Diffusion Constant of a Nonspecifically Bound Protein Undergoing Curvilinear Motion along DNA†

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The mechanism of a protein's diffusion along a DNA segment is a subject of much current interest because of the involvement of this diffusion in numerous biological processes, including the recognition of DNA sequences and chemical modifications of DNA. In this work we present a theoretical derivation of the diffusion coefficient of a nonspecifically bound protein, assuming that the protein follows a helical track along the DNA. It is shown that, for protein-sized molecules, the principal contribution to the total translational friction comes from the curvilinear motion along the helix, and this contribution is given by $6\pi\eta R R_{OC}^2 + 8\pi\eta R^3$, where R is the protein radius, R_{OC} is the distance of separation between the center of mass of the protein and the helical axis of DNA, and η is the viscosity of the medium. The translational diffusion of the protein along the helical track of DNA is thus predicted to have a nearly R^{-3} size dependence, not the R^{-1} dependence characterizing simple translational diffusion. It is shown that this expression gives rather good estimates of the translational diffusion coefficient measured in single molecule experiments.

I. Introduction

DNA-binding proteins find their target on DNA by nonspecifically binding to the DNA at a random location, then undergoing Brownian diffusion along DNA to the target site. This picture was initially derived from experimental observations of operator binding by the lac repressor showing that the binding rate constant was significantly larger than the expected Smoluchowski rate constant for a bimolecular reaction based on the known three-dimensional diffusion coefficient of the protein.[1−4] Recent single molecule tracking experiments have confirmed the existence of fast sliding by the DNA-repairing protein human oxoguanine DNA glycosylase (hOgg1) along DNA.[5]

Structures of nonspecific protein−DNA complexes have recently become available.[6−9] Although the nonspecific interaction is weaker than that of specific target-binding, it still requires particular molecular interactions and a defined orientation of the protein with respect to the DNA double helix. Therefore, one expects that the apparent one-dimensional translational diffusion along DNA observed in experiments must be coupled to the rotational motion corresponding to a helical path along the DNA.

Interestingly, recent single molecule experiments reveal a $1/R^3$ size dependence of diffusion, in contradiction to the usual Stokes−Einstein $1/R$ dependence expected, on hydrodynamic arguments, for one-dimensional translational diffusion.[10]

In an interesting early theoretical analysis, Schurr modeled protein diffusion along DNA as a translational diffusion accompanied by rotation such that the helical sense of the protein vis-à-vis the DNA is retained along the helix.[11] That is, the protein rotates by 2π after it traverses 10 base pairs, or one turn of the DNA helix. Note that what we measure in single molecule experiments is an apparent translational diffusion coefficient. Because of limitations of temporal and spatial resolution, the rotational motion of protein is not seen, but its effect is manifested in the measured translational diffusion coefficient. In Schurr's model, if the protein is approximated by a sphere of radius R, then the total friction is a sum of rotational and translational friction and is given by the following expression:

$$\zeta_{\text{Trans}}^{\text{Total}} = 6\pi\eta R + \left(\frac{2\pi}{10BP}\right)^2 8\pi\eta R^3 \qquad (1)$$

Here BP denotes the distance between two base pairs of DNA, equal to 3.4 Å (or, 0.34 nm). The diffusion constant can be obtained from eq 1 using Einstein's relation, $D = k_B T/\zeta$, where $k_B T$ is the Boltzmann constant times the absolute temperature. Schurr discussed that for the lac repressor, approximated by a sphere of radius 4.9 nm, the rotational contribution to the total friction, with R^3 dependence, is about 2 orders of magnitude larger than the translational component, and provides a much better agreement of protein diffusion value with the then known result. Recent single-molecule experiments have provided detailed estimates which seem to support Schurr's assertion that diffusion of a protein along DNA is more controlled by the rotational friction rather than by the translational friction. These single-molecule experiments also seem to provide evidence for the mechanism that proteins spin while sliding along DNA.[10]

The simplicity of Schurr's model, however, limits quantitative comparison with diffusion data from real systems. It assumes that during the sliding, the protein engulfs the DNA such that the center of mass of the protein always remains on the DNA axis. However, it has since been discovered that many proteins in nonspecific complexes with DNA are significantly offset from the DNA axis.[6−9] This is illustrated pictorially in Figure 1. Therefore, we need to generalize Schurr's expression to include the helical track along which diffusion takes place.

Here we derive such an expression, one that describes the total friction of a spherical body moving along a straight segment of DNA. The derivation has two steps. First we derive an expression of the form of the friction. Second, we perform a

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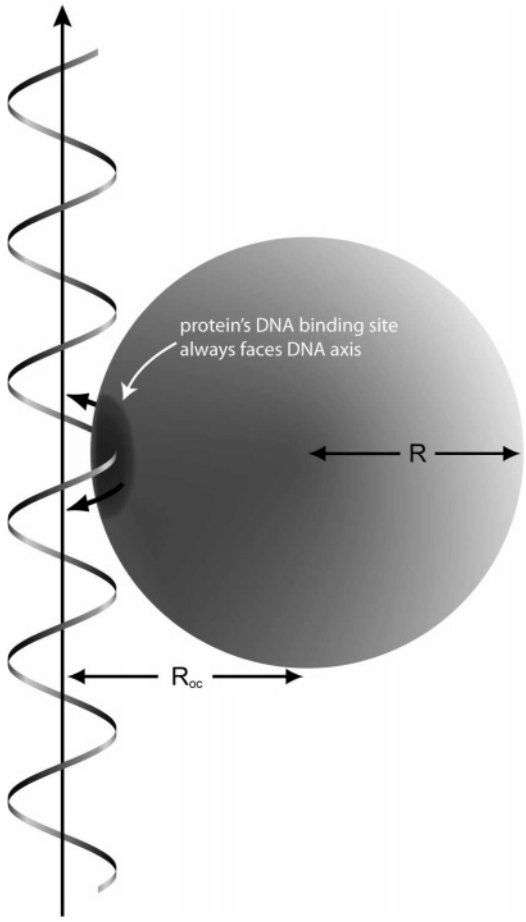

Figure 1. Diagram illustrating model parameters R and R_{OC}. Diagram is approximately to scale showing a ribbon with the parameters of the B-form DNA helix and a sphere representing a sphere (comparable in size to the lac repressor) with $R = 4.9$ nm and $R_{OC} = 5.5$ nm.

hydrodynamic calculation to obtain an explicit expression for the friction. Last, we show that the generalized expression provides a rather good estimate of the diffusion coefficient.

II. Friction for Motion Along a Helical Path

We start with the parametric equations for a helix:

$$x = r\cos(\theta) \tag{2}$$

$$y = r\sin(\theta) \tag{3}$$

$$z = b\theta \tag{4}$$

where θ is the angle the position vector (with components x,y,z) makes with the X-axis (projected to the XY-plane), and b is a constant. r is the radius of the circle in the $X-Y$ plane, and b is the pitch of the helix, and, for B-form DNA, $b = 10\text{BP}/2\pi$, where BP is the distance between the centers of two adjacent base pairs.

Thus, since displacement along Z is a function of θ, we can write a Langevin equation for z as follows:

$$\ddot{z}(t) = b\ddot{\theta}(t) \tag{5}$$

$$\ddot{\theta}(t) = -\varsigma_{\text{Rot}}\,\dot{\theta}(t) + T(t) \tag{6}$$

where T is the random torque on the motion of the sphere along the circle in the $X-Y$ plane. The rotational friction and the torque are related by the fluctuation−dissipation theorem. Note that this torque can include the rotation of the sphere on its own axis. This rotation of the sphere on its own axis does not alter the helical path, but increases the magnitude of the friction.

We now write down the Langevin equation for the projected linear motion in the Z-direction due to motion along the helix in terms of the translational friction ζ_{Trans} (which we want to calculate):

$$\dot{V}_z(t) = -\varsigma_{\text{Trans}}V_Z(t) + F_Z(t) \tag{7}$$

where V_Z is the velocity projected in the Z-direction due to the motion along the helix. $F_Z(t)$ is the random force related to the friction by the fluctuation−dissipation theorem. But this random force is related to random torque because of eq 5 and must obey the fluctuation dissipation theorem. Therefore, we need to express the fluctuating force in terms of fluctuating torque.

Let us consider a small displacement in θ, say $\delta\theta$, during a small time window δt. This will lead to small displacement in z. We now expand $\delta\theta$ in a power series in time δt:

$$\delta\theta = \omega\delta t + \frac{1}{2}T(\delta t)^2 \tag{8}$$

Similarly,

$$\delta z = V_Z\delta t + \frac{1}{2}F(\delta t)^2 \tag{9}$$

However, δz must be equal to $\delta\theta/b$. The terms in the above two equations must match term by term because of the equation of the helix. Therefore, we have the random force on the Z-axis as

$$F(t) = T(t)/b \tag{10}$$

The last equation implies that the translational friction *due to circular motion* around the helix is, by the fluctuation−dissipation theorem,

$$\varsigma_{\text{Trans}}^{\text{Helix}} = \frac{1}{b^2}\varsigma_{\text{Rot}} = \left(\frac{2\pi}{10\text{BP}}\right)^2\zeta_{\text{Rot}}, \tag{11}$$

The next step is the hydrodynamic calculation of this rotational friction of the motion of a sphere of radius R, which rotates on its axis and at the same time executes a circular translational motion around an axis, with a separation between the sphere's center of mass and the axis given by R_{OC}. An expression of this friction has already been derived by Happel and Brenner[12] in their well-known monograph on hydrodynamics. Here we just present the final result:

$$\varsigma_{\text{Rot}} = 8\pi\eta R^3 + 6\pi\eta R(R_{\text{OC}})^2 \tag{12}$$

Note that the second term in the right-hand side of the above expression is essentially a translational friction that is due to the curvilinear motion along the helix (see Figure 1).

In addition to the above friction, the sliding sphere will also experience random force along Z. This will give rise to a friction equal to $6\pi\eta R$. This needs to be added to account for the total friction for diffusion along a helical path. The total friction is then given by

$$\varsigma_{\text{Trans}}^{\text{Total}} = 6\pi\eta R + \left(\frac{2\pi}{10\text{BP}}\right)^2[8\pi\eta R^3 + 6\pi\eta R(R_{\text{OC}})^2] \tag{13}$$

This is our final expression of the translational friction on a protein sliding along a DNA while spinning (1) on its own axis and (2) about the DNA axis from which its center of mass is

projected by some distance. Expression 13 reduces to Schurr's expression if the center-to-center distance (R_{OC}) is zero. An alternative derivation of this formula is given in the Appendix.

An interesting aspect of the above expression is that the friction due to translational motion around the helical axis can be larger than the rotational contribution if R_{OC} is somewhat larger than R. For large proteins (such as hOgg1), however, we expect $R_{OC} \leq R$, so that the rotational contribution is larger. In any case, considering an offset of the protein from the DNA axis substantially increases the friction to movement along DNA.

III. Numerical Results and Discussion

We now consider quantitative implications of the new expression and compare the theoretical prediction with recent single-molecule experimental measurements. When modeling the lac repressor as a sphere with $R = 4.9$ nm, Schurr shows that on-axis rotation is expected to reduce the diffusion constant from the pure translational value, 4.45×10^{-11} m^2/s, ~110-fold to 4.04×10^{-13} m^2/s. Off-axis rotation of the lac repressor, modeled with $R = 4.9$ nm and $R_{OC} = 5.5$ nm according to the theory here presented, predicts an ~215-fold drop in the protein's diffusivity on DNA, to 2.09×10^{-13} m^2/s. The experimental value of LacI diffusion is $(4.6 \pm 1.0) \times 10^{-14}$ m^2/s.[13] Our second example is the diffusion of hOgg1, a smaller protein. For diffusion of hOgg1 ($R = 3.2$ nm and $R_{OC} = 2.5$ nm), Schurr's expression leads to a value of D equal to 1.43×10^{-12} m^2/s, our generalized expression gives 9.89×10^{-13} m^2/s, while the experimental value is $(4.92 \pm 1.0) \times 10^{-13}$ cm^2/s. In both the examples, inclusion of helical translation leads to diffusion constants closer to, but still larger than experimental values. The remaining gap can be attributed to the presence of small free energy barriers to sliding that arise from details of the protein-DNA interaction. It seems that these barriers can reduce the diffusion constant by a factor of 2−5 from the theoretical upper limits calculated using our generalized expression, which is based solely on hydrodynamic considerations. Additionally, the friction on the moving protein should also increase, and the diffusion rate should decrease as a result of the presence of the static DNA. It is known that the presence of a nearby static object modifies the hydrodynamic flow field to increase the friction. In reality, the DNA molecule shall perform small amplitude motions, which can again lower the effects of the presence of the static DNA.

In conclusion, we have derived an analytical expression of the translational diffusion of a protein moving along a helical path approximating the DNA helix. Comparisons between theoretical prediction and recent single-molecule experimental results seem to indicate correspondence consistent with a spinning mechanism of protein diffusion along DNA.

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Appendix

Here we present an alternative derivation of the total friction. The derivation outlined below follows that of Schurr closely, but provides additional insight into the use of the Happel−Brenner expression in a compact way. We shall follow the Landau−Lifshitz notation, which is more commonly known.[14]

Let Q_k and P_k be a set of generalized coordinates and momenta. In our case, they will be Z, θ, and Φ, where Z is the displacement coordinate along the DNA helix, θ denotes the helical angle in the $X-Y$ plane, and Φ denotes the rotation of the spherical diffusing particle on its own axis, which is taken to be parallel to the DNA axis (which is chosen as Z).

We then have a set of generalized equations of motion

$$\dot{P}_i = -\frac{\partial U}{\partial Q_i} - \sum_{k=1}^{3} \zeta_{ik} \frac{\partial K}{\partial P_k} \tag{A1}$$

where U is the potential energy, K is the kinetic energy, and ζ_{ik} are the friction coefficients. Since kinetic energy is quadratic in momenta, we have the standard Langevin equation

$$\dot{P}_i = -\frac{\partial U}{\partial Q_i} - \sum_{k=1}^{3} \zeta_{ik} \dot{Q}_k \tag{A2}$$

We now neglect off-diagonal coupling among friction terms. The total dissipation is then given by

$$f = \sum_{i=1}^{3} \zeta_i \dot{Q}_i \dot{Q}_i \tag{A3}$$

We have absorbed the factor of 2 here, so that rate of total energy dissipation is f and not $2f$, as in Landau−Lifshitz (page 381).[14]

So, total friction is the sum of three terms, each comes with the product of the square of the respective velocities.

Now comes the crucial part. We have to find the dissipation associated with motion along the Z-axis. Because of the helix equation (see equations earlier), both the angular terms have an effective velocity along Z which we need to pick up. Thus, both velocities of θ and Φ are to be $1/b = 2\pi/(10\text{BP})$, where BP is the base pair distance.

However, dissipation comes as the square of the velocities. That is, all three terms are multiplied by velocity along Z, so that they can easily be combined. This justifies the final expression given below.

So, we combine all this to obtain

$$\zeta_{\text{Trans}}^{\text{Total}} = 6\pi\eta R + \left(\frac{2\pi}{10\text{BP}}\right)^2 [8\pi\eta R^3 + 6\pi\eta R (R_{OC})^2]$$

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