ULTRAVIOLET DICHROISM OF fd BACTERIOPHAGE

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ABSTRACT The internal structure of the bacterial virus fd was investigated by ultraviolet dichroism of virus solutions oriented by flow through a small capillary tube. The dichroism was found to be positive for wavelengths longer than 262 mμ and shorter than 239 mμ, and negative for the intermediate wavelengths. The magnitude of the effect was at all times small, with dichroic ratios of 1.22 and 0.83 at 280 mμ and 250 mμ, respectively. The intuitive interpretation that this was the result of the addition of negative DNA dichroism and positive protein dichroism was confirmed by the application of a simple theory which allowed the calculation from protein and DNA absorption data of a dichroism curve closely approximating the experimental one. The parameters arrived at by this procedure indicate a semiangle of 25° ± 5° for a cone described by the normals to the DNA base planes inside the virus. The protein absorbers tryptophan and probably tyrosine were found to be oriented on the average relatively parallel to the longitudinal axis of the virus.

INTRODUCTION

The filamentous bacteriophage fd (Marvin and Hoffmann-Berling, 1963 *a*) is of interest because its physical and biological properties are quite different from most other phages. It has the shape of a flexible rod 760 × 5 mμ, a molecular weight of 11.3×10^6 (Marvin and Hoffmann-Berling, 1963 *b*), and is normally released from its host without inducing lysis or markedly affecting the growth of the bacterium (Hoffmann-Berling and Mazé, 1964). Its single stranded DNA comprises 12.2% of the virus weight (Hoffmann-Berling et al., 1963), and is believed to be in the form of a ring (Marvin and Schaller, 1966). While X-ray diffraction (Marvin, 1966) and optical rotary dispersion (Day, 1966) studies have suggested that the protein moiety of this bacteriophage is mostly in the form of an α-helix, little information has been forthcoming concerning the configuration of the DNA within the virus particle.

Anisotropic absorption, or dichroism of oriented macromolecules, is a phenomenon particularly well suited to the investigation of the orientation of absorbing groups within viruses. The extreme asymmetry of fd bacteriophage allows it to be easily oriented in gels or flowing solutions. The flow method offers the advantage that the optical properties of the molecules can be studied under desired solvent conditions, but suffers from the disadvantage of imperfect orientation of the in-

dividual particles. Although the technique has not been employed extensively, some interesting results have been obtained. The base planes of the RNA inside tobacco mosaic virus (TMV) have been shown to be oriented "approximately" parallel to the longitudinal axis of the virus (Schachter et al., in press), and the dichroic spectrum of potato virus X has suggested a similar configuration for its RNA base planes (Schachter, personal communication). F-actin, however, has the plane of its adenine, in the form of ADP, arranged nearly perpendicularly to the axis of the helical polymer (Higashi et al., 1963).

This study was undertaken to investigate the internal structure of the semirigid rod-shaped bacteriophage fd using UV flow dichroism.

MATERIALS AND METHODS

Virus

fd bacteriophage was obtained from Dr. Hoffmann-Berling at the Max Planck Institut für Medizinische Forschung, Heidelberg. It was grown principally on *Escherichia coli* K12, strain 112, an F^+ bacteria obtained from Dr. David Pratt at the University of Wisconsin. The bacteria were grown at 37° in 3XD media (Fraser and Jerrel, 1953) with vigorous aeration. Final titers of 10^{12} infective particles per milliliter were typical when the bacteria at a concentration of 3×10^8 cells/ml were infected at a multiplicity of one phage per 50 cells and assayed after an incubation period of 10 hr.

The final crude preparation consisted of phage and intact bacteria in growth medium. The first step in the concentration and purification of the phage consisted of nearly saturating this solution with ammonium sulfate; the resulting flocculent precipitate which floated to the top of the solution was carefully removed and resuspended in distilled water. This suspension of virus and bacteria was centrifuged at low speed to remove the bacteria, and then centrifuged at 80,000 g for 5 hr to pellet the phage. The pellets were resuspended in distilled water, and the differential centrifugation cycle repeated. After the second resuspension the density of the virus solution was brought to 1.3 g/ml with CsCl, taking care that the final virus concentration did not exceed 3.3 mg/ml, and the solution was centrifuged for 24 hr at 35,000 RPM in a Spinco SW39 rotor (Beckman Instruments, Inc., Palo Alto, California). The phage formed a single broad band which was carefully removed, and then either diluted two or three times for storage in the cold or diluted and dialyzed for use. Pelleting of the phage resulted in some loss of biological activity, but the dichroism manifested by pelleted and unpelleted virus was not significantly different. Biological assays generally were in an order of magnitude agreement with concentration measurements. All assays were performed using the standard double agar layer technique described by Adams (1959).

Virus Concentration

Weight concentration of the virus was determined by measuring the ultraviolet absorption at 260 mμ and using the absorptivity at that wavelength, 3.74 $(\text{mg/ml}-\text{cm})^{-1}$ (Hoffmann-Berling et al., 1963).

Protein Preparation

10 ml of purified virus at a concentration of 1.5 mg/ml in pH7, 0.1 μ, phosphate buffer was shaken with 10 ml of freshly distilled water-saturated phenol for 10 min, and then centrifuged

at 8000 g for 30 min to separate the phases. The water phase was removed and washed with another 10 ml of phenol by shaking for 2 min, and was centrifuged as before. The two phenol fractions were combined and washed twice by shaking for 2 min with phosphate buffer, followed by low speed centrifugations. Three volumes of methanol were added to the final washed phenol fraction, which was then dialyzed exhaustively against successive aqueous solutions of 60, 50, and 40% methanol. During this dialysis, the protein precipitated and all traces of phenol were removed. The precipitated protein was centrifuged for 40 min at 12,000 g and the resulting pellet resuspended in 6 M urea in 0.01 N HCl (pH 3.5). The protein dissolved readily in this reagent, and was subsequently dialyzed against 0.001 N HCl to remove the urea. In this solution the protein seemed quite stable and showed little tendency to precipitate at room temperature.

DNA Preparation

The phenol extraction of the DNA proceeded in a manner similar to that employed for the purification of the protein, except that the water phase was saved and washed with phenol. The final water fraction was shaken for 2 min with twice its volume of freshly distilled ethyl ether. The ether phase was removed and the procedure repeated three additional times. Then 2.5 volumes of ethyl alcohol together with two drops of 3 M sodium acetate were added to the remaining water phase. The DNA precipitated and was pelleted by centrifuging at 27,000 g for 30 min. The supernatant fluid was discarded and the DNA pellet resuspended in pH7, 0.1 μ, phosphate buffer.

Dichroism Apparatus

The apparatus was essentially that used and described by Schachter et al. (in press), and consisted of a 1 mm square cross-section quartz capillary mounted in the sample chamber of the Cary model 14 M recording spectrophotometer (Cary Scientific Instruments, Applied Physics Corp., Monrovia, California). The total volume required by the system was about 11 ml. A small peristaltic tubing pump in conjunction with two small reservoirs provided regulatable continuous flow through the capillary, and in all experiments flow gradients at the wall of the cell were calculated to be of the order of 3×10^4 sec^{-1}.

Definitions

$D(\lambda)$, dichroism at wavelength λ; $R_i(\lambda)$, dichroic ratio of component i at wavelength λ; $a_{u_i}(\lambda)$, absorptivity of component i at wavelength λ with unpolarized light; $a_{\parallel_i}(\lambda)$, absorptivity of component i at wavelength λ with light polarized parallel to the long axis of the virus; $a_{\perp_i}(\lambda)$, absorptivity of component i at wavelength λ with light polarized perpendicular to the long axis of the virus.

RESULTS

The results of the dichroism experiments on solutions of fd are presented in Figs. 1, 2, and 3. Light scattering in the region observed is very small, with absorbances of 0.1 or less at 320 mμ and extrapolated values of 0.5 or less at 230 mμ. All data were corrected for light scattering by assuming a λ^{-4} dependence of turbidity in all regions, and extrapolating the absorbance data between 400 and 320 mμ to shorter wavelengths where absorption occurs.

The absorbance data of a typical experiment are plotted in Fig. 1. The solid and dashed curves represent the absorptivity of the flowing solutions when the electric vector of the incident light is polarized parallel and perpendicular, respectively, to the direction of alignment of the particles. Dichroism is defined as the difference between these two curves, and the dichroic ratio is defined as their ratio. The dichroism of fd is positive for all wavelengths longer than 262 mμ with a maximum at 279 mμ, and negative for all wavelengths between 262 and 239 mμ with a mini-

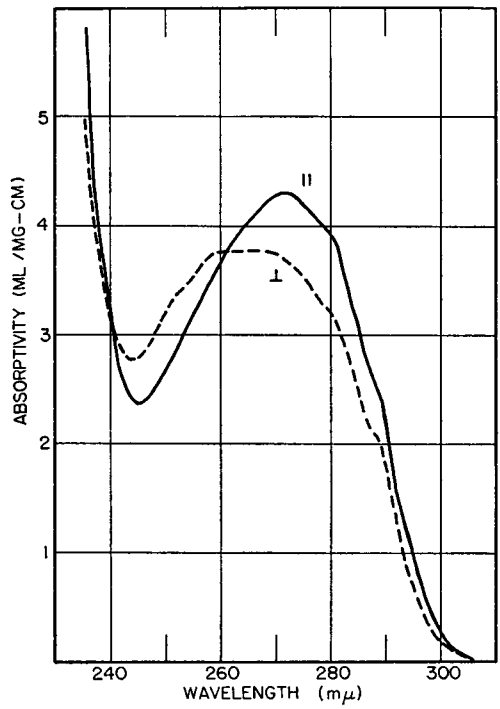

FIGURE 1 UV dichroic spectra of fd bacteriophage. The solid and dashed curves represent the absorption spectra of oriented fd with the incident light polarized parallel and perpendicularly, respectively, to the long axis of the virus.

mum at 250 mμ (Fig. 2). Six repeated experiments exhibited reproducible dichroism curves varying by less than 10% at 280 mμ. Salt concentrations of from 0 to 5 molal NaCl had no effect on the appearance of the curves.

INTERPRETATION AND DISCUSSION

The results of Schachter et al. (in press) on TMV showed that ultraviolet dichroism can be used as a powerful tool to investigate the internal structure of a rod-shaped virus. That work was successful because the authors were able to compare the dichroism of repolymerized protein with that of the whole virus and, therefore,

were able to separate out the dichroic contribution of the nucleic acid. Since in this study attempts to repolymerize the fd coat protein were unsuccessful, a different method was employed to calculate the dichroic contribution of the DNA within the virus. This treatment is by nature approximate but accounts qualitatively for the observed results and supports the intuitive interpretation that the DNA dichroism is negative and the protein dichroism is positive.

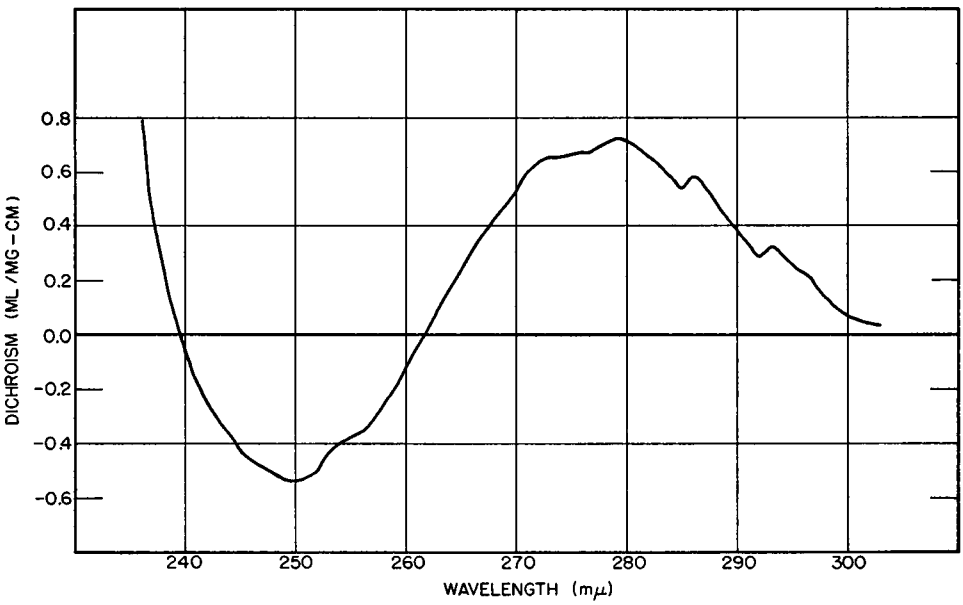

FIGURE 2 Dichroism of fd bacteriophage.

By assuming that the total absorption is the sum of the absorptions of each kind of chromophore, the following identity may be written for each wavelength λ:

$$D(\lambda) = a_{\parallel}(\lambda) - a_{\perp}(\lambda) = \sum_i a_{u_i}(\lambda) \frac{a_{\parallel_i}(\lambda) - a_{\perp_i}(\lambda)}{a_{u_i}(\lambda)}$$

$$= \sum_i a_{u_i}(\lambda) \left[\left(\frac{a_{\parallel_i}(\lambda) - a_{\perp_i}(\lambda)}{a_{\perp_i}(\lambda)} \right) \Big/ \left(\frac{\frac{1}{3}[a_{\parallel_i}(\lambda) + 2a_{\perp_i}(\lambda)]}{a_{\perp_i}(\lambda)} \right) \right] \quad (1)$$

$$= 3 \sum_i a_{u_i}(\lambda) \frac{R_i(\lambda) - 1}{R_i(\lambda) + 2}$$

(after Higashi et al., 1963). Assuming, too, that $R_i(\lambda)$ is constant with respect to wavelength, one may write:

$$D(\lambda) = 3 \sum_i a_{u_i}(\lambda) K_i \quad (2)$$

where $K_i = (R_i - 1)/(R_i + 2)$.

Since, in the simplest case the contributions to $D(\lambda)$ arise from only two kinds of chromophores, protein and DNA, one may write:

$$D(\lambda) = a_{u_1}(\lambda)K_1 + a_{u_2}(\lambda)K_2 \tag{3}$$

where subscript 1 refers to protein and subscript 2 refers to DNA. Implicit in this equation is that the dichroic ratios of adenine, thymine, cytosine, and guanine in the virus are the same, and that the dichroic ratio of tyrosine is the same as that of tryptophan. This assumption seems reasonable for the DNA because of the similar spectra of its bases and because each species of base most likely has the same average

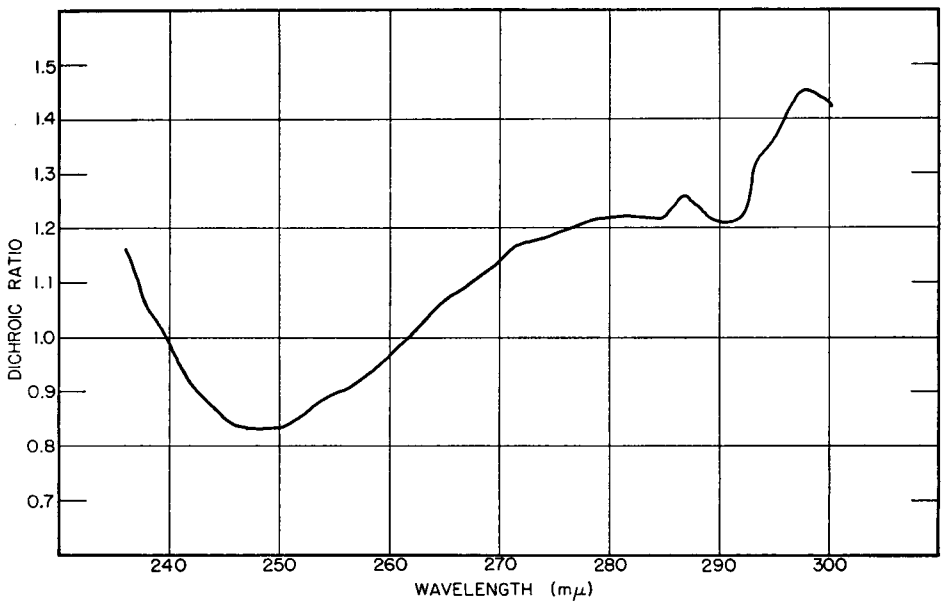

FIGURE 3 Dichroic ratio of fd bacteriophage.

orientation as the others, but it must be considered an approximation for the protein.

The two constants were selected by simultaneously solving equation 3 at two wavelengths using the absorption spectra of isolated DNA and protein corrected for viral composition. For a DNA absorptivity at 260 mμ of 23.8 $(\text{mg/ml} - \text{cm})^{-1}$ as calculated from the data of Hoffmann-Berling et al. (1963), and a protein absorptivity at 280 mμ of 1.60 $(\text{mg/ml} - \text{cm})^{-1}$ (R. Knippers, personal communication), the wavelengths of 250 and 282 mμ yield values of $K_1 = 0.828$ and $K_2 = -0.360$. If these values are substituted at all wavelengths back into equation 3, one can produce the artificially constructed dichroism curve shown in Fig. 4. It has a shape similar to that of the experimentally arrived at dichroism curve, but

appears shifted to longer wavelengths by as much as 5 mμ. This discrepancy may be attributed to spectral changes of either the DNA or protein occurring as a result of the virus disruption. In support of this interpretation, if either the protein spectrum is shifted a few millimicrons to the blue or the protein spectrum is repaced by those of tryptophan and tyrosine and equation 3 solved for three unknowns or the DNA in the virus is assumed to exhibit an $n \rightarrow \pi^*$ transition accounting for more than 20% of the absorption at 280 mμ (Fresco et al., 1961; Brahms, 1963; Gellert, 1961), then artificial dichroism curves which are more nearly congruent with the experimentally measured one can be constructed. Such calculations, how-

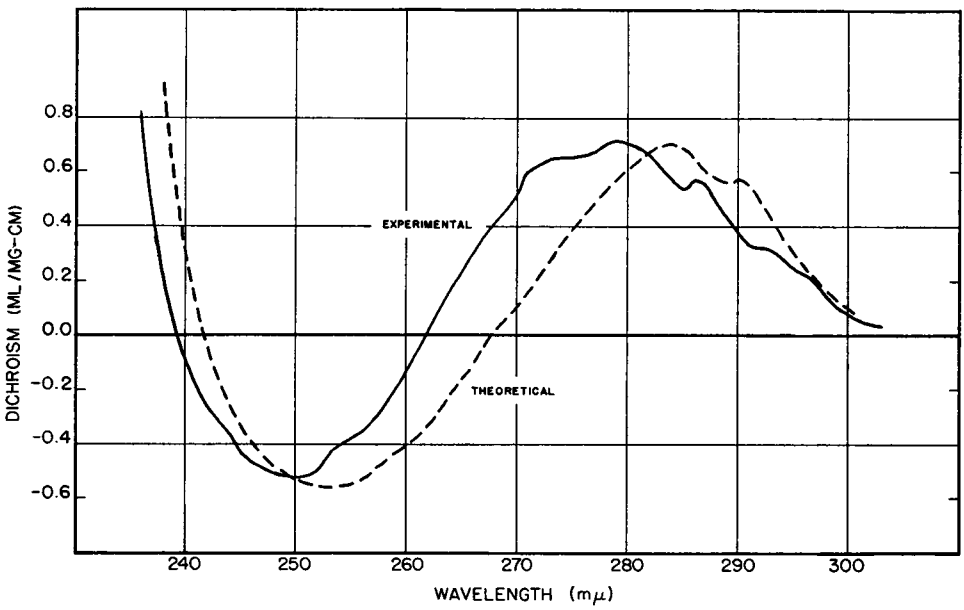

FIGURE 4 Experimental dichroism (solid curve) compared with the calculated dichroism (dashed curve). The solid curve was obtained experimentally while the dashed curve was calculated as described in the text.

ever, introduce a great deal of uncertainty and do not appreciably alter the conclusions arrived at in the following paragraphs.

For perfectly oriented linear absorbers in a fiber,

$$R = 2 \tan^2 \theta \tag{4}$$

where 90°-θ is the angle between the axis of the absorber and the axis of the fiber, whereas for planar absorbers, such as benzene,

$$R = \frac{\sin^2 \theta}{1 - \frac{1}{2} \sin^2 \theta} \tag{5}$$

where θ is the angle between the normal to the absorbing plane and the fiber axis (Fraser, 1953). If a flowing solution is considered to be composed of a fraction, f, of completely oriented virus and a fraction, $(1 - f)$, of completely disoriented virus, then the equations become,
for the linear case:

$$R = \frac{f \sin^2 \theta + \frac{1}{3}(1 - f)}{f/2 \cos^2 \theta + \frac{1}{3}(1 - f)} \tag{6}$$

for the planar case:

$$R = \frac{f \sin^2 \theta + \frac{2}{3}(1 - f)}{f(1 - \frac{1}{2} \sin^2 \theta) + \frac{2}{3}(1 - f)} \tag{7}$$

where $R = (3 + 2K_i)/(3 - K_i)$.

TABLE I
VALUES OF θ

$f =$	0.4	0.5	0.6	0.7
	°	°	°	°
DNA, linear	22	25	27	28
DNA, planar		36		
Protein, linear	63	57	53	50

The dichroic ratio of T2 DNA at 260 mμ was determined to be 0.4 in our apparatus. When this value is combined with the known value of the base plane tilt for the B form of DNA, one obtains a value for f of 0.5. T2 DNA is unusually "stiff" hydrodynamically (Wada, 1964) and since fd is probably more rigid than DNA, $f = 0.5$ would seem to be a conservative estimate for this parameter. Table I presents the values of θ calculated from equations 6 and 7 for the linear and planar possibilities, assuming various degrees of complete orientation.

The effect of errors in the assumed absorptivity on the angle θ should be considered. Hoffmann-Berling et al. (1963) indicated that the DNA in fd exhibits little hypochromism relative to the isolated DNA in solution. Even so, assuming an error in K_2 of $\pm 20\%$, due to inaccurate absorptivity data, the range of θ calculated from equation 6 for $f = 0.5$ is 22–27°, indicating that this constitutes a potential error of the same order of magnitude as that due to the uncertainty of the virus orientation.

No mention has specifically been made of the obvious fact that all the chromophores of one kind may not have the same angle θ. For example, there is no reason to suppose that the three tyrosine residues in each protein subunit are oriented in exactly the same way. The observed dichroism must always be considered to be a

net effect. For DNA, however, it is possible to imagine highly ordered configurations where the angle θ would be very nearly the same for each base in the virus.

With the possible exception of tyrosine, the aromatic absorbers (DNA bases and tryptophan) possess a sufficiently low degree of symmetry so that one would expect essentially linear absorption. Thus, while the degree of virus orientation can only be guessed at, a tilt angle of 20–30° for the DNA base planes would appear to be a reasonable estimate. Interpretation for the protein is more difficult, but it is clear that the residues responsible for the dominant absorption at 280 mμ are oriented on the average more parallel than perpendicular to the long axis of the virus, a result also found for all other rod-shaped proteins thus far studied, including TMV protein, type I pili of *E. coli*, and the flagella of *Salmonella typhimurium* (Schachter, personal communication).

The authors are grateful to Dr. R. Knippers at the Max Planck Institut für Virusforschung, Heidelberg, Germany for his helpful suggestions concerning the preparation of the virus protein and to Dr. Lauffer and Dr. Schachter for their participation in several valuable discussions.
This work was supported by grants from the United States Public Health Service (GM 10403 and 5 F1-GM-25, 558-02) and from the National Science Foundation (G-24046).

Received for publication 17 October 1966.

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