Capsid Polypeptides in a Group III Virus from *Gaeumannomyces graminis* var. *tritici* Are Related

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SUMMARY

Peptide mapping and amino acid analysis have shown that the two capsid polypeptides of a group III virus, 87-1-H, from *Gaeumannomyces graminis* are closely related.

Isometric double-stranded RNA (dsRNA) virus particles are common in field isolates of the wheat take-all fungus, *Gaeumannomyces graminis* var. *tritici* (Ggt), and have been divided into four groups based on their physical and serological properties and the number and sizes of their dsRNA segments and capsid polypeptides (Buck *et al.*, 1981; McFadden *et al.*, 1983; Buck, 1984). Viruses in groups I and II have a divided genome of two dsRNA segments and have been placed in the family *Partitiviridae* (Brown, 1986). A recently described group III virus, designated 87-1-H, which has an undivided dsRNA genome of mol. wt. 4.2 x 10⁶ and two capsid polypeptides, P1 and P2, mol. wt. 84000 and 78000 (Jamil & Buck, 1984), is a possible member of the family *Totiviridae* (Brown, 1986). However, viruses in the *Totiviridae* have only a single capsid polypeptide. To determine whether the 87-1-H capsid polypeptides are unrelated or whether one could be derived from the other we have compared them by peptide mapping and by determining their amino acid compositions.

Ggt isolate 87-1 was cultured and virus 87-1-H was isolated and purified as described by Jamil & Buck (1984). For the separation of capsid polypeptides P1 and P2, a virus suspension was first made 1% in SDS and 0.1% in 2-mercaptoethanol and heated to 100 °C for 3 min. The denatured capsid polypeptides were then separated by polyacrylamide gel electrophoresis (PAGE) at 5 V/cm for 24 h using a 4% stacking gel, a 10% resolving gel and the Laemmli (1970) discontinuous buffer system. The gel was stained for 15 min with 0.1% Coomassie Brilliant Blue in 50% (v/v) methanol with 10% (v/v) acetic acid and then destained for 1 h in 5% (v/v) methanol with 10% (v/v) acetic acid. A typical separation is shown in Fig. 1. Individual protein bands were excised, further purified by a second cycle of PAGE using 16% resolving gels and then electroeluted (Leibowitz & Wang, 1984) into dialysis bags containing 0.05 M-Tris-HCl, 0.24 M-glycine, 0.1% SDS, pH 8.3. The solutions were adjusted to 0.2 M-KCl and stored at 0 °C for 15 min. The precipitated polypeptides were collected by centrifugation, washed with 0.1 M-HCl in acetone and then with acetone, and then dried in vacuo.

For peptide mapping, polypeptides (5 to 10 μg) were dissolved in 0.5 M-sodium phosphate, 0.1% SDS, pH 7.5 and iodinated with 125I using Iodogen (Pierce Chemical Company, Rockford, Ill., U.S.A.) as described by Fraker & Speck (1978). Iodinated polypeptides were digested at 37 °C for 19 h either with trypsin (100 μg/ml) in 0.1 M-ammonium bicarbonate, or with pepsin (100 μg/ml) in 5% (v/v) formic acid, then diluted fivefold with water and lyophilized. Peptides

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Fig. 1. Separation of polypeptides P1 and P2 by PAGE. Electrophoresis was from left to right.

Fig. 2. Autoradiograms of $^{125}$I-labelled peptides produced by digestion of polypeptides P1 (a, c, e) and P2 (b, d, f) with trypsin (a, b, c, d) or pepsin (e, f) and separated first in the horizontal dimension by electrophoresis at pH 6.5 from the origin marked O with the cathode on the right, and then in the vertical dimension from bottom to top by chromatography (a, b) or electrophoresis at pH 2.1 with the cathode at the top (c, d, e, f).
Table 1. **Molar ratios of amino acids released after acid hydrolysis of polypeptides P1 and P2**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Molar ratio*</th>
<th>S.E.M.</th>
<th>Molar ratio*</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>2.33</td>
<td>0.20</td>
<td>2.42</td>
<td>0.02</td>
</tr>
<tr>
<td>Threonine</td>
<td>1.57</td>
<td>0.05</td>
<td>1.57</td>
<td>0.02</td>
</tr>
<tr>
<td>Serine</td>
<td>1.55</td>
<td>0.04</td>
<td>1.52</td>
<td>0.09</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>2.10</td>
<td>0.04</td>
<td>2.12</td>
<td>0.17</td>
</tr>
<tr>
<td>Alanine</td>
<td>4.62</td>
<td>0.06</td>
<td>4.25</td>
<td>0.04</td>
</tr>
<tr>
<td>Valine</td>
<td>1.88</td>
<td>0.06</td>
<td>1.86</td>
<td>0.05</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.86</td>
<td>0.05</td>
<td>0.82</td>
<td>0.05</td>
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<tr>
<td>Leucine</td>
<td>2.00</td>
<td>2.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.00</td>
<td>0.02</td>
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<td>0.02</td>
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<tr>
<td>Histidine</td>
<td>0.58</td>
<td>0.06</td>
<td>0.60</td>
<td>0.07</td>
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<tr>
<td>Arginine</td>
<td>0.95</td>
<td>0.06</td>
<td>0.98</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* Average of five determinations. The value for leucine was taken arbitrarily as 2.00.

were separated on 35 x 45 cm sheets of Whatman 3MM paper either by two-dimensional electrophoresis [3 kV for 1.5 h in acetic acid:pyridine:water 1:2:18 (by vol.) pH 6.5, first dimension; 3 kV for 45 min in formic acid:acetic acid:water 1:4:45 (by vol.) pH 2.1, second dimension] or by electrophoresis (as for the first dimension above) followed by chromatography [20 h in n-butanol:acetic acid:pyridine:water 15:3:10:12 (by vol.)] for the second dimension. The papers were then dried and autoradiographed using Fuji X-ray film with an intensifying screen. For comparing amino acid compositions, polypeptides were hydrolysed in 6 M-HCl at 110 °C for 48 h and hydrolysates were analysed with a Beckman Model 121M amino acid analyser.

Tryptic and peptic peptide maps of polypeptides P1 and P2, which were reproducible when repeated, are shown in Fig. 2(a to f) and are clearly very similar. Peptide maps produced from mixtures of P1 and P2 confirmed that all the major peptides and most of the minor ones had identical mobilities in both dimensions. One peptide which appears in the peptide digest of P1, but not in that of P2, is marked by an arrowhead in Fig. 2(e). Minor spots may be the result of partial digestion of the polypeptides or weak iodination of some peptides.

Molar ratios of amino acids released after 48 h hydrolysates of polypeptides P1 and P2 are given in Table 1. The values are not a quantitative evaluation of the amino acid composition of each protein because of partial destruction of some amino acids, e.g. serine and threonine, during acid hydrolysis. However, they represent an accurate composition of the products of hydrolysis of the two proteins under identical controlled conditions. Values for tryptophan, cysteine and methionine, which require separate determination, were not measured. Glycine values are not given because Tris-glycine buffer was used for electrophoretic separation of the polypeptides and it was difficult to ensure that all traces of glycine had been removed from the sample. Variable values were obtained for proline, lysine and tyrosine, probably due to partial oxidation of these amino acids, and these are therefore not shown. However, it is clear that the compositions of polypeptides P1 and P2 with respect to the 11 amino acids for which reliable data were obtained are very similar.

There are a number of explanations which could account for the close similarities of polypeptides P1 and P2. One polypeptide could be a modified form of the other, e.g. a phosphorylated or glycosylated derivative. Alternatively, P2 could be derived from P1 by proteolytic processing in vivo or in vitro. Freshly purified virions usually contain P1 and P2 in approximately equal proportions as judged by visual observation of Coomassie Brilliant Blue-stained gels, but virions which have been kept at 4 °C for 1 month or longer often contain predominantly P2, suggesting that conversion of P1 to P2 can occur in vitro. Further investigations will be required to resolve these various possibilities. However, the close relationship established between P1 and P2 indicates that, as with members of the Totiviridae family, the capsid of virus 87-1-H is encoded by a single gene.
Short communication

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REFERENCES


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