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Detection and Assessment of Citrus Tristeza Virus Antigens by Computer-Aided Scanning Densitometry

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ABSTRACT

Citrus tristeza virus (CTV) extracted from Etrog citron (*C. medica L.*) was immunoprecipitated. The immunoprecipitate was fractionated by SDS-PAGE and western blotted onto nitrocellulose. The CTV antigens were determined by immunoblot analysis using rabbit anti-CTV IgG, and the protein-band pattern exhibited on the nitrocellulose was assessed by soft-laser scanning densitometry. The densitometric tracing revealed the presence of bands that were not visible to the naked eye. Using the superimposition mode of the instrument, it was also revealed that the protein-band patterns of different CTV samples were not identical. Computer-aided soft-laser scanning densitometry proved to be a powerful approach in the detection and assessment of protein bands revealed on nitrocellulose immunoblots, which we were previously unable to do employing conventional methods.

Index Entries: Etrog citron; immunobloting; soft-laser scanning densitometry; citrus tristeza virus antigens, detection and assessment of by computer-aided scanning densitometry.

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INTRODUCTION

Citrus tristeza virus (CTV) is composed of particles 2000 nm long and 10–12 nm wide (*1–3*), and is well known to cause a serious disease of citrous plants. We have previously developed a modified procedure for purification of CTV, and we observed three different viral protein bands on nitrocellulose immunoblots recognized by rabbit anti-CTV IgG (*4*). In the present study, an attempt has been made to analyze our preliminary data (*4*) and to describe the use of computer-aided soft-laser scanning densitometry (*5–11*) for detection and quantitation of viral protein bands directly as they appear on nitrocellulose immunoblots.

MATERIALS AND METHODS

The CTV was immunoprecipitated from Etrog bark homogenate according to the procedure described by Acey and Smith (*4*). Control homogenate was obtained from healthy bark. The immuoprecipitate was fractionated by SDS-PAGE and then blotted onto nitrocellulose. Immunoblotting of nitrocellulose strips was carried out by means of rabbit anti-CTV IgG, and the immunoblots were developed using goat anti-rabbit IgG conjugated with horseradish peroxidase (*4*).

Analysis of the protein-band pattern shown on the nitrocellulose immunoblots was made using a soft-laser scanning densitometer, model SL-TRFF (Biomed Instruments, Inc.). The analog signal of the laser beam was processed through an A/D converter, model ADC-1 (Biomed Instruments, Inc.), in conjunction with an Apple IIe computer. The immunoblots were scanned at a speed of 2.5 mm/s with the width of the laser beam adjusted to 10 μm. Direct comparison of the CTV antigenic bands was made using the superimposition mode of the "Videophoresis II" program (Biomed Instruments, Inc.). Hard copies of densitometric tracings were obtained through a dot-matrix printer (STAR SG-10).

RESULTS

The immunoblots illustrating proteins recognized by rabbit anti-CTV IgG are shown in Fig. 1. The samples obtained from healthy plants, lanes B and F, failed to react with the rabbit IgG, whereas those obtained from CTV-infected plants, lanes C, D, and E, revealed four different distinct protein bands that were visible to the naked eye. Band (I) appeared in lanes C and D, but was not present in E. Unlike band (III), which was observed in all three lanes showing immunoblotting of CTV proteins, band (IV) was observed only in lanes C and E.

The tracings obtained following soft-laser scanning densitometry of the immunoblots are shown in Fig. 2. Scanning lane C (of Fig. 1) revealed six peaks (Fig. 2.1), four of which, (a), (d), (e), and (f) corresponded to

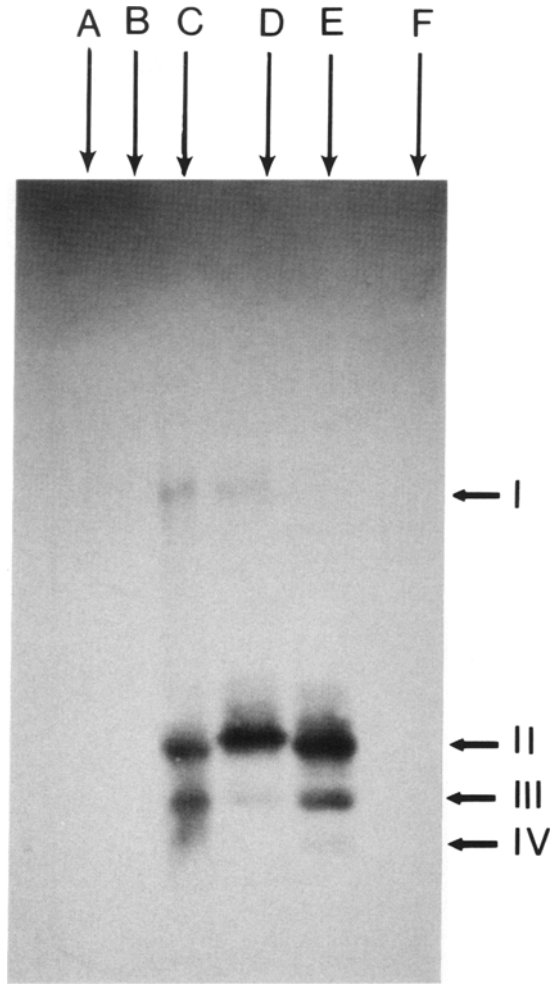

Fig. 1. Immunoblots of Citrus Tristeza Viral (CTV) proteins. The CTV was isolated from Etrog bark extracts and immunoprecipitated using goat anti-CTV IgG. The immunoprecipitate was fractionated by SDS-PAGE and then blotted onto nitrocellulose. Immunoblotting was performed using rabbit anti-CTV IgG. Development of the immuoblots was carried out by means of goal anti-rabbit IgG conjugated to horseradish peroxidase. Lanes C, D, and E are immunoblots of three different CTV samples; A, is a blank; and B and F are control extracts from healthy plants. The numbers I–V indicate visible stained protein bands.

bands (I), (II), (III), and (IV), respectively, of Fig. 6. Peak (b) fused with (a), (c) appeared distinct, and (d), (e), and (f) were defined by two intervening notches.

Figure 2.2 showed three distinct peaks, (g), (l), and (m), which appeared to correspond with bands (I), (II), and (III) on lane D. Closer review of the figure revealed three more peaks, identified as (h), (i), and (k), in the tracing.

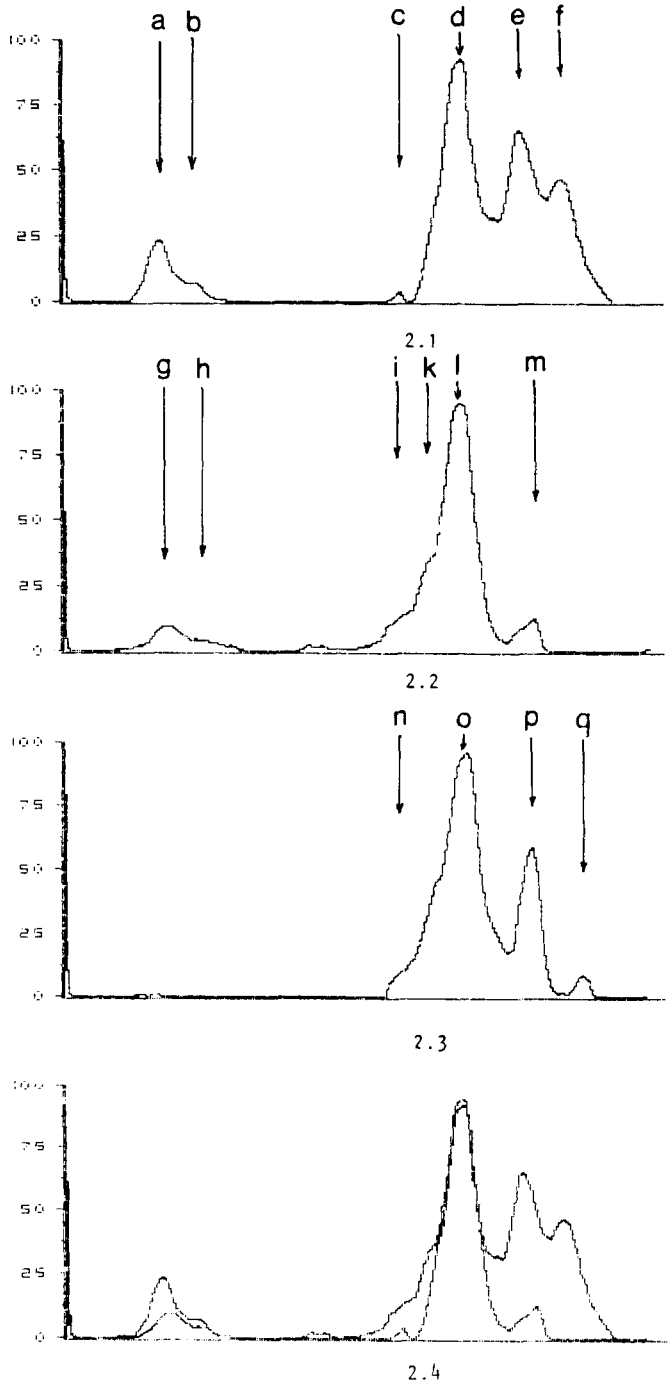

Fig. 2. Soft-laser scanning densitometry of immunoblots. Figure 2 (2.1–2.3) shows the tracings of lanes A, B, and C, respectively, as they appear in the prints produced by the computer. Figure 2.4 illustrates superimposition of Fig. 2.1 on 2.2 for direct comparison of the protein-band patterns of lanes C and D on the immunoblot.

The densitometric tracing of lane (E), shown in Fig. 2.3, revealed three distinct peaks, (o), (p), and (q), which appeared to correspond with bands (II), (III), and (IV) of lane E in the immunoblot. Peaks (o) and (p) were separated by a deep valley, whereas (p) was revealed as a small, but well resolved peak. On the other hand, peak (n) was observed as an abrupt elevation above the baseline, fusing immediately thereafter with peak (o).

Superimposition of the tracing obtained upon scanning lane E on that of C on the screen, using the "Videophoresis II" program, revealed that peaks (n), (o), (p), and (q) of E coincided with peaks (c), (d), (e), and (f) of C. Superimposition, however, of Fig. 1 onto Fig. 2 demonstated a different protein-band pattern between the two lanes, as indicated by the position of their respective peaks.

DISCUSSION

Computer-aided soft-laser scanning densitometry appears to be not only a powerful analytical modality, but also very convenient for the assessment of electrophoresed proteins. In this study, we have been able to obtain densitometric tracings by scanning directly viral proteins on nitrocellulose immunoblots and to discover the presence of proteins not visible to the naked eye. Furthermore, we were allowed to perform direct comparison of protein bands exhibited on different immunoblot strips and to determine whether their patterns are compatible, simply by superimposing their tracings on the screen of a computer monitor.

The densitometric tracings of the immuoblots demonstrated in Fig. 2 show that the soft-laser scanner has enough sensitivity to measure minute changes in density, such as when the protein resolved on the nitrocellulose strip is in trace amounts. A problem reflected by weak staining of that protein is that its quantitation or visual detection cannot be attained. Such a problem is clearly demonstrated in Fig. 1, in which no protein bands corresponding to the peaks (b), (c), (h), (i), (k), and (n) revealed in the densitometric tracings (Fig. 2) are visible in the immunoblot.

For instance, upon examining Fig. 2.1 it can be seen that peak (a) rises steeply, then midway before it returns to the baseline forms a plateau, peak (b), and finally descends at a steeper slope. Interpretation of the tracing suggests the presence of another protein band adjacent to band (I), which has a density approximately 60% less than that of band (I). Apparently, the protein band revealed as peak (b) in the densitometric tracing of lane C could not be detected by the naked eye for two reasons. The first one was that its concentration was too small to allow its staining and the second, that the interspace between the two protein bands was smaller than the width of the scanning light beam. The second problem was manifested in the densitometric tracing, Fig.

2.1, in which it can be seen that the descending side of peak (a), instead of reaching the baseline, fuses with the ascending side of peak (b), forming, in this manner, a plateau.

It should be noted that in order to attain complete resolution of two closely stacked bands by scanning densitometry, the scanning light beam should be narrower than the intervening gap between the two bands (*8,9*). In conventional scanners, resolution is improved by narrowing the slit, so that a thinner light beam than the interspace between adjacent bands is produced. Accordingly, as the slit width becomes narrower, resolution improves dramatically. However, controlling the width of the light beam in this manner is a self-limiting process, since the slit can be narrowed to a certain extent, beyond which, the light beam, after passing through it, starts diverging. That is to say, the width of the light beam becomes in this regard greater than that of the slit that controls it. In contrast, this problem is not encountered in laser scanners, since the light beam width is internally controlled. Adjustments of the laser beam can be made to produce a monochromatic light beam width of 1 μm or less, a feature required for faithful resolution of closely stcked bands. The potential of this advantage is that a scanning light beam, being thinner than the interspace of two adjacent bands, will allow their respective peaks in the densitometric tracing to reach the baseline, thus facilitating their quantitation.

The high sensitivity of the soft-laser scanner was also demonstrated upon review of peak (c) in Fig. 2.1. The height of the peak indicated that the respective protein band on the immunoblot was of very low density, that is, in trace amounts. However, the weakly stained protein, which could not be detected by the naked eye, was a sufficient stimulus for the scanner to produce a peak of small amplitude in the densitometric tracing.

Using the superimposition mode of the soft-laser scanner, we were able to make a direct comparison of the densitometric tracings and to determine a difference in the protein-band patterns obtained. By superimposing the tracing of lane C on that of D (Fig. 2.4), we observed that their protein-band patterns were different. On the other hand, it was found that the pattern of the tracing showing scanning of lane C was similar to that observed in lane E, except that no peaks corresponding to (a) and (b) were found in the latter.

Also of great interest was the observation that all CTV protein bands were recognized by a common antibody, regardless of their position in the immunoblot. Determination of CTV proteins is under investigation, and it will be the subject of another report. In this pilot study we were able to detect and to compare viral protein fractions by means of soft-laser scanning densitometry. First, we found the method of great convenience, since we were allowed to perform our assessments by scanning directly the nitrocellulose immunoblot. Second, we were able to detect, as a result of its high sensitivity, the presence of proteins not visi-

ble to the naked eye. And, finally, using the superimposition mode of the instrument, we were able to compare the densitometric peaks obtained after scanning different samples and to determine any existing differences between their patterns. In this regard, the presence or absence of a protein(s) of a sample, when examined in conjunction with a standard, could be easily determined at a glance of the eye.

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REFERENCES

1. Bar-Joseph, M., Loebenstein, G., and Cohen, J. (1972), *Virology* **50,** 821.
2. Kitajima, E. W., Silva, D. M., Oliveira, A. R., Muller, G., and Costa, A. S., (1964), in *Proc. 3rd Conf. Int. Organ. Citrus. Virol.* Price, W. C., ed., University of Florida, Gainesville, FL, pp. 1–9.
3. Price, W. C. (1966), *Virology* **29,** 285.
4. Acey, R., and Smith, C., *Detection of Citrus Tristeza Virus by Immunoblotting,* in preparation.
5. Barany, K., Barany, M., Gillis, M. J., and Kushmeric, J. M. (1979), *J. Biol. Chem.* **254,** 3618.
6. Barron, J. T., Barany, M., and Barany, K. (1979), *J. Biol. Chem.* **254,** 4954.
7. Scheff, G., Marrow, C., Pillay, V. K. G., and Zeineh, R. A. (1974), *Clin. Res.* **22** (3), 434A.
8. Zeineh, R. A., and Nijm, W. P. (1974), *Clin. Res.* **22** (3), 428A.
9. Zeineh, R. A., Nijm, P. W., and Al-Azzawi, F. (1975), *Am. Lab.* **7** (2), 51.
10. Zeineh, R. A. (1977), *Am. Lab.* **9** (6), 71.
11. Zeineh, R. A. (1977), *Proc. Intl. Symp.* August 2–4, 1976, Hamburg Germany, *Electrophocusing and Isotachophoresis,* pp. 147–151. Radola, B. J., and Graesslin, D., eds., Walter de Gruyter, Berlin, NY.