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Effect of Metals on Tobacco Mosaic Virus Infection

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담배모자이크 바이러스 감염성에 대한 금속의 영향

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The efficacy of various concentration of divalent copper and zinc ions was evaluated separately for the infectivity of tobacco mosaic virus. Infectivity of TMV was more enhanced by addition of zinc, while it was decreased by addition of copper. The number of local lesions were more produced on tobacco leaves inoculated with inoculum sap containing zinc than those inoculated with sap only. The effect of copper inhibited the infectivity of TMV is dependent on copper concentration. TMV particles treated with various concentration of zinc and copper, respectively, analyzed by electrophoresis, and appeared to be altered in electrophoretic behavior. When TMV was exposed to zinc concentration at more than 200 mM, the viral particles were completely degraded, and at 40-20 mM they were barely detectable, but at 2 mM they were quite stable. When TMV was exposed at less than concentration of 20 mM of copper, the viral particles remained structurally intact, but those treated at 100-200 mM of copper were degraded.

2가 금속인 구리와 아연을 다양한 농도로 각각 담배모자이크 바이러스 (TMV)에 처리하여 감염성에 어떤 영향을 주는 가를 조사하였다. TMV의 감염성은 아연의 첨가에 의해 활성화 된 반면, 구리의 첨가에 의해 감소되었다. 아연을 첨가한 바이러스 접종원을 담배 잎에 처리한 결과 바이러스 만을 접종한 잎보다 더 많은 국부반점이 생성되었다. TMV의 감염성을 감소시키는 구리의 효과는 구리의 농도에 관련되는 결과를 나타내었다. 또한 전기영동으로 다양한 농도의 구리와 아연에 처리된 TMV를 분석한 결과 그 특성이 변화됨이 관찰되었다. 아연 200 mM 이상의 농도에 노출시 바이러스는 완전히 분해되었고 40-20 mM 농도에서는 간신히 바이러스의 흔적을 찾을 수 있으나, 2 mM 농도에서는 상당히 안정되었다. 구리의 경우 20 mM 이하의 농도에 처리되었을 때 구조적으로 완전하게 남아있으나, 100 mM 이상의 처리에서는 완전히 분해되었다.

Key words : copper and zinc ions, infectivity, tobacco mosaic virus, local lesions, electrophoretic behavior

I. Introduction

Some divalent metal ions, Zn^{++} (Sehnke and Johnson, 1994) and Ca^{++} (Krüse et al., 1982), have a synergistic effect on the replication or

stability of plant viruses. A zinc finger nucleic acid binding motif was proposed for the coat protein of tobacco streak ilavirus (TSV) (Sehnke et al., 1989). The replicase activity of RNA-dependent RNA polymerase complex from alfalfa mosaic virus (AIMV) could be inhibited by treatment with a metal chelator and recovered by addition of zinc (Quadt and Jaspars, 1991). Direct binding by the coat proteins implies a functional role for zinc in viral replication (Sehnke and Johnson, 1994). Similarly for some isometric viruses whose structures are partially stabilized by Ca++ ions, but removal of these ions, by EDTA, leads to swelling of the virus particles (Kruse et al., 1982).

Other metal ions such as copper (Cu++) and silver (Ag+) have a inhibitory effect in bacterial and animal virus infectivity (Yahya et al., 1992). Copper at high concentration is one of the most toxic metals to microorganism and have been used successfully for years to control bacterial, algal, and fungal growth (Thurman and Gerba, 1989; Menkissoglu and Lindow, 1991; Gadd, 1993). Thurman and Gerba (1989) speculated that copper binds to sulfhydryl groups of respiratory enzymes in sensitive bacteria and impairs respiratory function. It may also bind to nucleic acids, resulting in cross-linking, or catalyze the formation of radicals, which cleave chemical bonds.

However, very little is known about the effect of these metal ions on plant viruses. This study was designed to evaluate the effect of zinc and copper ions on the infectivity and the viral integrity of tobacco mosaic virus (TMV).

II. Experimental

1. Virus source and plant materials

TMV was orginally isolated from tobacco in Chungnam province in Korea. After one single lesion transfer to *Nicotiana glutinosa*, the virus propagated in *N. tabacum* cv. Samsun and purified by previous description (McDaniel et al., 1995). For local lesion assays, *Nicotiana rustica* seedlings were transplanted into 11 cm pots and grown in a growth chamber at 16 hr photoperiod for a further 4 weeks. Assays are generally performed on uniform plants and growth stage, selected to produce a uniform response between plants and among leaves on the same plant.

2. Treatment of metals and local lesion ass ay

The sap for inoculum was prepared by grinding TMV-infected tobacco leaf tissues using a mortar and pestle. A 1:5 dilution and a 1:10 dilution (w/v), respectively, of the homogenous sap were made by 0.01 M NaPO4 buffer (pH 7.2) containing 0.2 mM or 2 mM of zinc. Same dilutions of the sap were made by phosphate buffer containing 0.2 mM or 2 mM of copper. Prior to inoculation, the inoculum sources were incubated for 5 min at room temperature. Each treatment was inoculated with cotton tips onto carborundum dusted leaves and replicated on 9 half leaves. The opposite half leaves were inoculated with identical dilution of sap without metals and served as a control. The additional plants inoculated with identical concentration of each metal in NaPO4 buffer. At this concentration, both zinc and copper did not affect the number of local lesions and did not damage the leaf tissues. Local lesions were counted 4 days after inoculation and the experiments were repeated 4 times.

3. Electrophoresis

Copper and zinc were tested for their ability to degrade TMV. Final concentration at 333 - 2 mM of zinc and 200- 2 mM of copper, respectively, mixed with the purified TMV (final conc. 20 μg). After incubation of the resulting mixture for 5 min, electrophoresis was performed in 1% agarose in 0.5 X TBE buffer, pH 8.3 and the gel was stained with Coomassie Brilliant Blue.

III. Results and Discussion

Infectivity of TMV was more enhanced by addition of zinc, while it was inhibited by addition of copper. The inoculum sap containing zinc at 2.0 mM increased more the number of local lesions in the inoculated tobacco leaves by 5.4 times than in those inoculated with sap without zinc. The effect of copper decreased the infectivity of TMV is dependent on the concentration of copper, as judged by increase in the number of local lesions. Dilution of copper from 2.0 mM to 0.2 mM was reduced the inhibitory effect and increased the number of local lesions by 4-5 times. The concentration of copper inactivated TMV is as low as 0.2 mM, resulted in about 50% reduction of infectivity. The effect of copper sulfate on poliovirus infectivity was proportional to its concentration (Totsuka and Ohtaki, 1974). For viruses, inactivation is thought to involve a modified site-specific Fenton mechanism (Samuni et al., 1984). It is assumed that the metal ion binds to a biological target and is reduced by superoxide radicals or other reductants and subsequently reoxidized by H2O2 generating hydroxide radicals. Repeated cyclic redox reactions may result in multihit damage as radical formation occurs near the target site. Another mechanism would be altering the adsorption of the virus to the cell. For example, zinc inhibits adsorption of M13 coliphage to its host bacterium (Tzagoloff and Pratt, 1964).

In order to investigate the possibility of a direct virucidal effect, TMV particles treated with various concentration of zinc and copper, respectively, and analyzed by electrophoresis. TMV treated with various concentration of metals appeared to be altered in electrophoretic behavior. The effect was even more distinguished when TMV treated with zinc. When TMV was treated with concentration at more than 200 mM of zinc, the viral particles completely degraded. At 40 mM and 20 mM concentration, the trace of viral particles barely detectable. However, viral particles are quite stable at 2 mM of zinc, of which concentration the viral infectivity was highly enhanced by the number of local lesions. Thus, zinc at this concentration may stabilize the structure of proteins and nucleic acids, preserve the integrity of the viral particles and play a important role in viral infectivity. Analysis by agarose gel electrophoresis revealed that viral particles

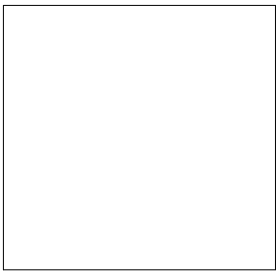

Fig. 1. Agarose gel (1%) electrophoresis of zinc treated TMV at constant 100 V for 1 hr at 4oC. Various concentration was treated at 333 mM (A), 200 mM (B), 40 mM (C), 20 mM (D), 4 mM (E), 2 mM (F) and no treated (G). The gel was stained with Coomassie Brilliant Blue.

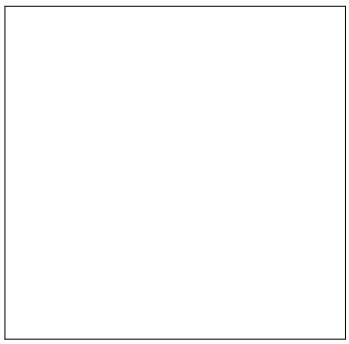

Fig. 2. Agarose gel (1%) electrophoresis of copper treated TMV at constant 100 V for 1 hr at 4oC. Various concentration was treated at 200 mM (A), 40 mM (B), 100 mM (C), 20 mM (D), 4 mM (E), 2 mM (F) and no treated (G). The gel was stained with Coomassie Brilliant Blue.

treated at less than concentration 20 mM of copper remained structurally intact but those treated at more than 100 mM of copper was enough to render viral particles undetectable (Fig. 2). We observed that treatment with 2 mM of copper resulted in significant reduction of viral infectivity more than 80% (Table 1).

Table 1. Effectiveness of metals on TMV infectivity in *Nicotiana rustica*

Virus dilution	Metal	Concentration	Number of local lesion (%) *
1:5	Zinc	2.0 mM	383
1:10	Zinc	2.0 mM	540
1:5	Zinc	0.2 mM	NT**
1:10	Zinc	0.2 mM	192
1:5	Copper	2.0 mM	10
1:10	Copper	2.0 mM	21
1:5	Copper	0.2 mM	39
1:10	Copper	0.2 mM	52

* # of local lesion = metal treated sap/ no metal treated sap X 100 (%)
All treated samples were inoculated on to 9 half leaves of N. rustica as described in the Experim ental. Means are the result of four separate exp eriments.

** NT, no test

but of which concentration the viral integrity was insignificantly damaged or not at all. These results *in vitro* are surprising, since *in vivo* assay TMV was inactivated by copper treatment.

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