The Coat Protein of the Alliaria Strain of Turnip Mosaic Virus: Molecular Weight and Degradation Products Formed During Purification and Upon Storage

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SUMMARY

Protein obtained by degradation of particles of the 'Alliaria' strain of TuMV (AllTuMV) was heterogeneous. Polyacrylamide gel electrophoresis experiments showed that this heterogeneity is greatly increased when purified virus suspensions are stored for 60 h in 0.02 M-borate buffer, pH 7.5, at 4 °C instead of being immediately degraded with 1% sodium dodecyl sulphate (SDS). When freshly purified virus particles were degraded, the protein preparations contained three components, I, II and III (estimated mol. wt. 38000, 30000 and 27500, respectively) whose relative amounts differed between samples of virus. It is suggested that components II and III, which both react against AllTuMV antiserum, are produced by degradation of component I. Electrophoresis in the presence of urea at different gel concentrations indicated that components I, II and III observed in the polyacrylamide-SDS system differ both in mol. wt. and in charge density.

The virus previously called alliaria mosaic virus (AlMV) (Papa, Michelin-Lauserot & Casetta, 1973; Papa & Michelin-Lauserot, 1973) is now thought, on serological evidence, to be better named the 'Alliaria' strain of turnip mosaic virus (AllTuMV; V. Lisa & O. Lovisolo, personal communication). Its particles are fragile and polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS) showed that the protein from the particles was heterogeneous in size at all stages of purification (Papa et al. 1973).

It is well known that the protein of viruses belonging to the potexvirus and potyvirus groups frequently displays a more or less pronounced heterogeneity when examined by polyacrylamide gel electrophoresis in the presence of SDS. Several authors have attributed this heterogeneity to breakdown of the protein subunit by host or microbial enzymes (Koenig et al. 1970; Shepard & Secor, 1972; Tremaine & Agrawal, 1972; Tung & Knight, 1972; Mayo & Cooper, 1973; Huttinga & Mosch, 1974) which are not inactivated or not removed during purification. Koenig (1972), suggested that some of the coat protein of potato virus X and cactus virus X behaved anomalously in polyacrylamide-SDS electrophoresis; when examined as described by Hedrick & Smith (1968) using polyacrylamide gels of different concentrations, the protein of these two viruses behaved as a mixture of two components with different charge density but equal mol. wt. Similarly Hiebert & McDonald (1973) reported that a strain of turnip mosaic virus and some other potyviruses contained two components separable by polyacrylamide-SDS electrophoresis and differing in charge density but not in mol. wt. In this paper investigations on the nature of the observed polydispersity of AllTuMV protein are described.

AllTuMV was purified from Petunia hybrida as described by Papa et al. (1973); this takes approx. 48 h. In some experiments the procedure was modified by adding, during extraction and/or the following stages of the purification, 0.001 M-EDTA, 1% NaHSO₃, 1 M-urea or 1% Na₂SO₃. Polyacrylamide gel electrophoresis in the presence of SDS was performed
Fig. 1. Densitometric tracings of SDS-polyacrylamide gels in which the protein of some representative AITuMV preparations was electrophoresed. Note that the relative quantities of components I, II and III vary greatly. The numbers shown under the peaks are proportional to their area. They were deduced by integrating the densitometric tracings and normalizing in the sum I + II + III to 100. Migration is from left to right.

according to Shapiro, Viñuela & Maizel (1967) with some minor modifications (Michelin-Lausarot et al. 1970). Virus protein samples were prepared by dialysing purified virus suspensions, to which 1% (w/v) SDS had been added, against 0.01 M-phosphate buffer, pH 7.5 containing 1% SDS and 1% (v/v) 2-mercaptoethanol (ME; sample buffer). The following mol. wt. markers were also dissolved in the sample buffer: bovine serum albumin (NBCo), ovalbumin (Calbiochem), trypsin, carboxypeptidase A, chymotrypsin and ribonuclease (Worthington). β-lactoglobulin was prepared from cow’s milk according to Ashaffenburg & Drewry (1955) and polymerized with diethylpyrocarbonate (Wolf et al. 1970). Electrophoresis in the presence of urea was performed in a discontinuous system modified from Duesberg & Rueckert (1965). Purified virus suspensions were dialysed overnight against 10 M-urea containing 1% (v/v) ME and 10% (v/v) glacial acetic acid. Electrophoresis was in 8, 10 and 12% polyacrylamide gels with an acrylamide/bisacrylamide ratio of 40/1. In immuno-electrophoresis experiments, polyacrylamide gels, immediately after electrophoresis in the phosphate-SDS system, were lined up in the grooves of an immunodiffusion box which will be described elsewhere and covered with a 1% agarose solution in Na-Veronal buffer, pH 8.2 (μ = 0.02) at 40 °C. The antiserum used in these studies was prepared as described by Papa et al. (1973) from highly purified virus whose protein had been examined by polyacrylamide-SDS electrophoresis.

Fig. 1 shows some typical scans obtained from different purified preparations which were degraded with SDS immediately after purification and analysed by polyacrylamide gel electrophoresis. The number of components and their relative quantities, but not their migration distances in the gels differed between preparations. In some of them, the slow component I represented the major fraction and low mol. wt. components were present only in trace amounts, while in others, this situation was reversed. When the purification method was modified as indicated above, the protein heterogeneity was only slightly decreased but when the virus was purified in the presence of NaHSO₃ and when isopycnic sedimentation in a CsCl gradient was added at the end of the standard purification procedure, the protein contained mainly components I and II and trace amounts of III. Changing the ionic strength, pH or ionic composition of the buffer and the concentration range of the sucrose gradient, on the contrary, had no effect on the heterogeneity of the protein. Ageing the virus suspensions in 0.02 M-borate buffer, pH 7.5 for 60 h at 4 °C prior to treatment with SDS greatly
Fig. 2. Degradation of AlTuMV protein during storage of purified virus: (a) sample treated with SDS and examined by electrophoresis immediately after purification; (b) sample stored for 60 h in 0.02 M-borate buffer pH 7.5 at 4 °C and subsequently treated with SDS and electrophoresed; (c) sample stored for 60 h in SDS before electrophoresis. I, II and III indicate, in order of decreasing mol. wt., the three protein components present in all AlTuMV purified preparations; IV and V are two lower mol. wt. fractions present in some preparations. IV and V represent a major fraction in the borate treated protein.

Fig. 3. Determination of the mol. wt. (arrowed) in preparations of AlTuMV protein. (a) Calibration curve obtained from bovine serum albumin (BSA), ovalbumin (OA), carboxypeptidase A (CPA), trypsin (TRY), ribonuclease A (RNase), chymotrypsin A chain (ACHY), chymotrypsin B chain (CHY); (b) calibration curve obtained from β-lactoglobulin polymers.
Fig. 4. Immunodiffusion against ALTuMV antiserum of protein components obtained from the degradation of purified virus suspensions and separated by electrophoresis in SDS-containing polyacrylamide gels. Diffusion into 1% agarose in Na-Veronal buffer, pH 8.2 (μ = 0.02) was for 48 h. The distance between the polyacrylamide gel and the groove containing antiserum was 4 mm. The slab was photographed before staining. Aligned to the photograph is a diagram showing the position of the bands on the polyacrylamide gel.

Fig. 5. Plot of log Rm against % acrylamide for components I, II and III deduced from the data of the electrophoresis in the pH 3.8 polyacrylamide–8 M-urea system.
increased the amount of low mol. wt. components as shown in Fig. 2. By comparing the distances migrated by AlTuMV protein components, when electrophoresed on polyacrylamide-SDS gels, with those of protein markers of known mol. wt. and of /3-lactoglobulin polymers (Fig. 3a, b), mol. wt. of components I, II and III were estimated to be 38,000, 30,000 and 27,500 respectively. Components I to V all reacted against AlTuMV antiserum in immunodiffusion tests (Fig. 4).

The heterogeneity detected by SDS-polyacrylamide electrophoresis was also found in the urea-containing polyacrylamide system, in which the separation is based on both charge and mol. wt. differences. When the logarithms of the migration distances relative to gentian violet (Rm) for components I, II and III were plotted versus acrylamide concentrations, the points lay on three convergent straight lines which intersected log Rm axis at different points indicating, according to Hedrick & Smith (1968), mol. wt. and charge differences (Fig. 5).

The heterogeneity of AlTuMV protein, evident on electrophoresis in SDS-containing polyacrylamide gels, could be attributed to: (a) impurities derived from the host plant; (b) aggregation of protein subunits; (c) existence in AlTuMV particles of more than one distinct kind of protein subunit; or (d) modification of protein subunits by enzymes which are not inactivated or not removed during virus purification.

Hypothesis (a) can be ruled out because all components shown on polyacrylamide gels react against AlTuMV antiserum and because components I to V do not migrate at the same rate as proteins in preparations obtained from healthy petunia (Papa et al. 1973). Hypothesis (b) is implausible because the mol. wt. of components I and II (38,000 and 30,000 respectively) are not integral multiples of that of component III (27,500). Hypothesis III would not explain the increase, occurring during storage in borate buffer at 4 °C, of the amount of low mol. wt. components at the expense of high mol. wt. components. However, the occurrence of components I to III in all preparations might suggest that all are integral parts of the virus particle. If this were true, one might expect that the relative quantities of the three components would be constant in all preparations, but they are not.

Our results fit best with the hypothesis that the heterogeneity in AlTuMV protein is caused by modifications of the protein subunit probably produced by enzymes which are difficult to remove or to inactivate. This postulated enzymatic activity was limited only by the use of NaHSO3 while variations of the ionic strength, composition of the buffers (pH 7.5–9.0) or the use of low concentrations of urea (Damirdagh & Shepherd, 1970; Hill & Shepherd, 1972) were ineffective. The enzyme action might result in degradation of the protein subunit and consequent appearance of low mol. wt. components and/or the production of charge isomers with identical mol. wt. Indeed the behaviour of components I, II and III in the urea system (Fig. 5) is considered to be evidence that AlTuMV protein becomes modified in both these ways.

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