NEUTRON SCATTERING STUDY OF THE SOLUTION STRUCTURE OF BACTERIOPHAGES Pf1 AND fd

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1. Introduction

The single-stranded circular DNA containing cylindrical bacteriophages are among the simplest of known viruses [1,2]. Their capsid is composed largely (~99%) of a major coat protein plus about 4 copies of a minor protein, the A-protein [3–5]. In addition, there may be a few copies of other minor coat proteins [6,7]. There are several strains of these phages and they have been grouped into two classes on the basis of their X-ray diffraction patterns [3,8]. The strains fd and Pf1 belong, respectively, to class I [8] and II [3]. Both have ~60 Å diam. [3,8] and Pf1 which of 19 600 Å length [9] is more than twice as long as fd, 8900 Å [11,12]. Surprisingly, their DNA contents are not very different: in Pf1 there are 7400 nucleotides [10] whereas in fd there are 6400 [12,13]. The major coat protein of Pf1 is composed of 46 amino acids [16] while that of fd has 50 [14,15]; both are comprised of an acidic NH_2-terminus, a central hydrophobic region and a basic COOH-terminus [14–16]. Only the X-ray fibre diffraction pattern of Pf1 has been interpreted and a model proposed in which the DNA of unknown structure is encapsulated in a helical shell of major coat protein subunits [3,17,18]. The X-ray patterns do not reliably give the no. subunits/axial repeat and so 22 was chosen over the alternative 27 on the basis of indirect arguments [3,18].

Conversely, physio-chemical measurements on solutions strongly favour a structure with 27 subunits/axial repeat [9,10]. The X-ray measurements also indicate that Pf1 and fd are very similar in structure [8] which is again contrary to the physio-chemical results [9,10].

This paper presents the mass/unit length and cross-sectional radius of gyration of Pf1 and fd in solution as determined by low angle neutron scattering. The results support the view that the phage are similar in structure and partly favour a model for Pf1 which has 22 subunits/axial repeat. It is also shown that Pf1 undergoes a reversible temperature-induced structural change in solution.

2. Materials and methods

The phage, prepared as in [3] were a gift from D. A. Marvin. The buffer used was 100 mM Tris–HCl which gave a pH-meter reading of 7.5 when the solvent was H_2O and 7.1 when the solvent was D_2O. Solutions containing different D_2O contents were produced by dialysis. The D_2O contents were accurately determined from neutron transmission measurements. The phage concentrations (2–12 mg/ml) were determined by ultraviolet A_{270} using $\epsilon = 2.07$ cm^2/mg for Pf1 [9] and 3.84 cm^2/mg for fd [11]. The neutron scattering from 3 concentrations in H_2O and D_2O solvent were recorded. At intermediate D_2O solvent contents usually 2 concentrations were used.

Freshly prepared haemoglobin in H_2O buffer supplied by H. B. Osborne was employed as reference. The concentration was estimated by converting it at the cyanide form which has an ϵ_{mM}/haem of 10.92 at 540 nm [19]. The neutron flux thus estimated was very close to that obtained from the incoherent

scattering from water. The water spectrum was also used to correct for detector responses.

In this paper frequent reference will be made to the axial subunit distance. This is the projection onto the virus of the distance from one subunit to the next along the virus length.

The neutron scattering profiles were measured on the D11 small-angle camera at the Institut Laue-Langevin, Grenoble [20]. The sample–detector distance was 2.55 m and the wavelength was 10 nm with a bandwidth of 8% fullwidth at half-maximum. The experiments were conducted at ambient temperature ~22°C unless otherwise stated. The samples were contained in a holder which had a circulating-fluid temperature control device. The scattering profile of the phage was obtained by subtracting the scattering curve of the solvent from that of the solution, taking into account any difference between the transmissions of solution and solvent. The scattering curves were circularly symmetric and could therefore be reduced to a single curve of intensity $I(Q)$ against Q, $Q = 4\pi\sin\theta/\lambda$, λ is the wavelength and θ is half the scattering angle.

Over a limited range ($QR_c \sim 1.5$) the neutron scattering from a solution of monodispersed rods is given by eq. (1) which is adopted from the X-ray formulation [21,22]:

$$I(Q)Q = \Pi N \mu \left(\frac{\Sigma b_i}{M} - \frac{V}{M}\rho_s \right)^2 \exp -R_c^2 Q^2/2 \qquad (1)$$

$I(Q)$ is the scattering intensity on an absolute scale, having been divided by the neutron flux, the particle concentration and the solution transmission and thickness; N is Avogadro's number; μ is mass/unit length in daltons/Å; $\Sigma b_i/M$ is scattering length/unit mass; V/M is dry volume or solvent excluded volume/unit mass; ρ_s is solvent scattering density; R_c is cross-sectional or axial radius of gyration. ln $[I(Q)Q]$ is linearly dependent on Q^2 and the slope is equal to $R_c^2/2$. From the value of $I(Q)Q$ at $Q = 0$, $[I(Q)Q]_o$, μ can be estimated and it is accomplished most accurately in H_2O solvent.

Normally Σb_i varies linearly with solvent D_2O content so $([I(Q)Q]_o)^{1/2}$ also change linearly. As the ratios $\Sigma b_i/M$ and V/M appear in eq. (1) it is not necessary to consider the whole particle but only subunit values. Therefore knowledge of the no. subunits/virus is not required and polydispersity in length has no effect provided the rod-like approximation is valid for all the particles. The contrast, $\overline{\rho}$, between the particle and the solvent is given by $\overline{\rho} = \Sigma b_i/V - \rho_s$. In the contrast-matched condition $\overline{\rho} = 0$, $V = \Sigma b_i/\rho_s$ and $[I(Q)Q]_o = 0$.

The cross-sectional radius of gyration of a cylinder made up of an inner core of radius r_o surrounded by n contiguous and concentric shells of radius r_i is given by:

$$R_c^2 = \frac{f_o r_o^2 + \sum_{i=1}^{n} f_i (r_i^2 + r_{i-1}^2)}{2} \qquad (2)$$

where the f is the fraction of total scattering due to each region. $\sum_{i=o}^{n} f_i = 1$. For a compact cylinder at infinite contrast $R_c^2 = r_n^2/2$ and if the core has the same scattering density as the solvent $R_c^2 = (r_o^2 + r_n^2)/2$, r_n is the outer radius of particle. Thus from R_c at infinite contrast only an upper limit equal to $2^{1/2} R_c$ can be set to the outer radius.

3. Results and discussion

Figure 1 shows an example of the linear variation of ln $[I(Q)Q]$ with Q^2 for both Pf1 and fd. From the

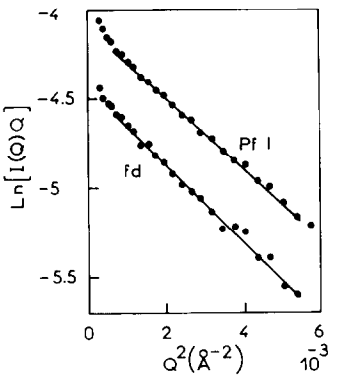

Fig.1. The logarithm of the scattering intensity, $I(Q)$, times Q plotted against Q^2. The slope of the least squares-fitted straight line is equal to half the square of the cross-sectional radius of gyration, R_c. The solvent contained 58% D_2O and the concentrations were 9.9 mg/ml and 7.9 mg/ml for Pf1 and fd, respectively.

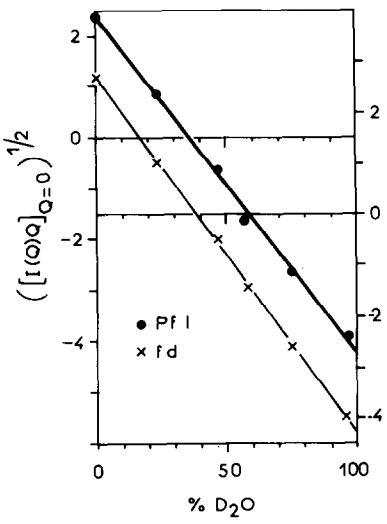

Fig.2. The variation of the square root of the intensity times Q extrapolated to $Q = 0$ as a function of the solvent D_2O content. The left scale refers to Pf1 and the right scale to fd.

slope of the least squares-fitted straight line R_c is obtained and by extrapolation to $Q = 0$ the value of $[I(Q)Q]_0$ is found. In the range examined $[I(Q)Q]_0$ is independent of concentration and, as shown fig.2, the square root of $[I(Q)Q]_0$ varies linearly with solvent D_2O content. These two observations are evidence for the monodispersity of the phage. The intercepts on the abscissa in fig.2 (36.0% for Pf1 and 38.8% for fd) correspond to the solvent D_2O content in the contrast matched condition.

There are 1 and 2.3 DNA bases/major coat protein in Pf1 and fd, respectively [11–13]. Therefore 1 subunit is considered to be composed of a coat protein plus the appropriate number of DNA bases. The amino acid [15,16] and base [9] compositions are known and assuming the H–D exchange of labile proteins is equal to the proportion of D_2O in the solvent Σb_i can be calculated. Hence the dry volumes/subunit are found from the match point solvent density to be 6700 Å^3 for Pf1 and 7900 Å^3 for fd. These will be overestimates if H–D exchange is less than assumed. The values calculated by summing the volumes of the amino acid residues [23–25] and DNA bases [26] are ~7% smaller as is the dry volume divided by the number of subunits reported [12] for fd.

The values of μ can be determined from $[I(Q)Q]_0$ in H_2O solvent. The dry volumes used are the mean between the measured and calculated values which gives rise to a possible error of ± 1.5% in μ. In daltons/Å, μ = 1570 ± 130 for Pf1 and 1840 ± 140 for fd which correspond, respectively, to rather similar, axial intersubunit distances of 3.15 ± 0.25 Å and 3.25 ± 0.25 Å. This axial spacing for Pf1 is substantially larger than that 2.6 Å obtained by dividing the length in the electron microscope by the number of protein subunits [10]. The principle axial subunit repeat determined from X-ray fibre diffraction patterns of Pf1 is 72 Å in dry fibres and 75 Å in wet fibres [3]. Over these distances the data presented here give 23 ± 2 and 24 ± 2 subunits, respectively. This data therefore mildly favours a structure with 22 subunits/axial repeat.

The axial intersubunit distance reported here for fd is in good agreement with that in [12] and corresponds to 5 ± 0.4 subunits in a distance of 16.1 Å. In X-ray fibre diffraction patterns the meridional reflections correspond to orders of 16.1 Å spacing [29]. The lower limit is close to the 4.5 proposed in the X-ray model. However, the data do support the suggestion that fd could contain 5 subunits/16.1 Å axial length [12].

The ratio of the mass/unit lengths Pf1/fd found from the data reported here is 0.85 ± 0.04 the estimated error is reduced because the neutron flux cancels out. This ratio can also be estimated by dividing the mass/subunit as defined earlier by the subunit axial spacing found by other techniques. In this way the X-ray fibre value is estimated also to be ~0.85 while the data in [10,12] gives $\gtrsim$1 and as there is an approximate general agreement about the subunit repeat in fd it seems that the most probable source of disagreement lies with Pf1. The length of Pf1 is known to be altered by temperature (see later) and other factors less well defined such as ionic conditions and pH [27]. Therefore it is possible that the preparation of samples for electron microscopy induces a change in length.

As the temperature is lowered from ~16°C to 6°C the value of $[I(Q)Q]_0$ given by Pf1 in H_2O and 97% D_2O solvent decreases reversibly by 5.5% (fig.3). As this change is the same at both these widely differing contrasts the dry volume, V, is constant. Therefore the relative decrease in mass/unit length

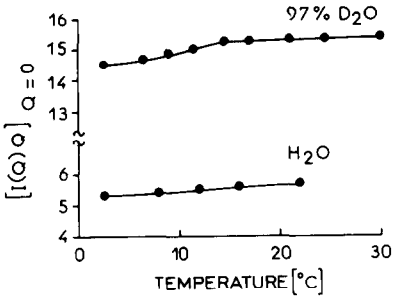

Fig.3. Variation of $[I(Q)Q]_0$ (intensity times Q extrapolated to $Q = 0$) with temperature from Pf1. The concentration was 7.7 mg/ml in H_2O and 4 mg/ml in 97% D_2O.

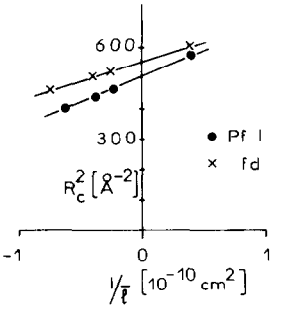

Fig.4. The square of the cross-sectional radius of gyration, versus the reciprocal of the contrast. The intercepts at infinite contrast ($1/\bar{\rho} = 0$) are 22.5 ± 0.5 Å for Pf1 and 23.5 ± 0.5 Å for fd and the slopes, α, are, respectively, 1.7×10^{-4} and 1.1×10^{-4}.

is accurately 5.5%. A similar change has been recorded from fibres formed at 20°C and 5°C for X-ray diffraction [28]. At the lower temperature the intersubunit axial spacing is increased to 3.30 ± 0.25 Å. The radius of gyration in 97% D_2O solvent was not temperature dependent and with H_2O solvent, although there was an apparent decrease with temperature, the data are not reliable. Magnetic birefringence measurements have shown that the α-helical major coat protein becomes more parallel to the phage long axis as the temperature is lowered [30].

The plot of the square of the radius of gyration, R_c^2, against the reciprocal of contrast, $1/\bar{\rho}$, is a straight line with a positive slope, α, for both phage fig.4. The value of R_c at infinite contrast $1/\bar{\rho} = 0$ corresponds maximum possible outer radius of 32 Å for Pf1 and 33 Å for fd. These are larger than the radii 27 Å and 27.7 Å, respectively, obtained from the smallest spacing found in dry fibres [3,29] but the relative difference remains. The hydrodynamic diameter for fd is 90 ± 10 Å [12] and therefore combined with the neutron radius the hydrodynamic boundary layer, contrary to the conclusion in [12], is rather large, which suggests that there is a larger counter ion cloud associated with the virus.

For a particle in which all sites of H–D exchange attain equilibrium with the solvent a positive α indicates that the region of highest scattering density is near the outer surface [31] which would mean that the DNA is, surprisingly, near the surface, however, if this were so α should be, contrary to observation (fig.4), larger for fd because it contains more DNA/coat protein. Equation (2) was used to obtain a qualitative estimate of α for a number of cylindrical models consistent with the volumes, subunit axial repeat and R_c at infinite contrast. These are very approximate calculations; for example, the unknown penetration of solvent into surface interstices is not easily taken into account. A positive α which is larger for Pf1 than for fd was obtained if the phage core contained hydrated DNA which only underwent partial H–D exchange with the solvent. Thus the coat protein may be tightly packed and rather inaccessible to solvent.

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