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Properties of viruses of the potyvirus group. 3. A comparison of buoyant density, S value, particle morphology, and molecular weight of the coat protein subunit of 10 viruses and virus isolates

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Abstract

A comparison of six isolates of bean yellow mosaic virus, pea seed-borne mosaic virus, two isolates of potato virus Y, and lettuce mosaic virus revealed that differences exist with respect to buoyant density and S value, but that the differences between isolates of one virus can be as big as those between different viruses. There is no correlation between buoyant density and S value on the one hand and pathogenicity on the other hand. All viruses and virus isolates, except the potato virus Y isolates, show the Mg^{++} effect. The molecular weight of the coat protein subunits of all viruses was 34,000 daltons. In 8 out of 10 cases the protein was easily degraded into a 28,000 daltons component. Only the potato virus Y isolates did not show this effect.

Introduction

In a previous article (Huttinga and Mosch, 1974) some physical properties of four viruses of the potyvirus group were described. We now extended the work to more isolates of these viruses and to another virus to see whether or not differences between isolates of one virus are smaller than differences between viruses.

Materials and methods

Virus isolates and host plants. In our previous studies (Huttinga, 1973; Huttinga and Mosch, 1974) we distinguished between bean yellow mosaic virus (BYMV) B25 and pea mosaic virus E198. Like Bos et al. (1974) we consider them now as isolates B25 and E198 of BYMV. Besides these we used four other isolates of BYMV: Kow14, Kow28, E212, and E221. These and the pea seed-borne mosaic virus (PSMV) E210 were provided by Dr L. Bos, who has described their properties and their relationships (Bos, 1970; Bos et al., 1974). The potato virus Y^O (PVY^O) was supplied by Dr J. A. de Bokx. It was described as PVY^OIdB (De Bokx et al., 1975). Potato virus Y^N (PVY^N) and lettuce mosaic virus (LMV) were the same as used before (Huttinga, 1973). The cryptograms of each of the viruses used is */*:*/*:E/E:S/Ap. The BYMV isolates and PSMV E210 were propagated in *Pisum sativum* 'Koroza', PVY^O in *Nicotiana tabacum* 'Samsun NN' (Huttinga, 1973).

Virus purification. The viruses were purified by differential centrifuging using relatively low centrifugal forces (Huttinga, 1973).

Buoyant-density measurements. Buoyant densities were determined according to Szybalski (1968) using BYMV B25 as an internal marker. Determinations were performed in CsCl at 20 °C.

S-value measurements. Centrifugal analyses were done in a Spinco Model E ultracentrifuge using Schlieren optics. Sedimentation coefficients at infinite dilution were determined by the graphical method of Markham (1960).

Particle morphology. The particle morphology was studied from electron micrographs made of purified virus preparations which were negatively stained with 1 % potassium phosphotungstate pH 6.5 in water.

Determination of the molecular weight of the coat protein subunit. The viruses were degraded, the proteins reduced and carboxymethylated according to Geelen et al. (1972). Molecular weights were determined by polyacrylamide gel electrophoresis as described before (Huttinga and Mosch, 1974). The coat protein of BYMV B25 was used as an internal marker.

Results

Virus purification. With BYMV Kow14, Kow28, E221, and PSMV E210 it was easy to obtain large amounts of unaggregated virus. However, BYMV E212 and PVY^{O} always aggregated to a large extent.

Table 1. Buoyant density, S value, molecular weight of the coat protein subunit, and the influence of Mg^{++} on the particle morphology of six strains of BYMV, PSMV E210, two strains of PVY, and LMV.

Virus	Buoyant density (g/cm^3)	S value (S)	Mw protein (daltons)	Mg^{++} effect[1]
BYMV B25	1.318	143	34,000	+
BYMV E212	1.318		34,000	
BYMV E198	1.321	140	34,000	+
BYMV Kow28	1.319	142	34,000	+
BYMV E221	1.325	145	34,000	
BYMV Kow14	1.318	144	34,000	+
PSMV E210	1.329	154	34,000	
LMV	1.330	143	34,000	+
PVY^{O}	1.323		34,000	−
PVY^{N}	1.326	145	34,000	−

[1] + = particle morphology can be influenced by Mg^{++}; − = particle morphology can not be influenced by Mg^{++}.

Tabel 1. Zweefdichtheid, S-waarde, molecuulgewicht van de manteleiwiteenheid en de invloed van Mg^{++} op de deeltjesvorm van zes stammen van BYMV, PSMV E210, twee stammen van PVY en van LMV.

Fig. 1. Equilibrium-centrifuging pattern in CsCl of (from left to right) BYMV Kow14, PVY°, and BYMV E221 after 22 h at 145,000 *g*. Bar angle 70°. Temperature 20°C.

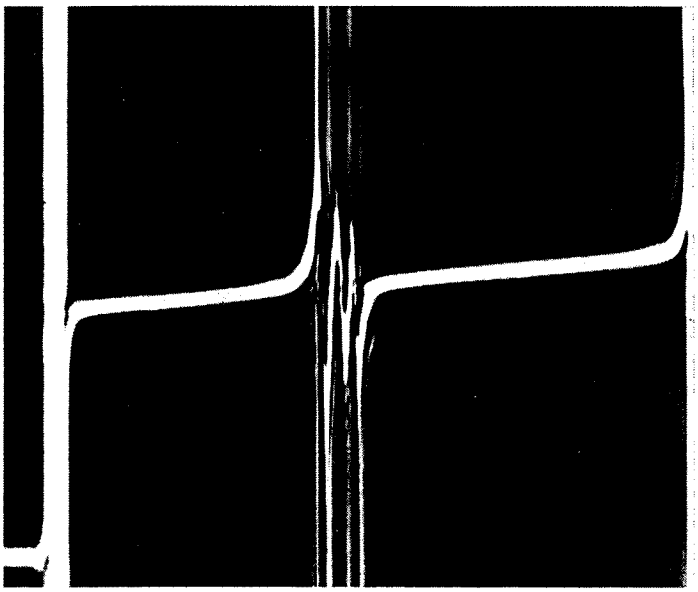

Fig. 1. Patroon van een evenwichtscentrifugering in CsCl van (van links naar rechts) BYMV Kow14, PVY° en BYMV E221 na 22 uren bij 145.000 g. Spleethoek 70°. De temperatuur bedroeg 20°C.

Buoyant densities. All isolates of BYMV, PSMV, and PVY reached equilibrium in a single band. However, they differed considerably in buoyant density as can be seen from Fig. 1, representing the results of an equilibrium centrifuging in CsCl of a mixture of BYMV Kow14, BYMV E221, and PVY°. The buoyant densities of BYMV Kow14, BYMV Kow28, BYMV E212, BYMV E221, and PSMV E210 were 1.318, 1.319, 1.318, 1.325, and 1.329 g/ml respectively. That of PVY° was 1.323. These results are presented in Table 1 together with data obtained previously for other viruses (Huttinga and Mosch, 1974).

S values. BYMV Kow14, BYMV Kow28, BYMV E221, and PSMV E210 each sedimented as a single sharp peak. Their S values at infinite dilution in 0.1 M tris-HCl buffer pH 9 at 20°C were 144, 142, 145, and 154 S (Table 1). BYMV E212 and PVY° sedimented in a very broad peak, indicating that severe aggregation had occurred. We therefore were not able to determine their S values accurately.

Particle morphology. The morphology of BYMV Kow14 and BYMV Kow28 could be influenced by environmental conditions. If purified preparations were dialyzed overnight against 0.05 M ethylenediaminetetraacetic acid (EDTA), flexuous particles were seen in the electron micrographs. Subsequent addition of 0.05 M $MgCl_2$ straightened the particles. When the $MgCl_2$ was removed by dialyzing against EDTA the particles became flexuous again. The morphology of PVY° was not affected by addition or removal of $MgCl_2$.

Coat protein subunit. The molecular weight of the coat protein subunit of BYMV Kow14, BYMV Kow28, BYMV E212, BYMV E221, PSMV E210, and PVY^{O} had to be the same because in co-electrophoresis experiments with BYMV B25 protein their migration proved to be identical. So their molecular weight has to be 34,000 daltons. For all viruses, except PVY^{O}, we also found the breakdown product of 28,000 daltons, described earlier for BYMV B25, BYMV E198, and LMV (Huttinga and Mosch, 1974).

Discussion and conclusions

According to Bos et al. (1974) five of the six BYMV isolates can be divided into three major groups. B25 and E212 belong to the so-called bean yellow mosaic isolates, E198 and Kow28 are isolates of the pea yellow mosaic strain, and E221 is a pea necrosis strain isolate. Kow14 does not fit in any of these groups.

It is obvious from Table 1 that the values found for the buoyant density and the sedimentation coefficient of the BYMV isolates are not correlated with the pathogenic groups. On the contrary, Kow14 which is the most deviating isolate with respect to pathogenicity has the same buoyant density and almost the same S value as the B25 isolate which is considered as the 'type isolate' of BYMV. Differences in buoyant density between the typical bean yellow mosaic virus isolates and the pea necrosis strain isolate are even bigger than that between BYMV B25 and PVY^{N}. It can be concluded that the variation in buoyant densities and S values among BYMV isolates is as great as between the different viruses of the potyvirus group.

The molecular weight of the coat protein is the same for all isolates and viruses tested so far. The breakdown of the 34,000 daltons component into a 28,000 daltons component is found for all isolates except for the two PVY isolates. We are not sure whether this is an intrinsic property of PVY, because these isolates were purified from *N. rustica* 'Samsun NN' and these preparations may have been free of proteolytic activity.

The Mg^{++} effect, as described by Govier and Woods (1971), was found for all isolates and viruses tested, except for PVY isolates. Here again, PVY behaves differently from the other members of the potyvirus group. It is remarkable that PVY^{O} and PVY^{N}, both isolates of the type member of the potyvirus group (Harrison et al., 1971), can clearly be distinguished from the other members of the group by two characteristics.

In general our data indicate that the viruses under investigation are rather closely related. Bos et al. (1974) came to the same conclusion using symptomatology, serology and electron microscopy. Furthermore it is obvious that the intrinsic properties we investigated are no suitable tools to identify isolates of one virus or even to identify viruses of one morphological group because there is no correlation at all with pathogenic properties of the viruses. Mosch et al. (1973) came to a similar conclusion after an investigation of some chemical and physical properties of 18 tobacco mosaic virus isolates. Identification of viruses on the basis of intrinsic properties, is an identification on the expression of one part of the polycistronic virus RNA. The pathogenic properties, however, are the expression of a completely different part of the RNA. Differences in this latter part, which are noticed as differences in pathogenicity, will not necessarily be reflected in the intrinsic properties. For the identification of different

pathogenic strains of one virus, other than on symptomatology, one has to determine the base sequence of the RNA. However, this method is rather time consuming, and there is still much work ahead before we can predict from the base sequence of an RNA the pathogenic effect of the virus concerned.

Samenvatting

Eigenschappen van virussen van de potyvirusgroep. 3. Een vergelijking van de zweefdichtheid, de S-waarde, de deeltjesvorm en het molecuulgewicht van de mantele iwiteenheid van 6 isolaten van het bonescherpmozaïekvirus, het erwterolmozaïekvirus, twee isolaten van het aardappelvirus Y en het slamozaïekvirus

Uit een vergelijking van 10 virussen en virusisolaten van de aardappelvirus Y-groep bleek dat er weliswaar duidelijke verschillen bestaan met betrekking tot de zweefdichtheid en de S-waarde, maar dat de verschillen tussen isolaten van één virus even groot kunnen zijn als die tussen verschillende virussen. Er werd geen correlatie gevonden tussen zweefdichtheid en S-waarde enerzijds en pathogeniteit anderzijds.

Alle bestudeerde virussen en virusisolaten, met uitzondering van de aardappelvirus Y-isolaten, vertoonden het Mg^{++}-effect. Het molecuulgewicht van de manteleiwiteenheid bedraagt voor al de virussen 34.000 daltons. In 8 van de 10 gevallen werd het eiwit gemakkelijk omgezet in een component met een molecuulgewicht van 28.000 daltons. Alleen de aardappelvirus Y-isolaten vertoonden dit effect niet.

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Book review

D. L. Hawksworth: Mycologist's Handbook. An introduction to the principles of taxonomy and nomenclature in the fungi and lichens. 231 pp., 22 figures. Commonwealth Mycological Institute, Kew, Surrey, England, 1974. Obtainable from Commonwealth Agricultural Bureaux, Farnham Royal, Slough, SL2 3BN, England. Price £ 5.50 cased, £ 2.75 paperback.

The 'Mycologist's Handbook' is as a completely rearranged, revised version of Bisby's 'An Introduction to the Taxonomy and Nomenclature of Fungi'. It is a source of background information and a guide to the literature in fungal taxonomy (including lichens). It contains chapters on collecting and preservation (herbaria, culturing) – taxonomic ranks – naming, describing and publishing (i.a. illustrating, monographs and revisions, keys, literature) – nomenclature – authors of fungi and lichens (with abbreviations) – title abbreviations of classic publications – a glossary to nomenclatural terms – references (14 pp.) and an index.

The book reflects the guiding principles of the taxonomic work carried out at the Commonwealth Mycological Institute at Kew: preservation and careful examination of numerous herbarium specimens and, sometimes, cultures and, whenever possible, comparison with type specimens – thorough documentation of the literature – naming in strict adherence to the Code of Nomenclature – careful editing of manuscripts with a good deal of standardization. Particularly remarkable are the instructions for preparation of manuscripts, a reproduction of all but four articles of the Botanical Code of Nomenclature (as far as relevant to Fungi) with newly chosen mycological and lichenological examples, and the most complete list available of mycological authors with the location of their herbaria.

The text is very concise, perhaps sometimes too much so, and much of the information must be sought in the comprehensive and up-to-date literature references. To make full use of the book, the reader must have a large library at hand. It would also have been desirable to include a list of publications referring to larger taxonomic monographs, check-lists, etc., since none of those available is really up-to-date. The book is carefully prepared and only a few minor errors were discovered. In addition to taxonomic mycological work, the handbook will also be invaluable in composing and editing mycological publications.

W. Gams

Alfred Kaestner: Lehrbuch der speziellen Zoologie. Band I: Wirbellose. Teil 3B, p. 229–905, 405 figs. with Index of part 3A en B. Cloth bound. Gustav Fischer Verlag, Jena 1973. Price 35 Mark.

In this part of the textbook a good introduction to general biology and taxonomy of insects is given. The book is descriptive and contains a wealth of facts about various aspects of insect biology in systematic arrangement. For those interested in a certain group of insects this is an advantage, but it limits the usefulness of the book for insect ecologists and physiologists since the information is scattered over many chapters.

The reference list of 30 pages, according to orders of insects, is up-to-date. It is well illustrated with figures derived from literature. Many are from well-known textbooks like those of H. Weber.

The book will be useful for applied entomologists and entomologists who need a first introduction into a certain group of insects. The price is low.

G. W. Ankersmit