한국식물병리학회지 3(4): 239-244(1987)
Korean J. Plant Pathol. 3(4): 239-244(1987)

Determining an Effective Electrophoretic Gel System for Separation of the Circular and Linear Potato Spindle Tuber Viroid RNA Molecules

Jai Youl Lee and Han Jip Kim*

Department of Microbiology, Kyungpook National University, Taegu 635, Korea

**Department of Biology, Yeungnam University, Gyeongsan 632, Korea*

環狀 및 線狀감자 갈쭉바이로이드 RNA분자의 電氣泳動的 分離를 위한 효과적인 조건에 관한 연구

李在悅 · 金翰集*

경북대학교 자연과학대학 미생물학과
*영남대학교 이과대학 생물학과

ABSTRACT

Low molecular weight plant ribonucleic acids including potato spindle tuber viroid(PSTV) RNA were electrophoresed in 0M to 8M urea-gradient polyacrylamide gels. The electrophoresis was carried on in a urea-gradient gel system with 1/40 and 1/10 dilution of TBE buffer at three different temperatures, 17°C, 37°C and 57°C. The most effective separation of PSTV-RNA molecules into circular and linear forms was achieved at the highly denaturing temperature of 57°C and at 1/40 dilution of TBE buffer. The electrophoretic mobility of the denatured circular viroid-RNA molecules is dependent mainly on the concentration of urea. In addition, a low concentration of TBE buffer would increase the separation distance between the circular and linear forms of PSTV-RNA molecules in the denaturing urea-gradient gel system

Key words : potato spindle tuber viroid, urea-gradient polyacrylamide gel electrophoresis.

要 約

감자 갈쭉바이로이드(PSTV) RNA분자를 포함한 低分子量의 식물성 RNA분자에 대해서 0M부터 8M까지의 尿素濃度勾配를 가진 폴리아크릴아마이드겔을 이용하여 1/40, 1/10로 희석한 완충액조건과 17°C, 37°C, 57°C의 온도조건에서 電氣泳動을 실시하였다. 바이로이드의 RNA분자가 環狀과 線狀의 두가지 분자로 나뉘어지는 변성조건에서는 1/40로 희석한 TBE완충액을 포함하는 尿素濃度勾配겔을 57°에서 전기영동을

실시하는 것이 효과적이었다. 變性된 環狀의 바이로이드분자에 대한 전기 영동적 이동성은 주로 요소의 농도에 따라 영향을 받는 것으로 보이는데, 이와 더불어 저 농도의 TBE완충액이 尿素濃度勾配겔에서 環狀과 線狀바이로이드분자 사이에서 전기영동적 분리를 증가시켜 주었다.

INTRODUCTION

Viroids are the smallest known agents of infectious diseases. So far as is known, viroids definitely are known to exist only in higher plants. Potato spindle tuber viroid(PSTV) RNA is single-stranded, and has both linear and convalently closed circular forms consisting of 359 nucleotides(1, 3, 11).

Polyacrylamide gel electrophoresis(PAGE) has been used for routine diagnosis of viroid infection to host plants. PAGE also can be used for the purification and the characterization of both the circular and linear viroid-RNA molecules(4, 7, 8, 11). The two forms of PSTV-RNA molecules migrate together and reveal a single band in 5% polyacrylamide gels under non-denaturing conditions. However, under the denaturing conditions provided by formamide and urea in the gel and by high temperatures during electrophoresis, the two forms of PSTV-RNA would be separated into two different bands(4, 6).

In this study, various temperatures and buffer concentrations were used to improve separation between the circular and linear PSTV-RNA molecules for the denaturing PAGE system. The purpose of this research is to determine the best combination of a temperature and a buffer concentration in a continuous urea-gradient gel for identification and separation of the two forms of PSTV-RNA molecules. In addition, the migration patterns of low molecular weight plant RNAs which were similar to those of the viroid-RNA molecules were identified and distinguished each other.

MATERIALS AND METHODS

Viroid-RNA preparation. Tomato leaves were inoculated mechanically with a isolate of potato spindle tuber viroid(PSTV) at the two or three leaf stage of plants(*Lycopersicon esculantum* Mill. cv. "Rutgers") using 600-mesh carborundum. The inoculated plants were grown in the greenhouse for about one month until the typical viroid symptom, epinasty, appeared.

A partial purification of PSTV-RNA from deep frozen or fresh tomato leaves involved phenol extraction, bentonite treatment, fractionation with 2M lithium chloride and removal of polysaccharide and DNA as described in the previous report(5). Each sample for electrophoresis contained 150 ug of partially purified RNA in 100 ul of TBE buffer(0.89 M Tris, 0.89M boric acid, and 0.025M Na-EDTA ; pH 8.3). The concentration of the buffer was twice that used for the corresponding gel system.

Electrophoresis. A horizontal urea-gradient gel system was used in the experiments. The direction of the electric field is perpendicular to the urea-gradient in the gel. The gradient of urea ranged from 0M to 8M. Procedures for preparing the urea-gradient polyacrylamide gel system were described in the previous report(6).

Gels were prerun with 0.01% bromphenol blue for about one hour to improve the quality of the gradient gel system. The 100 ul RNA sample was mixed with 90 ul of H_2O and 10 ul of 0.1% xylencyanol FF containing 10% sucrose, then heated for 1 min in boiling water, followed by slow cooling. Samples were electrophoresed at 20 mA for 2-3 hours until xylencyanol FF ran to the end of the gradient gel system in the urea-free region. The TBE buffer for the electrodes contained the same concentrations of salts as those in the corresponding gel system. The electrophoresed gels were stained with silver nitrate. Since low concentrations of salts in gels are required for a silver staining, a serial washing was performed by incubating gels in solutions of ethanol and acetic acid mixtures to wash out the salts according to the modified silver staining method of Goldman and Merril(2).

RESULTS

Naturally existing cicular and linear viroid-RNA molecules co-migrate as a single band when electrophoresed in 5% polyacrylamide gels under non-denaturing conditions(e.g. 185 mM Tris, 185 mM boric acid, 8M urea and temperature at 37°C), the viroid-RNA molecules were separated into the fast migrating linear molecules and the slow migrating circular molecules(Fig. 1B and 1b). Since the samples loaded on the urea-gradient gel were not highly purified, various low molecular weight RNAs from plants including 4s RNA, 5s RNA and both forms of circular and linear viroid-RNA molecules were detected form viroid-infected plants (Fig.1, 2 and 3).

In the urea-gradient gel electrophoresis, most bands of the nucleic acids gradually migrated faster in the region of low urea concentration but slightly slower in the region of high urea concentration(Fig. 2 and 3). Under the denaturing condition provided by 1/40 dilution of TBE buffer at 37°C and high urea concentration, both bands of circular and linear viroid-RNA molecules were apparent in the urea-gradient gel(Fig.2). The circular viroid-RNA band showed a sudden shift of mobility at the mid-range of the gradient system and appeared showing another shift of mobility in the region of low urea concentration in the same gel(Fig.2B). However, and extra plant RNA band was detected from both viroid infected and healthy plants in all gel systems with 1/40 dilution of TBE buffer at three different temperatures, 17°C, 37°C and 57°C(Fig.2). The band was apparent only in the low urea concentration region. This extra RNA band appeared to cause an other shift of mobility in the low urea concentration region of the gel 37°C(Fig.2B).

In urea-gradient gel containing 1/10 dilution of TBE buffer at the temperature of 37°C, a band whose migration pattern was similar to that of viroid-RNA was observed close to the viroid-RNA band(Fig.3B and 3b). This band also was detected from both viroid-infected and healthy plants, and seemed to be plant RNA molecules.

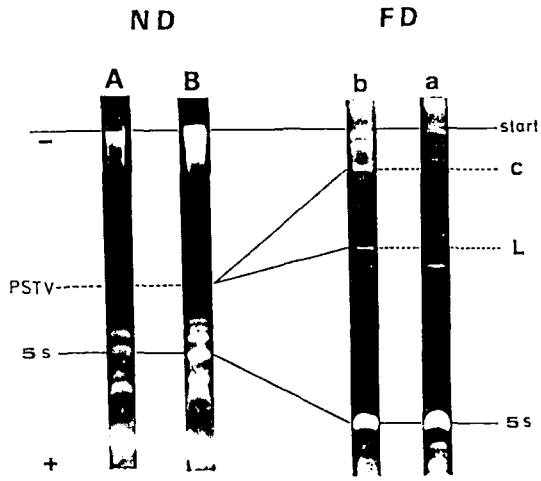

Fig. 1. Electrophorectic separation of potato spindle tuber viroid(PSTV) RNA molecules under non-denaturing(ND) and full-denaturing(FD) conditions.
(A) and (a) : low molecular weight RNAs from healthy plants.
(B) and (b) : low molecular weight RNAs from PSTV infected plants.
Separation of PSTV-RNA molecules into circular and linerar forms under the denaturing condition provided by 8M urea in the gel and electrophoresis at 37°C. The PSTV-RNA bands are absent from healthy plants(a).

DISCUSSION

Native viroid-RNA molecules existing as circular and linear forms co-migrate as a single band under non-denaturing conditions(Fig.1A). A full-denaturing condition was provided when samples were electrophoresed at 37°C in the gel system containing 8M urea. Under this condition, the viroid-RNA molecules can be separated into linear and circular viroid-RNA molecules can be separated into linear and circular viroid-RNA molecules(Fig.1b). Since high concentration of urea disrupts the non-covalent bonds in the circular viroid-RNA molecules, retardation of mobility for the circular RNA would be increased resulting in separation of the circular RNA band from the linear ones(4, 8).

Under partial denaturing conditions provided by low concentration of urea in the urea-gradient gel system, many different conformations of viroid-RNA molecules were detected as various indistinct bands, due to the difference in migration pattern

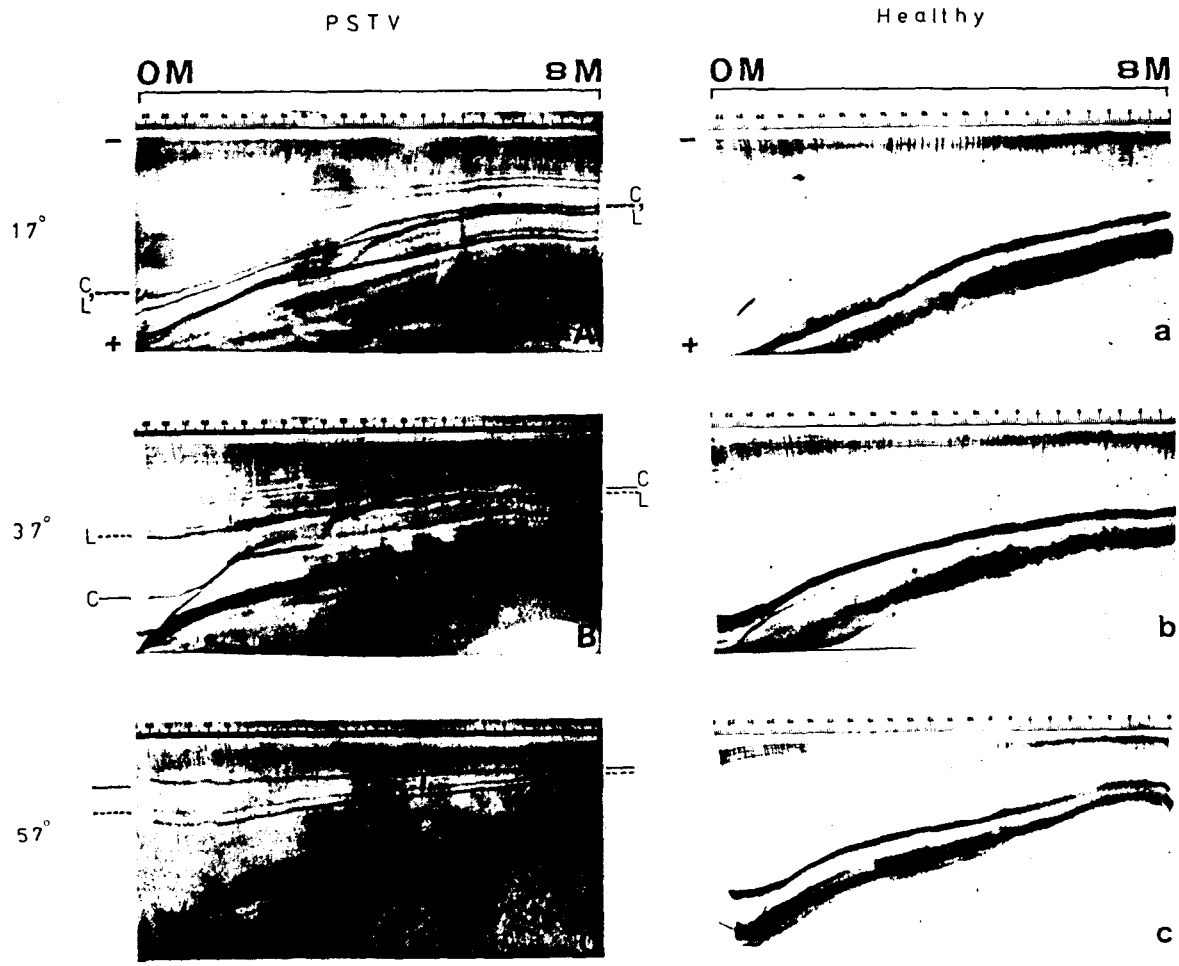

Fig. 2. Electrophoretic migration of the low molecular weight RNAs extracted from PSTV infected(A, B, C) and healthy(a, b, c) plants in an urea-gradient gel containing 1/40 dilution of TBE buffer at three different temperatures, 17°C, 37°C and 57°C. (C : circular PSTV-RNA molecules, L : linear PSTV-RNA molecules)

among the RNA molecules of different conformations. In the urea-gradient gel with 1/40 dilution of TBE buffer and the temperature of 37°C (Fig.2B), the band of circular viroid-RNA molecules migrated across the band of linear viroid-RNA molecules and showed a cross-over formation at the mid-range of the gradient system. Non-denatured and partially denatured circular RNAs would migrate faster than the linear viroid-RNA at low urea concentration region causing the cross-over formation in the mid-range of the urea-gradient gel at 37°C as shown in Fig.2B.

An extra RNA band detected in the region of low urea concentration with 1/40 dilution of TBE buffer at three different temperatures(Fig.2) appeared to be of plant origin, since the band is common for both the viroid-infected and healthy plants. Whether the plant RNA band in Fig.2 is identical to the RNA band detected in the gel system with 1/10 dilution of buffer at 37°C(Fig.3B and 3b), remains to be studies. Although the nature of the extra plant RNA molecules is not known, the cross-over form which appeared in the region of low urea concentration at 37°C seems to be caused by the presence of the plant RNA band that interferes with the migration of the circular viroid-RNA band.

A high concentration of salts which was made by 1/10 dilution of TBE buffer was used for the non-

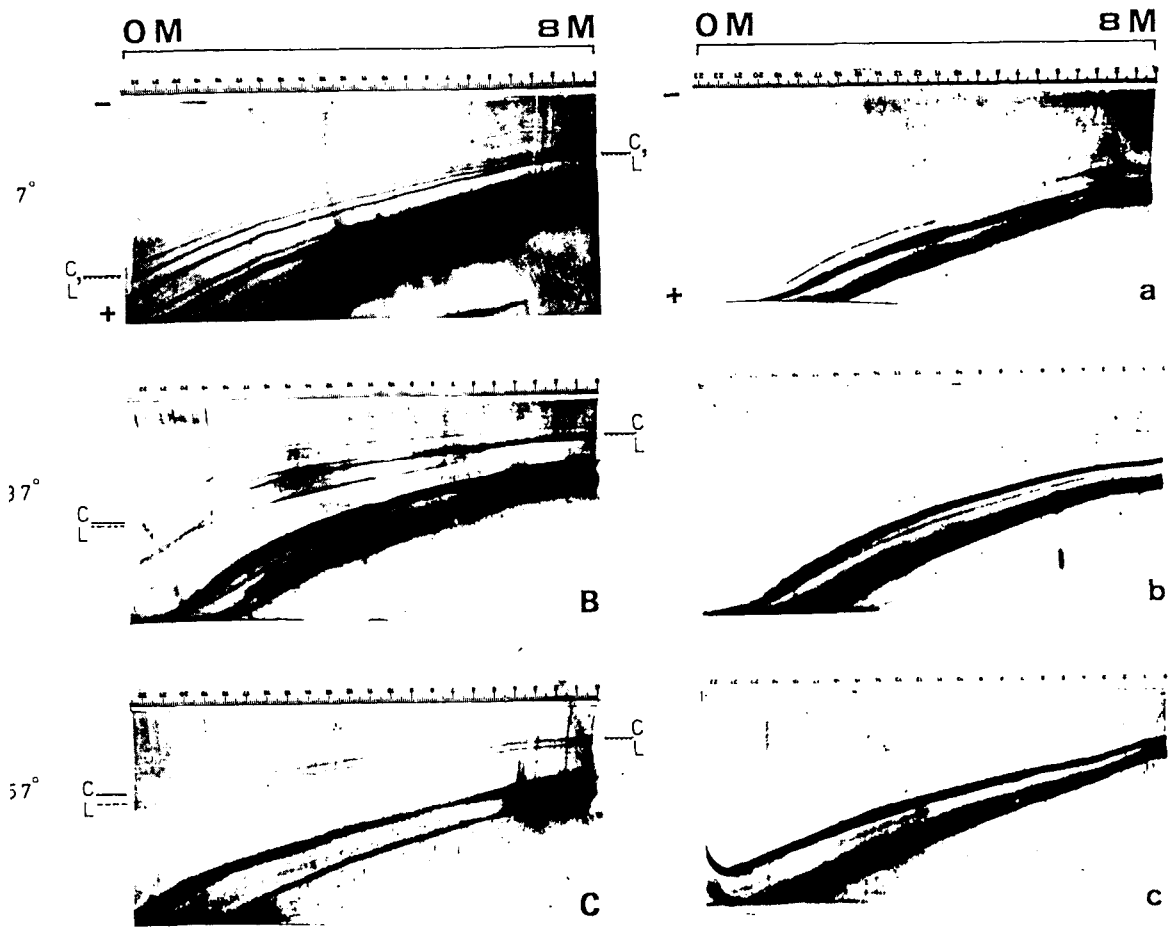

Fig. 3. Electrophoretic mobilities of the low molecular weight RNAs in a urea-gradient gel containing 1/10 dilution of TBE buffer at three different temperatures, 17°C, 37°C and 57°C. The RNAs were extracted from PSTV infected(A, B, C) and healthy(a, b, c) plants. C and L indicate circular and linear PSTV-RNA molecules, respectively.

denaturing gel system of viroid-RNA molecules, and a low concentration of salts by 1/40 dilution of TBE buffer was used for the fully denaturing gel system. When gels containing 1/10 dilution of TBE buffer were electrophoresed at three different temperatures, 17°C, 37°C and 57°C, a distinct separation of the viroid-RNA molecules into circular and linear forms was not shown despite the higher temperature for electrophoresis comparrtrations such as 1/10 dilution of TBE buffer appear to hinder the denaturation of the circular viroid-RNA molecules. Higher concentration of salts in the gel(Fig.3B) compared to that in Fig. 2B, may explain the absence of a cross-over form in highly salt concentrated gel system in the mid-range of the urea-gradient since both gel systems were prepared under the same conditions except for the difference in salt concentration. The separation of viroid-RNA molecules into two different forms is dependent mainly on the denaturing effect of urea in the gel(6). In addition, a low concentration of buffer would enhance the effective separation of two viroid-RNA molecules in the denaturing gel system at 37°C.

Full denaturation of the PSTV-RNA molecules was reported at the temperature of 53°C from the thermodynamic studies(9, 10). Since electrophoresis at 57°C would be sufficient for the denaturation of the circular PSTV-RNA, its separation from the linear RNA band was expected. However, the

separation distance between two bands was greater in the gel with 1/40 dilution of TBE buffer compared to that with 1/10 dilution of the buffer. The more effective separation condition for the circular viroid-RNA molecules from the linear molecules would be provided by 1/40 dilution of the buffer even at the highly denaturing temperature of 57°C.

In conclusion, an effective denaturing gel system for the separation of the viroid-RNA molecules into circular and linear forms can be accomplished at the high temperature of 57°C, low concentration of TBE buffer, and at low urea concentration.

ACKNOWLEDGEMENT

This research was supported in part by the Korea Science and Engineering Foundation. The authors express their appreciation to Prof. H.L. Sänger at Max-Planck Institut für Biochemie in München, Germany for his kind donation of the viroid isolate.

REFERENCES

1. DIENER, T.O.(1971). Potato spindle tuber "virus". IV. A replicating, low molecular weight RNA. *Virology* 45 : 411-428.
2. GOLDMAN,D. & MERRIL,C.R.(1982). Silver staining of DNA in polyacrylamide gels : Linearity and effect of fragment size. *Electrophoresis* 3 : 24-26.
3. GROSS, H.J., DOMDEY, H., LOSSOW, C., JANK, P., RABA, M., ALBERTY, H. & SÄNGER H.L.(1978). Nucleotide sequence and secondary structure of potato spindle tuber viroid. *Nature* 272 : 203-208.
4. HADIDI,A. & DIENER, T.O.(1978). *In vivo* synthesis of potato spindle tuber viroid : kinetic relationship between the circular and linear forms. *Virology* 86 : 57-65.
5. LEE,J.Y.(1982). Untersuchungen zur Übertragung des Viroids der Spindelknollensucht der Kartoffel(PSTV) durch Insekten und zu seinem Nachweis durch Polyacrylamid-Gel-Elektrophorese. Doctoral Thesis, Justus Liebig Univ., Giessen. pp. 1-91.
6. LEE,J.Y.(1987). Electrophoretic mobilities of the potato spindle tuber viroid RNA molecules in the ureagradient gels. *Kor. Jour. Microbiol.* 25 : 23-27.
7. MORRIS, T.J. & WRIGHT, N.S.(1975). Detection on polyacrylamide gel of a diagnostic nucleic acid from tissue infected with potato spindle tuber viroid. *Am. Potato J.* 52 : 57-63.
8. OWENS, R.A., ERREBE, E., HADIDI, A., STEERE, R.L. & DIENER, T.O.(1977). Separation and infectivity of circular and linear forms of potato spindle tuber viroid. *Proc. Natl. Acad. Sci. USA* 74 : 3859-3863.
9. RIESNER, D., HENCO, K., ROKOHL, U., KLOZ,G., KLEINSCHMIDT, A. K., GROSS, H. J., DOMDEY,H., & SÄNGER H.L.(1979). Structure and structure formation of viroids. *J. Mol. Biol.* 133 : 85-115.
10. RIESNER,D., STEGER G., SCHUMACHER, J., GROSS, H.J., RANDLES, J.W., & SÄNGER,H.L.(1983). Structure and funcion of viroids. *Biophysics. Struct. Mech.* 7 : 240-241.
11. SÄNGER,H.L., RAMM,K., DOMDEY, H., GROSS, H.J., HENCO, K. & RIESNER, D.(1979). Conversion of circular viroid molecules to linerar stands. *FEBS Letters* 99 : 117-122.