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# Electrophoretic Mobilities of the Potato Spindle Tuber Viroid RNA Molecules in the Urea-Gradient Gels

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# 감자 갈쭉바이로드(PSTV) RNA 분자의 요소농도기울기겔에서 전기영동적 이동성에 관하여

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**ABSTRACT: Low molecular weight plant ribonucleic acids including viroid-RNA molecules which are soluble in 2 M lithium chloride were electrophoresed in the 0 M to 8 M urea -gradient polyacrylamide gel. Although the linear viroid-RNA molecules migrated at a similar rate across the urea-gradient gel under the denaturing temperature, the circular viroid-RNA molecules moved more rapidly at low urea-gradient region than at high urea-gradient region. Consequently, the migration of the circular viroid-RNA molecules showed a sudden shift across the band of linear forms in the midrange of the urea-gradient gels. Electrophoretic mobilities of the circular viroid-RNA molecules seemed to depend mainly on the concentration of urea in the denaturing urea-gradient gels.**

**KEY WORDS □ viroid-RNA molecules, urea-gradient polyacrylamide gel.**

Viroids are the smallest ones of the known pathogens to higher plants. They have only ribonucleic acid lacking capsid compared to the conventional forms of viruses. The RNA of potato spindle tuber viroid(PSTV) is single - stranded and consists of 359 nucleotides(Gross *et al.* 1978). The RNA strand has two forms, a covalently closed circular form and a linear one caused by a single nick(Hadidi and Diener, 1978). Both forms of the viroid molecules would yield a same single band in 5% polyacrylamide gels under non-denaturing conditions(Sanger *et al.*, 1976). However, under denaturing conditions the two forms of viroid-RNA molecules would separate into two different bands(3, 10). The separation of circular and linear forms of PSTV-RNA on polyacrylamide gles in the presence of formamide and urea was reported by Owens *et al.*(1977).

The conformational changes of the viroid -RNA molecules has been studied in detail(Riesner *et al.*, 1979 : 1983). The authors reported that 8 M urea concentration at 37℃ would be sufficient in providing the denaturing electrophoresis system for viroid -RNA molecules. However, the physico-chemical and biological characteristics of the circular and linear viroid-RNAs have not been explained sufficiently.

Urea-gradient polyacrylamide gel electrophoresis have been used to study the character-

istics of nucleic acids. In this study, some modifications were made in preparing gradient gels to achieve more practical use of the electrophoresis system. The purpose of this research is to study the electrophoretic mobilities of circular and linear viroid-RNA, and particularly of the partially denatured circular forms under the different urea concentrations.

## MATERIALS AND METHODS

**Ribonucleic acid preperation**

Tomato plants(*Lycopersicon esculantum* Mill. cv. "Rutgers") were infected with the severe isolate of potato spindle tuber viroid(PSTV) using the method proposed by Singh and Sanger(1976). The partial purification of PSTV from deep frozen tomato leaves involved 1) phenol extraction of RNA; 2) bentonite treatment to inhibit nuclease; 3) fractionation of low molecular weight forms of nucleic acids with 2 M Lithium chloride solution; and 4) removal of polysaccharides and deoxyribonucleic acids to isolate RNA. Detailes of the purification procedures for plant nucleic acids were described in the previous report(Lee, 1982)). The nucleic acid samples were dissolved(1,500 $\mu$g of the purified RNA per 1 m$l$) in the 1/20 diluted TBE buffer(0.89 M Tris, 0.89 M boric acid, 0.025 M Na-EDTA, pH 8.3). The concentration of the buffer is two fold excess of that used in the gel system.

Acrylamide, N,N'-methylene-bis-acrylamide, TEMED and ammoniumpersulfate for electrophoresis gel were purchased from Bio-Rad Laboratories. All other chemicals were of reagent grade.

**Horizontal denaturing urea-gradient gel**

In the horizontal denaturing urea-gradient gel, the direction of the electric field is perpendicular to the urea-gradient. Urea-gradient gel was prepared according to Fischer and Lerman (1979) method with some modifications. The modified method is as follows. Gels were prepared by mixing a urea-free acrylamide gel solution with a gel solution containing 8 M urea. Each solution was poured into a gradient former to produce a continuous urea-gradient gel system. A syringe connected to the gradient former was inserted into the open top side of the assembly to pour the gradient solution into it. The assembly was made of a 1.5 mm thick Teflon U-frame spacer and two glass plates (220×105 mm). One of the glass plates has a slot former facing the inner part of the sandwich. The slot is made by adhering four layers of scotch tape(215×2×0.2 mm) to the plate, 1 cm beneath the U-frame. The capacity of the slot was approximately 200 $\mu l$. The flow rate of the gradient solution was about 10 m$l$ per a minute. Consequently, the denaturing gel system was composed of 1/40 diluted TBE buffer and of the urea-gradient 0 M to 8 M.

**Electrophoresis**

Two hundred microliter solution of 0.01% bromphenol blue containing 10% sucrose was prerun to improve the quality of the gradient gel system. One hundred fifty $\mu$g of the purified nucleic acid for a sample to load dissolved in 100 $\mu l$ of the corresponding TBE buffer at double salt strength of the gel system, were mixed with 90 $\mu l$ of $H_2O$ and 10 $\mu l$ of 0.1% xylencyanol FF containing 10% sucrose. The 200 $\mu l$ sample was heated for 1 min at 100℃ and then cooled solwly for 1 hour. The sample was applied to the slot and electrophoresis was carried out at 20 mA for 2-3 hours until the xylencyanol FF migrated to the end of urea-free side of the gel system. TBE buffer was used in the electrode buffer system with the same strength of salt as in the gel. After electrophoresis, the gel was stained with silver nitrate by the method of Sammons *et al.*(1981) with some modifications.

## RESULTS

Both types of circular and linear viroid-RNA molecules migrated together and rerealed a single band in 5% polyacrylamide gels under non-denaturing conditions(Fig.1a). Since many different conformations of viroid molecules could exist under partially denaturing condi-

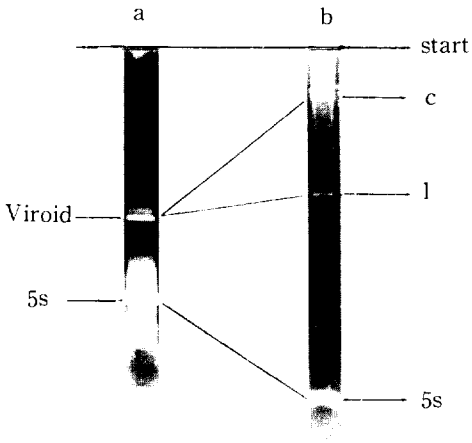


**Fig.1.** *Electrophoretic difference of the viroid -RNA bands under the nondenaturing condition and fully denaturing condition.* (a): electrophoretic separation between the viroid-RNA and the plant RNA under the nondenaturing condition. (b): separation of viroid-RNA molecules into the linear and circular forms under the high concentration of urea and the denaturing temperature, 37℃. C: circular viroid molecules, L: linear viroid molecules

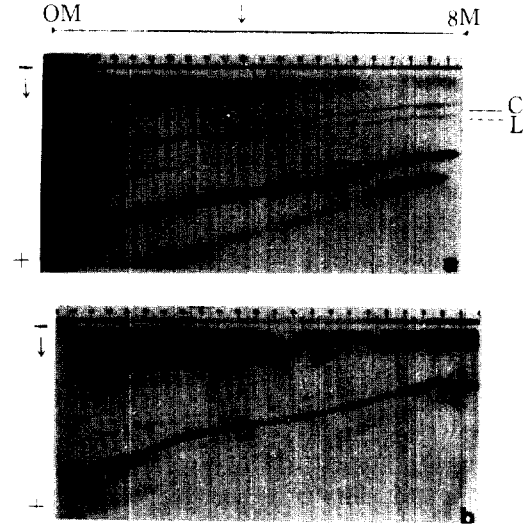


**Fig.2.** *Migration of 2 Mlithium chloride soluble RNAs on a denaturing urea-gradient perpendicular to the electric field.*

The ribonucleic acids molecules from viroid-infected tomato(a) and healthy plants(b) were loaded on the 5% polyacrylamide urea-gradient ranging from 0 M on left to 8 M on right. The arrow at the top indic ates the shift of the mobilities between circular and linear viroid-RNA molecules.

tions, only a smeared band was obtained and clear separation of the individual bands were not possible in these denaturing conditions(data not shown). Under the denaturing gel system the viroid-RNA molecules were separated into the fast migrating linear molecules and the slow migrating circular molecules(Fig.1b). Since the samples loaded on the denaturing gels were low molecular weight ribonucleic acids extracted from plants, the bands of 4s RNA and 5s RNA were detected together with both forms of viroid-RNA molecules.

In the denaturing urea-gradient gel electrophoresis, ribonculeic aicds extracted from both viroid-infected and healthy plants migrated at a similar rate across the urea-gradient gel system(Fig.2a and 2b). However, the bands of low molecular weight ribonucleic acids migrated slightly faster at the region of low urea concentration than at the region of high urea concentration. Two bands were detected distinctly from viroid-infected plants(Fig.2a) which were absent in healthy plants(Fig.2b). The bands were designated as C(circular) and L(linear) based on their mode of separation identified under denaturing gels(Fig.1b). The migration pattern of linear viroid-RNA was consistent with those of other low molecular weight ribonucleic acids. However, the band of circular viroid-RNA molecules migrated faster at low urea-gradient region than at high urea -gradient region. As a results, the band of circular viroid-RNA crossed over the band of linear molecules at the midrange of the urea -gradient gel(Fig.2a). Besides the two distinct bands of circular and linear viroid-RNAs many other bands of unidentified ribonucleic acids were detected from both viroid-infected plants and healthy plants.

## DISCUSSION

Polyacrylamide gel electrophoresis(PAGE) analysis may be used for routine diagnosis for

viroid infection to the host plants(Morris and Wright, 1975). Silver staining method has been used to detect the viroid molecules in polyacrylamide gels since the viroid-RNA is a minute fraction of the total ribonucleic acids extracted from diseased plants. In this study, the small fraction of viroid molecules and other unidentified low molecular weight ribonucleic acids were detected clearly by using the silver staining method in the denaturing gels.

Many single-stranded RNA molecules contain "hairpin loops" where the RNA chain folds back upon itself and forms short helical sections held together by hydrogen bonds. The loops of the hairpins might be attacked by nuclease not only during RNA extraction and purification but also *in vivo* system. By cooperating $^{32}P$ into PSTV, Hadidi and Diener(1978) reported that *in vitro*, the linear forms did not arise by nicking circles with nucleases. However, in the presence of $Mg^{++}$, viroid circles would be nicked to yield linear molecules during the viroid-RNA purification procedures(Sanger *et al.*1979). The linear viroid molecules presented in Fig.1 and 2 were derived from the viroid-infected plants in which the linear forms existed originally, since no $Mg^{++}$ containing chemical was added during the viroid-RNA extraction procedure.

The migration of circular and linear viroid molecules under denaturing urea-gradient gels showed unique patterns. The migration rates of the linear viroid-RNA molecules were consistent across the entire range of urea-gradient, indicating thermodynamic stability of the linear viroid-RNA. The thermodynamic characteristics of the linear viroid-RNA was known to be similar to that of multibranched single-stranded RNAs. Denaturation of multibranched single-stranded RNAs would not depend greatly on the temperature. Since other low molecular weight RNA bands on the gels were multi-branched single-stranded RNAs, the migration pattern of the linear viroid-RNA was similar to that of other low molecular weight RNAs.

The migration of the circular viroid-RNA molecules was different from that of linear forms in the denaturing urea-gradient gel. Teh melting temperature of PSTV, 53℃, is relatively low compared to that of a homogeneous double stranded RNA. Due to the covalently closed circular viroid-RNA molecules, the urea-gradient gel was a gradual denaturing system for the circular viroid molecules. Partly base-paired circular viroid molecules would reach a point where the concentration of a denaturing agent is sufficient for changing the conformations of the viroid-RNA, as they migrate through the urea-gradient gels. Since high concentration of urea would denature the circular viroid-RNA completely, retardation of the circular viroid mobility would be enhanced at the high urea concentration region on the gel system. However, covalently closed circular viroid-RNA molecules might be remanied intact or partially denatured at the low concentration of the denaturant. The retardation of the circular viroid-RNA mobility would be reduced as the concentration of urea decreases in the urea-gradient gel system. A sudden shift of the circular viroid-RNA molecules' mobility would be observed in the midrange of the urea-gradient where partially denatured conformations predominantly existed. The denaturation of the circular viroid-RNA molecules is mainly dependnet on the concentration of urea at temperature of 37℃ in the polyacrylamide urea-gradient gel system.

## 적 요

바이로이드 RNA 분자를 포함한 저분자량의 식물성 RNA 분자에 대해서 0M부터 8 M까지의 요소구배를 가진 폴리아크릴아미드겔을 이용하여 전기영동적 이동성을 조사하였다. 막대모양의 바이로이드 RNA 분자는 요소구배 겔에서 전반적으로 다른 저분량의 식물성 RNA 등과 마찬가지로 요소구배에 수직인 방향으로 비슷한 수준의 전기영동적 이동성을 보여주었다. 이와

달리 고리모양의 바이로이드 RNA 분자는 요소구배 겔에서 고농도의 요소구배 부분에서 보다도 저농도의 요소구배 부분에서 더 빠르게 이동하였다. 따라서 고리모양의 바이로이드 RNA 분자는 요소구배 겔의 중간부분에서 막대모양의 바이로이드 분자의 밴드를 가로 지르는 기울기를 가진 특이한 전기영동적 이동을 나타내었다. 결과적으로 고리모양의 바이로이드 RNA 분자의 전기영동적 이동성은 0M-8M의 요소구배를 가지는 바이로이드 RNA 분자의 변성겔에서 주로 요소의 농도에 따라서 영향을 받는 것으로 보인다.

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