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LOCALIZATION AND DIRECT QUANTITATION OF ^{3}H-LABELED PROTEINS AND RNAs IN SLAB GELS BY A NEW DETECTION SYSTEM

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The distribution patterns of ^{3}H-labeled proteins and RNAs in dehydrated polyacrylamide and agarose gels have been recorded with a newly developed instrument: the Linear Analyzer which has a position-sensitive detector that counts a whole gel lane simultaneously. The high sensitivity makes the Linear Analyzer a suitable instrument for direct quantitation of tritium labeled macromolecules in gels without pretreatment by impregnating reagents. The very low detection limit of the Linear Analyzer reduces substantially the time of evaluation in comparison to the relativly long exposure times needed in fluorography. The resolution of radioactivity profiles monitored by the Linear Analyzer reaches the quality attained by fluorography.

Due to the low energy of the beta particles of tritium, ^{3}H-labeled compounds cannot be detected by conventional autoradiography, in spite of the development of films of high sensitivity. For a long time the technique of liquid scintillation counting of dissolved gel slices was the only procedure to monitor the distribution of ^{3}H-labeled macromolecules in gels (1). This method has a limited resolution, is time consuming, and results in the destruction of the sample. The development of fluorographic procedures greatly facilitated the detection of ^{3}H-compounds in gels (2-5). Organic scintillators are incorporated into the gel matrix in order to convert the energy of the beta particles into light which has, compared to the emitted electrons, an increased penetration range producing an image on a blue-sensitive X-ray film. The response between ^{3}H-radioactivity and the absorbance of the film image is linear, if the X-ray film is presensitized with a flash of visible light (3). In spite of all the advantages of fluorography there remain several drawbacks, e.g. 2,5-diphenyloxazole and salicylate, which are used for impregnation, are potential hazards. An additional disadvantage is the fact that the sample can only be recovered with great difficulties from impregnated gels.

Since dehydrated gels are similar to thin-layer plates it should be possible to use thin-layer scanners for the localization and quantitation of beta particle emitting compounds.

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In a previous report we described the localization of ^{3}H-labeled proteins in SDS polyacrylamide gels by the Betacamera, which has a multi-wire spark chamber as detection system (6). In the following study the results with the Linear Analyzer are presented. This system was used for the rapid localization and directly quantitative evaluation of ^{3}H-labeled macromolecules in dehydrated gels without any additional pretreatment.

MATERIALS AND METHODS

Reagents - Acrylamide, N,N'-methylenebisacrylamide, N,N,N',N'-tetramethylenediamine, Coomassie brilliant blue, sodium dodecylsulfate were purchased from Serva. Triton X-100 was from Sigma. All other chemicals were analytical grade and came from Merck. High specific activity ^{3}H-amino acid mixture was purchased from Amersham Buchler and contained Leu (spec. act. 197 Ci/mmol), Lys (spec. act. 90 Ci/mmol), Phe (spec. act. 104 Ci/mmol), Pro (spec. act. 100 Ci/mmol), and Tyr (spec. act. 86 Ci/mmol) at a concentration of 1 mCi/ml. Joklik modified minimal essential medium was supplied by Seromed. Newborn calf serum came from Boehringer Mannheim. Oligo(dT)-cellulose was purchased from PL biochemicals.

Preparation of ^{3}H-labeled proteins and RNAs - A suspension culture of HeLa cells, grown in Joklik medium with 10 % (v/v) calf serum, was infected with VSV at a multiplicity of 10 plaque forming units per cell. Four hours after infection, when viral protein synthesis is maximal, 250 ml of cells were labeled with 250 µCi of a ^{3}H-amino acid mixture of high specific activity for 15 min. Cells were homogenized with a Dounce homogenizer and either a total cytoplasmatic extract or a membrane fraction was prepared as previously described (7). Membranes were dissolved with 1 % Triton X-100 in buffer A (10 mM KCl, 10 mM Tris-HCl, pH 7.5, and 2.5 mM $MgAc_2$). In order to prepare ^{3}H-labeled RNA, MPC 11 cells were infected with VSV (30 PFU/cell) and labeled with ^{3}H-uridine (5 µCi/ml) for 2 h in the presence of actinomycin D (5 µg/ml), beginning at 1.5 h after infection. Total RNA was extracted with guanidinium thiocyanate (8). Messenger RNA was purified by two successive affinity chromatography runs on oligo(dT)-cellulose (9, 10). Radioactivity was determined in an aliquot of the extracts by TCA precipitation according to the procedure of Mans and Novelli (11).

Gel electrophoresis - Aliquots of the cell extracts were routinely analysed on 10 % polyacrylamide gels of 1 mm thickness using the SDS buffer system of Laemmli (12). The gels were fixed and stained with 0.025 % (w/v) Coomassie brilliant blue in 40 % (v/v) methanol and 7.5 % (v/v) acetic acid. Destaining was in the same solvent mixture. The composition of RNA fractions was analysed on 1 % agarose gels in the presence of 6 M urea at neutral pH (13). After impregnation with PPO (3) the gels were dehydrated and exposed to Kodak XAR film at -80°C or monitored by the Linear Analyzer without impregnating. Blotting of proteins was performed according to Towbin et al. (14).

Evaluation of gels by the Linear Analyzer - The Linear Analyzer LB 282 (Labor Berthold, Wildbad, FRG) was used in connection with the calculator HP 97 S (Hewlett-Packard, Palo Alto, USA). The radioactivity distribution of proteins in dehydrated polyacrylamide gels was monitored by the Linear Analyzer counting the whole length of one gel lane simultaneously with a position-sensitive detector. The very low energy beta particles of tritium were detected with an open entrance window (250 mm x 15 mm). The count pulses were collected and stored in a multichannel analyzer which has 1024 channels for the 250 mm of the window. The tracings shown in the figures reflect the count distribution after the total registration period mentioned in the legends. Total radioactivity of individual peaks was calculated by electronically integrating the selected areas of the gel profile. The percentage contribution of peaks to the total counted activity was obtained after background substraction. All measurements were performed with a gain of 3.

RESULTS AND DISCUSSION

The detector of the Linear Analyzer registers electronically the count pulses which are produced by gas amplification at the position of the incident beta particles which are emitted from the dehydrated polyacrylamide gel matrix. Thus ^{3}H-labeled proteins of VSV (72.800 cpm) gave rise to well defined peaks in the Linear Analyzer profile after recording the gel lane for 60 min. The peaks appeared at the same positions, at which the ^{3}H-labeled bands showed up in the fluorogram after an exposure time of 5 days (Fig. 1A). The site of registration and position of the radioactively labeled gel band are only colinear when the beta particles leave the gel perpendicular to the detection wire of the proportional counter. Due to the low range of the ^{3}H-beta particles in air (5 mm) their primary ionization products are recorded by the instrument, giving rise to a particularly high spatial resolution (approximately 0.5 mm).

In order to determine the counting efficiency of the Linear Analyzer for tritium in polyacrylamide gels, serial dilutions of ^{3}H-labeled VSV proteins were electrophoretically separated in slab gels. The input ^{3}H-radioactivity of the different proteins was deter-

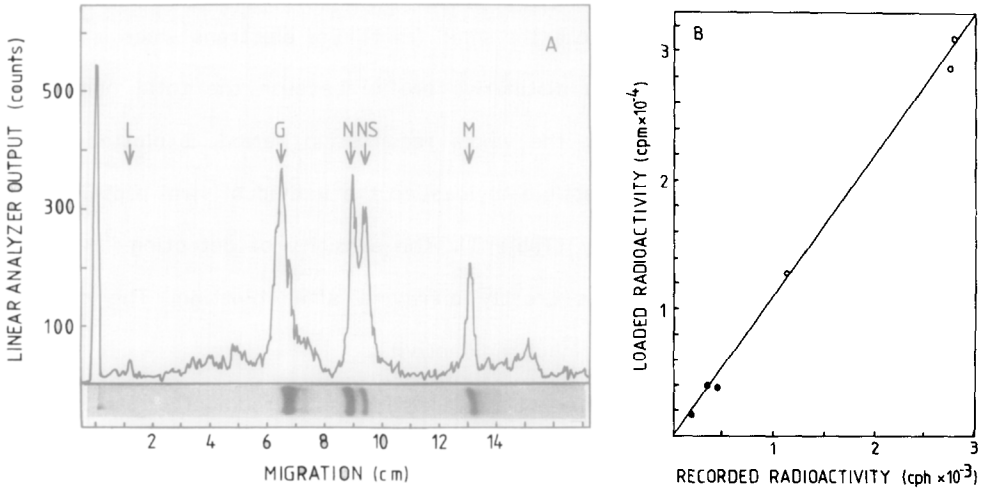

Fig. 1 Localization of ^{3}H-labeled viral proteins in polyacrylamide gels
^{3}H-labeled VSV proteins (72.800 cpm) were separated on a 10 % polyacrylamide gel (1 mm thick) according to Laemmli (12). Fluorogram of the gel lane after 3 days of exposure to X-ray film at -80°C (A, bottom). The radioactivity profile of the same gel lane was monitored with the Linear Analyzer for 60 min (A). Since a cellular fraction of VSV infected HeLa cells was analysed the viral protein composition differs from the protein ratios observed in VSV particles. The input radioactivity of the individual viral proteins was determined by scintillation counting and compared to the total impulses recorded over the period of 1 h (cph) by the Linear Analyzer (B). The values of the graph were obtained by integration of the peaks of Panel A (see Materials and Methods) and quantitation of an additional gel lane with lower input radioactivity.

Table 1: Counting efficiency of the Linear Analyzer

viral protein	^{3}H-radioactivity (a) (cpm)	(%)	^{3}H-radioactivity (b) (cph)	(%)	counting efficiency (c) (%)
G	28700	40	2755	41	0.160
N, NS	31400	43	2788	42	0.146
M	12700	17	1125	17	0.150
total	72800	100	6688	100	0.152

A membrane extract of ^{3}H-labeled VSV proteins (same experiment as in Fig. 1) was separated on a 10 % polyacrylamide gel of 1 mm thickness and the radioactivity was measured after oxidation of the gel slices (a) or directly recorded by the Linear Analyzer for 1 h (b). Total number of impulses which accumulated over the 1 h period are given (cph). The counting efficiency (c) was calculated dividing the cph value registered by the Linear Analyzer by 60 and by the corresponding input radioactivity. Since a cellular fraction of VSV infected Hela cells was analysed the viral protein composition differs from the protein ratios normally observed in viral particles.

mined by scintillation counting of the corresponding gel slices after oxidation. A high percentage of the low energy beta particles of tritium is absorbed by a 10 % polyacrylamide gel of 1 mm thickness, which remains still approximately 100 µm thick after vacuum drying. Thus only 0.1 - 0.2 % of the input ^{3}H-radioactivity was registered by the Linear Analyzer (Table 1). Nevertheless, the small fraction of electrons which is emitted from the gel suffices to produce a distinctive profile because the total number of electrons, which are counted during the whole registration period, is plotted by the instrument. Therefore, it was still possible to localize the individual viral proteins (Fig. 1A) and quantify their radioactivity (Table 1). The sensitity of detecting ^{3}H-labeled proteins by the Linear Analyzer is greatly enhanced after blotting. The counting efficiency increased to 2 - 3 % of the input radioactivity.

The Linear Analyzer should allow the quantitative evaluation of radioactive bands, if the gel matrix has a uniform thickness after drying and if the ^{3}H-labeled compounds are uniformly distributed across the gel depth after electrophoresis. Under these circumstances one would assume that the yield of emitted electrons from the gel should be proportional to the amount of radioactivity in the various bands. The relationship between loaded and recorded counts was linear (Fig. 1B) as long as recordings from gels with the same thickness were compared. The Linear Analyzer is therefore suitable for direct quantitative measurements of ^{3}H-labeled macromolecules in polyacrylamide gels.

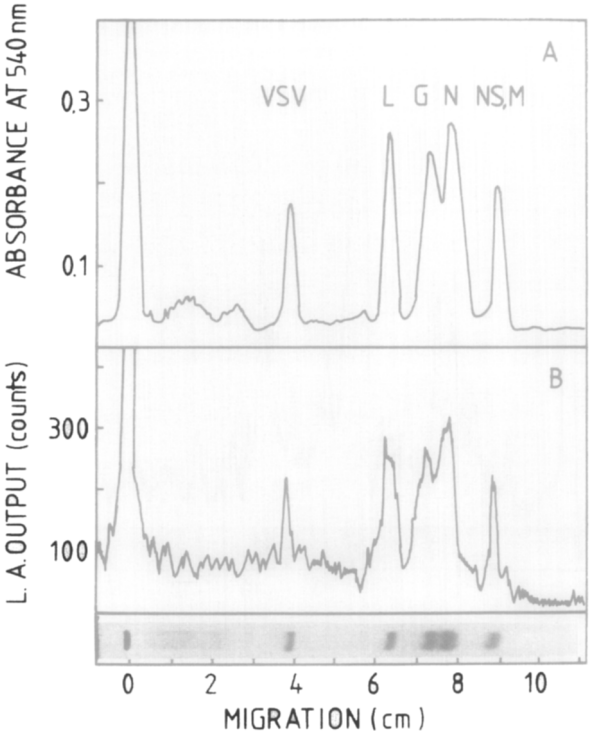

Fig. 2 Detection of ^{3}H-labeled viral RNAs in an agarose gel
An oligo(dT)-cellulose purified fraction of ^{3}H-labeled VSV RNA (6.500 cpm) was separated on a 1 % agarose gel according to Locker (13). Fluorogram of the gel lane after 20 days of exposure to X-ray film (bottom). The radioactivity profile of the dehydrated gel monitored by the Linear Analyzer for 14 h (B). A scan of the fluorogram is shown for comparison (A).

^{3}H-labeled RNAs which were separated on low percentage agarose gels (1 %) were also successfully quantified by the Linear Analyzer (Fig. 2). The radioactivity distribution was determined by fluorography after 20 days of exposure (bottom of Fig. 2). The optical scan of the fluorogram (A) and the direct quantitation of the same gel lane by the Linear Analyzer (B), which took only 14 h, are compared in Fig. 2. The detection of tritium in low percentage agarose gels is very efficient, because agarose gels shrink much more than polyacrylamide gels during dehydration, thereby reducing the absorption of beta particles.

The high sensitivity and resolution of the new detection system is demonstrated by recording a gel lane loaded with a ^{3}H-labeled cytoplasmic extract of VSV infected HeLa cells (Fig. 3). The Linear Analyzer profile which was recorded by direct measurement of the dry gel shows the same complex pattern as the corresponding fluorogram. The quantitative evaluation of the gel lane took only 45 min by the Linear Analyzer and more

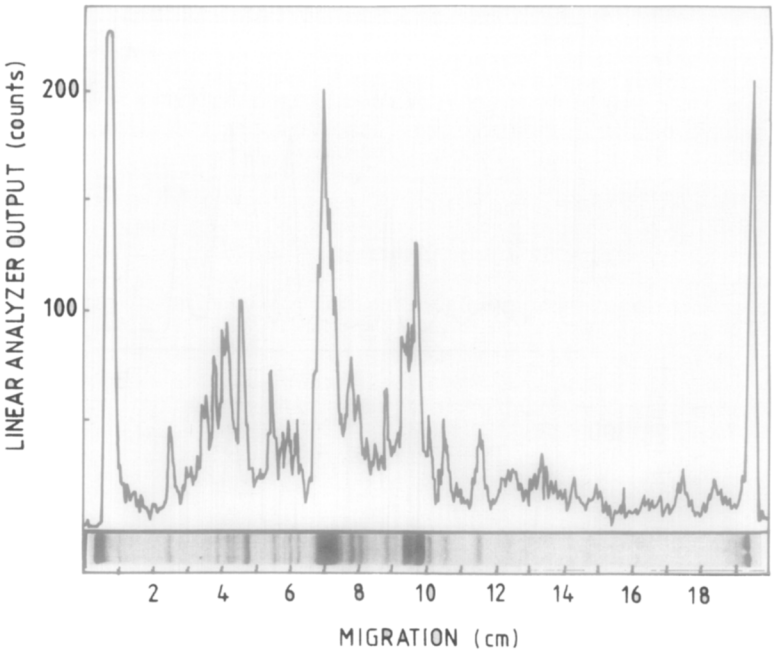

Fig. 3 Evaluation of a complex ^{3}H-labeld protein extract by the Linear Analyzer
VSV infected HeLa cells were labeled with a ^{3}H-amino acid mixture of high specific activity and an aliquot of the total cytoplasmic extract (140.000 cpm) was separated on a 10 % polyacrylamide gel. The radioactivity profile was monitored with the Linear Analyzer for 45 min. Fluorogram of the fixed and PPO impregnated gel after 24 h of exposure (bottom).

than 1 day by fluorography. Other radioisotopes which are frequently used in biochemical investigations, e.g. ^{14}C, ^{35}S, ^{32}P, ^{135}I, etc. can also be detected by the Linear Analyzer. The resolution as well as the detection limits for these isotopes are lower compared to tritium, since their radiation energy is higher.

A few advantages of monitoring gels by the Linear Analyzer may be mentioned. There is no need for staining, destaining, and impregnating the gel which takes 6 - 8 h in fluorography (3). The gel has simply to be vacuum dried. This fact eliminates the costs for the impregnation reagents. Also the handling and disposing of these hazardous chemicals is avoided. Since the gels have not been treated with chemical reagents the material is not altered and can easily be extracted. The detection time is reduced considerably. The profile of the Linear Analyzer reflects quantitatively the radioactivity distribution of the gel bands, which can be automatically integrated and thus easily evaluated. The pattern is continuously displayed during the time of recording, so that the investigator can visually follow and immediately judge the outcome of his experiment. Quantitative evaluation of

gels by the Linear Analyzer appears therefore to be a faster and simpler procedure than conventional fluorography.

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