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Hydrodynamic studies of a DNA-protein complex

Elongation of single stranded nucleic acids upon complexation with the gene 32 protein of phage T4 deduced from electric field-induced birefringence experiments

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Short DNA and RNA fragments complexed with the helix destabilizing protein of bacteriophage T4, GP32, have been studied in solution by electric birefringence and circular dichroism. The birefringence of the complexes is positive and the magnitude indicates that the DNA and RNA fragments become linear and rigid upon protein binding. The field free decay is biphasic. On the basis of a rigid rod approximation the slow relaxation time leads to a base-base distance along the helix axis in the complex from 4.3 to 5.6 Å, an elongation of at least 50% compared to single-stranded DNA.

DNA-protein complex *Helix destabilizing protein* *Bacteriophage T4* *Electric birefringence*
Axial increment determination

1. INTRODUCTION

The protein encoded by gene 32 of bacteriophage T4 (GP32) is a so-called helix destabilizing protein. It has an essential function in the replication of T4 DNA [1], due to its tight and cooperative binding to single-stranded (ss) nucleic acids. In the complex with ss DNA a protein monomer covers about 7–10 nucleotides [2,3]. Extensive studies have supplied quantitative data for the thermodynamic and kinetic parameters of the binding [2,4]. On the basis of results from electron microscopy it has been suggested that in the complex the axial increment of the DNA, i.e., the projection of the base-base distance on the helix axis, is 4.6 Å [5], an increase of more than 50% relative to uncomplexed ss DNA. However, these experiments are not necessarily relevant to the conformation in solution, since the GP32–DNA complex was spread on top of a hypophase, with the possibility that forces on the molecule may have caused some increase of its length (H. van Heerikhuizen, personal communication).

To obtain a more direct estimate of the base-base distance in the complex in solution we have used electric field-induced birefringence. Because these experiments must be performed at a relatively low salt concentration to obtain sufficient orientation by the applied electric field, we have compared the circular dichroism (CD) spectra of the complex at various salt concentrations.

This work suggests that in solution the axial increment of a nucleic acid increases dramatically upon the binding of GP32, irrespective of the salt concentration.

2. MATERIALS AND METHODS

GP32 was isolated and purified as in [6]. The following nucleic acid fragments were used (number of nucleotides given in parentheses): a mixture of different tRNAs of baker's yeast (76), $tRNA^{Phe}$ of brewer's yeast (76), $tRNA^{Tyr}$ of *E. coli* MRE600 (83), all purchased from Boehringer Mannheim; 5 S RNA of *Bacillus licheniformis* (116) was a kind gift of Dr H.A. Raué (Dept. of

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Biochemistry, Free University, Amsterdam); the 145 base-pair fragments were isolated according to [7] and denatured by heating to 90°C for 5 min. Gel electrophoresis showed negligible heterodispersity for all samples apart from $tRNA^{Tyr}$, in which about 10% shorter fragments were present. The buffer in all experiments contained 1 mM Na_2HPO_4, 0.1 mM Na_2EDTA (pH 7.7) and the NaCl concentration as indicated. The GP32 protein used in the birefringence experiments was dialysed against a buffer containing 15 mM NaCl. The desired salt concentration was established by dilution with a buffer without NaCl. The nucleotide-GP32 ratio was about 5 in all cases, ensuring that no uncomplexed nucleic acid fragments were present.

CD spectra were recorded on a Cary 61 spectrapolarimeter. The experimental set up for the birefringence experiments is essentially the same as in [8]. The signal-to-noise ratio was enhanced by the use of a cell with a light path of 4.9 cm and by accumulation of 10–20 pulses. The pulse length of the electric field was adjusted at such a value that the steady-state birefringence was just obtained. Relaxation times were corrected to standard conditions (25°C, water viscosity).

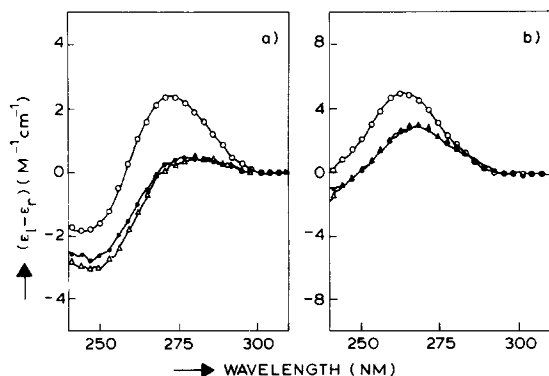

Fig.1. CD spectra of 145 b ss DNA–GP32 and tRNA–GP32 complexes at different salt concentrations. (a) 145 b ss DNA at 5 mM NaCl (○), 145 b ss DNA–GP32 complex at 50 mM (△) and 5 mM NaCl (●). [DNA] = 61.0 μM (nucl), [GP32] = 10.5 μM, T = 17°C. (b) tRNA of baker's yeast at 10 mM NaCl (○), tRNA–GP32 complex at 50 mM (△) and 10 mM NaCl (●), [tRNA] = 50 μM (nucl), [GP32] = 10.4 μM, T = 25°C. The spectra of the complexes were corrected for the protein contribution.

3. RESULTS

3.1. *CD spectra*

Fig.1 shows the CD spectra of complexed DNA and tRNA at different salt concentrations, after subtraction of the protein contribution. For comparison the CD spectrum of free ss DNA and free tRNA have been added. Since the CD of the complex between 310 and 240 nm is mainly due to the configuration of the bases [9] the presented spectra strongly indicate that the conformation of the DNA and the tRNA in the complex are independent of the salt concentration. Moreover, a CD titration of 145 base (b) ss DNA with GP32 shows that even at low NaCl concentrations the association constant is sufficiently strong to saturate the DNA fragments almost completely at a slight excess of GP32 ($K_{app} \gtrsim 10^7\ M^{-1}$). The same result was obtained for the tRNA fragments (not shown).

3.2. *Electric birefringence*

The birefringence of short, homodisperse DNA and RNA fragments complexed with GP32 shows the following features: (i) The magnitude is rather large, in particular if compared with the small signals of the isolated protein and free ss DNA or RNA as is shown in fig.2a for the 145 b fragment. (ii) The birefringence is positive in contrast to that of single- and double-stranded DNA. (iii) In spite of the homodispersity of the nucleic acid fragments a sum of two exponentials is minimally required to represent the field free decay of the signal for the DNA–protein complex adequately (fig.2b). In contrast, strictly monoexponential decays are obtained with samples of double-

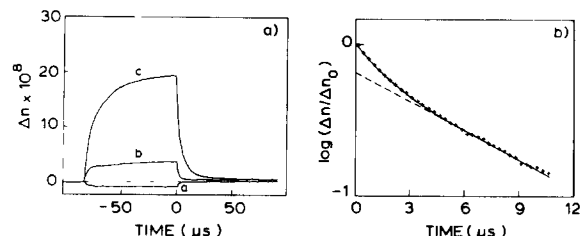

Fig.2. (a) Transient electric birefringence at 4 mM NaCl and 10°C. Field on at $t = -80\ \mu s$ and off at $t = 0$. (trace a) 145 b ss DNA, 39.5 μM (nucl); (trace b) GP32, 8.1 μM; (trace c) DNA–GP32 complex, concentrations as for a and b. (b) Semilogarithmic plot of field-free decay of DNA–GP32 complex.

stranded 145 base pair DNA, leading to relaxation times in agreement with previous measurements [10]. Analysis of the decay curves of the complexes results in a slow and fast relaxation time, τ_s and τ_f, respectively, together with their relative contribution (A_s, A_f). For the 4 complexes studied these are presented in table 1. Note that both relaxation times depend on the number of bases in the complex. In the following we will focus on the data concerning the slow relaxation time. A full discussion of the fast decay phase is beyond our present purpose and is postponed to a subsequent manuscript. The value of τ_s is independent of the applied field (1.4–4.0 kV/cm), indicating that the structure of the complex, as far as reflected in the field free decay, is not influenced by the applied electric pulse. At a constant nucleotide-GP32 ratio τ_s does not vary over the nucleotide concentration range 10–40 μM for the 145 b–GP32 complex. For the other fragments similar results were obtained. Obviously the interaction between particles is negligible. After correction for temperature effects on viscosity and Brownian motion the value of τ_s is not dependent on the temperature of measurement (7–37°C). Moreover, it remains constant over the salt concentration range, where accurate experiments are possible (3–15 mM NaCl).

3.3. *Calculation of the axial increment*

The relaxation time of the field free decay for the birefringence of monodisperse cylindrical particles is inversely proportional to the rotational diffusion coefficient D_R. For a rigid rod D_R is given by the following expression [10]:

$$D_R = \frac{3kT}{\pi\eta_0 L^3}[\ln(\frac{L}{d} - \gamma_R] \quad (1)$$

where η_0 is the solvent viscosity, kT is the thermal energy, L and d are the length and diameter of the cylinder, respectively, and γ_R is a parameter accounting for end effects.

In the absence of interactions τ_s is related to the end-to-end rotation of the complex. If the complex is approximated as a rigid rod and its diameter is assumed to be 60 Å as suggested in [5], the τ_s values given in table 1 lead to the lengths of the complexes using eqn.1, where the parameter γ_R is taken from the empirical expression of [11] (table 2). Dividing the length by the number of interbase distances in the complex gives the value of the axial increment (ax). It is 4.4 Å for the largest fragment in agreement with the value suggested on the basis of electron micrographs [5], and even 5.6 Å for the shortest ones. It is obvious that the axial increment is much larger than the value of 2.9 Å suggested for single-stranded DNA [5] and that of 3.4 Å for double-stranded DNA [11].

This conclusion is independent of the assumptions made above: (i) If the shape of the complex deviates in any way from a rigid rod, the actual axial increment would even be larger. (ii) The calculated length appears to be rather insensitive to assumptions about the diameter of the complex. Determination of the translational diffusion coefficient of the 1.45 b DNA–GP32 complex by quasi-elastic light scattering [12] indicates that the diameter is possibly around 45 Å. This would result in about 5% larger, axial increments than

Table 1

Parameters of a two-exponential fit for DNA–GP32 and RNA–GP32 complexes, averaged for different salt concentrations at 10°C

N	A_f (%)	A_s (%)	τ_f (μs)	τ_s (μs)
145	31 ± 2	69 ± 2	0.88 ± 0.07	4.20 ± 0.10
116	30 ± 5	70 ± 5	0.49 ± 0.08	2.14 ± 0.05
83	47 ± 5	53 ± 5	0.48 ± 0.05	1.81 ± 0.06
76	44 ± 2	56 ± 2	0.34 ± 0.11	1.46 ± 0.08

N, number of bases; τ_f and τ_s, corrected fast and slow relaxation time; A_f and A_s, relative contribution of τ_f and τ_s. Indicated errors are standard deviations

Table 2

Calculated length, L, and axial increment, ax, for DNA–GP32 and RNA–GP32 complexes from the data in table 1

N	L (Å)	ax (Å)
145	635	4.4 ± 0.1
116	493	4.3 ± 0.1
83	460	5.6 ± 0.1
76	423	5.6 ± 0.2

N, number of bases. Indicated errors are standard deviations

those given above. (iii) Several models have been proposed to calculate the parameter γ_R in eqn 1. However, in our particular case the different models have only minor influence on the calculated lengths.

For these reasons the presented values of the axial increment are in fact lower bounds.

4. DISCUSSION

This work shows that electric birefringence can supply valuable data concerning the conformation of the complex between GP32 and single-stranded DNA or RNA in solution. Although the experiments have been performed at low ionic strength, the CD measurements make it plausible that the data obtained are also relevant to the complex conformation at higher salt concentrations.

The positive birefringence of the complex is probably due to a substantial protein contribution to the signal, possibly in combination with an increase in tilt angle of the bases relative to the helix axis resulting in a reduction of their negative birefringence in the case of orientation of the long axis of the complex parallel to the electric field.

From the experiments described here it is clear that the nucleic acid in the complex with GP32 forms a rigid, more or less linear structure. For the RNA fragments the binding of GP32 apparently removes almost all tertiary structure. The shorter fragments (76, 83 b) lead to an axial increment of about 5.6 Å. It should be mentioned that the maximum value as determined by bond lengths is 7 Å. The axial increment in the other complexes is somewhat smaller. The reason for this is not quite clear. The difference between the values of the 145 b DNA complex and the tRNA complexes cannot be explained by applying the weakly bending rod model [13]. Moreover, the independence of τ_s from temperature (after correction to standard conditions) and salt concentration indicates that the flexibility in the complex must be of minor importance. Possibly the difference between the axial increments is caused by the different sugar moiety, which has an important function in the binding process as reflected in the association constant [2]. In conclusion the binding of GP32 to single-stranded nucleic acids induces an elongation to such an extent that one must expect a considerable change in position and orientation of the bases relative to one another. This is confirmed by a characteristic change of the CD spectrum.

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