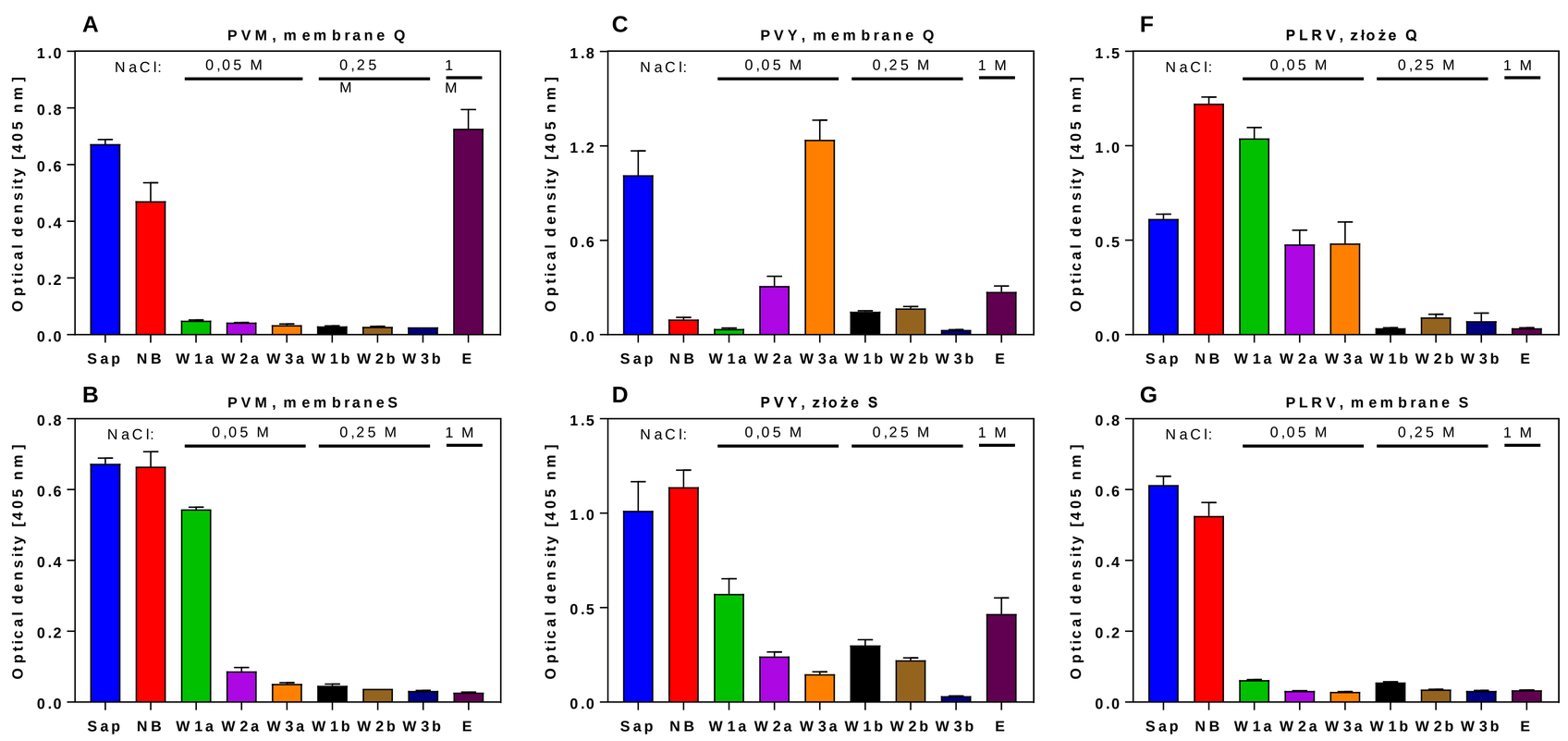

Fig. 1. Evaluation of the binding efficiency of PVM virus particles (A, B), PVY (C, D) and PLRV (E, F) to the Q (A, C, E) and S (B, D, F) membrane centrifugal filters using the ELISA test. Sap - initial sap, 20-fold diluted with the assay buffer, extracted from leaves of plants infected with the test viruses. Fraction not bound to the membrane - NB. Fractions eluted with assay buffer with 0.05 M NaCl - W1-3a. The fractions eluted with a buffer with 0.25 M NaCl - W2-3b. The fraction eluted with 1 M NaCl buffer, concentrated and desalted on a spin filter with a ten kDa cut-off point - E.

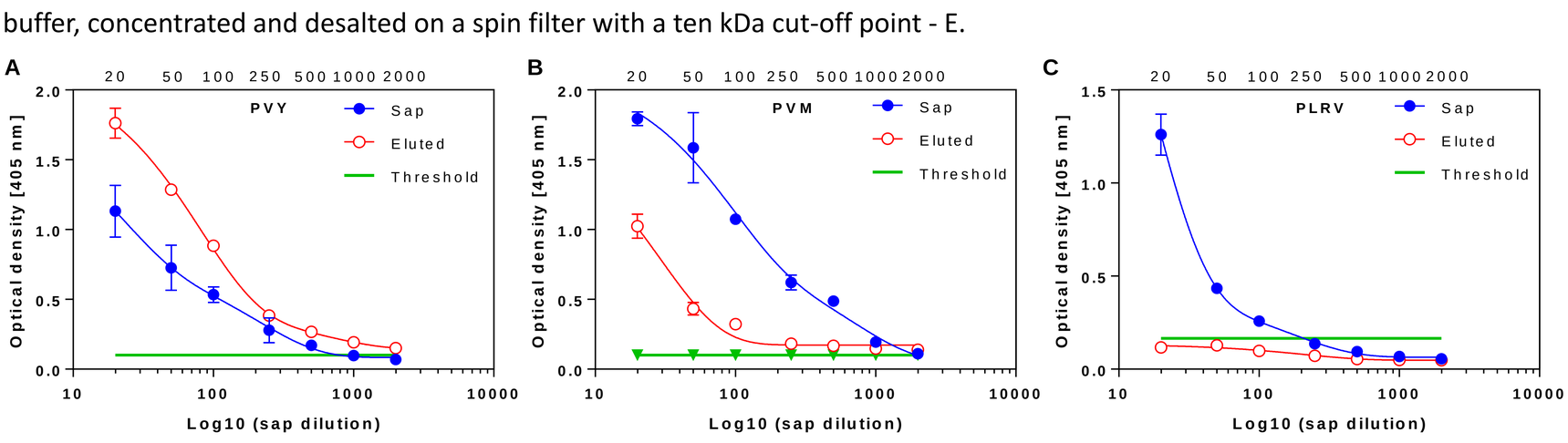

Fig. 2. Efficacy of concentration of PVY (A), PVM (B) and PLRV (C) particles on the filtration microplate with Q membrane Dilutions of extract from infected plants - Sap. The same dilutions after binding on membrane Q, elution with 1 M NaCl, and concentration on centrifugal filters with a cut-off point of 10 kDa - Eluted. The numbers above the graphs (20-2000) indicate the dilution of juices. The sum of the mean absorbance at 405 nm and the three times the standard deviation calculated for the virus-free samples - Threshold. Error bars represent standard deviations.

Size exclusion on Sepharose S1000

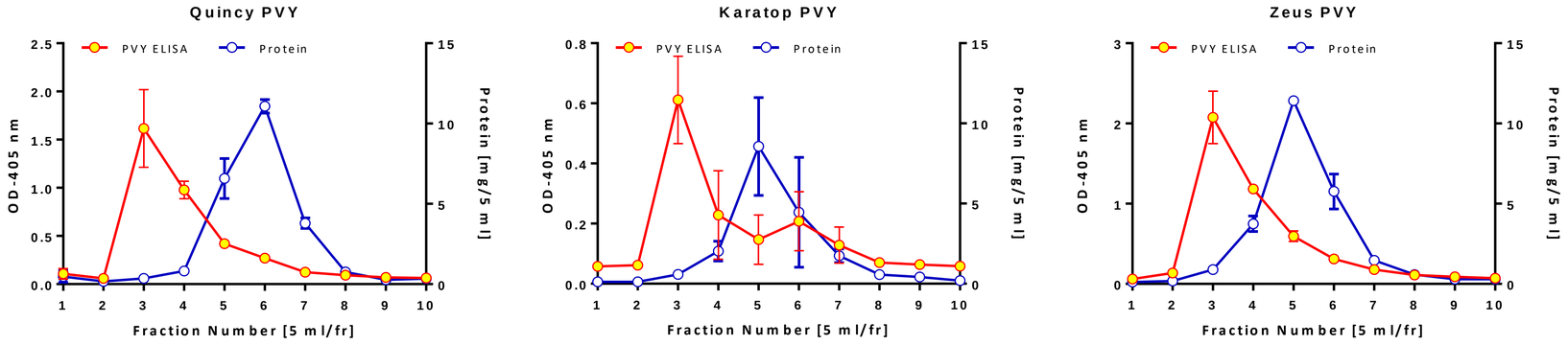

Fig. 3. Rapid separation of virus particles with associated proteins by Size Exclusion Chromatography on Sepharose S1000. The column was loaded with homogenates extracted from plants of the varieties Quincy, Karatop and Zeus infected with PVY applied to the column. Protein complexes with a mass equal to or greater than 1 million Da eluted in the empty column volume (Fractions 2-4, Peak 1) and proteins and peptides (1-900 kDa) as a combined large peak 2, equal to the volume of gel in the column. Each experimen was triplicated. Error bars represent standard deviations.

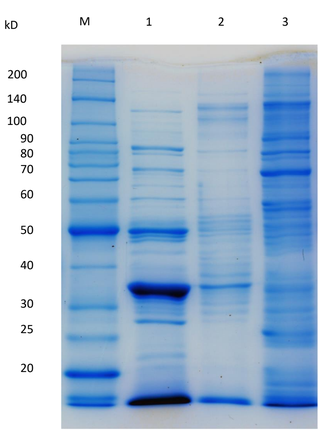

Fig 4. Analysis of protein composition in Pik 1 (fractions 2-4, Fig. 3, Karatop) by SDS-PAGE. Molecular mass standard - M. Peak 1 after concentration on the ten kDa filter – lane **1**. Peak 1 collected from the column (before concentration) – lane **2**. Homogenate before S1000 – lane **3**.

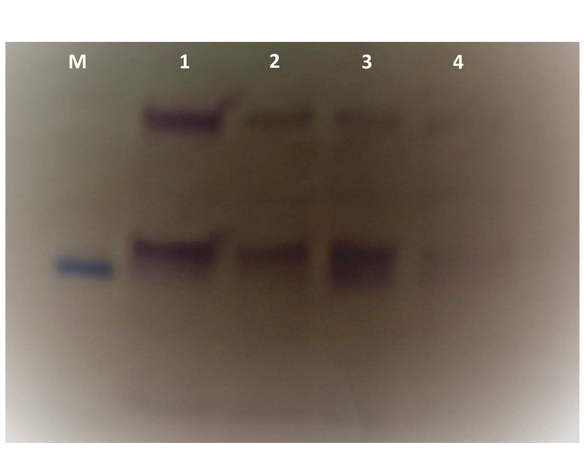

Fig 5. Detection of the coat protein of PVY in Peak 1 (fractions 2-4, Fig. 3, Karatop) by Western Blotting. 30 kDa molecular mass standard – lane **M**. Fractions 2-5 eluted from the column - lanes **1** to **4**.

MATERIALS AND METHODS

Concentration of viruses on IEX membranes. A total of 1 ml of sap extracted from leaves of potatoes infected with viruses Y, L and M were loaded to the spin columns with a positive (Q) or negative (S) charge membrane. After a 5 minute incubation, the columns were centrifuged and the juice collected into Eppendorf tubes. The columns were rinsed 3x with 1 ml of assay buffer with 0.05 M NaCl rinsing W1-3a), 3x with 1 ml of assay buffer with 0.25 M NaCl (W1-3b) and 3x with 1 ml of assay buffer with 1 M NaCl (elution, E). Samples eluted with 1 M NaCl were combined and concentrated to 1 ml on ten kDa centrifugal filters. The viruses Y, L, and M, were monitored by ELISA in sap before applying to the columns, sap that passed through the bed Q or S, and in the fractions eluted with a buffer with different concentrations of NaCl. The optical density at 405 nm obtained for samples isolated from healthy sap was used to calculate the threshold below which the samples were considered to be virus-free. The threshold was the sum of the mean absorbance at 405 nm obtained for the tests of healthy plants and three times the standard deviation calculated for this average.

Efficacy of PVY (A), PVM (B) and PLRV (C) concentration on the filtration microplate with Q bed. Two dilution series of sap collected from infected leaves were prepared for each virus (20, 50, 100, 250, 500, 1000, and 2000-fold dilutions). The standard DAS-ELISA tested 200 µl of each dilution for virus level. One ml of each dilution was applied to wells of a microplate (Pall) with a Q or S membranes. The dilution flow through the bed located on the bottom of the microplate wells was forced using negative pressure (about -25 mm Hg). The samples were collected into the wells of the receiver microplate under the microplate with the ion exchanger. After collecting independent samples, the wells of ion-exchange microplates were rinsed with buffer without salt (5x1ml). Viral particles were eluted with 1 M NaCl buffer (3x1ml / well). After collecting, elutes obtained from the same wells were combined and concentrated to 0.5 ml on centrifugal filters with a ten kDa cut-off point. Buffer without salt (3 ml) was then added and concentrated again to 0.5 ml to lower the NaCl concentration in the pooled samples. The DAS-ELISA tested the initial level of viruses in the sap and in the concentrated samples to estimate the concentration efficacy.

Fast separation of virus particles by Size Exclusion Chromatography. Leaves from infected plants and from healthy were homogenized to produce 30% homogenates. Homogenates were loaded to a 2.5 cm diameter column containing 50 ml of Sephacryl S1000 gel. Fractions were collected at 1 ml/min. The presence of PVY virus was determined by ELISA and the protein by BCA test. Fractions composing viral peak (Fractions 3-4) were collected and concentrated. The control preparations from healthy plants were prepared in the same way. Each isolation was repeated three times.

CONCLUSIONS

- The particles of all the viruses tested were associated with the Q membrane.
- PVM bond strength was high, and PLRV was low.
- PVY is heterogeneously charged and occurs in the form of fractions with different binding strength to the Q and S membranes.
- The developed method effectively concentrated PVY and PVM, but not PLRV.
- Size Exclusion Chromatography on Sepharose S1000 facilitates fast (5-15 min) separation of viral particles and accompanying host protein complexes from a bulk of host proteins.

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