Biophysical Chemistry 14 (1981) 283–291
Elsevier/North-Holland Biomedical Press



# STRUCTURE AND ARCHITECTURE OF THE BACTERIAL VIRUS fd. AN INFRARED LINEAR DICHROISM STUDY

Hartmut FRITZSCHE

*Academy of Sciences of the G.D.R., Central Institute of Microbiology and Experimental Therapy, DDR-6900 Jena, G.D.R.*

Timothy A. CROSS, Stanley J. OPELLA and Neville R. KALLENBACH

*University of Pennsylvania, Department of Chemistry and Biology, Philadelphia, PA 19104, U.S.A.*

Received 27 March 1981
Revised manuscript received 1 June 1981

Oriented gels of intact bacterial virus fd have been investigated by infrared linear dichroism. Infrared absorption band maxima and dichroism indicate an α-helix content of the major coat protein of 95–100%. The α-helical rods of the coat protein are aligned parallel to the long axis of the virion with an inclination roughly estimated to ≈37°. The presence of DNA infrared bands at 968, 885, 830 and 799 $cm^{-1}$, the absence of a band at 860 $cm^{-1}$ and the perpendicular polarization of the symmetric $PO_2^-$ stretching vibration at 1085 $cm^{-1}$ are all indicative of a B-type backbone conformation in the single-stranded DNA. We find no evidence for specific interaction between aromatic side groups (phenylalanine, tyrosine) and the DNA bases. Our results independently confirm most features of the model of Marvin and co-workers [2,15] based on low-resolution X-ray diffraction studies. However, our findings contradict their suggestion of an A-type DNA in the bacterial virus fd. Two results are consistent with rigid and stable order in the virus. First, over a 4-day period, 65% of the peptide hydrogens remain unexchanged with deuterium. Second, changes in the relative humidity of the sample do not result in any shifts in the DNA spectrum that are characteristic of free DNA.

## 1. Introduction

Filamentous bacterial viruses are the smallest known DNA phages. They consist of long rods of coat protein enclosing a single-stranded circle of DNA [1,2]. The strain fd has a diameter of ≈9.0 nm and a length of 890 nm [3–6] and contains a DNA of 6400 nucleotides [6]. The capsid is composed largely (≈99%) of one major coat protein (2710 subunits) plus about 4 copies of a minor protein, the absorption protein or A-protein [7–10]. There may be a few copies of other minor coat proteins [11,12] as well. There are 2.36 DNA bases per major coat protein subunit in the virus [6]. Amino acid [13] sequences are known as well as base composition [2,3]. Filamentous viruses can form oriented gels suitable for X-ray diffraction analysis [15] and ultraviolet linear dichroism studies [14]. Low-resolution X-ray diffraction data and molecular model building have resulted in a structural model of the virus in which small helical rods of coat protein are organized so as to overlap like shingles or fish scales [15].

However, the available diffraction data are at low resolution, and the DNA has not yet been detected in the diffraction pattern. * Thus, there is an important role in elucidating details of the structure and organization of both the protein and DNA components of fd for independent alternative spectroscopic studies, including NMR and Raman spectra [16] as well as the infrared measurements we report here. We have studied oriented gels of bacterial virus fd by infrared linear dichroism under different conditions. By this tech-

* Very recently, layer lines of the X-ray diffraction pattern of magnetically aligned fd virus have been assigned to DNA [15a].

0301-4622/81/0000–0000/$02.50 

nique the alignment of the major constituents with respect to the particle axis can be determined. Moreover, the conformation of both coat protein and DNA can be analyzed both by the dichroism as well as characteristic absorption band maxima.

To our knowledge, this is the first infrared dichroism study of an intact organism.

## 2. Experimental section

### 2.1. Materials

Bacterial virus fd was isolated and purified by methods described previously [7]. Several drops of a concentrated purified aqueous suspension were placed on an infrared transparent KRS-5 (TlBr-TlI) disk. Excess water was removed by equilibrating in an atmosphere of 75% relative humidity controlled by a saturated NaCl solution.

### 2.2. Methods

Gels were oriented by unidirectional stroking with a spatula. By this procedure, the filamentous virus particles are aligned preferentially in the stroking direction.

The oriented virus sample on the KRS-5 disk was placed inside an infrared cell equipped with a second KRS-5 disk and a glass vessel containing saturated salt solution to control the relative humidity of the cell. Infrared spectra were recorded on a Perkin Elmer model 325 spectrophotometer equipped with a wire grid polarizer. Details of the measurement and the calculation of the dichroic ratio have been described previously [17,18]. Hydrogen-deuterium exchange was carried out by exposing the oriented gel to a $D_2O$ atmosphere of controlled humidity. This was done by replacing the saturated $H_2O$ salt solution in the cell with an equivalent saturated $D_2O$ salt solution. Temperature studies were performed using a variable temperature cell manufactured by Perkin Elmer.

## 3. Results

The infrared spectrum of the oriented virus gel is dominated by amide group vibrations of the coat protein. Beside these strong bands, several weak and less clearly resolved bands can be identified. Variation of the relative humidity between 96 and 24% has no significant influence on the spectrum.

The polarized spectrum of the oriented gel of virus fd is shown in fig. 1. The dichroism of the sample is low but well pronounced. Amide A at 3300 $cm^{-1}$ as well as amide I at 1650 $cm^{-1}$ are polarized parallel in contrast to amide II at 1540 $cm^{-1}$ which is perpendicularly polarized.

Most of the bands have been assigned on the basis of the known primary structure of the coat protein [13] together with the known spectra of DNA in different conformations [19,20] (table 1).

Change of the $H_2O$ atmosphere inside the in-

Table 1

Infrared absorption maxima, dichroism behavior and assignment of an oriented gel of bacterial virus fd (97% relative humidity, $H_2O$ atmosphere)

| Maximum position ($cm^{-1}$) | Dichroism behavior | Assignment |
|---|---|---|
| 3 290 | ∥ | amide A |
| 3 060 | ∥ | amide B |
| 2 960 | | CH stretching |
| 2 955 | | CH stretching |
| 2 870 | | CH stretching |
| 1 651 | ∥ | amide I |
| 1 542 | ⊥ | amide II |
| 1 453 | | CH deformation |
| 1 415 | | CH deformation |
| 1 395 | | CH deformation |
| 1 385 | | CH deformation |
| 1 295 | ∥ | amide III |
| 1 230 | | antisymmetric $PO_2^-$ |
| 1 165 | | |
| 1 085 | ⊥ | symmetric $PO_2^-$ |
| 968 | | DNA backbone |
| 935 | | amide C-C-N stretching |
| 885 | | DNA backbone |
| 830 | | DNA backbone |
| 800 | | |
| 775 | | DNA backbone |
| 752 | | phenylalanine and/or tyrosine |
| 697 | ⊥ | |
| 660 | | |
| 620 | ⊥ | amide V |

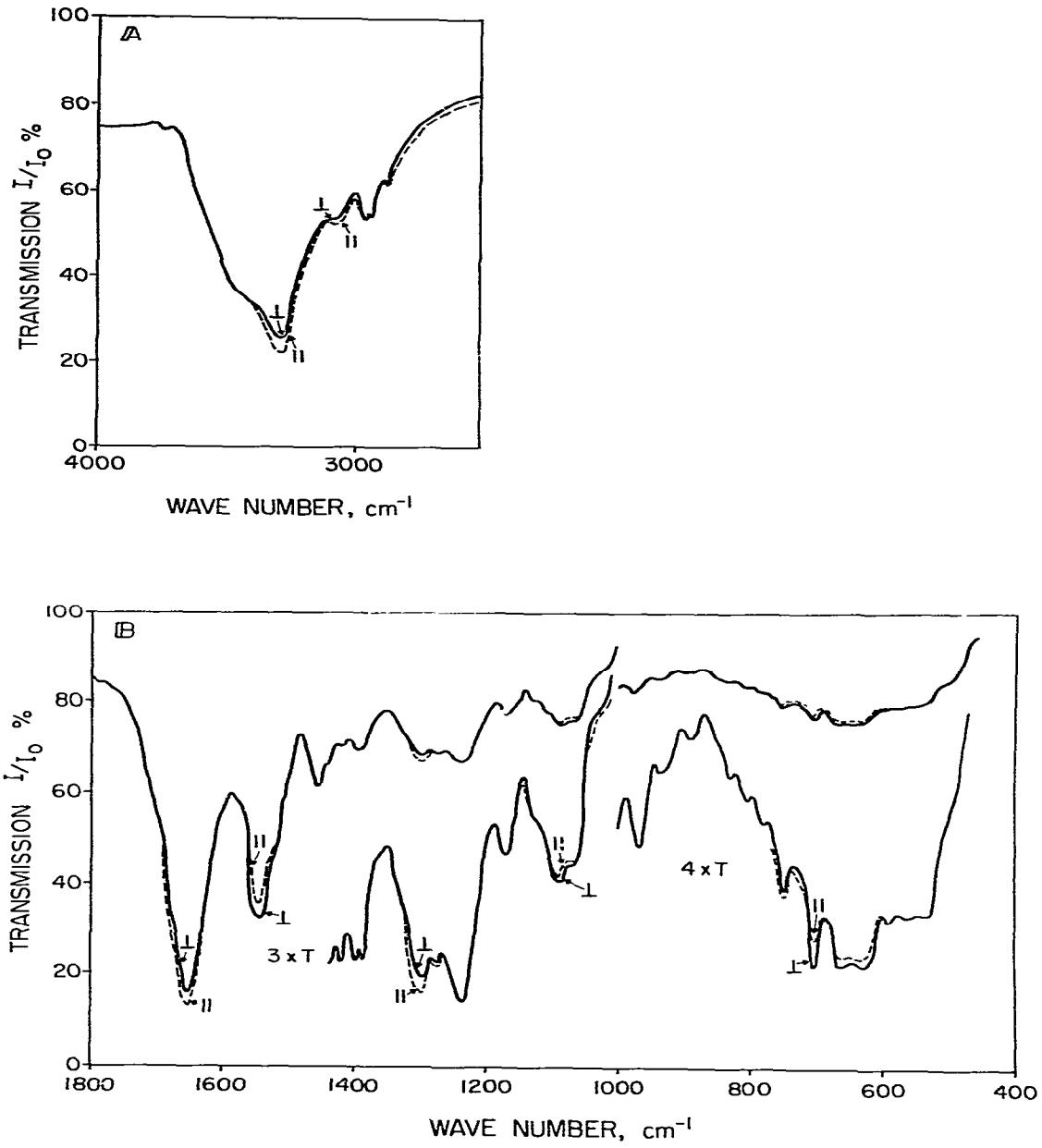


Fig. 1. Polarized infrared spectrum of an oriented gel of bacterial virus fd. The gel is equilibrated with an $H_2O$ atmosphere of 97% relative humidity. Transmission is plotted against wavenumber in $cm^{-1}$. 3×T and 4×T are spectra expanded by a factor of 3 and 4, respectively, on the transmission scale. Full line: electric vector of the polarized radiation perpendicular to orientation direction; broken line: electric vector parallel to orientation direction. (A) The region around 3000 $cm^{-1}$. (B) The region from 400 to 1800 $cm^{-1}$.

frared cell to a $D_2O$ atmosphere is accompanied by a hydrogen-deuterium exchange of exchangeable OH and NH groups. At pH 7 the peptide backbone NH groups are exchanged incompletely as monitored by intensity changes of amide A, amide II and amide II' (table 2); after 4 days of incubation the fraction of nonexchanged peptide NH groups has been determined to be $\approx 65\%$. This partial hydrogen-deuterium exchange is accompanied by changes in the infrared spectrum (table 3). These changes are related to peptide vibrations: amide A, amide II, amide III, and amide C-C-N stretching. The amide I shifts only slightly from 1651 to 1648 $cm^{-1}$. This behavior is consistent with $\approx 35\%$ H-D exchange of the amide NH of the peptide groups of coat protein over the time period monitored.

Temperature variation between 30 and 75°C is accompanied by a small shift of the amide II band from 1536 to 1533 $cm^{-1}$ ($\pm 0.5$ $cm^{-1}$) with increasing temperature. The shift is reversible.

Incubation in $D_2O$ vapor at 55°C results in a complete hydrogen-deuterium exchange of the peptide NH within $\approx 3$ h. (table 2). The changes in the infrared spectrum are consistent with the complete replacement of the NH by ND.

Table 2

Intensity changes of amide bands of virus fd coat protein in the course of H-D exchange of a gel exposed to $D_2O$ vapor.

| Infrared band (position in $cm^{-1}$) | Exposition time (h) | Absorbance [a] | %of exchanged amide protons |
|---|---|---|---|
| Amide II | 0 | 0.340 | 0 |
| (1545 $cm^{-1}$) | 2 | 0.261 | 23.2 |
| | 72 | 0.218 | 35.9 |
| Amide II' | 0 | 0.040 | |
| (1440 $cm^{-1}$) | 24 | 0.225 | |
| | 72 | 0.224 | |
| Amide A | 0 | 0.378 | 0 |
| (3290 $cm^{-1}$) | 1 | 0.287 | 24.1 |
| | 4 | 0.269 | 28.8 |
| | 72 | 0.264 | 30.2 |

[a] Amide II and amide II': relative absorbance refers to the absorbance of amide I at 1650 $cm^{-1}$; amide A: absolute absorbance.

Table 3

Changes of the polarized infrared spectrum of an oriented gel of bacterial phage fd by hydrogen-deuterium exchange

| | Bands (position in $cm^{-1}$) |
|---|---|
| Intensity decrease | 3290 (amide A), 3060 (overtone of amide II), 1545 (amide II), 1300 (amide II), 935 (amide C-C-N stretching) |
| New bands | 2375 (amide A'), 1440 (amide II') |

## 4. Discussion

### 4.1. Secondary structure of the coat protein

The virus fd consists of 88% coat protein, 12% DNA and a neglible amount of other proteins [6]. The characteristic amide group vibrations reflect the secondary structure of the coat protein. The wavenumbers of amide I, amide II, amide III and C-C-N stretching (table 1) are in full agreement with the values expected for $\alpha$-helices which are $1650 \pm 5$, $1545 \pm 5$, $1285 \pm 15$ and $935 \pm 5$ $cm^{-1}$, respectively [21]. Significant $\beta$-pleated sheets and random-coiled forms can be excluded by our findings; they would absorb at (in brackets are the values of the random coil) $1665 \pm 5$ ($1665 \pm 5$), $1530 \pm 5$ ($1525 \pm 5$), $1235 \pm 5$ ($1248 \pm 5$) and ($960 \pm 5$) $cm^{-1}$, respectively [21]. If $\beta$-sheets or random coils are present, their fraction should be less than 5%, otherwise a shoulder at 1665 $cm^{-1}$ as well as new bands between at 1520 and 1530 $cm^{-1}$ would occur.

In previous studies the $\alpha$-helical structure of the coat protein has been inferred from different spectroscopic studies [22–24]. Our results fully support these earlier findings.

### 4.2. Orientation of coat protein

According to the model of the $\alpha$-helical rods of Marvin and his co-workers [15] the coat proteins run almost parallel to the phage long axis and overlap radially like shingles or fish scales. The orientation of the $\alpha$-helical rods of coat protein with respect to the particle axis can be established independently by infrared linear dichroism of oriented gels of the virus.

Beetz et al. [24a] recently disputed the reliability of structural information obtained from infrared linear dichroism. Their criticism holds true in the absence of reliable values of the direction of the transition dipole moments of the vibrations. In this paper we avoided deducing any quantitative structural parameters of the DNA from infrared linear dichroism data. Nevertheless, the dichroism data are very convenient to determine the conformation of the DNA backbone. On the other hand, structural information on the coat protein can be deduced from infrared linear dichroism data, since reliable values of the direction of the transition moments of the peptide vibrations are available.

The angle $\theta$ between the phage axis and the transition moment of a vibrational transition can be calculated from the measured dichroic ratio $R = A_\perp / A_\parallel$ using the expression of Fraser [25]:

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

where $A_\perp$ and $A_\parallel$ are the absorbances with perpendicular and parallel polarized radiation relative to the phage axis, respectively. In eq. (1), $f$ is the fraction of perfectly oriented phages, and both $\theta$ and $f$ represent unknown quantities. However, reasonable limits can be estimated from the experimental $R$ values as follows.

The transition moment of the amide I vibration is tilted 29–34° with respect to the helix axis [26]. Hence, from the $R$ values in table 4 we obtain $f = 2$–$10\%$ for the degree of orientation in the sample. The moment of the amide II vibration on the other hand is inclined between 75 and 77° from the $\alpha$-helix axis [25], and from this $f = 15$–$17\%$. Finally, if we assume a B DNA conformation for the DNA molecule (see below), the observed value $R = 1.15$ for the symmetric phosphate stretching vibration at 1085 $cm^{-1}$ yields $f = 21\%$, since the transition moment is inclined by 64.3° from the helix axis [18]. Each of these estimates assumes that both the DNA and coat protein

Table 4

Angle $\vartheta$ between the helix axis of the coat protein subunits and the particle axis of the filamentous virus fd calculated from the infrared linear dichroic ratios $R = A_\perp / A_\parallel$ by eq. (2) under the assumption of $f = 21$ and 36% of sample I and sample II, respectively, where $f$ is the fraction of perfectly oriented particles of the investigated virus preparations. Amide I' and amide II' are the respective vibrations under conditions of complete hydrogen-deuterium exchange of the amide groups of the coat protein, amide $A^-$ and amide $I^-$ are the respective vibrations under conditions of an incomplete hydrogen-deuterium exchange after 3 day exposition to $D_2O$ vapor at room temperature (cf. text). $\theta$ is the angle between the transition moment of the vibration and the $\alpha$-helix axis; the $\theta$ values are taken from the literature [25,26].

| Vibration | $\theta$ (°) | Dichroic ratio [a] ($R = A_\perp / A_\theta$) | | $\vartheta$ (°) | |
|---|---|---|---|---|---|
| | | Sample I | Sample II | Sample I | Sample II |
| Amide A | 28 [b] | 0.84 | 0.79 | 38.3 | 41.6 |
| Amide $A^-$ | 28 [b] | 0.78 | 0.72 | 30.6 | 36.2 |
| Amide I | 31.5 ± 2.5 [c] | 0.94 | 0.84 | 48.1 | 43.8 |
| Amide $I^-$ | 31.5 ± 2.5 [c] | 0.92 | | 45.9 | |
| Amide I' | 31.5 ± 2.5 [c] | | 0.88 | | 46.7 |
| Amide II | 76 ± 1 [c] | 1.22 | 1.43 | 24.7 | 24.1 |
| Amide II' | 76 ± 1 [c] | | 1.34 | | 30.1 |
| Mean value | | | | 37.5 ± 11.1 | 37.1 ± 9.5 |
| DNA $PO_2^-$ (1085 $cm^{-1}$) | 64.3 [d] ± 0.3 | 1.15 | 1.28 | (0) | (0) |

[a] Error less than 4%.
[b] Ref. [25].
[c] Ref. [26].
[d] Ref. [18].

subunits are perfectly parallel to the long axis of the virion. While it is difficult to assess the validity of this assumption for the DNA molecule, it must be noted that any deviation of the protein subunits from this parallel orientation will lead to an increase in the true value of $f$. Thus, $f = 21\%$ represents the *minimum* value for the oriented fd sample.

A second preparation of an oriented gel of the virus fd resulted in a slightly better degree of orientation. This is indicated by $f = 36\%$ calculated from the dichroic ratio $R = 1.28$ of the symmetric phosphate vibration at 1085 $cm^{-1}$ under the same assumptions made above.

The mean angle $\vartheta$ between the axis of the $\alpha$-helical coat protein subunits and the particle axis can in fact be estimated by an equation deduced from the general relation [25a]:

$$\frac{1-R}{2+R} = \left\langle \frac{3\cos^2\theta - 1}{2} \right\rangle \left\langle \frac{3\cos^2\vartheta - 1}{2} \right\rangle \times \left\langle \frac{3\cos^2\alpha - 1}{2} \right\rangle \qquad (2)$$

where $\alpha$ is the angle of the virus particles with respect to the orientation direction. It has been shown that the orientation function $\langle 3\cos^2\alpha - 1/2 \rangle$ equals $f$, the fraction of perfectly oriented molecules [26a]. Substituting $f$ into eq. (2), the following equation results:

$$R = \frac{A_\perp}{A_\parallel} = \frac{\frac{1}{2}\sin^2\theta + \dfrac{\sin^2\vartheta + g}{2 - 3\sin^2\vartheta}}{\cos^2\theta + \dfrac{\sin^2\vartheta + g}{2 - 3\sin^2\vartheta}} \qquad (3)$$

with $g = \frac{3}{2}(1-f)/f$. With $f = 0.21$ for sample I and $f = 0.36$ for sample II, respectively, eq. (3) yields $\vartheta = 37 \pm 11°$ (table 4). The considerably scattering values of $\vartheta$ may be explained by the arbitrariness of the base-line procedure to obtain the absorbances $A_\perp$ and $A_\parallel$ instead of the more correct integral intensities of the infrared bands. This explanation is supported by the fairly good agreement of corresponding $\vartheta$ values of sample I and sample II in table 4; the difference is only 0.6–5.6°. The mean values of $\vartheta$ are in good agreement also, and underline the reproducibility of the infrared dichroism experiments despite the low orientation of both samples.

The angle $\vartheta = 37 \pm 11°$ is slightly greater than the $\approx 30°$ of Marvin's model [15]. It should be emphasized, however, that the dichroism method implies two assumptions: (i) the DNA is aligned parallel to the virion axis, i.e., $\vartheta = 0°$. (ii) The phosphate group of the single-stranded circular DNA has the same geometry as double stranded DNA in its standard B form.

The first assumption is generally accepted [2,15a]. The second assumption is the crucial point. We used $\theta = 64.3°$ for the inclination of the transition moment of the 1085 $cm^{-1}$ phosphate vibration to the DNA helix axis, the mean value found in an extensive study of calf thymus DNA films in the B form [18]. It can easily be shown by the use of eqs. (1) and (3) that the experimental results of the amide I and amide II dichroism (table 4) are consistent with $\theta$ of the 1085 $cm^{-1}$ phosphate vibration only within the limits $58° < \theta_{1085} < 68°$. Outside these limits, either $f$ exceeds 100% ($\theta_{1085} < 58°$) or nondefined $\vartheta$ values of amide II ($\theta_{1085} > 68°$) are found. These hold for both samples I and II. The limits of $\theta_{1085}$ simply given by the consistency of the data suggest a phosphate geometry very near to that of B-DNA. As discussed below, infrared and Raman data support a backbone conformation very similar to the standard B form of DNA. These findings justify a tentative value of $\theta = 64.3 \pm 0.3°$ to estimate roughly the angle $\vartheta$ by eq. (3).

Due to all implied uncertainties, the calculated angle $\vartheta \approx 37°$ can be regarded only as a very rough estimation. Nevertheless, the data of table 4 clearly demonstrate a significant inclination of the coat protein subunits with respect to the virus axis.

### *4.3. Packing and interaction of the coat protein and DNA*

Indirect information on the packing of DNA and coat protein can be obtained (i) by variation of the controlled relative humidity to which the oriented film of the virus is exposed and (ii) from hydrogen-deuterium exchange measurements.

The antisymmetric $PO_2^-$ vibration of DNA normally shifts to higher wavenumbers with decreas-

ing relative humidity. To a first approximation the shift is independent of whether the DNA is native or denatured, single or double stranded. Native double-stranded DNA in its B form (predominant at high relative humidity $>90\%$) absorbs at 1222 ($\pm 0.5$) $cm^{-1}$, in its A form (predominant at 60–80% relative humidity) at 1237 $cm^{-1}$ [32]. Further shift of the antisymmetric $PO_2^-$ vibration occurs at further dehydration of the DNA when the ordered structure is successively replaced by disordered forms.

The band maximum of the single-stranded circular DNA in situ of bacterial virus fd occurs at ($1230 \pm 1$) $cm^{-1}$ under high humidity conditions (97%). No significant shift can be found even at relative humidities as low as 23%. Free accessibility of DNA to water should result in a shift to higher wavenumber of at least 10–15 $cm^{-1}$ going from 97 to 23% relative humidity. The constant position at 1230 $cm^{-1}$ indicates a hydration sphere equivalent to an atmosphere of $\approx 80$–85% relative humidity, constrained so as to be incapable of the alterations in structure with relative humidity that occur in free DNA. The hydrogen-deuterium exchange of amide NH comprising the backbone peptide groups is limited to 35% in our samples, over a period of 4 days at room temperature. Hydrogen-deuterium exchange in the case of most known proteins has been observed to involve peptide hydrogens that exchange at rates ranging from the so-called "free" peptide rate to peptide hydrogens that exchange at rates which can be eight or nine orders of magnitude slower [27]. If we use the opening-limited scheme of Englander and Englander [27],

$$[\text{N-H}]_{\text{internal}} \underset{k_{\text{cl}}}{\overset{k_{\text{op}}}{\rightleftharpoons}} [\text{N-H}]_{\text{open}} \overset{k_{\text{chem}}}{\rightarrow} \text{H exchanged}$$

the situation in native proteins near pH 7 corresponds to one in which the chemical rate is slow, and thus the observed exchange rate is

$$k_{\text{ex}} = k_{\text{op}}\ k_{\text{chem}} / (k_{\text{op}} + k_{\text{cl}} + k_{\text{chem}}) \approx \left(\frac{k_{\text{op}}}{k_{\text{chem}}}\right) k_{\text{chem}} = K_{\text{eq}}\ k_{\text{chem}} \qquad (3)$$

where $K_{\text{eq}}$ denotes the equilibrium constant for the opening event. Based on the data of Molday et al. [28], at pH 7 and 20°C the exchange rate of an average peptide is about 10 $s^{-1}$. Since in $\approx 100$ H 65% of the peptides have not exchanged, we can conclude that for these $K_{\text{eq}} \leq 10^{-7}$. Structural opening of the peptides of the coat protein is thus far more difficult than for any simple $\alpha$-helix structure by a factor of $\approx 10^3$ and thus probably reflects substantial additional stabilizing interactions present within the virion. The 35% of peptides which exchange include both "free" peptides groups present as well as those $\alpha$-helical groups that are not involved in quaternary interactions.

Under the more severe conditions of 55°C, both $K_{\text{eq}}$ and $k_{\text{chem}}$ increase and complete exchange occurs within 3 h, as seen in table 2.

The lack of variation in any bands with change in humidity and the hydrogen-deuterium exchange behavior suggest that the structure involves a rigidly held DNA molecule complexed within a stable coat protein matrix. This picture is in agreement with $^{31}$P-NMR studies of the phosphate chemical shielding tensor in fd [31] and with other solid-state NMR studies on the coat protein of the virion.

### *4.4. Structure of DNA*

The DNA of bacterial virus fd is circular and single stranded [1,2]. The circle is assumed to be folded in two, with the two strands oriented parallel to the long axis of the particle. Base composition and noncooperativity of melting rule out extensive base pairing [2]. A highly base-stacked structure of DNA with a base tilt of about 20° has been deduced from ultraviolet linear dichroism results [14] as well as from model building studies [2,15a]. Therefore, the A form of DNA has been considered as most likely [2]. A Raman study, however, failed to detect the Raman band at 810 $cm^{-1}$ characteristic for A-DNA [16].

We have made a further attempt to elucidate the DNA structure by infrared spectroscopy including linear dichroism. Unfortunately, the base vibrations at 1650–1700 $cm^{-1}$ of DNA are fully masked by the strong amide I vibration band of the coat protein. Therefore, the inclination of the DNA bases with respect to the long axis of the virion cannot be calculated from the dichroism

data. The symmetric $PO_2^-$ vibration at 1085 $cm^{-1}$, however, can be observed and is perpendicularly polarized. This is clearly consistent with the B-type backbone structure. A-DNA is characterized by a parallel polarization of the 1085 $cm^{-1}$ band [32]. Typical infrared bands of B-DNA are at 966, 890, 835 and 779 $cm^{-1}$, those of A-DNA at 860 and 815 $cm^{-1}$ [18,19]. We observe in the spectrum of bacterial virus fd bands at 968, 830 and 779 $cm^{-1}$ (fig. 1 and table 1) but no band between 830 and 885 $cm^{-1}$. This indicates absence of any A-type backbone structure of the viral DNA in situ. There is no contradiction between the postulated 20° base tilt of the viral DNA and the exclusion of A-DNA. Under special conditions, duplex DNA in aqueous solution may in fact assume a $\approx 20°$ base-tilted conformation as shown recently [33]. Single-stranded DNA is presumably even more flexible with respect to the base tilt angle than duplex DNA.

Exposure of the oriented gel to 23% relative humidity is not accompanied by any significant change in the infrared bands of the DNA. This agrees with the results discussed in the preceding paragraph concerning the lack of conformational freedom of the DNA molecule within the virion, in contrast to DNA free in solution.

Summarizing, our results are consistent with a single-stranded DNA molecule oriented parallel to the long axis of the virion, with a backbone conformation closer to the B type, than A.

### *4.5. Coat protein-DNA interaction*

An unresolved question concerning fd virus centers on the nature of the interaction between coat protein and the enclosed circle of single-stranded DNA. The basic C-terminal part of the coat protein (residues 40–50) contains 4 lysine, 3 serine + threonine and 2 phenylalanine side chains and is assumed to be attracted by DNA predominantly via electrostatic forces [22]. Specific interaction could occur by intercalation of aromatic side groups (3 phenylalanine, 2 tyrosine, 1 tryptophan) between DNA bases or by hydrogen bonds to the bases (4 serine, 3 threonine). The preference of tyrosine and tryptophan for single-stranded DNA has been demonstrated [34,35]. Regular arrangement of aromatic side groups as a consequence of specific interaction with DNA bases should be detectable by infrared dichroism of the respective infrared bands, particularly in the 700–900 $cm^{-1}$ region where the out-of-plane CH deformation vibrations occur [36]. We have located infrared bands in this region (fig. 1, table 1). The only candidates for phenylalanine and tyrosine vibrations are the bands at 800 and 745 $cm^{-1}$; the out-of-plane deformation is expected near 735 $cm^{-1}$ [36]. Neither observed band exhibits significant dichroism. Hence, involvement of any of the aromatic amino acid side chains in a specific interaction to DNA bases in the virion seems unlikely.

### *4.6. Molecular architecture of bacterial virus fd*

Our results provide a completely independent test of several features of the model for fd virus proposed by Marvin and Wachtel [15]. Thus, we confirm both the extremely high $\alpha$-helical content of the coat protein as well as its roughly parallel alignment of the long axis.

The only discrepancy with the model of virus fd concerns the backbone structure of DNA. We do not confirm the A-type DNA structure postulated by Marvin and Hohn [2]. Our results agree with those of a Raman spectroscopic study [16], in that the duplex is closer to a B form.

## Acknowledgements

We wish to thank Ms. Monika Richter for her skillful technical assistance and Dr. B. Reimann (ZIMET Jena) for helpful discussions.

## References

[1] A. Kornberg, DNA replication (W.H. Freeman and Co., San Francisco, 1974).
[2] D.A. Marvin and B. Hohn, Bact. Rev. 33 (1969) 182.
[3] C.A. Marvin, W.J. Pigram, R.L. Wiseman, E.J. Wachtel and F.J. Marvin, J. Mol. Biol. 88 (1974) 581.
[4] S.A. Berkowitz and L.A. Day, J. Mol. Biol. 102 (1976) 531.
[5] J. Newman, J.L. Swinney and L.A. Day, J. Mol. Biol. 116 (1977) 593.
[6] L.A. Day and S.A. Berkowitz, J. Mol. Biol. 116 (1977) 603.

[7] T.A. Cross and S.J. Opella, Biochem. Biophys. Res. Commun. 92 (1980) 428.
[8] D.A. Marvin, R.L. Wiseman and E.J. Wachtel, J. Mol. Biol. 82 (1974) 121.
[9] M.E. Goldsmith and W.H. Konigsberg, Biochemistry 16 (1977) 2686.
[10] J.L. Woolford, H.M. Steinman and E.R. Webster, Biochemistry 16 (1977) 2694.
[11] T.J. Henry and D. Pratt, Proc. Natl. Acad. Sci. USA 62 (1969) 800.
[12] G.F.M. Simons, R.N.H. Konings and J.G.G. Schoenmakers, FEBS Letters 106 (1979) 8.
[13] Y. Nakashima, R.L. Wiseman, W. Konigsberg and D.A. Marvin, Nature 253 (1975) 68.
[14] E.J. Bendet and J.E. Mayfield, Biophys. J. 7 (1967) 111.
[15] D.A. Marvin and E.J. Wachtel, Nature 253 (1975) 19.
a. D.W. Banner, C. Nave and D.A. Marvin, Nature 289 (1981) 814.
[16] G.J. Thomas, Jr and R. Murphy, Science 188 (1975) 1205.
[17] H. Fritzsche, W. Pohle and H. Lang, Stud. Biophys. 55 (1976) 113.
[18] W. Pohle and H. Fritzsche, Stud. Biophys. 82 (1981) 81.
[19] P. Champeil, T.P.L. Tran and J. Brahms, Biochem. Biophys. Res. Commun. 55 (1973) 881.
[20] W. Pohle and H. Fritzsche, in: DNA—recombination, interactions, and repair, eds. S. Zadrazil and J. Sponar (Pergamon Press, New York, 1980) p. 273.
[21] M. Shie, E.N. Dobrov and T.I. Tichhonenko, Biochem. Biophys. Res. Commun. 81 (1978) 907.
[22] F. Asbeck, K. Bayreuther, H. Kohler, G. Von Wettstein and G. Braunitzer, Hoppe-Seyler's Z. Physiol. Chem. 350 (1969) 1047.
[23] L.A. Day, J. Mol. Biol. 15 (1966) 395.
[24] L.A. Day, J. Mol. Biol. 39 (1969) 265.
a. C.P. Beetz, G. Ascarelli and S. Arnott, Biophys. J. 28 (1979) 15.
[25] R.D.B. Fraser, J. Chem. Phys. 21 (1953) 1511.
a. E. Fredericq and C. Houssier, Electric dichroism and electric birefringence (Clarendon Press, Oxford, 1973).
[26] A. Elliott, Infrared spectra and structure of organic long chain polymers (E. Arnold, London, 1969).
a. R.D.B. Fraser, J. Chem. Phys. 24 (1956) 89.
[27] S.W. Englander and J.J. Englander, Methods Enzymol. 496 (1978) 24.
[28] R.S. Molday, S.W. Englander and R.G. Kallen, Biochemistry 11 (1972) 153.
[29] W. Pohle, H. Fritzsche and V. Zhurkin, 12th FEBS Meet. Dresden (G.D.R.) Abstr. No. 3603 (1978).
[30] J. Torbet, FEBS Lett. 108 (1979) 61.
[31] S.J. Opella, T.A. Cross, J.A. DiVerdi and C.F. Sturm, Biophys. J. Biophys. Disc. 2 (1980) 421.
[32] T. Shimanouchi, M. Tsuboi and Y. Kyogoku, Adv. Chem. Phys. 7 (1964) 435.
[33] M. Hogan, N. Dattagupta and D.M. Crothers, Proc. Natl. Acad. Sci. USA 75 (1978) 195.
[34] F. Brun, J.J. Toulme and C. Helene, Biochemistry 14 (1975) 558.
[35] J.J. Toulme and C. Helene, J. Biol. chem. 252 (1977) 244.
[36] G. Varsanyi, Vibrational spectra of benzene derivatives (Akademial Kiado, Budapest, 1969).