Evidence for a Single Coat Protein in Southern Bean Mosaic Virus

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ABSTRACT

The coat protein of southern bean mosaic virus was shown to consist of a single polypeptide, as shown by polyacrylamide gel electrophoresis experiments. A single electrophoretic component was obtained with alkylated protein in two cationic gel systems containing urea, and one anionic gel system containing dodecyl sulfate. Phytopathology 61: 1408-1409.

Southern bean mosaic virus (SBMV) is a small spherical virus that has been well characterized, both chemically and physically. The significance of some chemical features of the virus which have been reported recently, e.g., amino acid analyses and tryptic peptide maps (4, 10), is based on the assumption that the virus has a single coat protein. This assumption may not be true, however, as other small spherical plant viruses have recently been found to contain two coat proteins (2, 12). We report herein that the capsid of southern bean mosaic virus is composed of monomeric units of a single polypeptide chain.

The severe bean mosaic strain of the virus (13) was purified by a procedure involving clarification of plant extracts at pH 4.5, precipitation with polyethylene glycol, and differential centrifugation. The final preparation was passed through a granulated agar-gel column (5% agar, 40-60 mesh), and the virus concentrated from the diluted solution by ultracentrifugation. In later studies, virus was centrifuged to equilibrium in CsCl density gradients, and the virus zone collected and freed of salt by dialysis.

Virus protein was prepared by the warm formic acid method of Miki & Knight (8), followed by precipitation with ammonium sulfate and resuspension in 8 *M* deionized urea. The protein was freed of any residual undegraded virus by ultracentrifugation, then dialyzed against water and lyophilized. This product was reduced and carboxymethylated under a nitrogen barrier by a method similar to that of Crestfield et al. (3), except that a reduction time of 15-20 hr using both mercaptoethanol and dithiothreitol was used, and the reaction time was 1 hr. The reaction was stopped with an excess of reducing agent and dialysis. Protein prepared in this way proved to be insoluble in 8 *M* urea, however, and it was found preferable to carboxymethylate whole virus in the same manner followed by subsequent preparation of the protein by the warm formic acid method. The aminoethylated product was prepared from formic acid degraded material, using the method of Jones (5) as modified by Tsung & Fraenkel-Conrat (11).

Viral protein was evaluated for homogeneity by electrophoresis on polyacrylamide gel using both cationic and anionic systems in which all reagents were made up in 8 *M* deionized urea. Two cationic systems with running gel pH levels of 2.3 and 4.3 (1, 9) and one anionic system with a running pH of 10.4 (12) were used with 0.6 cm diam columns of 7.5% polyacrylamide gel. Protein samples (50-100 μg) in 8 *M* urea were applied to each column in 0.1 ml of a sample gel identical to the 2.5% spacer gel. Electrophoresis was carried out for 30-180 min at a current of 5 mA/column. Gels were stained in 0.05% aniline blue-black in 5% trichloroacetic acid for 3-5 hr, and destained by washing in 7% acetic acid.

Only a single protein band was obtained in gels loaded with carboxymethylated protein using both cationic systems (Fig. 1-A, B). However, prominent staining at the origin was evident, which indicated that either one or more proteins were present, or that residual insoluble protein remained in the sample gel; hence, the results were of doubtful significance. In addition, multiple components were sometimes obtained with carboxymethylated protein unless the

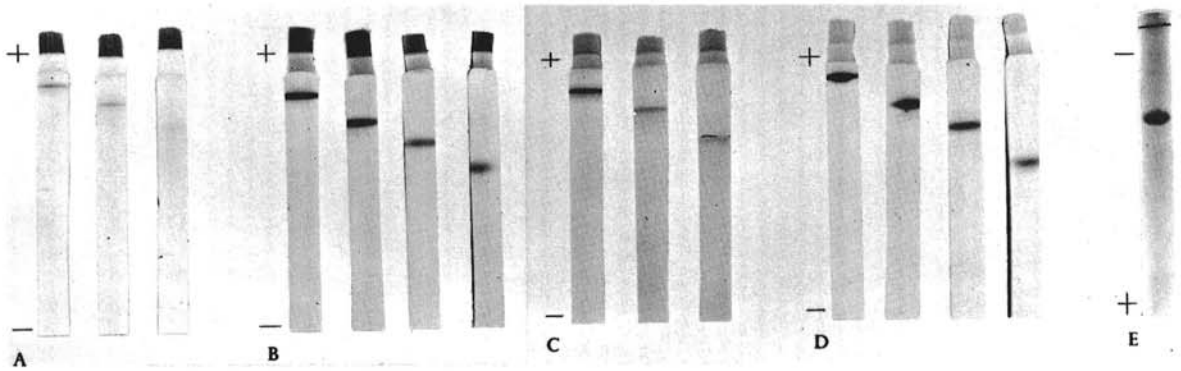

Fig. 1. Polyacrylamide gel electrophoresis of southern bean mosaic virus protein. Electrophoresis at 5 ma in 7.5% polyacrylamide gel of reduced-carboxymethylated protein at **A)** pH 2.3 for 1, 2, and 3 hr; and **B)** pH 4.3 for 0.5, 1.0, 1.5, and 2 hr; aminoethylated protein at **C)** pH 2.3 for 1, 2, and 3 hr; and **D)** pH 4.3 for 0.5, 1.0, 1.5, and 2 hr (left to right, respectively). **E)** Electrophoresis of reduced-carboxymethylated protein for 2.5 hr at 8 ma/gel in 10% polyacrylamide gel containing sodium dodecyl sulfate. A wire was inserted near the top of the gel.

virus had been purified further with CsCl density gradients. Aminoethylated protein in both cationic systems migrated as a single component without retention of material in the sample gel, indicating a single coat protein in the virus (Fig. 1-C, D). With the anionic system, however, both carboxymethylated and aminoethylated protein were retained in the sample gels. A succinylated derivative of aminoethylated protein prepared as described by Klotz & Keresztes-Nagy (6) did not move in any of the three gel systems.

Additional experiments to evaluate the number of proteins in the virus were done with carboxymethylated protein in polyacrylamide gels containing sodium dodecyl sulfate. The method used was similar to that used recently by Lesnaw & Reichmann (7) for virus coat proteins. With this technique, the intrinsic charge of the protein is apparently nullified by the detergent, and all proteins behave as cations. Separation occurs as the result of molecular sieving in the gel, and is based on size. In these experiments, only a single component was obtained, which indicated that the virus contains a single coat protein (Fig. 1-E).

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