Hydrodynamic studies of a DNA-protein complex

Dimensions of the complex of single-stranded 145 base DNA with gene 32 protein of phage T4 deduced from quasi-elastic light scattering

M.A. Scheerhagen, M.E. Kuil, R. van Grondelle and Joh. Blok

Department of Biophysics, Free University, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands

Received 13 March 1985

The translational diffusion coefficient of the saturated complex of single-stranded 145 base DNA and the helix-destabilizing protein of phage T4, GP32, can be measured at equilibrium by means of quasi-elastic light scattering. If the complex is considered as a rigid rod one can estimate its dimensions by combining the translational diffusion coefficient with earlier data on rotational diffusion. It was found that the average base-base distance of the 145 base DNA in the complex is between 4.3 and 4.7 Å, while the diameter of the complex is between 44 and 68 Å. This suggests that the conformation of the complex must be such that a large amount of water is trapped.

DNA-protein complex *Helix-destabilizing protein* *Bacteriophage T4* *Quasi-elastic light scattering* *Hydrodynamic dimensions*

1. INTRODUCTION

The interaction of single-stranded (ss) DNA with the 'helix-destabilizing protein' encoded by gene 32 of bacteriophage T4, GP32, has been studied using a variety of spectroscopic methods [1–3]. These studies have led to the following conclusions: (i) the protein binds strongly and cooperatively to ss DNA and homopolynucleotides [1] and the association constant is a strong function of the salt concentration, being maximal at about 50 mM NaCl [4]; (ii) one protein molecule occupies 7–10 bases [1,5]; (iii) the circular dichroism (CD) spectrum of the DNA and the polynucleotides in complex with GP32 points to relatively strong interactions between the bases and supports a rather rigid base configuration in the complex [6]; (iv) the base-base distance measured along the helix axis, i.e., the axial increment, of ss DNA seems to increase significantly upon complexation with GP32 [7]. Calculations show that this is not in contradiction with the CD spectrum poly(rA) adopts in the complex with GP32 [6].

In [8] we used electric field-induced birefringence (ELB) experiments to show that short DNA and RNA fragments complexed with GP32 behave as more or less rigid rods. From the field free decay time the axial increment was estimated to be at least 4.3 Å/base for 145 b ss DNA and 5.6 Å/base for a tRNA fragment of 76 nucleotides in complex with GP32.

Here we report the measurement of the translational diffusion coefficient of the 145 b ss DNA-GP32 complex using quasi-elastic light scattering (QELS), a technique which is well fitted to the study of biological macromolecules if (large) dust particles can be removed without impairing the sample. From a combined analysis of the rotational and translational diffusion coefficient estimates of the dimensions of the complex can be extracted.

2. MATERIALS AND METHODS

The gene 32 protein was prepared as described by Hosoda and Moise [9]. Nucleosomal double-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

stranded 145 base pair (bp) chicken erythrocyte DNA was purified using the method in [10]; the material was more than 95% homogeneous as shown by polyacrylamide gel electrophoresis and the ELB field free decay of the 145 bp fragment was monoexponential with a relaxation time of 3.17 μs at 8.9°C and at an Na^+ concentration of 2.2 mM. The 145 bp DNA was denatured by heating at 90°C for 5 min.

QELS experiments with homodyne detection were performed using an apparatus extensively described in [11]. Both the 488 nm and 514 nm lines of an argon ion laser were used for the scattering experiments. An essential part of the QELS experiments is the preparation of a dust-free sample. To this purpose a closed circuit containing a Millipore filter (pore size 0.15 μm) was designed to purify very small volumes. First the system was extensively cleaned using distilled water and buffer. Then about 1 ml of a solution of the complex was circulated for 10 min. After filtration the sample was collected in a measuring cuvette that had been rinsed with acetone. This cuvette was centrifuged at 65000 × g for 30 min. Measurement of the CD and absorption spectrum showed that the complex was still intact. Unless large fluctuations in the scattered light were detected indicating the presence of large particles, the thus prepared sample was used for the QELS experiments.

All experiments were performed with the GP32-145 b DNA complex in 2 mM Na_2HPO_4, 0.2 mM Na_2EDTA (pH 7.2) and NaCl at the concentration indicated. The temperature was kept close to 20°C. The value of the translational diffusion coefficient, D_T, was always corrected to 20°C and water viscosity.

3. RESULTS AND DISCUSSION

3.1. *Determination of the translational diffusion coefficient*

For the conditions relevant to this work gene 32 protein binds strongly and cooperatively to 145 b ss DNA as indicated by the characteristic change in the DNA CD spectrum. This implies that even at a slight excess of protein almost complete saturation of the ss DNA molecules will be obtained.

Because the scattering intensity of a molecule is proportional to the square of its M_r value, the contribution of the DNA-GP32 complex to the detected signal will greatly exceed that of the unbound components in the sample.

The scattered intensity, I, of the (polarized) laser light was almost independent of the scattering angle θ for $30° \lesssim \theta \lesssim 150°$, if the angular dependence of the scattering volume was taken into account. The intensity was indicative for a complex with $M_r > 200000$, but no exact estimate was possible because of the properties of the current equipment. For $\theta \lesssim 30°$ an increase in I was observed in most cases.

In fig.1 a representative example of the autocorrelation function $C(\tau)$ of the scattered intensity $I(t)$ is shown. It should be noted that the concentration of DNA used in these experiments was extremely low. Because the scattering particles are much smaller than the wavelength, they behave like Rayleigh scatterers. Furthermore, the contribution of diffusion anisotropy and/or flexibility to the angular dependence of $C(\tau)$ is negligible in view of the expected dimensions of the complex, and therefore the function $C(\tau)$ is to a first approximation given by eqn 1 for homodyne detection [12]:

$$C(\tau) = A \exp(-2D_T q^2 \tau) + B \quad (1)$$

where q is the scattering vector, τ is the correlation time and B is a constant in part determined by the correlator and in part by additional uncorrelated scattered light. A fit of the experimental data based on eqn 1 is shown in fig.1.

In the absence of contaminating dust particles the calculated value of D_T should be independent of θ. This is shown in fig.2 for θ between 15 and

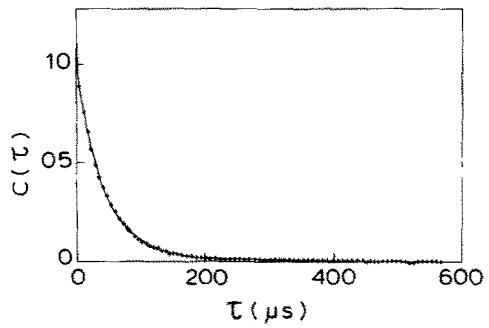

Fig.1. Autocorrelation function of intensity variations in the light scattered at 90° from a solution of the 145 b ss DNA-GP32 complex after subtraction of the baseline. [DNA] = 73.8 μM (nucl.), [GP32] = 23.4 μM, [NaCl] = 50 mM, T = 20.4°C.

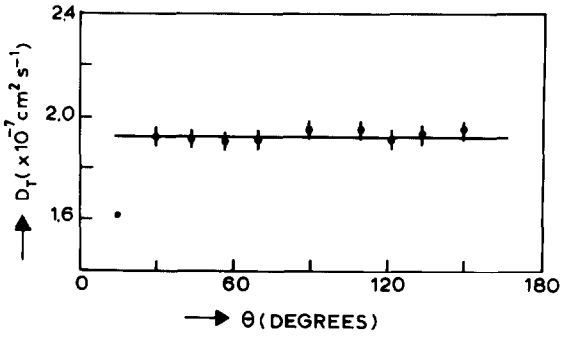

Fig.2. D_T as a function of the scattering angle θ. [DNA] = 61.1 μM (nucl.), [GP32] = 21.0 μM, [NaCl] = 50 mM, T = 20.4°C. Bars indicate estimated errors.

150°. Only for θ = 15° was a considerable decrease of D_T observed, probably due to a small amount of remaining dust.

We have determined the value of D_T at 3 different salt concentrations. At 50 and 200 mM NaCl the experimentally obtained D_T value is independent of the GP32-DNA ratio over the range 0.1–0.6 protein molecules per nucleotide. In addition, at a constant GP32-DNA ratio, D_T does not depend on the DNA concentration between 70 and 200 μM, which shows that interaction between particles is not significant. At 10 mM NaCl, only two experiments were performed. However, at this salt concentration no significant dependence of the rotational diffusion coefficient on GP32-DNA ratio and DNA concentration was observed in ELB measurements, even at concentrations comparable to those used in the QELS experiments. Therefore it is concluded that interparticle interactions are undetectable under these conditions. The results at the different salt concentrations are shown in table 1, in which the D_T value given is the average obtained from a number of experimens at various DNA and GP32 concentrations. The weak but significant decrease of D_T observed upon lowering the salt concentration may be due to: (i) an increase in the hydrodynamic volume of the complex caused by increased electrostatic interactions between different charged parts of each individual protein or between distinct, bound GP32 molecules; (ii) a decrease in the flexibility and the overall bending of the complex, resulting in an increase of its persistence length and thereby of its effective dimensions; or (iii) a small change in the binding stoichiometry as a function of the salt concentration. In addition, the D_T value obtained at 10 mM NaCl may be slightly influenced by electrostatic particle-particle interactions.

Table 1

Averaged D_T value of the 145 b ss DNA-GP32 complex for various salt concentrations (given errors are possible errors)

NaCl (mM)	D_T ($\times 10^{-7}$ $cm^2 \cdot s^{-1}$)
10	1.78[a]
50	1.92 ± 0.05
200	2.05 ± 0.05

[a] Average of two experiments

Application of cumulant analysis on the measured decay curves yielded a positive second cumulant in most cases, but its value did not show any correlation with the obtained D_T values. The second cumulant, μ_2/Γ^2 as defined in [13], was about 0.05. This may point to some polydispersity of the sample, possibly due to a certain length distribution of the complexed molecules in spite of the homodispersity of the original 145 b ss DNA. Analysis of these curves using a multi-exponential approach is currently in progress. However, the biphasic character of the electric birefringence decay [8] appears not to be manifested in the autocorrelation function. In fact, as will be shown below, the obtained value of D_T corresponds to the slow phase of the ELB decay.

3.2. *Combination of* D_T *and* D_R

For the case that we approximate the 145 b DNA-GP32-complex as a rigid rod we can estimate the dimensions of the complex from a combination of the measured values of D_T and D_R obtained from the QELS and ELB experiments, respectively. To do so we use the following equations for D_T and D_R:

$$D_T = \frac{kT}{3\pi\eta L}(\ln(L/d) + \gamma_T) \quad (2)$$

$$D_R = \frac{3kT}{\pi\eta L^3}(\ln(L/d) - \gamma_R) \quad (3)$$

where L is the length of the complex, d the

diameter of the complex and γ_T and γ_R are parameters that take into account the end effects. We found that applying the different existing models for γ_T and γ_R [14–16] had only a small effect on the ultimately calculated values of L and d. For this analysis we have chosen the D_T value at 50 mM NaCl and the D_R value at 15 mM NaCl, mainly because these measurements were the most accurate. QELS experiments at lower salt concentrations may possibly be affected by interparticle interactions, while ELB experiments at higher salt concentrations are severely hindered by the low degree of orientation that can be attained. In principle, the obtained D_T values could have been affected by some association, especially if longer aggregates were present. However, this seems very unlikely because: (i) D_T does not depend on the concentrations of the components; (ii) at 15 mM NaCl such an association is not seen in the ELB decay, although these experiments are very sensitive to the presence of longer particles due to their relatively high degree of orientation ($\sim L^2$) and their much lower D_R value ($\sim L^{-3}$); (iii) the low value of the second cumulant does not correspond to a broad distribution of dimensions; and (iv) sedimentation experiments under identical conditions did not give any indication for larger aggregates [17]. Finally, we emphasize that even if some aggregation had been present, this will not affect the conclusions discussed below significantly. In fig.3 we show how various combinations of L and d may be used to fit the experimentally obtained values for D_T and D_R, including the error margins. This leads to a length of the complex between 625 and 670 Å, i.e., an axial increment between 4.3 and 4.7 Å, and a diameter between 44 and 59 Å.

We mentioned before that the measurement of D_T at 10 mM NaCl resulted in a value of 1.78×10^{-7} cm$^2 \cdot$s^{-1}. Therefore the correct D_T value at this low salt concentration, i.e., in the absence of possible interparticle interactions, will be between 1.78 and 1.92×10^{-7} cm$^2 \cdot$s^{-1}). Because at 10 mM NaCl and at almost identical concentrations ELB experiments did not point to interactions between distinct complexes [8], the correct D_T value will probably be close to 1.78×10^{-7} cm$^2 \cdot$s^{-1}. This indicates that the length given above may be slightly too large and that the actual diameter may be as large as 68 Å, a value in close agreement with that

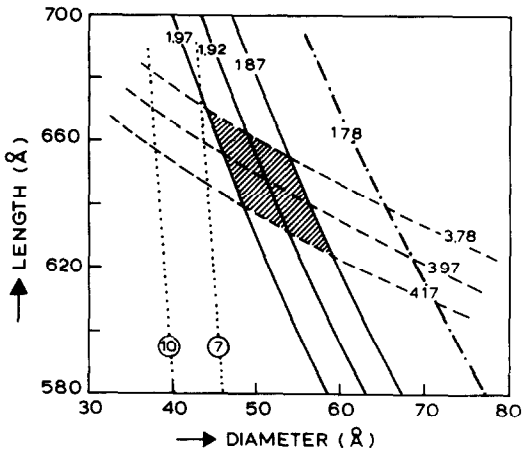

Fig.3. Combination of D_T and D_R of the 145 b ss DNA-GP32 complex. Combinations of length and diameter that fit $D_T = (1.92 \pm 0.05) \times 10^{-7}$ cm$^2 \cdot$s^{-1} (——), $D_T = 1.78 \times 10^{-7}$ cm$^2 \cdot$s^{-1} (-·-·-), $D_R = (3.97 \pm 0.20) \times 10^4$ s^{-1} (– – –) and the volume calculated for $n = 7$ and $n = 10$ (···).

obtained from electron microscope experiments [7].

Finally, we can estimate the volume of the complex on the basis of the specific volumes of both the protein (= 0.73 cm^3/g) and the DNA (= 0.50 cm^3/g), together with the size of the binding site, n. In fig.3 it can be observed that the calculated volume of the non-hydrated complex leads to combinations of L and d that are far too small, in particular for $n = 10$, a value that was given by Bobst et al. [5] and for which we have also supporting evidence [17]. Therefore, we must assume a substantial contribution of water to the hydrodynamic volume. A calculation shows that δ, i.e., weight H_2O/weight complex, must be between 1 and 2 implying that 60–75% of the hydrodynamic volume consists of water. For native DNA δ is about 0.6 g H_2O/g DNA [18], but the value for the ss DNA in the complex could be rather different. However, because the DNA forms at most 10% of the weight of the complex, its hydration can probably be neglected. For most proteins the value of δ is around 0.5 g H_2O/g protein [18] and probably even lower for GP32 [19]. Therefore this suggests that the GP32-ss DNA complex is in a special conformation, that traps a large amount of water, for instance like a hollow cylinder, as recently proposed for the complex of

the helix destabilizing protein of phage fd, GP5, and ss DNA [20]. Even if we assume that all the hydration water corresponding to $\delta = 0.5$ is on the outside of the hollow cylinder, the diameter of the water core will still be between 30 and 50 Å.

We remark that the rigid rod approximation for a complex that may have some flexibility leads to lower bounds for the value of the axial increment and the amount of trapped water.

In conclusion, single-stranded 145 b DNA complex with the helix-destabilizing protein GP32 forms a more or less rigid complex that can be very well studied at chemical equilibrium with quasi-elastic light scattering. A combination of D_T and D_R leads to realistic estimates of the length and diameter of the complex.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Netherlands Organization for the Advancement of Pure Research (ZWO). The authors are indebted to Mrs C.A. Vlaanderen for preparation of the GP32 samples.

REFERENCES

[1] Newport, J.W., Lonberg, N., Kowalczykowski, S.C. and Von Hippel, P.J. (1981) J. Mol. Biol. 145, 105–121.
[2] Toulmé, J.J. and Hélène, C. (1980) Biochim. Biophys. Acta 606, 95–104.
[3] Anderson, R.A., Nakashima, Y. and Coleman, J.E. (1975) Biochemistry 14, 907–917.
[4] Jensen, D.E., Kelly, R.C. and Von Hippel, P.H. (1976) J. Biol. Chem. 251, 7215–7228.
[5] Bobst, A.M., Langemeier, P.W., Warwick-Koochaki, P.E., Bobst, E.V. and Ireland, J.C. (1982) J. Biol. Chem. 257, 6184–6193.
[6] Scheerhagen, M.A., Van Grondelle, R. and Blok, J. (1985) J. Biomol. Struct. Dyn., in press.
[7] Delius, H., Mantell, N.J. and Alberts, B. (1972) J. Mol. Biol. 67, 341–350.
[8] Scheerhagen, M.A., Van Amerongen, H., Van Grondelle, R. and Blok, J. (1985) FEBS Lett. 179, 221–224.
[9] Hosoda, J. and Moise, H. (1978) J. Biol. Chem. 253, 7547–7555.
[10] Lutter, L.C. (1978) J. Mol. Biol. 124, 391–420.
[11] Hopman, P.C. (1980) PhD Thesis, Free University, Amsterdam.
[12] Berne, B.J. and Pecora, R. (1976) in: Dynamic Light Scattering, 1st edn, pp.38–52, John Wiley, New York.
[13] Koppel, D.E. (1972) J. Chem. Phys. 57, 4814–4812.
[14] Broersma, S. (1960) J. Chem. Phys. 32, 1632–1635.
[15] Tirado, M. and Garcia de la Torre, J. (1979) J. Chem. Phys. 71, 2581–2587.
[16] Elias, J.G. and Eden, D. (1981) Biopolymers 20, 2369–2380.
[17] Scheerhagen, M.A., Vlaanderen, C.A., Van Grondelle, R. and Blok, J. (1985) in preparation.
[18] Cantor, R.C. and Schimmel, P.R. (1960) in: Biophysical Chemistry, vol.2, 1st edn, pp.550–555, W.H. Freeman, San Francisco.
[19] Caroll, R.B., Neet, K.E. and Goldthwait, D.A. (1972) Proc. Natl. Acad. Sci. USA 69, 2741–2744.
[20] Torbet, J., Gray, D.M., Gray, C.W., Marvin, D.A. and Slegrist, H. (1981) J. Mol. Biol. 146, 305–320.