Improved Method for Virus Structural Polypeptide Analysis on Dissociating Acrylamide Gel

P. A. BOULANGER AND R. WAROCQUIER
Unité de Recherches sur la Biochimie des Protéines de l'Institut National de la Sante et de la Recherche Medicale, Lille, and Laboratoire de Virologie de l'Institut Pasteur, Lille, France

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A method is described in which polypeptides can be separated, with a high band resolution, by electrophoresis through "pore gradient" acrylamide gel (15 cm in length) in a sodium dodecyl sulfate-urea dissociating system. The applicability of this technique to the analysis of virus structural components was examined with the adenovirus type 2 model system.

Dissociation of virus particles in 2-mercaptoethanol-sodium dodecyl sulfate (SDS)-containing medium and subsequent analysis of polypeptide components in SDS-acrylamide gels (8) is now a method widely employed for structural analysis of a number of viruses (1, 3, 9-11). Nine major proteinic components have been thereby detected in labeled adenovirion by staining the peptide bands and slicing the gels for counting in a liquid scintillator (10, 11). To improve the electrophoretic separation of adenovirus polypeptides, Laver recommends the use of acrylamide gels 35 cm in length soaked in reductive buffer for a prolonged period to remove the ammonium persulfate catalyst and the un-cross-linked polymer material (5).

All of these techniques have the same major drawback: polypeptides of a wide molecular weight range (e.g., from 10,000 to 120,000 daltons in adenovirus) are subjected to electrophoresis in a molecular sieve of the same porosity. The usual uniform acrylamide concentrations in standard systems are 5 or 7.5%, which do not provide a good resolution of low-molecular-weight polypeptides. It therefore appeared suitable to employ "pore gradient" electrophoresis through a linear gel gradient from low to high gel concentration. The behavior of charged macromolecules in both linear and nonlinear polyacrylamide gel gradients and also the factors affecting the resolution of bands have been recently analyzed (7, 12). On the other hand, the velocity of polypeptides in SDS-containing gels depends only upon their molecular size (13).

We describe here a method of acrylamide gel gradient electrophoresis in an SDS-urea dissociating system with gels of a reasonable length, which permits the polypeptide analysis of multi-component mixtures or complex structures such as virus particles. This new technique was successfully applied to analysis of adenovirus type 2 polypeptide components.

MATERIALS AND METHODS

Reagents. Acrylamide was purified by recrystallization from chloroform (6). N',N'-diallyltartardiamide (DATD) was used mole for mole in lieu of N',N'-methylenebisacrylamide in the preparation of polycrylamide gels. DATD was synthesized as follows (2): 1 mole of diethytlartrate was dissolved in 10 volumes of ether, 2.5 moles of allylamine was added, and the mixture was refluxed for 8 to 10 hr. The crystals thus obtained were filtered with suction, washed with ether-alcohol (90:10), and dried under vacuum. SDS was recrystallized from ethyl alcohol.

The following solutions were used: (i) acrylamide-DATD (30:1, per 100 ml of water); (ii) N',N',N',N'-tetramethylethylenediamine (TEMED), added as undiluted liquid; (iii) 1 M sodium phosphate buffer (pH 7.0); (iv) 10% SDS (w/v); (v) 10% ammonium persulfate (w/v), freshly prepared; (vi) 10 M urea.

Preparation of acrylamide gel gradient. The gradients connected to a peristaltic pump (flow rate, 20 ml/hr). For 10 ml of 5% acrylamide solution the composition was 1.7 ml of reagent (i), 0.005 ml of reagent (ii), 1.0 ml of reagent (iii), 0.1 ml of reagent (iv), 0.050 ml of reagent (v), 5.0 ml of reagent (vi), and distilled water to 10 ml. For 10 ml of 10% acrylamide solution, the composition was 3.4 ml of reagent (i), 0.005 ml of reagent (ii), 1.0 ml of reagent (iii), 0.1 ml of reagent (iv), 0.050 ml of reagent (v), 5.0 ml of reagent (vi), and distilled water to 10 ml.

Final urea concentration was 5 mM throughout the gel gradient. To prevent early polymerization, acrylamide solutions, gradient apparatus, and electrophoresis tubes were kept in ice while casting the gels. The acrylamide gradient was cast in a glass tube (16 cm in length, 6 mm in diameter) and completed when it was 15 cm in length. It was then carefully overlayered with 0.2 ml of 3 M urea solution. Polymerization was achieved in 30 min.

Virus growth and purification. Type 2 adenovirus
was grown on KB cells, extracted with fluorocarbon, and purified by banding three times in a CsCl gradient by conventional techniques (4).

**Dissociation and electrophoretic analysis of virus polypeptide components.** Adenovirus particle preparation was dissociated in 0.01 M sodium phosphate buffer (pH 7.0) containing 1% SDS and 2% (v/v) 2-mercaptoethanol (8). The dissociated virus sample (about 200 μg of protein contained in 150 to 200 μl) was applied on top of the acrylamide gel gradient, along with a trace of bromophenol blue marker, and gelled into Sephadex G-200. Electrode buffer (0.1 M sodium phosphate buffer, pH 7.0, containing 0.1% SDS and 0.01% 2-mercaptoethanol) was gently layered over the sample gel. Electrophoresis was carried out at a constant voltage of 2 V/cm for 22 hr. The gels were fixed in 20% (w/v) sulfosalicylic acid overnight, stained in 0.25% Coomassie Brilliant Blue in water for 4 hr, and destained in 7% acetic acid-20% ethanol. When labeled virus material was run, gels were sliced, and gel portions were easily dissolved in 2% (w/v) periodic acid solution in 30 min at room temperature; the radioactivity was determined in Bray’s solution.

**RESULTS**

Stained pore gradient acrylamide gels reveal 11 polypeptides in adenovirion (Fig. 1, right). This electrophoretic pattern, repeatedly obtained with various adenovirus preparations, is composed of three major (labeled 1, 8, and 10) and eight minor polypeptides (labeled 2-7, 9, and 11) visible as sharp bands. Parallel electrophoresis was carried out with the same adenovirus preparation run on a conventional neutral SDS-5% acrylamide gel (8). Figure 1 (left) shows that separation of minor neighbor components is barely obtained on gels 15 cm in length and the bands appear diffuse. Thus, electrophoresis on gel gradient detects two components (10 and 11) in the wide major anodic-moving band visible on 5% gel and corresponding to internal arginine-rich proteins (5).

Isotopic analysis of 14C-valine-labeled adenovirus polypeptides eluted after dissolution of the gel slices in periodic acid showed that the degree of quenching was similar to that observed with elution of labeled material from acrylamide-bisacrylamide gel in NH4OH as in conventional techniques (3).

**DISCUSSION**

Our adenovirus polypeptide pattern corresponds to that obtained by fractionation of gel and isotopic analysis of labeled adenovirus polypeptides (10). A similar band pattern was also obtained by subjecting dissociated type 2 adenovirus to electrophoreses on 35-cm-long SDS-containing 6% acrylamide gels in another buffer system (5).
Analytical electrophoresis through an SDS-DATD-urea-containing polyacrylamide gel gradient offers several advantages over usual dissociating systems. (i) Acrylamide concentration gradient provides a better resolution of minor low-molecular-weight components. (ii) The presence of high urea concentration increases the viscosity, thereby reducing the dispersion of the peptide bands. (iii) The use of DATD in place of bisacrylamide results in a gel solubilizable in 2% periodic acid, which would facilitate the recovery of polypeptides after dissolution of the gel (2). (iv) The adenovirus polypeptide patterns on dissociating pore gradient acrylamide gels are similar to those obtained with much longer gels; gels 15 cm in length are easier to handle and do not require special acrylamide gel electrophoresis equipment.

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