Journal of Chromatography, 418 (1987) 345–357
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3625

REVIEW

AGAROSE GEL ELECTROPHORESIS OF BACTERIOPHAGES AND RELATED PARTICLES

PHILIP SERWER

Department of Biochemistry, The University of Texas Health Science Center, San Antonio, TX 78284-7760 (U.S.A.)

(First received October 31st, 1986; revised manuscript received January 27th, 1987)

CONTENTS

1. INTRODUCTION

To understand the assembly and function of a multimolecular complex, attempts are made to isolate and characterize the complex's partially assembled precursors, referred to as assembly intermediates. Subsequently, the assembly intermediates' kinetics of progression in the assembly pathway are determined. To help detect short-lived intermediates and to determine the function of known gene products in the assembly pathway, kinetics are sometimes determined after alteration of assembly by either a mutated gene product or an assembly-inhibiting compound. The number of samples that are analyzed for these experiments can be large enough so that procedures of ultracentrifugation, electron microscopy and other procedures of detection–quantitation–characterization consume too much time and resource to be practical. In addition, if a procedure of frac-

0378-4347/87/$03.50

tionation is performed at several different times, time-dependent changes in either the samples analyzed or the procedure will influence the kinetics measured. One procedure for overcoming these limitations is electrophoresis in a gel, followed by either staining of the gel or, in the case of radioactively labeled particles, autoradiography. Slab gels have been used for the analysis of hundreds of samples simultaneously subjected to electrophoresis in a single electrophoresis apparatus (details for some of the procedures used are in refs. 1–3). As described below, gel electrophoresis can also be used to obtain information about the surface electrical charge of any particle, the radius (R) of a sphere and even the shape of a non-spherical particle.

Bacteriophages are viruses whose hosts are bacteria. These viruses are comparatively inexpensive to grow and purify in milligram quantities in the laboratory and are assayed by the formation of plaques after 3–10 h of growth [4–6]. Bacteriophages have a multimolecular protective outer shell (capsid) of protein that encloses and protects the bacteriophage's genome. The genome is either a single- or a double-stranded nucleic acid, either RNA or DNA (reviewed in refs. 4–12). The capsid consists of a genome-enclosing outer shell and in some cases internal proteins and/or external projections. In the case of bacteriophages f1, fd, M13 and other related single-stranded DNA coliphages, the outer shell is rod-shaped (a helical array of identical subunits) without external projections [6,7]. In the case of most studied double-stranded DNA bacteriophages, the capsid is either spherical (an icosahedral array of subunits) or elongated with two hemispheres connected by a comparatively short cylinder. These double-stranded DNA bacteriophages have an external projection (tail) with attached fibers, used to bind the bacteriophage to its host. Bacteriophages T2 and T4 have an elongated outer shell with a tail even longer than the outer shell. Bacteriophages P22, T3 and T7 are spherical with a tail less than a fifth the extension of the outer shell; ϕ29 has an elongated outer shell with a short tail and additional spikes projecting from the outer shell; λ has a spherical shell with a tail longer than the outer shell [5–7]. Spherical bacteriophages that contain single-stranded nucleic acid have also been studied. Spherical RNA bacteriophages (R17, MS2, QB, for example) contain no external projection detected by electron microscopy; bacteriophage ϕX174, a single-stranded DNA bacteriophage, has twelve external spikes (reviewed in refs. 5–7). The bacteriophages discussed above are some of the more frequently studied bacteriophages. Many others exist, but have been omitted above because they apparently have not been studied by the procedures discussed here.

Assembly of bacteriophages from their component proteins and nucleic acid is studied to learn the basic physical principles of assembly, the mechanisms for the genetic control of assembly and the evolution of assembly mechanisms. Advantages of using bacteriophages for these studies include: (i) the comparative ease (and, consequently, advanced development) of manipulation of bacteriophage genes, (ii) the comparatively short life cycle (13 min at 37°C for bacteriophages T7 and T3) of bacteriophages and (iii) the comparatively simple and inexpensive procedures with which bacteriophages are grown and radiolabeled. Because each of the bacteriophages has unique parameters of size and shape, and because size and shape vary among the different bacteriophages, the above bacteriophages

have also been used, as described below, to quantitate the electrophoretic sieving of gels.

The above considerations suggest that the use of gel electrophoresis for the study of bacteriophage assembly was an obvious development that should have occurred as soon as it was technically possible. Agarose gels were first used for electrophoresis in 1961 [13]; agar gels (agarose is the least ionic component of agar [14,15]) had been used for electrophoresis a decade earlier (reviewed in ref. 16). As described below, agarose gels can be routinely cast with both large enough pores to admit all known bacteriophages and sufficient strength to be used for electrophoresis as multisample slab gels. Within the next five years studies of bacteriophage assembly pathways accelerated when procedures were developed for: (i) using conditional–lethal mutations to identify the functions of bacteriophage gene products [17] and (ii) conducting assembly in cell-free extracts [18]. However, the use of agarose gel electrophoresis for the study of bacteriophage assembly did not begin until fifteen years later. The development of gel electrophoresis for detecting, characterizing and quantitating non-bacteriophage multimolecular particles has also occurred more slowly than it could have developed. In the next section of this review the history of gel electrophoresis of bacteriophages will be described. Subsequently, procedures for the use of agarose gel electrophoresis to characterize virus-sized particles will be presented. Current hypotheses for the sieving of agarose gels will be discussed. Finally, some ways in which the information obtained by agarose gel electrophoresis is used to understand the assembly of bacteriophages, function of bacteriophages and the virulence of disease-causing viruses will be discussed.

2. HISTORY

In 1967, electrophoresis of bacteriophage ϕX174 (spherical, with spikes, radius at the outer end of the spikes = 18 nm [7]) in 2.6–5.3% cylindrical polyacrylamide gels was used to characterize mutants of ϕX174 with altered capsids [19]. Using similar procedures, several different spherical RNA bacteriophages (radius = 13.3 nm) were separated from each other [20,21]. Bacteriophage T4 has an effective hydrodynamic radius of 74.8 nm [22] and has been subjected to electrophoresis through cylindrical polyacrylamide gels [23,24]. However, the gels used must be dilute enough (1.85–2.0%) to be practically liquid in order to have pores large enough to admit T4. Bacteriophage T4 is, to the author's knowledge, the widest particle (85 nm [25]) that has ever been subjected to polyacrylamide gel electrophoresis.

Bacteriophage M13 (radius = 4 nm; length = 800 nm [7]) was also subjected to polyacrylamide gel electrophoresis and end-to-end dimers were separated from monomeric rods [26]. Because the dimer has the same surface composition and, therefore, presumably same average electrical surface charge density (σ) as the monomer, it is assumed that the electrophoretic mobility (μ; μ = particle velocity/voltage gradient) when measured in the absence of a gel (μ_0) for the monomer is the same as the μ_0 of the dimer [27]. Therefore, the separation of dimer from monomer is presumed to be the result of a gel-induced, size-dependent reduction

in $|\mu|$, referred to as sieving [27,28]. Based on the data obtained with bacteriophage T4 (above), the pore sizes of the polyacrylamide gels used for the electrophoresis of M13 are too small to admit M13, unless the rods of M13 are on the average oriented in the direction of electrophoresis (referred to as reptation). Confirmation of the reptation of the rod-shaped virus, tobacco mosaic virus (radius = 9 nm; length = 300 nm [29]), has been obtained by X-ray diffraction after electrophoretically driving tobacco mosaic virus into a polyacrylamide gel [30].

The utility of polyacrylamide gel electrophoresis for fractionating mature viruses suggests use of this technique for analysis of viral assembly pathways. However, this use was never made of polyacrylamide gel electrophoresis, apparently because of the weakness of polyacrylamide gels dilute enough for this purpose. Thus, multisample slab gels could not be used.

Because bacteriophages are assayed by formation of plaques in agar overlays (usually 0.7%) that are rigid enough to form slab gels, it should have been suspected from the start that bacteriophages can be made to enter agarose gels rigid enough to be used as slabs for electrophoresis. Quantitation of the sieving of plant viruses smaller than most bacteriophages was performed during diffusion through agar gels and this conclusion was confirmed [31]. Agarose gel electrophoresis in cylindrical tubes was performed with the animal viruses, African horsesickness virus and pantropic Rift Valley fever virus in 1967 [32]. There were subsequent reports of the agarose gel electrophoresis of other plant, insect and animal viruses (reviewed in ref. 33). The use of agarose gel electrophoresis for diagnostic plant virology [34,35] and analysis of the disassembly of tobacco mosaic virus [36] have been reported.

In 1978, agarose gel electrophoresis of a bacteriophage (T7) and it assembly intermediates was first performed [37]. In this study both cylindrical gels and horizontal multisample slab gels were used. The horizontal slab gels used were poured and used for electrophoresis by procedures almost identical to these previously used [2] for fractionation of restriction enzyme digests of double-stranded DNA. The only alteration made for use with bacteriophages was the circulation of buffer [3] to prevent the formation of pH gradients. Because double-stranded DNA does not detectably titrate hydrogen atoms with a pK_a between 5 and 10 [38], electrophoresis of double-stranded DNA at neutral pH usually does not require circulation of the buffer. In contrast, proteins and nucleo–protein complexes do require recirculation.

In subsequent studies, assembly of T7 was studied by electrophoresis in agarose slab gels. During the assembly of T7, a DNA-free proteinaceous capsid (procapsid) is assembled and subsequently binds and packages T7 DNA. Such procapsids have been found in the assembly pathway of all studied double-stranded DNA bacteriophages, including $\phi 29$, λ, P2, P22, T3, T4 and T7 (reviewed in refs. 8–12). The appearance kinetics in infected cells of the T7 and T3 procapsid and its conversion products were determined by agarose gel electrophoresis of infected cell lysates, sometimes in conjunction with procedures of centrifugation (ref. 39 and references therein). A host that slows the DNA packaging pathway was found [39]. Application of agarose gel electrophoresis to detecting and quantitating

procapsids and other DNA packaging intermediates has also been made for bacteriophages P22 [40,41] and ϕ29 [42].

3. SIZE OF PORES IN AGAROSE GELS

Lack of knowledge of the comparatively large size of pores in agarose gels has in the past inhibited attempts to use agarose gel electrophoresis for the analysis of multimolecular complexes. In 1982 Righetti et al. [43] defined the average radius of a gel's pores (P_E) to be the radius (R) of the biggest sphere that can enter the gel during electrophoresis. The result for a commercial preparation (Isogel, Marine Colloids [44]) that consisted of both agarose and galactomannan was (P_E in nm):

$$P_E = 70.4\, A^{-0.7} \tag{1}$$

A, the percentage of gel-forming material in the gel, was between 0.16 and 1.0. Using a revised procedure based on the same concept as the procedure in ref. 43, it was subsequently found [45] for unadulterated agarose that (P_E in nm):

$$P_E = 118\, A^{-0.74} \tag{2}$$

A was between 0.2 and 4.0. However, evidence was found for a ca. 20% increase in the exponent of eqn. 2 as A increased [45]. Such a change in exponent introduces inaccuracy to extrapolations beyond the range of A values used for measurement of P_E. Extrapolation of a P_E versus A relationship previously obtained [46] by use of a 6.5% hydroxyethylated agarose, a different definition of P_E and a comparatively narrow range of A values (2.2–3.6) yields P_E values for A below 1.0 that are too high when compared to values calculated [47] from the sieving [46] of gels of the same agarose with $A < 1.0$.

A P_E versus A relationship has also been obtained by use of random coils of double-stranded DNA and by making the following assumptions [48]: (i) the radius of a DNA random coil in solution is also its radius during agarose gel electrophoresis with results extrapolated to a voltage gradient of 0 and (ii) the A which halves the magnitude of μ has a P_E twice the root-mean-square radius of the random coil. The result is (P_E in nm):

$$P_E = 25 + 70/A \tag{3}$$

Values of P_E calculated from eqn. 2 are 3–24% higher than values calculated from eqn. 3 for A between 0.2 and 4.0. This difference is surprisingly small, especially when you consider that the gel characteristics derived by use of solid spheres as size standards differ from those derived by use of random coils [48]. By comparison of the sieving of solid spheres with the sieving of non-reptating random coils, it has been found that random coils of linear double-stranded DNA with molecular weights between $2.9 \cdot 10^6$ and $26.4 \cdot 10^6$ shrink in radius by a factor of 1.9–2.6 during electrophoresis in agarose gels [49]. Thus, the closeness of P_E values calculated by eqn. 2 to the P_E values calculated from eqn. 3 does not have an obvious basis.

Eqn. 1 yields P_E values 0.56–0.63 times smaller than the P_E values obtained

from eqn. 2. In comments made in one of our previous studies [45], it was assumed that the pore size defined in ref. 43 was a radius. From the source of eqn. 1 in ref. 43, it appears that this pore size was a diameter. Assumption of a radius in ref. 45 produced an apparent contradiction to the observation that Isogel sieves more than DNA grade agarose. However, there appears to be no such contradiction.

The most dilute agarose gel used in a published experiment had an A value of 0.035 [50]. By use of eqns. 1–3, it is concluded, therefore, that P_E values as big as 1500 nm can be achieved with agarose gels. Therefore, pore size exclusion does not limit the use of agarose gels for the electrophoresis of any virus. The most serious limitation thus far has been adherence of particles either to agarose gels or to each other during electrophoresis [15,28,45]. The cause of adherence to agarose gels is not well enough understood to routinely and systematically prevent it.

4. CHARACTERIZATION BY AGAROSE GEL ELECTROPHORESIS

Semilogarithmic plots of μ versus A are linear (slope $= K_R$) for spherical particles (viruses and subviral components of viruses) with R values between 13 and 42 nm, if $A \leq 0.9$ (reviewed in ref. 15). That is:

$$\mu = (\mu_0 + \mu_E)\, e^{-K_R \cdot A} \tag{4}$$

μ_E is a term that quantitates the electrical field-induced motion of buffer through the gel (electro-osmosis). By subtracting μ_E, values of μ extrapolated to an A of 0 are converted to μ_0. For particles the size of bacteriophages and other viruses in buffers usually used for electrophoresis, μ_0 is proportional to σ and independent of R [27,37]. The value of K_R increases as the R of a sphere increases and K_R can be used to determine R; the accuracy is $\pm 8\%$ (reviewed in ref. 15). Procedures for determining R with greater accuracy, as accurate as $\pm 1\%$, have been developed [28,46,51]). For all of the above procedures, spheres of known size must be used to calibrate the gel.

The linearity of semilogarithmic μ versus A plots described above is lost as A values increase above 0.9; the magnitude of the slope of this plot progressively increases as A increases in this region (convex curvature; reviewed in ref. 15). In contrast, semilogarithmic μ versus A plots for the rod-shaped viruses, bacteriophage fd (radius = 4.5 nm; length = 895 nm [52]) and tobacco mosaic virus have concave curvature for A above a critical value that decreases as the length of the rod-shaped virus increases. Below the critical value of A, the plot is linear [53]. Thus, a rod can be easily distinguished from a sphere by the use of a semilogarithmic μ versus A plot. Furthermore, the above observations suggest that the critical value of A can be used to determine the length of a rod. However, additional rods must be investigated and a more accurate determination of the critical A made in order to accomplish this goal. If the rod's length were known, then K_R should reveal the rod's radius. However, the details of procedures for doing this have not yet been developed.

An obvious possible explanation for the concave curvature of semilogarithmic μ versus A plots for rods is that: (i) at the critical A, the P_E is one half the rod's

length and (ii) for higher, but not lower, values of A the rod reptates during electrophoresis. Using P_E values from eqn. 2 and the data from ref. 53, statement (i) is true within a factor of 2–3. More accurate analysis of critical A values will improve the reliability of this comparison.

5. THEORIES OF SIEVING

To quantitatively understand the sieving of gels, at least two types of assumption can be made for spherical particles: (i) the gel sieving-induced decrease in the magnitude of μ is equal to the probability that a randomly placed sphere intersects a cylindrical fiber of gel (this assumption will be referred to as the assumption of steric sieving); (ii) this decrease in the magnitude of μ is the result of alterations in hydrodynamics introduced by stationary gel fibers in a fluid phase (this assumption will be referred to as the assumption of hydrodynamic sieving). For studies of gel electrophoresis, the assumption of steric sieving was introduced in 1970 [54] and has been used in most quantitative studies of sieving during gel electrophoresis (for instance, see refs. 55–58). The implications of this assumption have been determined quantitatively [59,60] for a gel of randomly oriented cylindrical fibers. The expression in ref. 60 after algebraic rearrangement [53] is:

$$K_R = \frac{\pi(R+r)^2}{P} + \frac{2\pi(R+r)^3}{3PL_f} \tag{5}$$

P is the mass per unit length of fibers in the gel and equals A/L_t; L_t is the total length of fibers per unit volume; r is the average radius of a fiber of gel; L_f is one half the length of individual fibers.

If, as previously proposed [61] and supported by electron microscopy of agarose gels [62,63], agarose gels consist of linear fibers joined with branching to form a network, the L_f of eqn. 5 would decrease with increasing A. Therefore, according to the theory represented by eqn. 5, the linearity of semilogarithmic μ versus A plots at the lower A values suggests (but does not prove) that $L_f \gg R+r$ and, therefore, that the first term of eqn. 5 predominates (one-dimensional, or 1-d, gel in the terminology of ref. 54). The development of convex curvature in these plots at the higher A values can be explained by either one or both of the following: (i) for the lower A values $L_f \gg R$, but as A increases above 0.9 L_f approaches R and the second term of eqn. 5 contributes to K_R, thereby causing convex curvature; (ii) either P, r or both vary with A. If the second term in eq. 5 should ever predominate, the gel is referred to as zero-dimensional, or 0-d [54]. Assuming that agarose gels are 1-d for $A \leq 0.9$, the value of r determined from a plot of $K_R^{1/2}$ versus R is 25 ± 1 nm [15]. The 25-nm fiber observed by sieving must be an aggregate of smaller chains of agarose [15,61].

To explain the convex curvature of semilogarithmic μ versus A plots quantitatively, a 1-d gel and possibility (ii) of the previous paragraph have been assumed; possibility (i) and the possibility of a 0-d gel were ignored [64]. The result was the finding that the convex curvature of semilogarithmic μ versus A plots can be explained by a progressive increase in r from 21.2 to 54.4 nm as A decreased from

1.6 to 0.4 for a 6.5% hydroxyethylated agarose gelled in 0.05 *M* sodium phosphate, pH 7.4, 0.001 *M* magnesium chloride. The total volume of fibers per gram of agarose remained constant, implying that L_t decreased as r increased. The logical way to test the model of steric sieving is to measure r and L_t by electron microscopy as a function of A and to compare then with values obtained from measurement of sieving. A rigorous procedure for measuring L_t by electron microscopy has been presented [63]. However, the agarose used in ref. 63 was not hydroxyethylated and gels were cast either in water or in borate buffer. Borate buffers increase the sieving of underivatized and hydroxyethylated agarose in relation to the sieving in acetate buffers [65] and possibly also in relation to the sieving in the phosphate buffer used to obtain the sieving data of ref. 64. Thus, the appropriate measure of r and L_t by electron microscopy for comparison with r and L_t measured by sieving has not yet been obtained. The gels used for electron microscopy must be made of the same agarose and cast in the same buffers as the gels used for sieving.

After further development of the model in ref. 64, it was concluded that the R of spherical viruses must decrease as A increases to keep the data self-consistent [66]. The decrease observed for bacteriophage P22 requires closer than hexagonal close packing for the DNA of bacteriophage P22, clearly impossible. The decrease observed for turnip crinkle virus, a spherical RNA plant virus, leaves the virus without enough room for its anhydrous RNA and protein. Thus, there is an error in either the use of data or the model in refs. 64 and 66. In contrast to the conclusions of ref. 66, the observation that sieving of both hollow and filled bacteriophage T7 capsids (i.e. capsid II and mature T7 bacteriophage in ref. 67) is consistant with R obtained by X-ray scattering [46] suggests that R does not change during agarose gel electrophoresis. This observation also suggests that sieving is determined primarily by R and is independent of mass (see also ref. 51). Thus, it appears at present that no valid test of the assumption of steric sieving for spheres has been made. No attempt has been made to use the assumption of hydrodynamic sieving for the explanation of sieving during gel electrophoresis. The most objective approach to understanding sieving would be to test both of these assumptions.

6. EFFECT OF μ_0 ON ADSORPTION OF VIRUSES TO CELLS

All studied bacteriophages have a negative μ_0 during agarose gel electrophoresis at neutral pH (references are in ref. 33). Of the double-stranded DNA bacteriophages, all have an external projection (tail) with fibers. The tail is necessary for attachment to a host cell (reviewed in refs. 7–12). In the case of at least bacteriophages P22, T4 and T7, the tail fibers have a positive σ, in contrast to the negative σ of the rest of the bacteriophage [67]. This observation has led to the proposal that, during attachment to a host, the tail fibers form a positively charged bridge to overcome charge–charge repulsion between the negatively charged bacteriophage and its negatively charged host. By retracting fibers when experiencing conditions not suited for infection, the bacteriophage reduces the chance of non-productive attachment and resultant inactivation [67].

For a virus to be pathogenic to a multicellular organism, presumably this virus must, like the bacteriophage in the wild, select surfaces to which it will absorb. If so, then μ_0 will influence virulence. It has been shown that the attenuation of both Rift Valley fever and African horsesickness viruses is accompanied by a decrease in the magnitude of a negative μ_0 [32]. This observation has been made more recently for mink Aleutian disease virus [68]. A possible explanation is that a negative μ_0 comparatively high in magnitude increases the virulence of these viruses by reducing adsorption to negatively charged, non-host cell surfaces in the infected organism. The surfaces of most cells are negatively charged [69,70]. During the process of attenuation, the virus is exposed to comparatively few non-host surfaces, thereby reducing selective pressure for maintaining a μ_0 comparatively high in magnitude. Further studies are needed to determine how well μ_0 correlates with virulence.

7. VARIABILITY OF THE SIZE OF BACTERIOPHAGE CAPSIDS

The procapsid that packages DNA in cells infected with the bacteriophages discussed above undergoes changes in several characteristics either before or during DNA packaging. These include: (i) a 10–15% increase in size for all of these procapsids, (ii) either loss or cleavage of one or more major capsid proteins (reviewed in refs. 8–12) and (iii) a decrease in the magnitude of μ_0 that accompanies expansion and has been observed for T7 [67], T3 [67], P22 [40,41] and ϕ29 [42]. In the case of T3, a 1–4% increase in the magnitude of μ_0 occurs before expansion, apparently because of the binding of an additional protein [71]. The universality of the capsid's expansion during packaging suggests that expansion is necessary to obtain packaging by a procapsid. A possible reason for this has previously been discussed [72].

If expansion of a capsid occurs during entry of DNA into the capsid, one of the DNA packaging intermediates should be a capsid that: (i) has an R intermediate to the R of the procapsid and mature capsid and (ii) contains a subgenomic length of DNA (a capsid with a subgenomic length of packaged DNA will be referred to as an spDNA-capsid). In the case of bacteriophage P22, an spDNA-capsid (Int capsid) whose μ in a 0.9% agarose gel is intermediate to the μ of the procapsid and mature bacteriophage capsid has been detected [41]. However, it is not yet known whether the Int capsid is intermediate in R, μ_0 or both. Because of the comparatively small amount of Int capsid isolated and its impurity [41], the conventional techniques of light and X-ray scattering cannot be used for determining R. However, a procedure of two-dimensional (2-d) agarose gel electrophoresis developed subsequent to the discovery of the Int capsid can be used to measure R with an accuracy of $\pm 1\%$ [51]. By this procedure a first electrophoresis is conducted in a comparatively dilute (first-dimension) gel and then a second, orthogonally oriented electrophoresis is performed. During the second electrophoresis, the sample migrates out of the first-dimension gel into a more concentrated (second-dimension) gel in which the first-dimension gel has been embedded. After this procedure, the R of a particle has been found to decrease as the angle (Θ) between the first-dimension gel and a line, referred to as a size

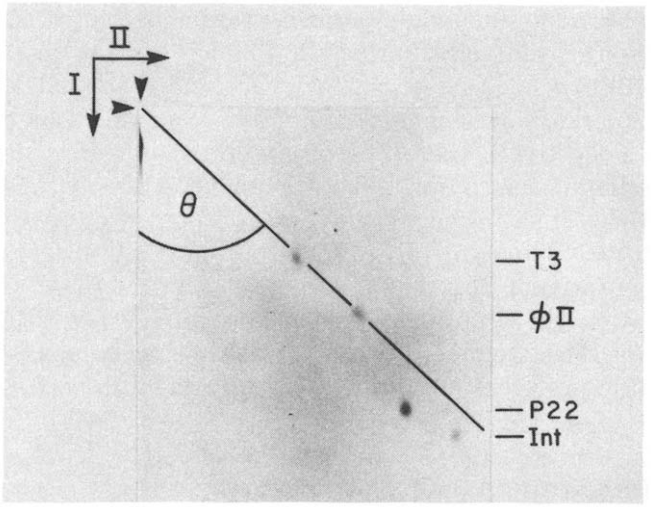

Fig. 1. Two-dimensional agarose gel electrophoresis of a bacteriophage P22 capsid. The Int capsid of bacteriophage P22 was isolated by centrifugation in density gradients of cesium chloride, as previously described [41]. To 38 μl of this preparation of Int capsid were added 3.8 μl each of bacteriophages T3 and φII and 5.6 μl of bacteriophage P22, containing 3–5 μg of protein each. After further addition of 12 μl of buffer containing 0.01 *M* sodium phosphate, pH 7.4, 0.001 *M* magnesium chloride, 9% sucrose and 200 μg/ml bromophenol blue, 2 μl of 2 mg/ml DNAase I were added to digest any DNA that was outside of capsids. Of this mixture 50 μl were subjected to two-dimensional agarose gel electrophoresis, as previously described [51], by use of the following conditions of electrophoresis. The first electrophoresis was conducted in a 0.15% agarose (Seakem LE agarose, purchased from the Marine Colloids Division of the FMC) gel at 2.0 V/cm, room temperature (20±3°C) for 9 h. The second electrophoresis was conducted through a 2.4% Seakem LE agarose second-dimension gel at 1.8 V/cm, room temperature for 40 h. After the second electrophoresis, the gel was stained with Coomassie blue [51]. The origin of electrophoresis is indicated by arrowheads. The directions of the first (I) and second (II) electrophoresis are indicated. The size line for particles 30.1 nm in *R* is drawn. Particles that form bands in the gel are indicated at the right. Inhomogeneity in the background was the result of supporting the gel on a Plexiglas plate during photography, instead of the glass plate usually used. The first-dimension gel is not shown.

line, increases. The size line passes through the effective origin of electrophoresis and the center of the particle's band. The magnitude of μ_0 increases as the distance from the origin along a size line increases [28,51]. The result of 2-d agarose gel electrophoresis suggests that the Int capsid (Int in Fig. 1) is smaller than mature bacteriophage P22 ($R=31.4$ nm [73]) and larger than bacteriophages T3 and φII ($R=30.1$ nm [51]). T3 and φII are 20 nm larger than P22's procapsid [51]. Thus, the apparent *R* of P22's Int capsid, calculated from Θ to be 30.8 nm, is intermediate to the *R* of P22 bacteriophage and P22's procapsid. The μ_0 of P22's Int capsid is higher in magnitude than the μ_0 of bacteriophage P22 (Fig. 1) and lower in magnitude than the μ_0 of P22's procapsid; the procapsid migrated out of the second-dimension gel in Fig. 1. Thus, the Int capsid is intermediate in both *R* and μ_0 to the mature P22 bacteriophage and procapsid. The Int capsid appears to be a procapsid that has only completed part of its eventual expansion to a mature capsid. If it is assumed that the *R*, μ_0 and amount of DNA packaged were not significantly altered during isolation of the above P22 capsids, in the case of

P22 at least some capsid expansion occurs during entry of DNA into the capsid. The accuracy of this assumption has not yet been determined.

8. ACKNOWLEDGEMENTS

For performing the experiment of Fig. 1, I thank Gary A. Griess and Shirley J. Hayes. For secretarial assistance, I thank Charlotte Farrell. Work in the author's laboratory was supported by grants from the National Institutes of Health (Grants AI22568 and GM24365) and the Robert A. Welch Foundation (Grant AQ-764).

9. SUMMARY

Viruses and related particles have been fractionated by electrophoresis through gels. For agarose gels, the radius at the exclusion limit for spheres varies from 1500 nm in a 0.04% gel to 3.6 nm in a 4.0% gel. Thus, the size of the gel's pores can be adjusted to sieve all known viruses. By measurement of electrophoretic mobility (μ) as a function of agarose concentration, the μ in the absence of a solid support (μ_0) can be determined for any particle. From the shape of a semilogarithmic plot of μ as a function of agarose percentage, a rod-shaped particle can be discriminated from a spherical particle. The sphere's radius can be determined from this plot with an accuracy of $\pm 8\%$. Accuracy of $\pm 1\%$ has been more recently achieved using two-dimensional agarose gel electrophoresis. Though bacteriophages have been the primary object of study, the above techniques of agarose gel electrophoresis have also been applied to plant viruses and should be applicable to animal viruses. The μ_0 values measured for bacteriophages with and without their tail fibers suggest a mechanism of controlling attachment to a host. A related mechanism is proposed for the control of the virulence of animal viruses. Measurement of outer radius for different forms of the capsid of bacteriophage P22 reveals variability in outer radius too small to be detected by electron microscopy.

REFERENCES

1 F.W. Studier, J. Mol. Biol., 79 (1973) 237.
2 M.W. McDonell, M.N. Simon and F.W. Studier, J. Mol. Biol., 110 (1977) 119.
3 P. Serwer, Methods Enzymol., 130 (1986) 116.
4 S.E. Luria, J.E. Darnell, Jr., D. Baltimore and A. Campbell, General Virology, Wiley, New York, 1978.
5 G.S. Stent and R. Calendar, Molecular Genetics, W.H. Freeman, San Francisco, CA, 1978.
6 E.C.C. Lin, R. Goldstein and M. Syvanen, Bacteria, Plasmids and Phages, Harvard University Press, Cambridge, MA, 1984.
7 A.S. Tikhonenko, Ultrastructure of Bacterial Viruses, Plenum, New York, 1970.
8 W.B. Wood and J. King, in H. Fraenkel-Conrat and R.R. Wagner (Editors), Comprehensive Virology, Vol. 13, Plenum, New York, 1979, p. 581.
9 W.C. Earnshaw and S.R. Casjens, Cell, 21 (1980) 319.
10 R.W. Hendrix, in S. Casjens (Editor), Virus Structure and Assembly, Jones and Bartlett, Boston, MA, 1985, p. 170.
11 A.C. Steven and B.L. Trus, in J.R. Harris and R.W. Horne (Editors), Electron Microscopy of Proteins, Vol. 5, Academic Press, London, 1986, p. 1.

12 S. Casjens, in S. Casjens (Editor), Virus Structure and Assembly, Jones and Bartlett, Boston, MA, 1985, p. 1.
13 S. Hjertén, Biochim. Biophys. Acta, 53 (1961) 514.
14 K.B. Guiseley and D.W. Renn, Agarose Purification, Properties and Biomedical Applications, Marine Colloids, Rockland, ME, 1975.
15 P. Serwer, Electrophoresis, 4 (1983) 375.
16 R.J. Wieme, Agar Gel Electrophoresis, Elsevier, Amsterdam, 1965.
17 R.H. Epstein, A. Bolle, C.M. Steinberg, E. Kellenberger, E. Boy de la Tour, R. Chevalley, R.S. Edgar, M. Susman, G.H. Denhardt and A. Lielausis, Cold Spring Harbor Symp. Quant. Biol., 28 (1963) 375.
18 R.S. Edgar and W.B. Wood, Proc. Natl. Acad. Sci. U.S.A., 55 (1966) 498.
19 C.A. Hutchison III, M.H. Edgell and R.L. Sinsheimer, J. Mol. Biol., 23 (1967) 553.
20 E.G. Strauss and P. Kaesberg, Virology, 42 (1970) 437.
21 R.J. Radloff and P. Kaesberg, J. Virol., 11 (1973) 116.
22 T.K. Lim, G.J. Baran and V.A. Bloomfield, Biopolymers, 16 (1977) 1473.
23 J.D. Childs and H.C. Birnboim, J. Virol., 16 (1975) 652.
24 J.D. Childs, J. Mol. Biol., 141 (1980) 163.
25 F.A. Eiserling, in C.K. Mathews, E.M. Kutter, G. Mosig and P.B. Berget (Editors), Bacteriophage T4, American Society for Microbiology, Washington, DC, 1983, p. 11.
26 J. Beaudoin, T.J. Henry and D. Pratt, J. Virol., 13 (1974) 470.
27 D.J. Shaw, Electrophoresis, Academic Press, London, 1969.
28 P. Serwer, in J. Jorgenson and M. Phillips (Editors), New Directions in Electrophoretic Methods, American Chemical Society, Washington, DC, 1987, p. 158.
29 A. Klug and D.L.D. Caspar, Adv. Virus Res., 7 (1960) 225.
30 B.V. Barkas, A.N. Kornev, V.V. Mesianzhikov, B.F. Poglazov and A.I. Turkin, Dokl. Akad. Nauk SSSR, 245 (1979) 736.
31 G.K. Ackers and R.L. Steere, Biochim. Biophys. Acta, 59 (1962) 137.
32 A. Polson and B. Russell, Methods Virol., 2 (1967) 391.
33 P. Serwer, ASM News, 50 (1984) 414.
34 P. Serwer, E.T. Moreno, S.J. Hayes, P. Berger, M. Langham and R.W. Toler, Electrophoresis, 5 (1984) 202.
35 A. Asselin and J. Grenier, Can. J. Plant Pathol., 7 (1985) 223.
36 R. Hogue and A. Asselin, Can. J. Bot., 62 (1984) 457.
37 P. Serwer and M.E. Pichler, J. Virol., 28 (1978) 917.
38 H.B. Bull, An Introduction to Biophysical Chemistry, F.A. Davis, Philadelphia, PA, 1971, p. 131.
39 P. Serwer and R.H. Watson, Virology, 140 (1985) 80.
40 R. Gope and P. Serwer, J. Virol., 47 (1983) 96.
41 P. Serwer and R. Gope, J. Virol., 49 (1984) 293.
42 D. Anderson, unpublished results.
43 P.G. Righetti, B.C.W. Brost and R.S. Snyder, J. Biochem. Biophys. Methods, 4 (1982) 347.
44 R.B. Cook and H.J. Witt, U.S. Pat., 4,290,911 (1981).
45 P. Serwer and S.J. Hayes, Anal. Biochem., 158 (1986) 72.
46 P. Serwer and J.L. Allen, Electrophoresis, 4 (1983) 273.
47 O.J. Lumpkin, P. Déjardin and B.H. Zimm, Biopolymers, 24 (1985) 1573.
48 N.C. Stellwagen, Biopolymers, 24 (1985) 2243.
49 P. Serwer and J.L. Allen, Biochemistry, 23 (1984) 922.
50 P. Serwer, Anal. Biochem., 112 (1981) 351.
51 P. Serwer, S.J. Hayes and G.A. Griess, Anal. Biochem., 152 (1986) 339.
52 J. Newman, H.L. Swinney and L.A. Day, J. Mol. Biol., 116 (1977) 593.
53 P. Serwer, G. Merrill, E.T. Moreno and R. Herrmann, in H. Peeters (Editor), Protides of the Biological Fluids, Vol. 33, Pergamon, Oxford, 1985, p. 517.
54 D. Rodbard and A. Chrambach, Proc. Natl. Acad. Sci. U.S.A., 65 (1970) 970.
55 A. Chrambach and D. Rodbard, Science, 172 (1971) 440.
56 D. Rodbard, C. Levitov and A. Chrambach, Sep. Sci., 7 (1972) 705.

57 S. Ghosh, K. Basu and J.S. Schweppe, Anal. Biochem., 50 (1972) 592.
58 N.C. Stellwagen, Biochemistry, 22 (1983) 6180.
59 J. Cornfield and H.W. Chalkley, J. Wash. Acad. Sci., 41 (1951) 226.
60 A.G. Ogston, Trans. Faraday Soc., 54 (1958) 1754.
61 S. Arnott, A. Fulmer, W.E. Scott, I.C.M. Dea, R. Moorhouse and D.A. Rees, J. Mol. Biol., 90 (1974) 269.
62 A. Amsterdam, Z. Er-el and S. Shaltiel, Arch. Biochem. Biophys., 171 (1975) 673.
63 S. Waki, J.D. Harvey and A.R. Bellamy, Biopolymers, 21 (1982) 1909.
64 D. Tietz and A. Chrambach, Electrophoresis, 6 (1986) 241.
65 S. Peats, S. Nochumson and F.H. Kirkpatrick, Biophys. J., 49 (1986) 91a.
66 D. Tietz, E. Gombocz and A. Chrambach, in M.J. Dunn (Editor), Electrophoresis '86, VCH, Weinheim, 1986, p. 253.
67 P. Serwer, R.H. Watson, S.J. Hayes and J.L. Allen, J. Mol. Biol., 170 (1983) 447.
68 B. Aasted, B. Avery and A. Cohn, Arch. Virol., 80 (1984) 11.
69 C.C. Brinton, Jr. and M.A. Lauffer, in M. Bier (Editor), Electrophoresis Theory Methods and Applications, Academic Press, London, 1959, p. 427.
70 G.V.F. Seaman, in D. Mac N. Surgenor (Editor), The Red Blood Cell, Academic Press, New York, 1975, p. 1135.
71 P. Serwer, R.H. Watson and S.J. Hayes, J. Virol., 55 (1985) 232.
72 P. Serwer, J. Mol. Biol., 138 (1980) 65.
73 W. Earnshaw, S. Casjens and S.C. Harrison, J. Mol. Biol., 104 (1976) 387.