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The Particle Weight, Hydration and Shape of the T_2 Bacteriophage of *Escherichia coli*[1]

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The diffusion coefficients of both the 1000 Svedberg and the 700 Svedberg components of the T_2 bacteriophage have been determined. Corrected to the values in water at 20° these are 3.46×10^{-8} cm.²/sec. and 2.96×10^{-8} cm.²/sec., respectively. The particle weights of the two components were calculated by means of the Svedberg equation to be 220×10^6 and 181×10^6, respectively. The hydrations of both components were determined, by sedimentation measurements in sucrose solutions, to be 0.60 g. of water per g. of dry bacteriophage. It is tentatively concluded that the two components do not differ in sedimentation rate because of aggregation or change in hydration. They may differ in shape.

Introduction

Sharp, Hook, *et al.*,[4,5] demonstrated that purified preparations of the T_2 bacteriophage of *Escherichia coli* contained either or both of two sedimenting components, with sedimentation constants of about 700 and 1000 svedbergs. They suggested the interesting possibility, based on the behavior of plastic models of the T_2 bacteriophage, that the slower sedimenting component was composed of aggregates of two single particles, oriented during sedimentation.

The work of Putnam[6] indicates that this theory may not be correct. He found evidence that the related T_6 bacteriophage is not oriented during sedimentation. Putnam suggested that, on the basis of usual sedimentation behavior, it is more likely that the faster sedimenting component is aggregated.

The question whether one of the components is an aggregate can be resolved by determining the particle weights, M, of both components. In principle, this can be deduced from the sedimentation coefficient s, the diffusion coefficient D, and the partial specific volume V, by means of the Svedberg equation

$$M = \frac{RTs}{D(1 - V\rho)} \qquad (1)$$

where R is the gas constant, T the absolute temperature and ρ the density of the solution. Putnam and Goldwasser[6,7] have determined the particle weight of the faster sedimenting component of T_6 bacteriophage.

The present study is a further investigation of the two components of T_2 bacteriophage. The particle weights and other properties of the two components have been determined by experiments with preparations in *p*H 5 and 7 buffers. In these buffers, the faster and slower sedimenting components, respectively, are present alone.

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(2) U. S. Department of Agriculture, Agricultural Research Service, Northern Utilization Research Branch, Peoria, Illinois.

(3) Brandeis University, Waltham, Mass.

(4) D. G. Sharp, A. E. Hook, A. R. Taylor, D. Beard and J. W. Beard, *J. Biol. Chem.*, **165**, 259 (1946).

(5) A. E. Hook, D. Beard, A. R. Taylor, D. G. Sharp and J. W. Beard, *ibid.*, **165**, 241 (1946).

(6) F. W. Putnam, *ibid.*, **190**, 61 (1952).

(7) E. Goldwasser and F. W. Putnam, *ibid.*, **190**, 75 (1952).

Materials and Methods

The stocks of T_2 bacteriophage and *Escherichia coli* bacteria were obtained originally from Dr. M. H. Adams. The bacteriophage was cultured by growing the bacteria in aerated broth at 37° to about 10^8 per ml. and seeding with 2 to 4 bacteriophage infectious units per bacterium. The culture was then aerated overnight. The resulting lysate containing the bacteriophage was purified by Herriott's procedure.[8] It was necessary to digest this broth-grown bacteriophage with desoxyribonuclease before differential centrifugation. Undigested preparations were highly viscous. The pellets from the high-speed centrifugation were resuspended by the convection procedure of Lesley, *et al.*[9] The purified bacteriophage preparations contained 1.0 to 1.6×10^{-15} g. of material per infectious unit. Infectivity was measured by the agar layer method of Hershey, *et al.*[10]

Viscosity and sedimentation rates were determined as described previously.[11] The densities of preparations of bacteriophage in buffers were measured in an oil gradient column. The densities of the sucrose solutions were determined in a pycnometer.

The *p*H 5 buffer was 0.1 *M* acetate, and the *p*H 7 buffer was 0.1 *M* phosphate. In addition, each contained 5 g. of NaCl per liter.

The diffusion coefficients were measured in an Aminco-Stern electrophoresis apparatus equipped with a Philpot-Svensson optical system. The constant temperature water-bath was insulated and cooled with ice without stirring. The boundaries were formed in two Tiselius electrophoresis cells placed in the bath. Preparations of bacteriophage in *p*H 5 and 7 buffers were placed in the two cells and the boundaries were moved into view by compensation. Photographs of the schlieren patterns were taken on infrared film with a Wratten 88A filter. Measurements were made on enlarged tracings of the photographs.

The concentration of bacteriophage in solution was determined with a Phoenix Precision Instrument Company differential refractometer. Each solution was compared with its dialyzing buffer, after at least 12 hours of dialysis at 5°. The refractive index difference between solvent and solution was divided by the specific refractive increment, 0.00183 (*vide infra*), to obtain the concentration of bacteriophage.

Results

Specific Refractive Increment of T_2 Bacteriophage.—A single determination of the specific refractive increment of T_2 bacteriophage was made with the differential refractometer on a preparation dialyzed against *p*H 7 buffer. The concentration of bacteriophage material in the preparation was determined by sedimentation and subsequent drying over P_2O_5 at 46°. The weight of the dry pellet was corrected for the weight of salts associated with the water which evaporated when the

(8) R. M. Herriott and J. L. Barlow, *J. Gen. Physiol.*, **36**, 17 (1952).

(9) S. M. Lesley, R. C. French and A. F. Graham, *Can. J. Res., E*, **28**, 281 (1950).

(10) A. D. Hershey, G. Kalmanson and J. Bronfenbrenner, *J. Immunol.*, **46**, 267 (1943).

(11) M. A. Lauffer, N. W. Taylor and C. C. Wunder, *Arch. Biochem. Biophys.*, **40**, 453 (1952).

pellet was dried. Calculated from the refractive index difference between the solution and solvent and the concentration of bacteriophage, the specific refractive increment was 0.00183 in units of 100 ml. of solution per g. of bacteriophage.

Sedimentation Rates of T_2 Bacteriophage in *p*H 5 and 7 Buffers.—The sedimentation rates of purified preparations of T_2 bacteriophage were determined after dialysis overnight against either *p*H 5 or 7 buffers. These preparations showed single sharp boundaries on centrifugation. In Fig. 1 the products of sedimentation rate s, and either solution viscosity η, or solvent viscosity η_0, are plotted against the concentration of bacteriophage, c, in the solution. For both the faster and slower sedimenting components the product, ηs, is independent of concentration of bacteriophage in the concentration range investigated. The average values of ηs for the two components were 1041 and 713 centipoise-svedbergs.

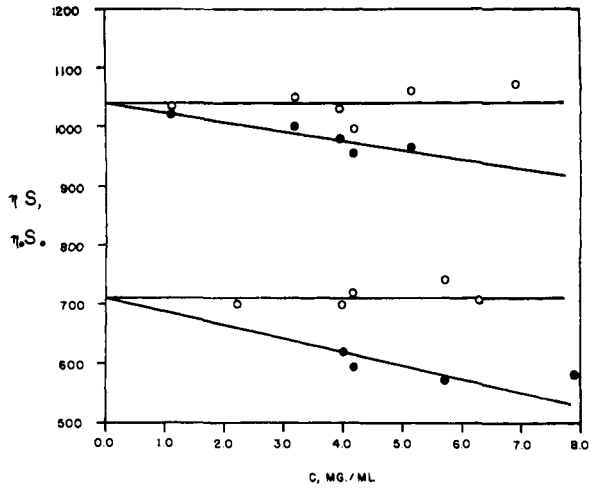

Fig. 1.—Sedimentation rates of T_2 bacteriophage in *p*H 5 and 7 buffers; ηs (O) and $\eta_0 s$ (●) are plotted against concentration of bacteriophage.

The products $\eta_0 s$ of both components are concentration dependent. It appears, from the limited data in Fig. 1, that the 700 svedberg component is the more concentration dependent. This is contrary to the results of Sharp, *et al.*,[4] for T_2 bacteriophage.

Each value of ηs was corrected for density of the solution making use of the relationship that ηs is proportional to $(1 - V_h\rho)$, where V_h is, in Fig. 2, the reciprocal of the intercept corresponding to zero sedimentation rate. The result was divided by the viscosity of water at 20° to obtain the corrected sedimentation constant, s^w_{20}. The average corrected values of s^w_{20} are 1066 Svedbergs for *p*H 5 and 751 svedbergs for *p*H 7 preparations. These values can be compared with those obtained by Sharp, *et al.*,[4] 1044 and 708 svedbergs, respectively.

Diffusion Coefficients.—In a typical experiment, the diffusion coefficients of two solutions of the same bacteriophage preparation, dialyzed against *p*H 4.94 and 6.84 buffers, were determined in separate cells at the same time. The concentration of bacteriophage in the *p*H 4.94 solution was 4.2 mg. per ml., and the concentration in the *p*H 6.84

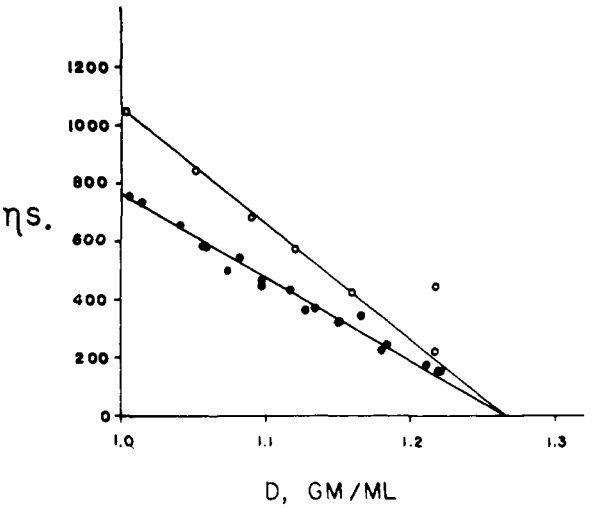

Fig. 2.—Sedimentation rates of T_2 bacteriophage in solutions of sucrose in *p*H 5 buffer (O) and in *p*H 7 buffer (●) plotted against solution density.

solution was 7.9 mg. per ml. Twelve photographs of each boundary were taken between 10 and 100 hours after formation of the boundaries.

The temperature varied during the diffusion experiment between 4.5 and 4.9°, and the average temperature was 4.8°. Although this is a relatively large temperature variation, there was no evidence of abnormal diffusion behavior in the data obtained. The plots of $1/H^2$ against t were linear. H is the maximum ordinate of the center of the slit image in the schlieren pattern, and t is the time after formation of the boundary. The schlieren patterns of the boundaries were symmetrical and essentially smooth curves. This is evidence that the temperature changed slowly enough that the boundaries were not appreciably disturbed.

The diffusion coefficients D_T were calculated from the equation

$$D_T = \frac{d(1/H^2)}{dt} \times \frac{A^2}{4\pi g^2} \qquad (2)$$

A is the area under the center of the slit image, and g is the linear magnification along the cell height. $d(1/H^2)/dt$ is the slope of the line determined from the plot of $1/H^2$ against t. The diffusion coefficients determined from the two boundaries (A and B) in each cell are given in Table I.

TABLE I

THE DIFFUSION COEFFICIENTS OF T_2 BACTERIOPHAGE IN *p*H 5 AND 7 BUFFERS

Boundary	*p*H	D_T, 10^{-8} cm.²/sec.	D^w_{20}, 10^{-8} cm.²/sec.
A	6.93	1.74	2.98
B	6.93	1.71	2.94
A	4.96	2.17	3.60
B	4.96	2.01	3.33

The diffusion coefficients were corrected by the usual method to the values which would be obtained in water at 20°, D^w_{20}.[12] These values are given in Table I. The average diffusion coefficient

(12) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, p. 410.

in pH 4.96 buffer was 3.46×10^{-8} cm.^{2}/sec. and that in pH 6.93 buffer was 2.96×10^{-8} cm.^{2}/sec.

Three other similar diffusion experiments were performed, each with preparations in pH 5 and 7 buffers containing 4 mg./ml. of bacteriophage. The mean ratio for the four experiments of the diffusion coefficient, D^{w}_{20}, at pH 5 to that at pH 7 was 1.21 with a standard error of ± 0.036. Thus, the diffusion coefficient in pH 5 buffer was reproducibly greater than that in pH 7 buffer. However, in these additional experiments, a diagonal knife edge was used in the schlieren optical system in place of the diagonal slit, and the actual values of the diffusion coefficients were about 20% less than those reported in Table I.

Goldwasser and Putnam[7] determined the diffusion coefficient of the faster sedimenting component of the T_6 bacteriophage. Their value corrected to 20° was 4.0×10^{-8} cm.^{2}/sec.

Sedimentation in Sucrose Solutions.—The sedimentation rates of the T_2 bacteriophage were determined in solutions containing various concentrations of sucrose, both in pH 5 and 7 buffers. The solutions contained 2 mg./ml. of bacteriophage. The viscosity η and the density d of each solution were determined. The products, ηs, are plotted against d in Fig. 2.[13] Straight lines were fitted to the data for each of the faster and slower sedimenting components by the method of least squares. The data obtained from the solution in pH 5 buffer with the greatest density were omitted from the computation, since two sedimenting components were observed in the ultracentrifuge.

$$\eta s = 5050 - 3993d \text{ for } p\text{H } 5 \text{ buffer} \quad (3)$$

$$\eta s = 3468 - 2720d \text{ for } p\text{H } 7 \text{ buffer} \quad (4)$$

At the density of water (0.9982 g./ml.) these equations extrapolate to ηs values of 1060 for pH 5 and 753 for pH 7 buffer. At a sedimentation rate, ηs, of zero, these equations extrapolate to densities of about 1.27 g./ml. for both pH 5 buffer and pH 7 buffer. This density can be identified with the solvated densities of the two components of T_2 bacteriophage in sucrose solution.

Discussion

Particle Weights.—The particle weight of each component was calculated by equation 1 in which the following values were used: for ρ, 0.9982 g./ml.; for T, 293°K.; for V,[14] 0.66. In pH 5 buffer, the calculated particle weight was 220×10^6 atomic weight units, and in pH 7 buffer the calculated particle weight was 181×10^6 atomic weight units.

Putnam and Goldwasser[6,7] have determined the sedimentation rate to be 1050 svedbergs and the diffusion coefficient to be 4.0×10^{-8} cm.^{2}/sec. for the faster sedimenting component of the related T_6 bacteriophage. The particle weight calculated from their data is 188×10^6 atomic weight units.

Of the various measurements involved in the determination of these particle weights, the diffusion coefficients are the least reliable. Difficulties in measuring the photographs and in controlling the conditions of the diffusion experiments make the absolute values of the diffusion coefficients uncertain. However, since the experiments on both boundaries in pH 5 and 7 buffers were performed in the same machine at the same time, the ratios of the diffusion coefficients of the two components are probably sufficiently reliable to differentiate between the three simple possibilities, namely, that the ratio of the two particle weights could be 2.0, 1.0 or 0.5.

It is obvious from equation 1 that, if V remains constant

$$\frac{M_5}{M_7} = \frac{s_5}{s_7} \times \frac{D_7}{D_5} \quad (5)$$

where the subscripts refer to the value at the pH indicated. When the values of 1066 and 751 are substituted for s_5 and s_7, respectively, it follows that for M_5/M_7 to be 2.0, D_5/D_7 would have to be 0.71. For M_5/M_7 to be $^1/_2$, D_5/D_7 would have to be 2.84, and for M_5/M_7 to be 1.00, D_5/D_7 would have to be 1.42. The experimentally determined ratio obtained from the four diffusion experiments had a mean value of 1.21 with a standard error of ± 0.036 for 7 degrees of freedom. From statistical tables, one can evaluate the probability that the value 1.42 differs from the experimental ratio due solely to errors of random sampling as less than 0.001. The probabilities that the values 0.71 and 2.84 differ from the experimental ratio due solely to errors of random sampling are much smaller. Thus, it is very unlikely that the ratio of the particle weights of the two components differs from any of the three possibilities due solely to random errors in measuring the diffusion coefficients.

The experimental ratio of the particle weights is 1.21. This value is in best agreement with the hypothesis that the two components are composed of particles of the same weight. Since the experimental mean ratio of the diffusion coefficients is significantly different from 1.42, it is possible that the two particle weights are in fact slightly different, or alternatively, that there is some systematic error in the ratio of the diffusion coefficients. In order for the first possibility to be the correct explanation, the particle weight at pH 7 would be 85% that at pH 5. Such a preparation, in order to be transformable into the heavier particle at pH 5, would have to consist of 85% principal component and 15% additional material. However, no second component has been detected at pH 7, and, therefore, the possibility that the particle weights differ by 15% at the two pH values is highly unlikely.

Hydration.—The solvated density of both components of T_2 bacteriophage has been determined by sedimentation measurements in sucrose solutions. From this figure and the partial specific volume V, the weight of excess water h, associated with 1 g. of dry bacteriophage can be calculated by means of the equation

$$h = \frac{1 - Vd_h}{d_h/d_w - 1} \quad (6)$$

In this equation, d_h is the solvated density of the bacteriophage, and d_w is the density of the water.

The solvated density of the two components was

(13) The authors acknowledge the assistance of Mr. Louis G. Swaby who was responsible for about half of the experimental values presented in Fig. 2.

(14) A. R. Taylor, *J. Biol. Chem.*, **165**, 271 (1946).

1.27 g./ml. Calculated from this value, the hydration was 0.60 g. of water per g. of bacteriophage. Strictly, this value is the excess water in addition to solvent associated with 1 g. of dry bacteriophage in sucrose solution.[11] If the assumption is made that the sucrose solution does not change the bacteriophage in any way, then it is also the value for the water of hydration of the virus in water.

Since the calculated hydrations of the two components are identical, it is unlikely that the different sedimentation rates of the two components of T_2 bacteriophage are the result of a difference in hydration of the particles.

Shape of the Particles.—In principle, the sedimentation rate of a substance depends on the weight, hydration and shape of the particles. A change in any one of these properties might change the sedimentation rate. It appears that the two components of T_2 bacteriophage probably do not differ as a result of an aggregation or a change in hydration. If such is indeed the case, then the different sedimentation rates of the two components can be attributed to a change in shape.

The friction ratio, f/f_0, for a component can be calculated from the experimental friction coefficient f, and the friction coefficient for a spherical anhydrous particle of the same mass f_0, by means of the equations

$$f = RT/ND^{\circ}_{20} \quad (7)$$

$$f_0 = 6\pi\eta_w r_0 \quad (8)$$

$$r_0 = \sqrt[3]{3MV/4\pi N} \quad (9)$$

in which N is Avogadro's number, η_w is the viscosity of water at 20°, and r_0 is the radius of the spherical anhydrous particle of the same mass M. The friction ratios are 1.59 and 2.00 for the faster and slower sedimenting components, respectively.

The friction ratio is a function of the hydration and shape of the particles of material. The higher friction ratio for the slower sedimenting component indicates that this component (a) is the more hydrated, or (b) departs more from the spherical shape. Since evidence has been given that the hydrations of the two components are identical, the second alternative is the more likely. Thus, from all the evidence given above, the most likely deduction is that, when the pH of the medium is changed from pH 5 to 7, the bacteriophage particles change in shape in such a way as to result in a slower sedimentation rate, a smaller diffusion coefficient, and a higher friction ratio.

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[CONTRIBUTION FROM THE DEPARTMENT OF INDUSTRIAL MEDICINE, NEW YORK UNIVERSITY POST-GRADUATE MEDICAL SCHOOL]

The Specific Interaction of Some Dinitrobenzenes with Rabbit Antibody to Dinitrophenyl–Bovine γ-Globulin[1]

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The specific binding of homologous haptens of different size to antibody has been studied at two temperatures by the method of equilibrium dialysis. The antibody was formed in rabbits against dinitrophenyl-bovine γ-globulin. Dinitroaniline and ε-dinitrophenyllysine were the haptens used in the binding studies. Relative binding affinities were obtained graphically. The hapten in which the determinant group was combined with amino acid in the same way as in the immunizing antigen, namely, ε-dinitrophenyllysine, was more strongly bound than the simpler hapten 2,4-dinitroaniline. Binding was somewhat higher at low than at high temperature. The antibody was found to exhibit considerable heterogeneity and a multiple number of binding sites. An antibody fraction which precipitated with the homologous antigen, showed greater homogeneity and a "valence" of approximately two.

The specific combination of antigen of high molecular weight with antibody has been studied extensively in the past. In most cases this combination leads to the formation of a precipitate. On the other hand, the specific interaction of antibody with certain homologous haptens of low molecular weight frequently results in the formation of soluble complexes. Because of their small size and solubility, these complexes are amenable to a physicochemical study from which information may be obtained concerning the number of binding sites (valence) of antibody, the free energy of binding of hapten and the specificity of the interaction of antibody with hapten.

(1) (a) We wish to acknowledge the support of this work by grants from the Standard Oil Co. (N. J.), New York City, and the National Microbiological Institute of the National Institutes of Health, United States Public Health Service (Grant No. RG-1713). (b) Presented in part at a meeting of the American Association of Immunologists, *Federation Proc.*, **13**, 488 (1954).

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In the present investigation the soluble complexes formed by rabbit antibody to 2,4-dinitrophenyl-bovine γ-globulin (DNP-bovine γ-globulin) with 2,4-dinitroaniline and ε-N-dinitrophenyllysine (ε-DNP-lysine) have been studied by the method of equilibrium dialysis. Marrack and Smith[3] were first to demonstrate by this method the specific binding of homologous hapten to antibody, after removal of serum albumin from the immune serum of rabbits immunized with an azoprotein. Haurowitz and Breinl[4] carried out a similar experiment without removing serum albumin, and instead compared the amount of hapten bound to immune serum with that bound to normal serum. In a quantitative study of purified antibody by this method, Eisen and Karush[5] determined the number of binding sites on an antibody and the free energy of binding of a hapten.

(3) J. Marrack and F. C. Smith, *Brit. J. Exp. Path.*, **13**, 394 (1932).
(4) F. Haurowitz and F. Breinl, *Z. physiol. Chem.*, **214**, 111 (1933).
(5) H. N. Eisen and F. Karush, THIS JOURNAL, **71**, 363 (1949).