

Chenopodium amaranticolor.の葉に含まれる植物ウイルス 感染阻害物質の純化

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Purification of an Inhibitor of Plant Virus Infection Occurring in the Leaves of *Chenopodium amaranticolor*

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谷口 武*・後藤孝男*: *Chenopodium amaranticolor* の葉に
含まれる 植物ウィルス感染阻害物質の純化

Many inhibitors of virus infection are known to occur in plant tissues¹⁾. The chemical nature of most inhibitors remains unsolved. Although the inhibitors from sap of some species of *Chenopodiaceae* have not been isolated, several workers have suggested that they are proteinaceous^{2,3,4,5,6,7)}. Gel filtration with Sephadex G 200 suggested that the inhibitors have molecular weight of 25,000-38,000⁶⁾. This paper describes on a purification procedure of the inhibitor in the sap of *Chenopodium amaranticolor* and on the estimation of the molecular weight.

The inhibitor was isolated from frozen leaves of *C. amaranticolor* grown in the greenhouse. The frozen tissue was homogenized in 0.1 M phosphate buffer at pH 7.0 (PB) (2 ml/g tissue) in a Waring Blender. The homogenate was filtrated through gauze and then centrifuged at 10,000×g for 20 min. To the supernatant was added equal volume of saturated ammonium sulfate solution with stirring. The resultant precipitate was collected by centrifugation and suspended in PB (0.5 ml/g original tissue). Insoluble materials were removed by low speed centrifugation at 10,000×g for 20 min. Precipitation by saturated ammonium sulfate was repeated again, and the precipitate was resuspended in PB (0.25 ml/g original tissue) and centrifuged. The supernatant was emulsified by one fifth volume of chloroform and mixture was shaken for 5 min. The emulsion was broken by centrifugation at 1,000×g for 10 min and the resultant aqueous phase was mixed with equal volume of saturated ammonium sulfate solution. The precipitate formed was collected by centrifugation and suspended in PB. The partially purified inhibitor was obtained by the precipitation with 50% ethylalcohol.

It was fractionated further by column chromatography⁷⁾. A column, 1.5 cm in diameter was packed with Sephadex G-50 (Pharmacia, Sweden) to 25 cm and equilibrated with PB. Four ml of partially purified inhibitor was applied to the top of the

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column and then eluted by PB. Each 4 ml of the effluent was assayed for its UV absorption at 280 nm. The result was shown in Fig. 1. The two separated peaks

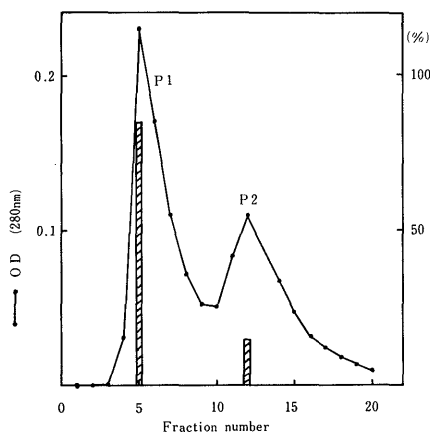


Fig. 1. Gel filtration chromatography of partially purified inhibitor on a Sephadex G-50 column and % inhibition of TMV infection by P1 and P2. Solvent: 0.1 M phosphate buffer pH 7.0 (PB).

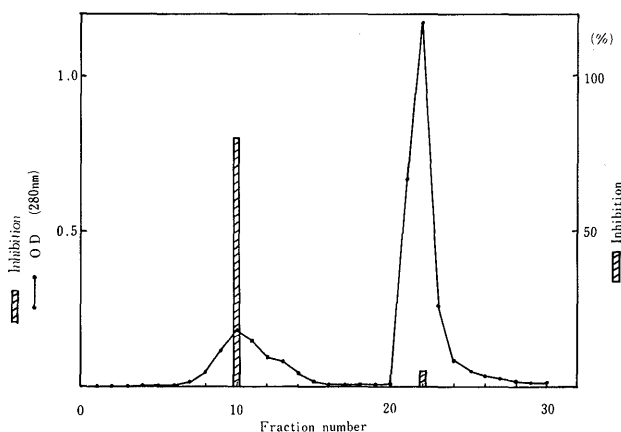


Fig. 2. ECTEOLA cellulose column chromatography of the P1 fraction obtained by the method of Fig. 1 and % inhibition of TMV infection by the eluates. Solvent for adsorption: PB. Eluent: 50 ml of PB and 100 ml of 1 M NaCl in PB.

were obtained. The fraction having higher optical density, P1 in Fig. 1, was tested for inhibitory activity on excised leaves of *Nicotiana tabacum* var. Samsun NN, using a half-leaf method. Each leaf was placed in petri dishes on wet filter paper. One half was inoculated with a mixture of equal volumes of TMV solution and the P1 solution to examine inhibitory activity, the opposite half was inoculated with a mixture of equal volumes of the same virus solution and an appropriate buffer solution. The petri dishes containing the inoculated leaves were transferred into a constant temperature room (about 22 C) and kept under continuous illumination (about 1,500 lux). As shown in Fig. 1 the first emerging peak (P1) had high inhibitory activity, whereas the other (P2) showed low inhibitory activity.

The fractions of P1 resulting from Sephadex G-50 gel filtration were combined and fed into an ECTEOLA cellulose (Brown Chemical Co.) column (1.5×15 cm) prepared by the method of earlier report⁸⁾. Then, 50 ml of the same buffer was passed through the column. Then the adsorbed materials were eluted from the column by 1 M NaCl in PB. The effluent from the column was collected as 4-ml fractions of which optical densities were determined at 280 nm (Fig. 2). The two well-separated peaks were obtained; the first emerging peak had high inhibitory activity, whereas the other showed low activity. The ultraviolet absorption spectrum of the fraction (purified inhibitor) showed the curve of a protein with a maximum absorption at 270 nm.

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The homogeneity of the purified inhibitor following the above isolation procedure was assessed by electrophoresis on polyacrylamide gels. Gels were prepared by the method of Summerville et al.⁹⁾ and consisted of 15% acrylamide, 0.2% N, N'-methylene-bisacrylamide, 0.1% N, N, N', N'-tetramethylethylenediamine, 0.05% ammonium persulfate, 0.1% SDS, and 0.1 M sodium phosphate buffer, pH 7.2. Samples of purified inhibitor in 0.1 M PB (pH 7.2) containing 1% SDS and 1% 2-mercaptoethanol, which had been heated at 100 C for 1 min and cooled¹⁰⁾, were electrophoresed at 6 mA per gel for 4.5 hours at room temperature. The buffer used for electrophoresis was 0.1 M sodium phosphate, pH 7.2, with 0.1% SDS. After electrophoresis, gels were stained with 0.25% Coomassie brilliant blue R 250 in 50% methanol and 10% acetic acid, and destained with a mixture of 50% methanol and 10% acetic acid. Marker proteins used were pepsin (mol. wt. 34,500), TMV-protein (mol. wt. 17,500) and ribonuclease (mol. wt. 13,500). The migrations were made relative to the migration of ribonuclease as 1.0.

SDS gel electrophoresis of the purified inhibitor revealed a major and some minor bands of proteins (Fig. 3). The molecular weight of a major band of the inhibitor was estimated to be 15,000 daltons in SDS containing gel electrophoresis (Fig. 4).

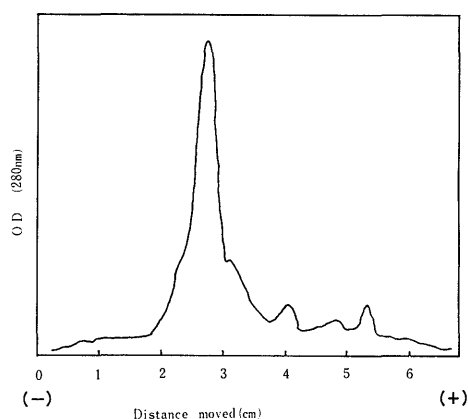


Fig. 3. Densitometric tracing of 15% polyacrylamide gel electrophoresis of the inhibitor. Purified material was degraded in 1% SDS and 1% 2-mercaptoethanol, and electrophoresed in 15% polyacrylamide gels containing 0.1% SDS at 6 mA per gel for 4.5 hours.

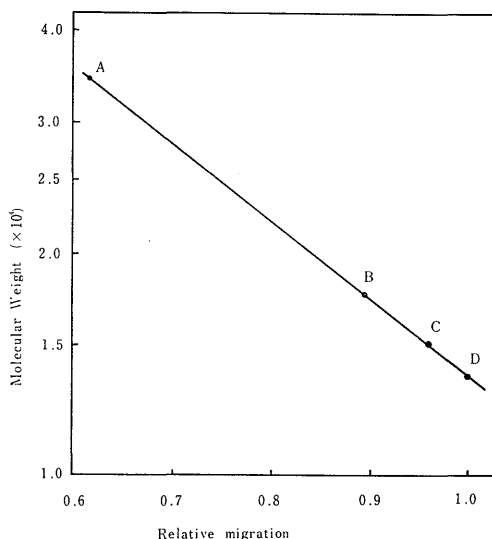


Fig. 4. Estimation of molecular weight of the inhibitor. Semilog plot of molecular weight against distance of migration relative to ribonuclease is indicated. A: Pepsin (MW 34,500), B: TMV-protein (MW 17,500), C: Inhibitor (MW 15,000), D: RNase (MW 13,500).

Smookler⁶⁾ suggested that several basic proteins showing virus inhibitory activity

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have molecular weight around 10,000-14,000, and the molecular weight estimations of 25,000-38,000 for species of the Chenopodiales possibly represent multiples of a basic molecular weight of 10,000-14,000. The molecular weight estimation of 15,000 for the inhibitor obtained by us approximately consistent with that suggested by Smookler.

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