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Review

Non-denaturing gel electrophoresis of biological nanoparticles: viruses

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Abstract

Although gel electrophoresis is usually used for the fractionation of monomolecular particles, it is also applicable to the fractionation of the multimolecular complexes produced during both cellular metabolism and assembly of viruses in virus-infected cells. Gel electrophoretic procedures have been developed for determining both the size of a spherical particle and some aspects of the shape of a non-spherical particle. Capsids bound to DNA outside of the capsid can also be both fractionated and characterized. The procedures developed will be used for screening viral mutants; they also can potentially be used for diagnostic virology. Sensitivity of detection, the major current limitation, is being improved by use of both improved stains and scanning fluorimetry. The gels used for fractionation sometimes approximate random straight fiber gels, but become increasingly biphasic as the gel concentration is decreased.

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1. Introduction

Because the minimal parts of living systems evolved while small enough to be influenced by thermal (Brownian) motion, two expectations initially developed from consideration of the fundamentals, without knowledge of details. (a) Chemically, the components of genetic information are linked to each other by forces larger than those between separate molecules, i.e., these components are part of a macromolecule [1] (in Ref. [1], what we now know as a macromolecule is approximated by the term aperiodic solid). (b) Physiologically, fixation of statistical fluctuations is the source of energy transduction [2]. Experimental determination of the details of chemistry has revealed the gene-carrying macromolecule, DNA (reviewed in Ref. [3]). Ex-

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perimental determination of the details of cellular physiology has revealed outlines of energy-transducing processes such as DNA replication, protein synthesis, protein transport and muscle contraction. However, the mechanisms have not been determined for the integration of Brownian fluctuations with energy-requiring processes to produce purposeful behavior. One common feature of these processes has been the participation of several macromolecules bound together in a multimolecular complex (reviewed in Refs. [3-6]). When compared with a single macromolecule, a multimolecular complex provides both reduced requirement for accuracy during protein synthesis and increased freedom of motion (including internal Brownian motion) during function.

Analysis of both the assembly and the structural transitions of multimolecular complexes has previously been performed to understand cellular physiology. For the following reasons, the assembly of bacteriophages has been used as a model for this purpose: (a) genetic perturbation is most easily performed with bacteriophages; (b) both bacteriophages and partially assembled bacteriophages (to be called assembly intermediates whether or not altered during isolation) are comparatively easy to purify in milligram amounts. To improve the fractionation of both viruses (bacterial, plant and animal) and their assembly intermediates, non-denaturing gel electrophoresis has been developed (last reviewed in Ref. [7]). In the case of all studied double-stranded DNA bacteriophages and some double-stranded DNA animal viruses, morphogenesis of the mature virus occurs in two stages: (a) a DNA-free, multimolecular capsid (procapsid) is assembled; (b) the procapsid packages DNA, and while so doing, changes in structure. For some viruses, the mature DNA is cut from a longer (concatemeric) DNA (reviewed in Refs. [8-11]). The change in procapsid structure usually includes loss of a protein (scaffolding protein) that is necessary for assembly of the procapsid. Both the procapsid and its DNA packaging-associated conversion products have a ring-shaped multimolecular complex (connector) that holds an external projection (tail) to the outer shell of the mature bacteriophage capsid. For bacteriophage T7, the following capsids are sketched in Fig. 1: (a) the procapsid (also called capsid I), (b) the bacteriophage-like DNA packaging-associated conversion product (capsid II) and (c) the mature bacteriophage capsid. Capsids that have analogous biological function have been found for all of the double-stranded DNA bacteriophages (reviewed in Refs. [9-11]). However, structural details vary among the bacteriophages. In Fig 1, proteins are labeled by p, followed by the number of the protein's gene [12].

Often by use of viral size standards, gel electrophoretic analysis has also been extended to other multimolecular complexes, including multi-enzyme complexes [13], chromatin [14,15], microtubles [16], polysaccharide-protein complexes used as vaccines [17], cellular ribonucleoprotein complexes of unknown function [18], ribosomes [19], whole (alive) bacterial cells [20], clathrin-coated vesicles [21], lipoproteins [22,23] and protein complexes that form active chaperonins [24]. Gel electrophoresis yields data

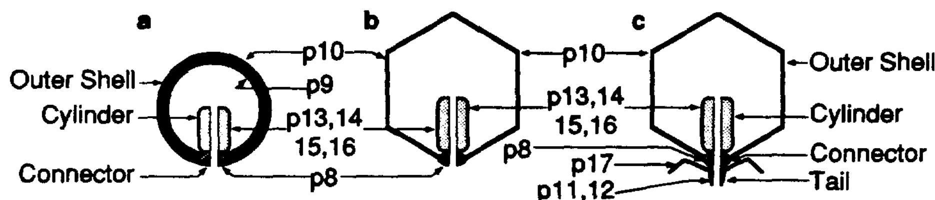

Fig. 1. The capsids of bacteriophage T7. (a) The T7 procapsid (capsid I); (b) the DNA packaging-associated conversion product of capsid I (capsid II); (c) the capsid of mature bacteriophage T7. The T7 scaffolding protein is p9; the T7 connector is made of p8; the T7 tail is made of p11, p12 and p17 (tail fiber) (see Refs. [12,28]).

that assist in the analysis of cellular physiology in the following ways. (a) For any particle with a radius between 1 and 200 nm, both the effective radius and the average electrical surface charge density (σ) are determined by comparing electrophoretic mobility (μ) at more than one gel concentration. Particles in this nanometer size range (called nanoparticles [25]) include the majority of known cellular multimolecular complexes. Changes in both the effective radius and σ reveal the dynamics of both assembly and conformation (e.g., [26]). (b) New assembly intermediates are identified. By use of nucleic acid-specific probing of gels, sensitivity of detection can be in the subpicogram range (e.g., [27,28]). (c) Information about both shape and flexibility are obtained by comparing μ both at more than one electrical field and at more than one gel concentration [29]. In this review, we discuss progress made since a previous review [7] in the application of gel electrophoretic analysis to both new biological systems and new biological problems. We project future applications. Although based on previously published research, the figures presented here are all originals.

2. Characterization of particles

When particles 5–1000 nm in diameter are subjected to electrophoresis by use of the buffers in the references described here (either in the presence or in the absence of a gel), μ is a function only of the properties of the surface of the particle. For example, a bacteriophage with packaged DNA has a μ equal to that of the bacteriophage capsid without the DNA, if the surface of the capsid has not been changed by release of the DNA. Experimental and theoretical background is presented in Refs. [30–32].

In the absence of detectable adherence to the gel, three factors determine μ: (a) the σ, known [30,31] to be directly proportional to the μ that would be measured in the absence of a solid support (μ_0), (b) retardation that depends on both the hydrodynamic and the steric effects of the fibers that form the gel (collectively called sieving) and (c) the electrical field-induced flow of buffer in the gel (electroosmosis) (reviewed in Refs. [19, 30, 31]). The electroosmotic μ (μ_E) is related to both μ_0 and μ extrapolated to a gel concentration of zero (μ_0') by the following [19]:

$$\mu_0' = \mu_0 + \mu_E \quad (1)$$

Analysis of the sieving of spheres (radius R) has yielded the relationship [33]

$$\mu/\mu_0' = (1 - R/P_E)^2 \quad (2)$$

where P_E is the radius of the effective pore of the gel. Given an empirically determined plot of μ vs. gel concentration for a sphere of known R, Eq. 2 yields a plot of P_E vs. gel concentration. This plot can be used to determine from μ/μ_0' the effective R of a particle of unknown dimensions; the accuracy is greatest when both the standard and the unknown are present in the same gel.

Eq. 2 is the simpler of two relationships found in Ref. [33] to describe the sieving of spheres. Eq. 2 provides a definition of P_E that agrees with the following alternative definitions: (a) the radius of the largest sphere that enters the gel during electrophoresis [33] and (b) half the length of the longest inflexible rod that migrates through the gel without detectable effects of end-first orientation [29] (reptation); reptation is a phenomenon originally studied for double-stranded DNA that is long enough to be a stretched random coil [34–36]. One effect of reptation is a μ vs. gel percentage plot that has the following characteristic: when measured by use of both Eq. 2 and P_E determined from an internal spherical standard, the effective R decreases as the gel percentage increases [15,29]. At lower gel percentages, the effective R obtained by application of Eq. 2 to a rod is best approximated by the R of a sphere that has the surface area of the rod [29]. The studies in Ref. [29] were performed by use of the rod-shaped viruses, bacteriophage fd (radius = 4.5 nm; length = 895 nm), tobacco mosaic virus (radius = 9.0 nm; length = 300 nm) and variants of these viruses that had either greater or lesser lengths. Correlation of sieving with surface area has also

been observed with rod-shaped particles of defined-length chromatin [15].

A second effect of reptation is a μ that depends on the electrical field (E). For example, the following procedure of two-dimensional electrophoresis identifies a reptating particle in the presence of several different spherical particles: E comparatively high in magnitude for a first-dimensional electrophoresis, followed by E comparatively low in magnitude for a second-dimensional electrophoresis; the same gel is used for both dimensions. The results of applying this procedure are shown in Fig. 2 for a mixture of both the rod-shaped bacteriophage fd-106/SM2 (a length variant of fd that is 1384 nm long) and the spherical viruses bacteriophage MS-2 (R = 13.3 nm [37]), bacteriophage P22 (R = 31.4 nm [38]) and tomato bushy stunt virus (TBSV; R = 15.5 nm [39]). The spherical viruses are all on a single straight line from which the position of the rod-shaped virus deviates. All spheres, independent of R, would be found on this line. Quantification of the deviation of rod-shaped particles from the line of spheres (i.e., the angle θ in Fig. 2) is being used [40] to test theories of reptation [41,42].

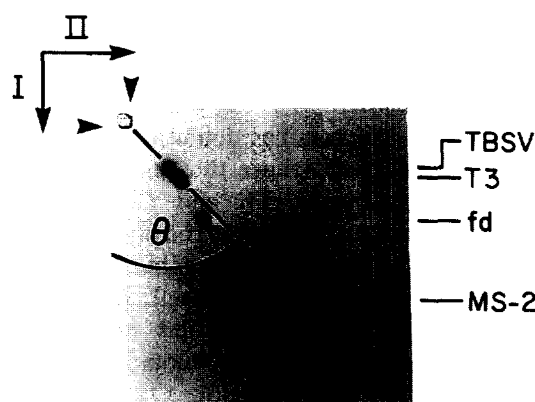

Fig. 2. Two-dimensional agarose gel electrophoresis for the detection of rod-shaped particles. A mixture containing 2–8 μg protein each of both the spherical particles bacteriophage MS-2 (R = 13.3 nm), tomato bushy stunt virus (a generous gift from Dr. T.J. Morris; R = 15.5 nm) and bacteriophage T3 (R = 30.1 nm) and the rod-shaped particle bacteriophage fd-106/SM2 (radius = 4.5 nm; length = 1384 nm [29]) was subjected to two-dimensional electrophoresis in a 0.8% agarose gel (Seakem ME agarose; FMC Bioproducts, Rockland, ME, USA) by use of procedures described in Ref. [29]. The first electrophoresis (direction indicated by I) was conducted at 2.0 V/cm for 5.0 h; the second electrophoresis (II) was conducted at 0.5 V/cm for 20.0 h. The gel was stained with the protein-specific stain Coomassie Brilliant Blue. The line defined by the spheres is indicated. Particles that form the bands are indicated with abbreviated names on the right.

For application to any particle of the procedures previously described in this section, the following properties are required for the particle (particles that have these properties will be called well behaved): (a) μ does not change during gel electrophoresis; for example, neither dissociation nor formation of aggregates occurs; (b) μ is not altered by adherence to the gel. To test for time-dependent changes in μ, either μ is measured as a function of time during a single electrophoresis or μ after a first-dimensional electrophoresis is compared with that after a subsequent, identical, orthogonally oriented second-dimensional electrophoresis. After this two-dimensional electrophoresis, a well behaved particle will form a band that is on a single line that subtends an angle of $\pi/8$ rad with the direction of either the first- or second-dimensional electrophoresis (dashed line in Fig. 3). A band formed by a particle homogeneous in μ is indicated by arrow 1 in Fig. 3. If a particle forms a single band, but is heterogeneous in μ_0, its band will be elongated in the direction of this line (arrow 2 in Fig. 3). In one case [43], adherence to the gel caused a capsid-like intermediate of bacteriophage T7 procapsid assembly to adhere to the first-dimensional gel (arrow 3 in Fig. 3). So far, adherence to an agarose gel is the only way to differentiate this particle from other T7 capsids.

Electrophoresis in a third dimension so far has not been found practical. To obtain a third dimension of information, the most productive approach appears to be use of information-yielding procedures for detection of particles after fractionation by gel electrophoresis. For example, nucleic acid hybridization (reviewed in Ref. [44]) yields information about the nucleotide sequences of fractionated nucleic acids. Stains specific for either the composition or the con-

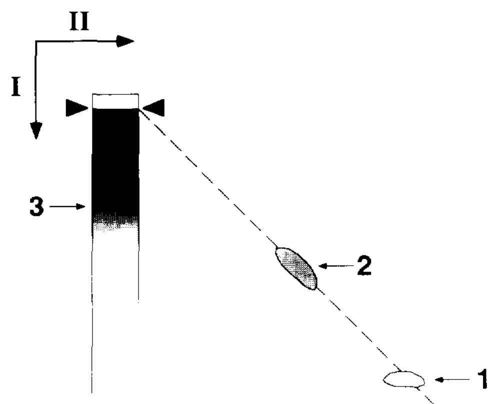

Fig. 3. Sketch of a test for a well behaved particle. The test consists of two-dimensional gel electrophoresis for which the first-dimensional electrophoresis is the same as the second-dimensional electrophoresis. The arrows indicate the direction of the first (I)- and second (II)-dimensional electrophoresis; the arrowheads near the top indicate the sample well at the origin of electrophoresis. The arrows labeled with arabic numbers point to (1) a band formed by a well behaved particle that has a μ_0 that does not detectably vary, (2) a band formed by a well behaved particle that has a variable μ_0 and (3) a band formed by a particle that adhered to the gel during the first-dimensional electrophoresis.

formation of particles fractionated should add to the needed third dimension of information. The authors project that the development of such stains will be a major growth area for obtaining information by gel electrophoresis.

3. Characterization of gels

Two gel-forming compounds, polyacrylamide and agarose, have been used for most of the gel electrophoretic work on both the macromolecules and the multimolecular complexes of biological systems. The chemistry of these two compounds has been reviewed previously (agarose [19,45,46]; polyacrylamide [47,48]). Agarose gels differ from polyacrylamide gels in several respects, including the following

(a) Acrylamide is polymerized during casting of polyacrylamide gels; agarose is present as a polymer during casting of agarose gel. Hence agarose gels are easier to cast unless the greater viscosity of precast agarose becomes a problem. The viscosity of agarose solutions can be decreased by lowering the molecular mass of the agarose [49,50]. However, lowering the molecular mass, in at least some cases, causes changes in the structure of the gels formed [50,51].

(b) Polyacrylamide gels adhere to glass plates sufficiently to be used routinely in a vertical orientation during electrophoresis [47,48]; agarose falls from between vertical glass plates unless action is taken either to improve adherence to the plates or to block slippage. Hence, polyacrylamide gels are usually used when electrophoresis is performed in a vertical orientation. Vertical gels are favored when minimization of heat dissipation is limiting for performance. Agarose gels are usually used in a horizontal orientation; the horizontal gel is also usually submerged beneath the electrophoresis buffer to prevent drying during electrophoresis.

(c) The most dilute polyacrylamide gels strong enough to use for electrophoresis have a concentration of 1.85–2.0% [52]; the equivalent concentrations for the strongest agarose gels are 0.03–0.4% [20]

(d) At any given concentration of gel, the size of the effective pore for a conventional agarose gel is larger than that for the polyacrylamide gels most conveniently used for electrophoresis [7,53].

Taken together, aspects (c) and (d) have increasingly promoted the use of agarose gels as the size of the particles fractionated increased. Agarose gels have been used to fractionate particles with an effective radius as large as 1.2 μm, including intact (alive) cells of the bacterium *Escherichia coli* [20]. For spheres with a radius above about 0.7 μm, gravitational descent during gel electrophoresis is the limiting factor in achieving fractionation [54].

(e) The fibers of polyacrylamide gels undergo sufficient Brownian motion to cause both spectral broadening of scattered light and interference with the measurement of the diffusion coefficients of particles in a gel; the fibers of conventional agarose gels are, by these criteria, stationary [55,56]. The mobility of polyacrylamide gel fibers is a possible source of the

stabilization of DNA–protein complexes that is sometimes essential for assay of these complexes by a gel mobility shift that can be observed in polyacrylamide, but not conventional agarose, gels (this assay is reviewed in Refs. [57–59]). That is, while migrating through a polyacrylamide gel, a DNA–protein complex would cause collapse of fibers around the complex (perhaps like an insect moving through a spider's web), thereby causing an increase in the effective concentration of the complex and stabilizing it. This type of ballistic effect possibly also contributes to the fractionation of bent from linear DNA; this fractionation occurs in polyacrylamide, but not agarose, gels [60].

(f) Polyacrylamide gels both shrink when dehydrated in organic solvents and reswell completely when rehydrated by transfer from either an organic solvent or air (after drying) [61]; agarose gels do not shrink in several organic solvents [50,62] and do not fully reswell when hydrated from a dried state. The stability of agarose gels to organic solvents is necessary for the preservation of structure during epoxy resin embedding that has been used to visualize the arrangement of fibers in agarose gels [50,62].

(g) The polyacrylamide gels usually used for electrophoresis are clear; conventional agarose gels are turbid.

Differences (c)–(g) all appear to have the same source: agarose gels are formed by fibers more massive than the fibers that form polyacrylamide gels. Each gel-forming fiber for agarose is a lateral aggregate of many (usually over ten [62,63]) primitive polysaccharide chains of agarose (reviewed in Refs. [19,45,49]). The mass of polyacrylamide gel fibers, although presumably much smaller than that of agarose gel fibers, has not, to the authors' knowledge, been determined.

Agarose and polyacrylamide are only two of many gel-forming compounds that are potentially useful for gel electrophoresis. For example curdlan, a β-(1$\rightarrow$4)-glucan [64], has been used for the gel electrophoresis of DNA [65]. In electron micrographs, curdlan gels have comparatively massive, agarose-like fibers [50]. In terms of properties (a)–(g) above, agarose and polyacrylamide appear to be at opposite poles. Other gel-forming compounds should provide gels that are intermediate to agarose and polyacrylamide with regard to these properties.

For both quantitative study of sieving and the use of sieving to characterize quantitatively the particles sieved, agarose gels are preferred because of both the comparative stability of their structure and the comparatively large size of their pores. Thus, comparatively large particles can be used as samples during agarose gel electrophoresis. For analysis of sieving, the two most influential aspects of an agarose gel appear to be P_E and the heterogeneity of the distribution of interfiber spacing.

For understanding heterogeneity of interfiber spacing, statistical analysis of the interfiber spacing observed in electron micrographs of thin sections has been performed. A 1.5% gel of underivatized agarose was found to have a distribution of interfiber spacing that was indistinguishable from that of a gel of straight fibers that are both randomly oriented and randomly placed [66]. However, some changes in procedures of gelation have yielded a gel that has a distribution of interfiber spacing that has the following characteristic: a phase of comparatively small spacings is distributed in zones throughout a phase of comparatively large spacings. These changes include both lowering the concentration of agarose [66] and using a derivatized agarose that has had its molecular mass decreased by irradiation [50]. Determining the effect of pore size heterogeneity on sieving is an area for future development.

4. Applications of gel electrophoresis to both viruses and solid virus-related particles

In the authors' experience, most bacteriophages and plant viruses are well behaved during agarose gel electrophoresis. The same appears to be true of animal viruses. Details were reviewed in 1987 [7]. When analyzed, the DNA-free capsids of viruses have also been well behaved during gel electrophoresis. The follow-

ing are post-1987 examples of gel electrophoretic analysis of both viruses and their capsids.

(a) The procapsid of Herpes virus (also called the B capsid) [67,68], a double-stranded DNA animal virus, has been fractionated by agarose gel electrophoresis, together with the capsid produced when guanidine hydrochloride is used [69] to remove selectively the scaffolding protein from the B capsid [70].

(b) Bacteriophage HK97, a recently isolated double-stranded DNA bacteriophage that has its major capsid subunits covalently cross-linked [71], has been fractionated by agarose gel electrophoresis, together with the HK97 procapsid and its DNA packaging-associated conversion product. In the case of HK97, both pentameric and hexameric ring-shaped subassemblies of the major HK97 capsid protein have also been fractionated by agarose gel electrophoresis [72]. The latter study appears to be the only isolation of such subassemblies for a bacterial virus. Assembly of pentamers and hexamers to form procapsids can be monitored by agarose gel electrophoresis; however, subviral assemblies made of multiple pentamers and hexamers were not observed [72].

(c) For bacteriophage T7, both unassembled major capsid protein and unassembled scaffolding protein have been both fractionated by agarose gel electrophoresis and observed to assemble to form a procapsid in vitro [73].

(d) In the case of bacteriophage ϕ29, a double-stranded DNA bacteriophage that has fibrous projections from its capsid, the following particles have been fractionated by agarose gel electrophoresis: the wild-type bacteriophage, the wild-type bacteriophage without its fibrous projections [74], the scaffolding protein, the (intact) connector and subassemblies of the connector and scaffolding protein [75].

(e) In the case of adenovirus, both the hexamic subcomponent of the capsid and multimers of the hexamers have been fractionated by polyacrylamide gel electrophoresis [76].

(f) In the case of both bacteriophage T3 [27] and T7 [28], capsids with incompletely packaged DNA have been fractionated by agarose gel electrophoresis after treatment with DNase to remove DNA that protruded outside of the capsid.

In addition to detecting and quantifying intermediates in assembly, gel electrophoresis can be used to characterize them. By increasing the sieving of a second-dimensional gel used in a procedure of two-dimensional electrophoresis, resolution by the radius of a bacteriophage capsid has been made as high as ±0.5% [77]. Eventually, quantification of reptation should yield shape. However, procedures for determining shape have not yet been applied to assembly intermediates.

5. Application to both viral DNA and DNA–protein complexes

Gel electrophoretic fractionation of both viral and other DNAs, a procedure previously reviewed thoroughly [35,78–80], separates both linear and circular DNA both by length and from each other. Viral DNA from unprefractionated infected cell lysates can be fractionated [81]. Thus, gel electrophoresis can be used for the analysis of pathways of DNA metabolism, without the use of additional procedures of fractionation. For this purpose, the major limitation can be the presence of host DNA that interferes with either the fractionation of the viral DNA (by causing overloading of the gel) or the detection of the viral DNA, unless DNA–DNA hybridization is used for detection.

The recent development that has had the greatest impact on the analysis of viral DNA is the use of an electrical field that varies in either magnitude or direction during electrophoresis [pulsed field gel electrophoresis (PFGE)]. By use of PFGE, stretching–reptation-induced loss of length resolution has been overcome for both linear double-stranded DNA as long as 10 000 kilobase pairs (kb) and open circular DNA as long as 300 kb (reviewed in Refs. [79] and [80]).

In addition to improved fractionation of DNA, improved fractionation of DNA–protein complexes has also been achieved by use of PFGE. A DNA–protein complex used as a test object was a complex that consists of the 39 936 kb

bacteriophage T7 DNA and a T7 capsid (28 nm in radius) from which the DNA had been expelled by incubation at elevated temperature. When subjected to constant-field agarose gel electrophoresis by use of a field of either 3 V/cm or more, this T7 DNA–capsid complex becomes sterically arrested (entrapped) by the network of agarose gel [82]. Presumably, arrest occurs because the DNA enters pores that are too small to permit passage of the capsid [83]. The arrest is reversed by either lowering the field (to 1 V/cm) or using PFGE (field inversion mode). The following separations can be performed; (a) DNA from DNA–capsid complex, (b) DNA–capsid complex according to position of the capsid on the DNA and (c) DNA–capsid complex according to the length of the DNA [82]. Subsequent to the work in Ref. [82], decreasing the length of the capsid-bound DNA was found, as expected [83], to increase the field needed to induce arrest [84]. For a 14 kb restriction endonuclease fragment of T7 DNA, binding of a T7 capsid was not sufficient to cause arrest in a 0.5–0.7% agarose gel, even when the field was raised to 18 V/cm. Fractionating DNA–capsid complexes by use of either PFGE or constant-field gel electrophoresis is useful for improving both the efficiency (in time and cost) and the sensitivity with which DNA–capsid complexes can be detected, characterized and quantified when isolated from virus-infected cells.

6. Biomedical perspective

When applied to either intracellular virus-related multimolecular particles or multimolecular particles normally present in a eukaryotic cell, gel electrophoresis is a quantitative assay for a broad spectrum of particles. In this respect, gel electrophoresis yields a biomedical determination of state that is more comprehensive than that of the more selective biochemical, immunological or biological procedures. Broad spectrum determination of state is desirable for determining disease status, particularly when more selective assays have failed to reveal the pathway for the causation of a disease. As this pathway becomes more complex, the value of a broad-spectrum analytical procedure, such as gel electrophoresis, increases.

The major limitation in the use of gel electrophoresis for broad spectrum analysis of cellular content is the sensitivity of detection. This limitation exists for both biomedical applications and basic science-oriented applications such as analysis of viral assembly pathways. Two current developments appear to be reducing this limitation: (a) for staining macromolecules, fluorescent dyes are being developed that have both increased yield and decreased background; (b) for forming an image of a gel stained with a fluorescent dye, scanning laser beams are being developed to increase the signal further and, via filtering, decrease the background. An example is the following gel electrophoretic detection of T7 capsid II-packaged DNA, after analysis of unprefractionated lysates obtained at several times after infection of *E. coli* with bacteriophage T7. After agarose gel electrophoresis, the agarose gel was stained with the newly developed dye SYBR Green I (Molecular Probes, Eugene, OR, USA). Next, DNA was detected by scanning digital fluorimetry by use of a Molecular Dynamics (Sunnyvale, CA, USA) Fluorimager. The resulting pattern included a band formed by mature bacteriophage T7 (ϕ in Fig. 4a) that progressively increased in amount as the time after infection increased [Fig. 4a; the time after infection (min) is indicated above the lanes]. In addition, a weaker band was formed by DNA packaged within capsid II (CII in Fig. 4a); no capsid I-associated DNA was detected; the position of capsid I is beyond the lowest shown region of the gel. When detection was performed by staining with ethidium, followed by photography of fluorescence, the sensitivity of detection was dramatically reduced (Fig. 4b).

By quantification of the data obtained with the Fluorimager, the amount of capsid II-associated DNA in Fig. 4a was always no more than 0.12 times the amount of mature bacteriophage-associated DNA. The sensitivity of detection in Fig. 4a is about 27 times that in Fig. 4b. In basic science, the future uses of improved staining efficiencies will include screening of mutants that

Fig. 4. Detection of DNA packaged in capsid II by use of scanning fluorimetry. Lysates of wild-type bacteriophage T7-infected *E. coli* BB/1 were obtained at several times after an infection (multiplicity = 3) that had been conducted with aeration, in broth medium (20 g of tryptone, 10 g of yeast extract and 5 g of NaCl in 1000 ml of water), at 30°C. The procedure for both stopping infection and lysing of cultures with non-ionic detergent is described in Ref. [82]; the time at which infection was stopped (min) is indicated above the lanes. After lysis, 8 μl of lysate were DNase digested by diluting into 40 μl of 0.15 *M* NaCl, 0.015 *M* $MgCl_2$ that contained freshly dissolved 200 μg/ml DNase I; this mixture was incubated for 1 h at 30°C. Subsequently, 8μl of 400 μg/ml bromophenol blue were added; 20 μl were layered in the sample wells of a horizontal, submerged 1.0% agarose gel (Seakem LE agarose; FMC Bioproducts). Electrophoresis was performed at 1.0 V/cm and 25 ± 2°C for 34 h through a gel both cast in and submerged beneath the electrophoresis buffer: 0.09 *M* Tris–acetate (pH 8.4) + 0.001 *M* $MgCl_2$. After electrophoresis, the gel was first stained with 1 μg/ml ethidium bromide for 2 h and then destained overnight in 0.001 *M* EDTA (pH 7.4). The ethidium-stained gel was photographed during illumination from the top with a 300-nm ultraviolet transilluminator (UV Products, San Gabriel, CA, USA). After photography, the gel was air dried. After storage for about 5 months, the gel was rehydrated in buffer [0.01 *M* Tris–Cl (pH 8.0) + 0.001 *M* EDTA] that contained a 1/10 000 dilution of a stock standard solution of SBYR Green I. After incubation for 10 min (without destaining), the gel was subjected to scanning fluorimetry by use of the 488-nm line of an argon ion laser; the output filter passed 515-nm and longer wavelength radiation. The resultant ca. 1 megabyte image was reproduced photographically. The figure shows the image of the gel stained with either (a) SYBR Green I or (b) ethidium. The arrow indicates the direction of electrophoresis; the arrowheads near the top indicate the origins of electrophoresis. Particles in the gel are mature bacteriophage T7 (ϕ) and capsid II (CII).

produce altered assembly intermediates. In biomedicine and biotechnology, the future uses should include broad-spectrum analysis of both viruses and virus-related particles in crude cellular lysates. Improvement in sensitivity of staining is the most critical need for developing gel electrophoresis for use in both diagnostic virology and other diagnostic disciplines.

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