Erysimum Latent Virus–Further Characterization as a Tymovirus

D. D. Shukla, R. Koening, K. H. Gough, W. Huth, and D.-E. Lesemann

First and third authors: Division of Protein Chemistry, Commonwealth Scientific and Industrial Research Organization, 343 Royal Parade, Parkville, Vic. 3052. Australia.

Second, fourth, and fifth authors: Institut fur Viruskrankheiten der Pflanzen, Biologische Bundesanstalt, Messeweg 11, D 3300 Braunschweig, German Federal Republic.

We are grateful to C. M. Roxburgh for the amino acid analysis, to A. J. Gibbs for the FITMOL analysis, and to Miss Angelika Sieg, Miss Petra Rähse, Mrs. B. Merkhoffer, and Mrs. U. Herzberg for reliable technical assistance. The work in Braunschweig was supported by a grant of the Deutsche Forschungsgemeinschaft.

Accepted for publication 29 October 1979.

ABSTRACT

SHUKLA, D. D., R. KOENING, K. H. GOUGH, W. HUTH, and D.-E. LESEMANN. 1980. Erysimum latent virus–further characterization as a tymovirus. Phytopathology 70:382-384.

Erysimum latent virus induced peripheral double membrane-bounded vesicles in chloroplasts of infected cells. Nuclei contained large masses of lightly staining shells thought to be empty shells of the virus. Electrophoresis in agarose gels at pH 7.0 caused the virus to migrate slowly towards the anode. It was a good immunogen, but serological relationships with other tymoviruses were extremely weak and found only sporadically. The capsid protein contained 208 amino acid residues determined by FITMOL analysis of the amino acid composition and had a molecular weight of 21.7×10^3 determined by SDS polyacrylamide gel electrophoresis. The base composition of the RNA was C 34.5, A 23.9, G 15.6, U 26.0.

Erysimum latent virus (ELV) was first described by Shukla and Schmelzer (21). Its in vitro properties, particle morphology, sedimentation behaviour (20), transmissibility by beetles (16,19), and a very weak reactivity with antisera to some tymoviruses (18) suggested that the virus may belong to the tymovirus group. The molecular weight and amino acid composition of the capsid protein, the base composition of the RNA, the results of electrophoretic and further serological studies, and observations on the cytopathological effects of the virus are now presented. These results further confirmed that ELV is a tymovirus.

MATERIALS AND METHODS

Leaves of turnip (*Brassica rapa* L. 'Just Right') and of *Brassica chinensis* L. systemically infected with ELV and showing fully developed mosaic symptoms were embedded for cytological studies. Small samples were fixed 1 hr in 0.5% O_3O_4 in 0.05 M phosphate buffer pH 7.0 (PB), postfixed 1 hr in 2.5% glutaraldehyde in PB, dehydrated in acetone, and infiltrated with acetone-Epon mixtures and with Epon (15) at 40 C in a rotator. During dehydration, uranyl acetate half saturated in 70% acetone was used for additional tissue staining. Ultrathin sections were cut with a diamond knife on an LKB Ultratome III. Sections were stained with lead citrate (17) and examined in a Siemens Elmiskop 1a electron microscope.

The fine structure of ELV particles was checked with preparations negatively stained with 2% uranyl acetate.

ELV was purified from systemically infected *Brassica chinensis* or *Brassica rapa* by the butanol/chloroform method (20) or by the bentonite method proposed for turnip yellow mosaic virus (3). Antiserum production, serological double diffusion tests, immunoelectrophoretic studies (11,12), and SDS polyacrylamide gel electrophoresis (13) were done as described previously. Viral capsid protein was separated from RNA using acetic acid (7). The protein was hydrolysed in 4N methanesulphonic acid containing tryptamine (22) and the hydrolysate analysed on a Model 120 C Beckman amino acid analyzer. Viral RNA was extracted with sodium dodecyl sulfate and phenol (10). The nucleotides obtained by alkaline hydrolysis of the RNA were separated by one-dimensional thin-layer electrophoresis in a 0.05 M triethylamine acetic acid-buffer at pH 3.8 (9).

RESULTS

Particle morphology. In negatively stained preparations from purified ELV and also from crude extracts of infected plants, 25- to 30-nm-diameter isometric particles that were either penetrated or unpenetrated by the stain were present. Penetrated particles appeared to occur in much higher numbers than unpenetrated ones. Unpenetrated particles showed morphological subunits which indicated a clustering of protein subunits similar to that described for turnip yellow mosaic virus (TYMV) (4).

Cytopathology. In cells of *Brassica rapa* and *Brassica chinensis,* ELV induced the formation of small, double-membrane-bounded vesicles at the chloroplast peripheries (Fig. 1). Chloroplasts often were rounded, but rarely showed the vacuolation characteristic of other tymoviruses (14). The nuclei of infected cells contained large masses of lightly staining small shells (Fig. 2), presumably virus protein shells (8). The cytoplasm of infected cells appeared to be dense and had an increased volume compared to uninfected cells. It contained many round, dark-staining granules thought to be virions of ELV.

Electrophoresis of intact virus in agarose gels. In 1% agarose gels made with 0.025 M phosphate buffer, pH 7.0, ELV migrated slowly towards the anode. Its migration rate was about one-sixth that of ononis yellow mosaic virus and one-seventh that of clitorea yellow vein virus (11).

Serology. ELV is a good immunogen. Three rabbits were immunized and antisera produced had homologous titres ranging from 1:512 to 1::4,096. These sera were tested with purified preparations of the 15 tymoviruses studied earlier (11). Early and late bleedings from two of the rabbits showed no reactivities with tymoviruses; bleedings from the third rabbit reacted up to dilutions of 1:4 and 1:2 with Andean potato latent and ononis yellow mosaic viruses, respectively.

Purified preparations of ELV were tested against a total of 70 antisera to the 15 tymoviruses that had been studied earlier (11) comprising early and late bleedings from different rabbits. Reactions up to a dilution of 1:8 were observed with different bleedings of two animals immunized with okra mosaic virus. None of the other antisera reacted.

Amino acid composition and protein molecular weight. The amino acid composition (Table 1) was subjected to the FITMOL analysis (6) and yielded a curve (Fig. 3) with a minimum integer

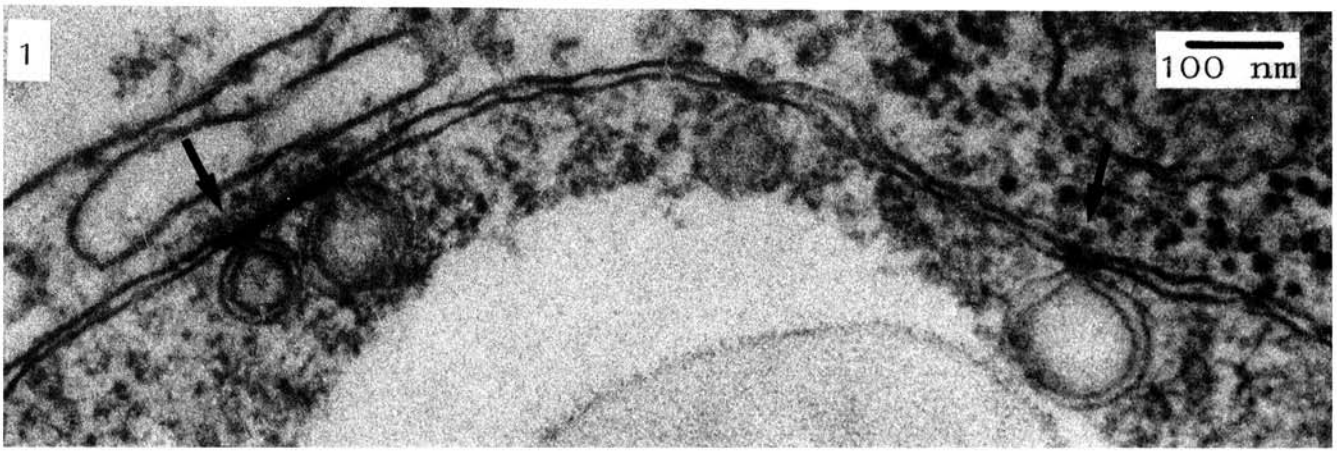
0031-949X/80/05038203/$03.00/0

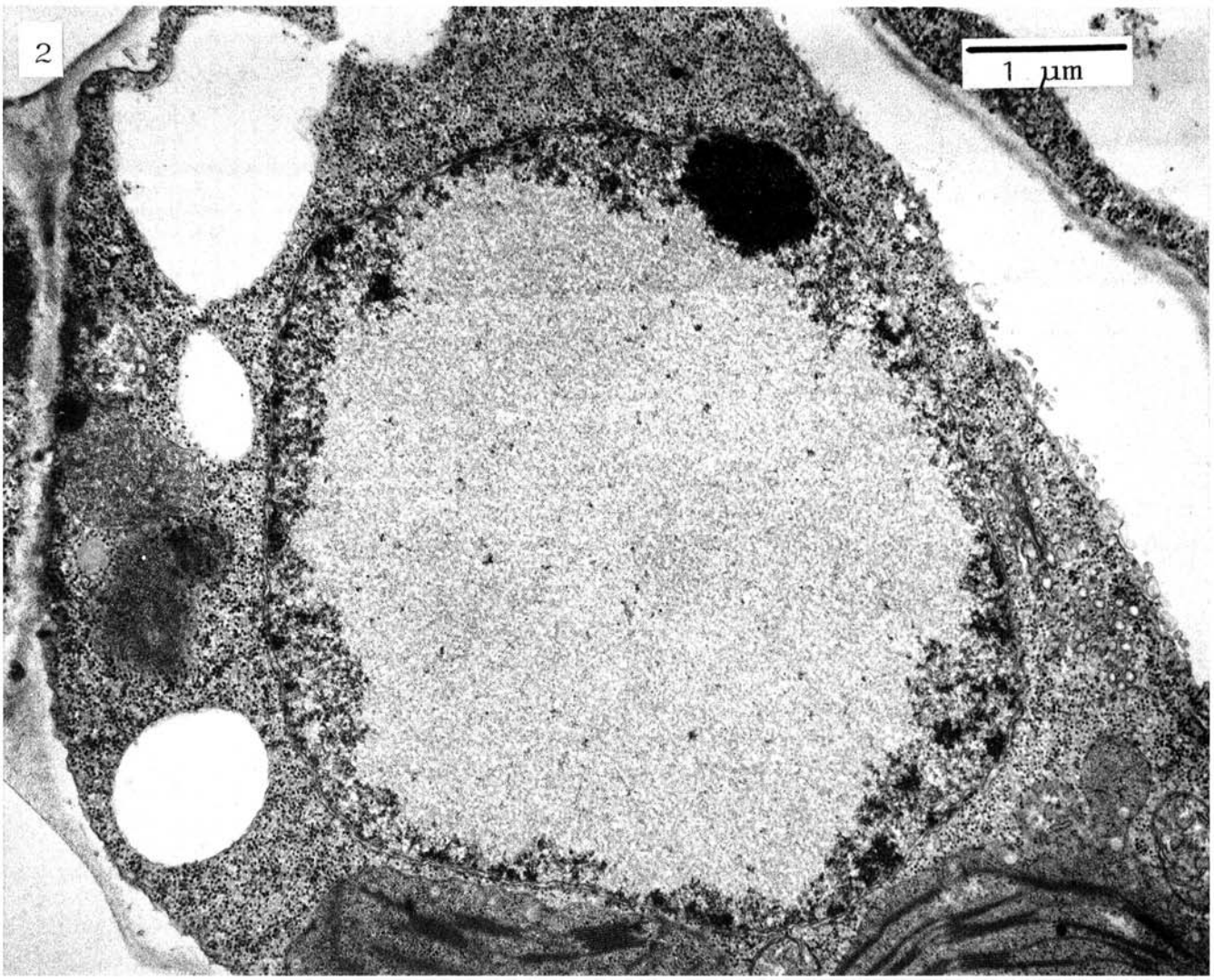
deviate index (IDI) corresponding to a polypeptide with 208 amino acid residues. This figure gave a molecular weight of 21.77×10^3 and agreed with the molecular weight of 21.7×10^3 calculated from 7.5% SDS polyacrylamide gels (average of eight experiments, standard error of mean $\pm$ 0.5).

Base composition of the RNA. The molar percentages base composition of ELV RNA was C 34.5, A 23.9, G 15.6, U 26.0, with a standard error of the mean of $\pm$ 0.5 in all cases. The dissimilarity of the values for A and G and for C and U suggests that the RNA is probably single-stranded. The ratio of purines to pyrimidines was 0.65. Because of conflicting literature reports (1,5) the base composition of cocoa yellow mosaic virus also was checked. It was found to be C 40.5, A 21.0, G 15.5, and U 22.7 with a standard error of the mean of $\pm$ 0.5 in all cases. These values resemble those reported for members of the Andean potato latent virus sub group of tymoviruses (5).

DISCUSSION

Of the new characters described, the cytopathological effects provide the strongest evidence that ELV is a tymovirus. Double membrane-bounded peripheral vesicles on the chloroplast





Figs. 1 and 2. Ultrathin sections of leaves from turnip infected with Erysimum latent virus. **1,** Periphery of chloroplast (lower part of micrograph) showing invaginations of the chloroplast double membrane (arrows) which form peripheral, double-membrane-bounded vesicles. **2,** Part of infected cell showing dense voluminous cytoplasm and the center of the nucleus filled by a mass of lightly stained shells, presumably virus protein shells.

peripheries so far have been seen only in cells infected by tymoviruses (14). The accumulation of large numbers of empty shells in the nuclei is another characteristic feature of tymovirus infections (14).

The ELV amino acid composition data agreed with that of the TYMV type strain (23) except for threonine 10.0 (14.0), serine 13.8 (8.6), half-cystine 0 (2.2) and isoleucine 3.8 (8.1) (values in parentheses are for TYMV). Similar to other tymoviruses, ELV has an RNA with a high percentage of cytidylic acid (5).

The serological relations of ELV to the rest of the tymovirus group are more distant than those of any other tymovirus. In previous studies (11,12) it had been found that all tymoviruses known at that time were serologically interrelated. Viruses which among themselves showed no or only weak serological cross-reactivities were stepwise interconnected by others which were more closely related. These observations formed the basis of a serological classification system (11). Since serological cross-reactivities of ELV with other tymoviruses are extremely weak or missing, ELV can not presently be given a definite position in this classification system. As previous experiences show, this situation may change however, when data for other tymoviruses become known. Before Kennedya yellow mosaic virus had been described (2), the serologically related viruses of okra mosaic, desmodium yellow mottle and cocoa yellow mosaic showed only very distant serological relationships, if at all, with the rest of the tymovirus group (12). Now, Kennedya yellow mosaic virus clearly connects them to turnip yellow mosaic virus (11).

TABLE 1. Amino acid composition of Erysimum latent virus capsid protein[a]

Amino acid	Residue (%)	FITMOL IDI of 0.38[d] No. of residues in protein	
Cystine	0	0	(0)
Aspartic Acid	5.53	11.16	(11)
Threonine[b]	10.00	20.18	(20)
Serine[b]	13.80	27.85	(28)
Glutamic Acid	10.20	20.58	(21)
Proline	10.34	20.86	(21)
Glycine	8.40	16.95	(17)
Alanine	7.02	14.17	(14)
Valine[c]	8.05	16.24	(16)
Methionine	1.44	2.91	(3)
Isoleucine[c]	3.80	7.67	(8)
Leucine	10.88	21.95	(22)
Tyrosine	1.50	3.03	(3)
Phenylalanine	3.50	7.06	(7)
Lysine	3.49	7.04	(7)
Histidine	2.04	4.12	(4)
Arginine	2.61	5.27	(5)
Tryptophan	0.48	0.97	(1)

[a] Values calculated from three analyses hydrolysed for 24, 48, and 72 hr.
[b] Extrapolated to zero hydrolysis time.
[c] Values obtained from 72 hr hydrolysis was taken as correct.
[d] FITMOL (6) is a computer program which assesses how well a particular amino acid composition fits every protein within a stipulated size range by calculating an 'integer deviate index' (IDI) for each protein within that size range. 0.38 is the minimum IDI obtained (see Fig. 3). Values in parentheses are integral values.

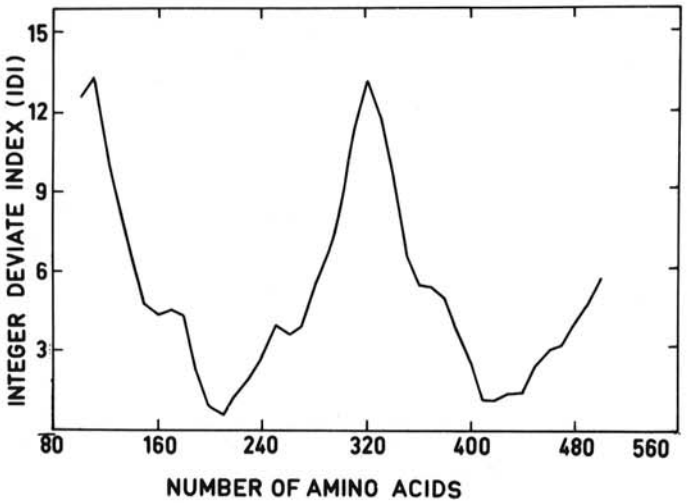


Fig. 3. FITMOL analysis of the Erysimum latent virus amino acid composition data presented in Table 1.

LITERATURE CITED

1. BRUNT, A. A. 1970. Cacao yellow mosaic virus. No. 11 in: Descriptions of plant viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England.
2. DALE, J., and A. GIBBS. 1976. Kennedya yellow mosaic virus: another tymovirus. Aust. J. Biol. Sci. 29:397-403.
3. DUNN, D. B., and H. J. HITCHBORN. 1965. The use of bentonite in the purification of plant viruses. Virology 25:171-192.
4. FINCH, J. T., and A. KLUG. 1966. Arrangement of protein subunits and the distribution of nucleic acid in turnip yellow mosaic virus. II. Electron microscopic studies. J. Mol. Biol. 15:344-364.
5. GIBBS, A. J., E. HECHT-POINAR, R. D. WOODS, and R. K. McKEE. 1966. Some properties of three related viruses: Andean potato latent, dulcamara mottle, and ononis yellow mosaic. J. Gen. Microbiol. 44:177-193.
6. GIBBS, A. J., and G. R. KNOWLES. 1977. A method for assessing the size of a protein from its composition: a correction. J. Gen. Virol. 38:167-168.
7. HARRIS, J. I., and J. HINDLEY. 1965. The protein subunit of turnip yellow mosaic virus. J. Mol. Biol. 13:894-913.
8. HATTA, T., and R. E. F. MATTHEWS. 1976. Sites of coat proteins accumulation in turnip yellow mosaic virus-infected cells. Virology 73:1-16.
9. HUTH, W., and H. L. PAUL. 1977. Two viruses isolated from *Cynosurus cristatus* compared with Lolium mottle virus and cocksfoot mottle virus. Ann. Phytopathol. 9:293-297.
10. JANKULOWA, M., W. HUTH, H. G. WITTMANN, and H. L. PAUL. 1968. Untersuchungen über ein neues isometrisches Virus aus *Atropa belladonna* L. II. Serologische Reaktionen, Basenverhältnisse der RNS und Aminosäurezusammensetzung des Proteins. Phytopathol. Z. 63:177-185.
11. KOENIG, R. 1976. A loop-structure in the serological classification system of tymoviruses. Virology 72:1-5.
12. KOENIG, R., and L. GIVORD. 1974. Serological interrelationships in the turnip yellow mosaic virus group. Virology 58:119-125.
13. KOENIG, R., H. STEGEMANN, H. FRANCKSEN, and H. L. PAUL. 1970. Protein subunits in the potato virus X group. Determination of the molecular weights by polyacrylamide electrophoresis. Biochim. Biophys. Acta 207:184-189.
14. LESEMANN, D.-E. 1977. Virus group-specific and virus specific cytological alterations induced by members of the tymovirus group. Phytopathol. Z. 90:315-336.
15. LUFT, J. H. 1961. Improvement in epoxy resin embedding methods. J. Biophys. Biochem. Cytology 9:409-414.
16. PROESELER, G., and K. SCHMELZER. 1977. Laboratory and field tests about the transmission of Erysimum latent virus by *Phyllotreta* species (Chrysomelidae). Zentralbl. Bakt., Abt. II, 132:716-721.
17. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208-212.
18. RICHTER, J., and H. KLEINHEMPEL. 1978. Das latente Erysimum-Virus (Erysimum latent virus)–ein Vertreter der Tymo-Virusgruppe. Arch. Phytopathol. Pflanzenschutz 14:65-66.
19. SHUKLA, D. D., G. PROESELER, and K. SCHMELZER. 1975. Studies on viruses and virus diseases of cruciferous plants. XVIII. Beetle transmission and some new natural hosts of Erysimum latent virus. Acta Phytopathol. Acad. Sci. Hung. 10:211-215.
20. SHUKLA, D. D., E. PROLL. K. SCHMELZER, and H. B. SCHMIDT. 1973. Studies on viruses and virus diseases of cruciferous plants. VIII. Purification, properties and serology of Erysimum latent virus. Acta Phytopathol. Acad. Sci. Hung. 8:91-103.
21. SHUKLA, D. D., and K. SCHMELZER. 1972. Studies on viruses and virus diseases of cruciferous plants. IV. The previously undescribed Erysimum latent virus. Acta Phytopathol. Acad. Sci. Hung. 7:157-167.
22. SIMPSON, R. J., M. R. NEUBERGER, and T.-Y. LIU. 1976. Complete amino acid analysis of proteins from a single hydrolysate. J. Biol. Chem. 251:1936-1940.
23. SYMONS, R. H., M. W. REES, M. N. SHORT, and R. MARKHAM. 1963. Relationships between ribonucleic acid and protein of some plant viruses. J. Mol. Biol. 6:1-15.