0273–2289/86/1302–0111$02.00

Soft-Laser Scanning Densitometry Performed on a 35-mm Slide Illustrating SDS-PAGE Separation of Adenovirion Polypeptides

RASHID A. ZEINEH AND GEORGE KYRIAKIDIS*

Biomed Instruments, Inc., 1020 S. Raymond Avenue, #B, Fullerton, CA 92631

AND

A. RASHID BHATTI

Preventive Medicine Section, Defense Research Establishment, Suffeld, Ralston, Alta., Canada TOJ 2NO

Received November 25, 1985; Accepted March 20, 1986

ABSTRACT

Virion polypeptides (35 S), methionine-labeled and purified by CsCl gradient centrifugation, were separated by SDS-gel electrophoresis. Analysis of their band pattern was performed by scanning the images of the SDS-gels shown on a 35-mm slide. The densitometric tracings revealed the presence of 17 protein bands, although only 15 of them were visible to the naked eye. The high sensitivity and resolving capacities of the soft-laser scanning densitometer enabled us to detect trace amounts of protein bands separated in SDS-gels and to obtain a resolution compatible to that of electrophoresis. Fourfold electronic amplification of the densitometric tracings, produced by a computer, generated new information regarding the pattern of the electrophoregram. The facility to amplify peaks of importance is particularly advantageous when faint or overlapping protein bands revealed on a gel are assessed.

Index Entries: Soft-laser scanning densitometry; SDS-PAGE separation, of adenovirion polypeptides; adenovirion polypeptides.

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

One of the major problems frequently encountered in the analysis of electrophoresed proteins is quantitative assessment of their components. The fine resolution obtained by new developments and methods in electrophoresis (*1–3*) are often attenuated by the limitations in resolving power of the conventional scanning densitometers. For instance, quantitative assessment of stained, separated, protein bands by isoelectric focusing on polyacrylamide gel requires a scanner than can offer a resolution in the order of 15 μm. Since the finest resolution obtained from scanners using white light is 120 μm, obviously, the separation of the protein components achieved by electrophoresis cannot be faithfully replicated on the densitometric tracing. Therefore, scientists using electrophoretic methods of high resolution are often unable to obtain densitometric tracings of high fidelity. Scanning of closely stacked bands in the electrophoregram invariably reveals tracings in which the respective peaks produced have been fused, a fact demonstrating the limit of the resolving capacity of a scanner. This is encountered when the width of the light scanning beam is greater than that of the gap separating two resolved components. In this regard, the peaks, instead of being separated by an intervening valley in which the sides touch the baseline, fuse, forming a plateau. Thus, quantitation of the bands of interest becomes, in this way, not feasible.

Another limitation of conventional densitometry is its low sensitivity. That is, weakly stained protein bands in the gel, because of small concentrations, may not be sensed by the scanner, though they are visible by the naked eye. In this manner, quantitative assessment of the electrophoregram becomes incomplete.

It has been reported that the use of the laser beam in scanning densitometry brought significant improvements in both resolution and sensitivity (*4–7*). One of its outstanding features is that the laser light can be adjusted internally, without the use of a slit, so that a light beam as narrow as 1 μm in width can be produced. In this manner, the scanning beam of the densitometer becomes thinner than the interspace between two adjacent bands, allowing the intervening valleys between the respective peaks to touch the baseline. In this regard, quantitation of the resolved components in the elctrophoregram may be accomplished by integrating the area occupied by the respective peaks exhibited in the densitometric tracing.

In this report, the resolving power and sensitivity of the soft-laser scanning densitometer coupled to a computer was evaluated by scanning the images of electrophoresed virion polypeptides (adenovirus type 2) shown on a 35-mm slide. In this manner, the interspace between the resolved components in the electrophoregram was reduced to such an extent that visual distinction of adjacent bands by the naked eye was virtually obliterated.

MATERIALS AND METHODS

Sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis (SDS-PAGE) was carried out as previously described (*13*). A black and white 35-mm slide showing the image of two electrophoregrams (Fig. 1) was scanned, using a soft-laser densitometer, model SL-TRFF (Biomed Instruments, Inc.). The analog signal of the densitometer was channeled to an A/D converter of 15-μs conversion time and then processed by a

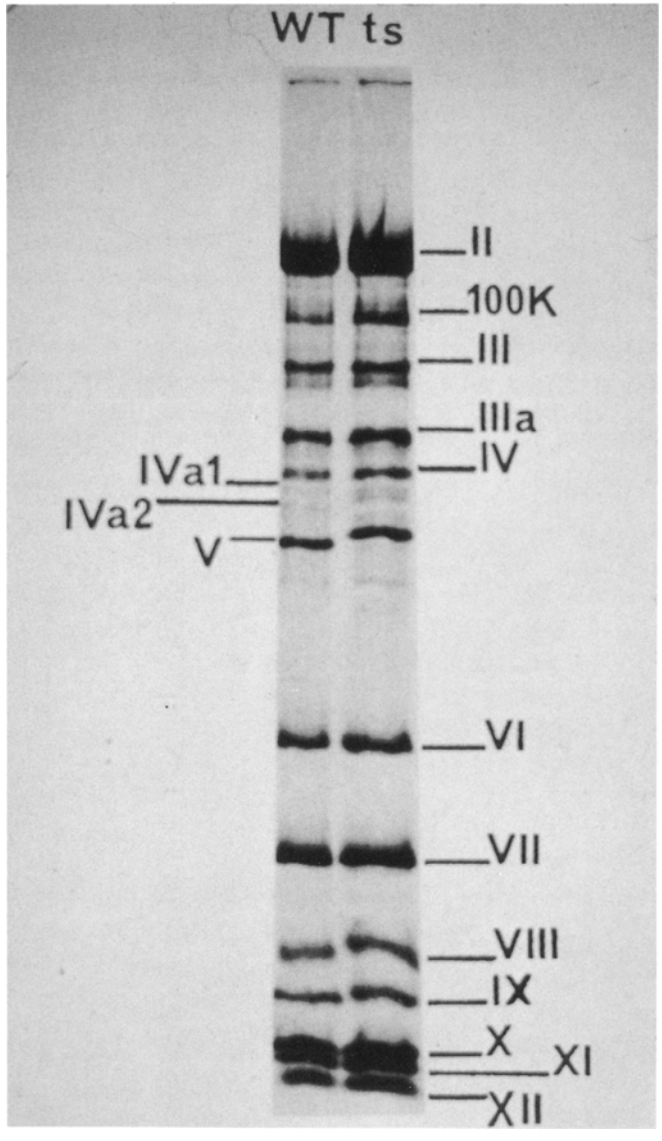
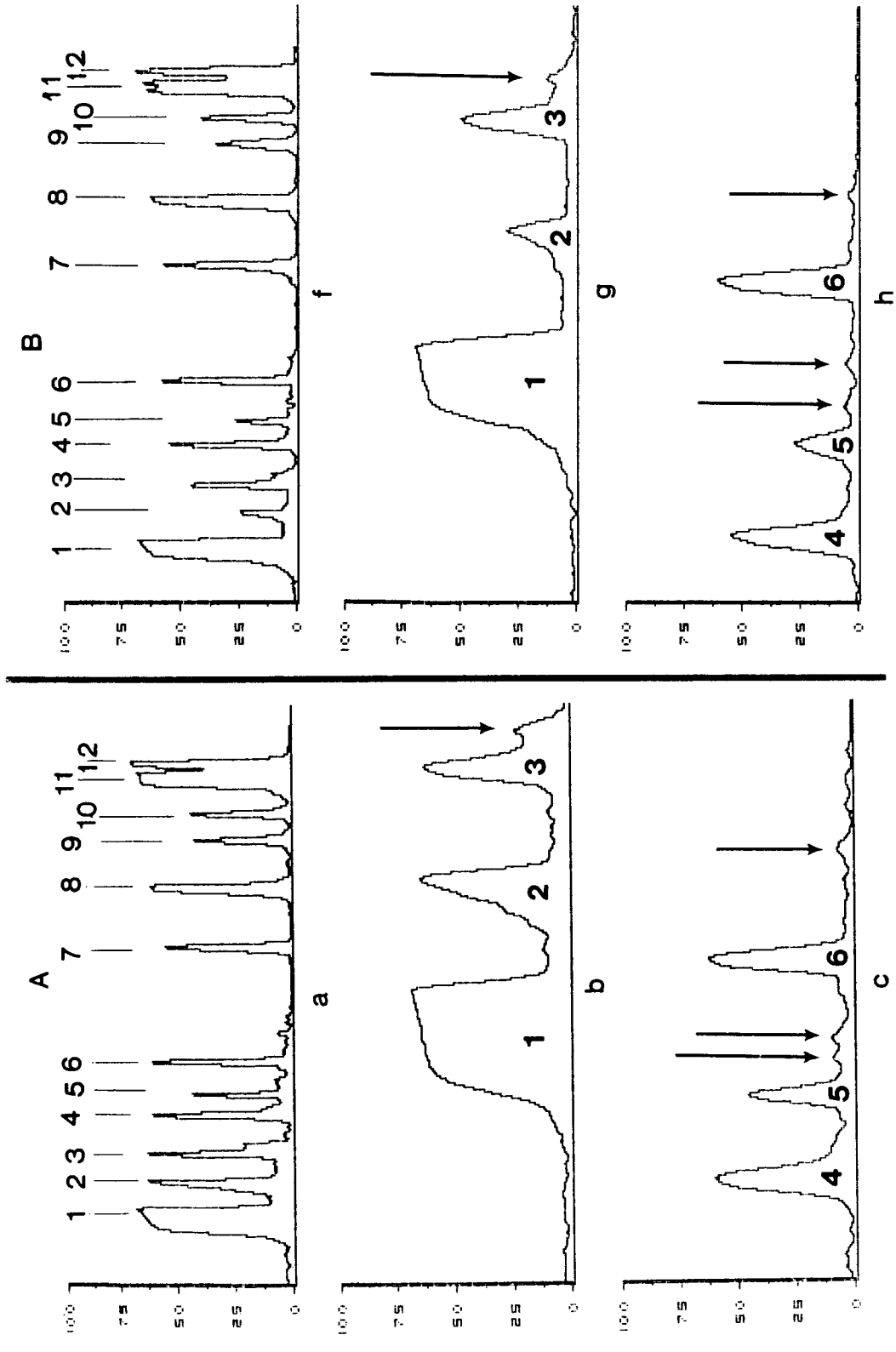


Fig. 1. Adenovirion polypeptides separated by SDS-gel electrophoresis. The illustration represents an eightfold magnification of a 35-mm slide scanned by a soft-laser scanning densitometer.


A
B
a
b
c
f
g
h
1 2 3 4 5 6 7 8 9 10 11 12
100 75 50 25 0

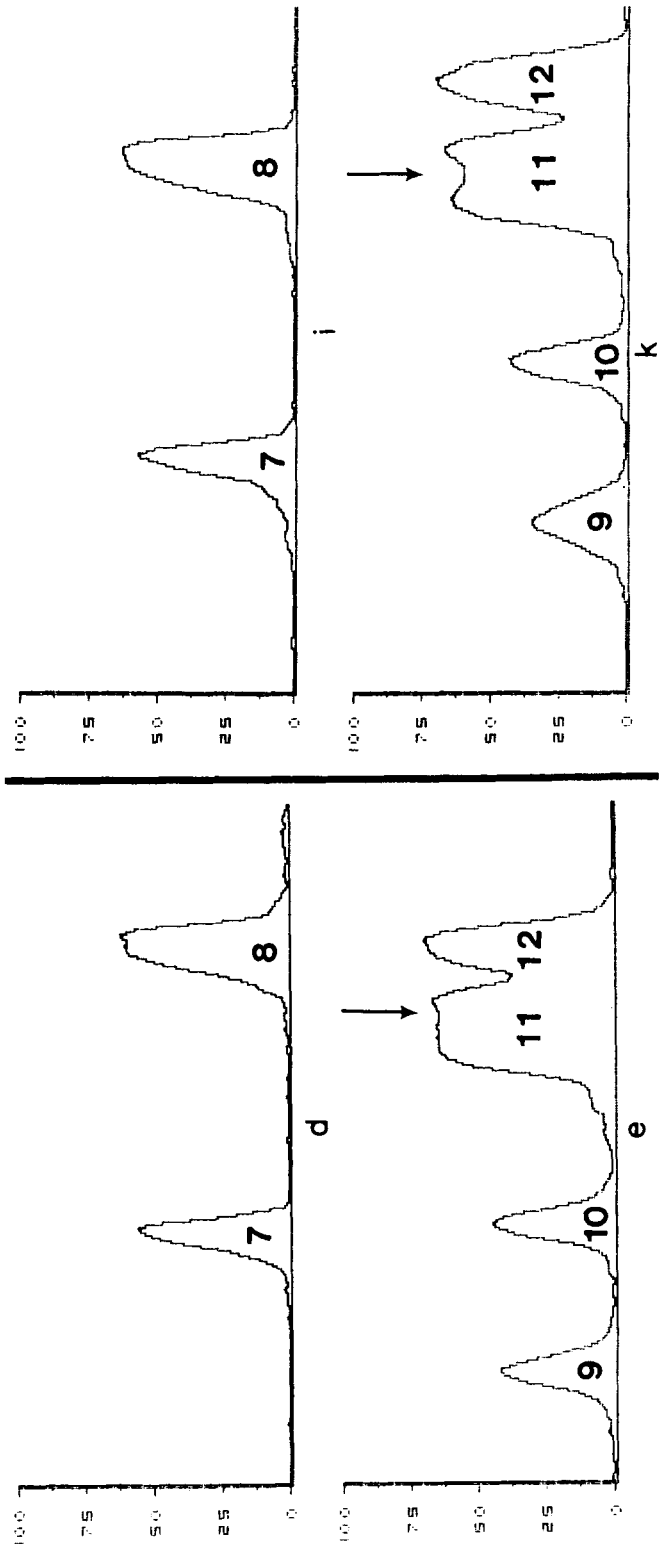


Fig. 2. Densitometric tracings obtained from a dot matrix printer connected to a computer-interphased soft-laser scanning densitometer. Figures (a) and (f) illustrate densitometric tracings of the gel images depicted on a 35-mm slide labeled, in Fig. 1, WT and ts, respectively. The figures below illustrate electronic amplification of the 12 peaks in accordance to their number order.

computer (Apple IIe), using the "Videophoresis II" program (Biomed Instruments Inc.). Hard copies of the densitometric tracings were obtained from a dot matrix printer (STAR SG-10).

RESULTS AND DISCUSSION

The high-resolving capacity and sensitivity of soft-laser densitometry becomes evident when closely stacked or faint bands shown on a gel are scanned. A typical illustration of the resolving capacity of the scanner is demonstrated in Fig. 2, (a) and (k). In each case, peak no. 11 is separated from peak no. 12 by a deep valley. Considering that the interspace between the two bands, XI and XII, when scanned on the 35-mm slide, was eight times thinner than that observed in Fig. 1, the resolution obtained from the densitometer was nearly complete.

The densitometric tracings obtained upon scanning the gel images depicted on the 35-mm slide are demonstrated in Fig. 2. Rows A and B refer to the scannings of gels labeled WT and ts in Fig. 1, respectively. A gross examination of Fig. 2, (a) and (f) reveals 12 distinct peaks in each of the tracings that appear to correspond to the dark bands on the gels shown in Fig. 1. It can be seen that almost all of the bands were well resolved, as evidenced by their respective peaks on the tracing. All of the sides of the peaks reached the baseline, with the exception of peaks no. 11 and 12, which were separated by a deep valley.

The tracings, shown in descending order below Fig. 2, (a) and (k), illustrate a 4-fold expansion of peaks no. 1–12. A process carried out electronically in four successive segments using the "Videophoresis II" computer program. Utilization of this mode enhanced significantly the analytical capacity of the scanner, since minute changes in peak contour, not visible by the naked eye, were revealed for further study. A typical example is illustrated in Fig. 2, (b) and (g) showing peak no. 3 resolved into two peaks, a major and a minor one, identified by arrows. It can be seen that electronic expansion of the peaks revealed more informative data than the original tracings illustrated in Fig. 2, (a) and (f).

The enhancing effect of electronic amplification of the densitometric tracing on the analytical capacity of the soft-laser scanner is also demonstrated in Fig. 2, (e) and (k). When peak no. 11 is observed, its contour reveals, in both sections of the figure, that it can be resolved into two peaks of approximately equal amplitude. This is more evident in (k) than in (e), since, in the former, the presence of a notch between them offers better separation than the plateau formed in the latter. This may be explained by the fact that bands X and XI exhibit a lesser degree of overlapping in gel ts than that observed in WT.

The high sensitivity of the soft-laser densitometer was evaluated by its capacity to detect protein bands resolved on a gel barely visible by the naked eye. This is clearly demonstrated in Fig. 2, (c) and (h), in which

three minor peaks have been revealed in each of the two tracings. As the arrows show, the two of them are enclosed between peaks no. 5 and 6, whereas the third one rises at a short distance after peak no. 6. The first two peaks appear to correspond to the two faint bands shown. In Fig. 1, IVa1 and IVa2, respectively. The peak on the right side of peak no. 6 has been apparently produced by the faint band revealed distally to band V.

The high sensitivity of the soft-laser densitometer was assessed by its ability to detect protein bands that were barely visible by the naked eye. An example of this is demonstrated in Fig. 2, (c) and (h), in which three small peaks are identified by three arrows in both illustrations. The two on the left appear to correspond to bands IVa1 and IVa2 shown in Fig. 1, whereas no corresponding component to the third peak was identified in the electrophoregram by the naked eye.

The present communication demonstrated the capacity of the soft-laser scanning densitometer to resolve with remarkable fidelity protein components separated by SDS-gel electrophoresis and that, unlike conventional scanners, it was found superior to the naked eye. The relative sensitivity of the scanner was also evaluated, and it was found to be comparable to that offered by radioisotope techniques. However, soft-laser densitometry offers a greater level of confidence than that offered by radioisotope techniques, since the high level of noise in the latter may offset its sensitivity, particularly when dealing with data reflecting minute changes in protein concentration. Small peaks, in this regard, may require extensive statistical analysis in order to evaluate their significance, or they may escape observation since they may be confused with noise. In sharp contrast to the radioisotope techniques, which are tedious, complex, and time consuming, soft-laser scanning densitometry was found to be easy to perform, accurate and rapid in execution.

REFERENCES

1. Anderson, G. N., Anderson, L. N., and Tollaksen, L. S. (1979), *Clin. Chem.* **25,**(7) 1199.
2. Diezel, W., Kopperschlager, G., and Hoffman, E. (1972), *Anal. Biochem.* **48,** 617.
3. Weber, J., Begin, M., and Carstens, E. B. (1977), *Virology* **76,** 709.
4. Scheff, G., Marrow, C., Pillay, K. G., and Zeineh, R. A. (1974), *Clin. Res.* **22**(3) 434A.
5. Zeineh, R. A., Mbawa, E. H., Pillay, V. G., Fiorella, B., and Dunea, G. (1973), *J. Lab. Clin. Med.* **82,** 326.
6. Zeineh, R. A., and Nijm, W. P. (1974), *Clin. Res.* **22**(3) 428A.
7. Zeineh, R. A., Nijm, P. W., and Al-Azzawi, H. F. (1975), *Am. Lab.* 7(2) 51.
8. Porro, M., Viti, S., Antoni, G., and Saletti, M. (1982), *Anal. Biochem.* **127,** 316.