# DIFFUSION-CONTROLLED REACTIONS ON SPHERICAL SURFACES

## APPLICATION TO BACTERIOPHAGE TAIL FIBER ATTACHMENT

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ABSTRACT We have explored the kinetic implications of a model that may account for the acceleration of tail fiber (F) attachment to baseplates (B) by whiskers (W) on bacteriophage T4. The model assumes that a W-F complex is formed initially, and that the tethered fiber then undergoes rotational diffusion until a B-F encounter takes place. In the absence of whiskers, B-F complexes must form unassisted. Formation of a W-F intermediate will accelerate F attachment to B if (*a*) the bimolecular rate constant for W-F complex formation is larger than that for direct B-F interaction and (*b*) subsequent rotational diffusion of the tip of F to B is not much slower than the dissociation of W-F. Condition *a* was investigated by applying a recent theory of orientational effects on translational diffusion-controlled reactions. This theory suggests that substantial rate enhancement is expected if the reaction half-angle $\theta_0$ is larger for W-F than for B-F complex formation. Condition *b* was investigated by calculating the mean and the variance of the time required for the diffusion of a molecule (the proximal tip of the fiber) on a spherical surface (whose radius is the distance from the tip to the whisker tethering point) into a circular sink (the baseplate site). The mean time is on the order of the inverse rotational diffusion coefficient, $D_R$, of the fiber, but is sensitive to $\theta_0$. Both conditions are satisfied for plausible choices of parameters. The solution to the diffusion equation we have obtained should have application to other physical situations, such as the rate of quenching of a fluorophore as it diffuses on the surface of a spherical membrane into proximity with a quencher.

### INTRODUCTION

The attachment of tail fibers to T-even bacteriophage is the last step in the assembly of these complicated viruses (1, 2). A striking recent discovery is that, in bacteriophage T4, fiber attachment is considerably accelerated by the presences of whiskers attached to the phage tail at the head-tail junction (3, 4). It has been proposed that the whiskers, fibrous proteins ~400 Å long (5, 6), serve as jigs to facilitate proper alignment of fibers with the phage body (6, 7). In this paper we examine theoretically some quantitative aspects of this proposal.

Specifically, we first consider the kinetics of the simplest plausible mechanisms for fiber attachment in the absence and presence of whiskers. In the absence of whiskers, a fiber (F) attaches irreversibly to a site on the baseplate (B) of the tail (Fig. 1 *a*):

$$B + F \xrightarrow{k_1} B - F. \tag{1}$$

With whiskers (W) attached, the fiberless phage is denoted BW. We hypothesize that a whisker binds reversibly to a fiber, probably near the kink in its middle. The tethered fiber

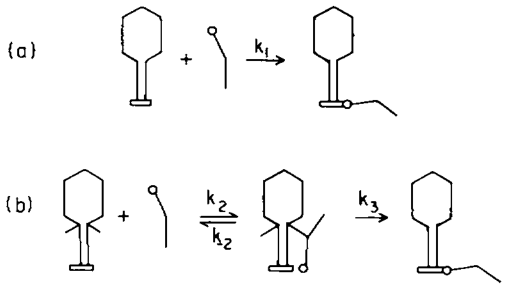

FIGURE 1 Hypothetical mechanisms for the attachment of tail fibers to baseplates (*a*) in the absence of whiskers; (*b*) in the presence of whiskers.

then undergoes rotational diffusion until it encounters and reacts with the baseplate site (Fig. 1 *b*):

$$B + F \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} BW - F \xrightarrow{k_3} WB - F. \tag{2}$$

In the next section, we analyze these two mechanisms using recently constructed theories of orientation effects on translational diffusion-controlled reactions (8, 9, and references cited therein). We conclude that the accelerated production of WB-F compared to B-F is due to more rapid formation of BW-F compared to B-F.

We then undertake an estimate of the magnitude of $k_3$. The conversion of BW-F to WB-F involves the rotational diffusion of the fiber until it encounters and then reacts with the baseplate attachment site. Although it is known that the formation of the B-F bond is catalyzed by the phage-coded protein gp63 (10, 11), an upper limit on $k_3$ can be estimated by assuming the reaction to be diffusion controlled. From a mathematical point of view, this problem is identical with the rate of diffusion of a molecule (the proximal tip of the fiber) on a spherical surface (whose radius is the distance from the tip to the whisker-tethering point) into a circular sink (representing the baseplate site). Therefore, the solution we arrive at has application to other situations currently of interest in molecular biophysics, such as the rate of quenching of a fluorescent molecule as it diffuses on the surface of a spherical membrane into proximity with a quencher molecule (12–15).

## KINETIC ANALYSIS

The rate of formation of B-F according to Eq. 1 is

$$\frac{d[BF]}{dt} = k_1[B][F], \tag{3}$$

whereas steady-state analysis of Eq. 2 yields

$$\frac{d[WB\text{-}F]}{dt} = \frac{k_2 k_3}{k_{-2} + k_3}[BW][F]. \tag{4}$$

Assuming $[BW] = [B]$, the acceleration due to the presence of whiskers is $k_2k_3/(k_{-2} + k_3)k_1$. Because $k_3/(k_{-2} + k_3) < 1$, the acceleration must result from $k_2/k_1 \gg 1$.

Recent work on the effects of orientation on bimolecular diffusion-controlled reactions has shown that requirements of proper alignment can dramatically decrease reaction rates. Schmitz and Schurr (8) considered an artificial but tractable model system: the reaction between a diffusing sphere and a stationary site on a plane. For reaction to occur, the center of the sphere, whose hydrodynamic radius is $R_H$, is required to lie on a target hemisphere of radius $R_T$, whose center is a distance $R_H$, above the site on the plane; and the orientation vector of the sphere, from its center to the reaction site on its surface, must lie within a cone of half-angle $\theta_0$ about the perpendicular to the plane. The combined translational-rotational diffusion equation with reaction boundary conditions was solved numerically in the steady state. The calculated bimolecular diffusion-controlled rate constant, $k_{eff}$, relative to the rate constant $k_{diff}$ for a sphere uniformly reactive over all its surface, is a function only of $R_t/R_H$ and of $\theta_0$. If, furthermore, the plausible (for small $\theta_0$) condition $R_T = \theta_0 R_H$ on the target radius is imposed, then $k_{eff}/k_{diff}$ is a function only of $\theta_0$. This ratio is calculated to run from $2.97 \times 10^{-5}$ at $\theta_0 = 0.048$ rad = 2.75°, to $2.50 \times 10^{-4}$ at $\theta_0 = 0.098$ rad = 5.61°, to $1.98 \times 10^{-3}$ at $\theta_0 = 0.2$ rad = 11.5°. Thus $k_{eff}/k_{diff}$ is a very sensitive function of $\theta_0$ in this region. Such reaction cone half angles are probably not unrealistically small. It was also found that the ratio of $k_{eff}$ to $k_{\theta_0}$, the rate constant in the absence of rotational diffusion, was within 20% of 2 over a wide range of $\theta_0$.

Although these results were obtained for the sphere-plane geometry described above, Schmitz and Schurr (8, 9) conjectured that similar results would hold for reactions between two spheres. We may extend the conjecture even further, at least qualitatively, to the fiber attachment reactions of interest here. Thus we would expect that if the reaction cone half-angle $\theta_{BF}$ for insertion of the proximal end of the fiber into the baseplate is rather small, whereas that for attachment of the fiber to the whisker, $\theta_{WF}$, is larger, $k_2$ may be much greater than $k_1$. The numbers in the previous paragraph suggest that $\theta_{WF}/\theta_{BF} = 4.2$ could lead to $k_2/k_1 = 67$. For small $\theta_0$, in fact, the diffusion-controlled rate constant varies approximately as $\theta_0^3$ (9) (Eq. 4). Of course, if reaction (1) has a significant "chemical" component, or activation energy, as seems likely to be the case, whereas BW-F is a looser complex, then $k_2/k_1$ may be even greater.

Inspection of Fig. 2*a* in reference 3 suggests that whiskers produce a 20–40-fold acceleration of the production of infectious particles. This is only a very crude estimate of the acceleration of attachment of a single fiber, since several fibers are required to make a phage particle infectious, and phage with three or four fibers are not as infectious as those with five or six (10). Recognizing this uncertainty, an acceleration of 20–40 suggests $\theta_{WF}/\theta_{BF} = 2.7\text{–}3.4$.

Even if $k_2/k_1 \gg 1$, whiskers cannot accelerate fiber attachment if $k_3/(k_{-2} + k_3) \ll 1$; that is, if $k_{-2} \gg k_3$. Because $k_2/k_{-2} = K$, the equilibrium constant for fiber-whisker attachment, we make a very rough estimate of $k_{-2}$. If the fiber-whisker attachment is rather loose and geometrically unrestrictive, one might expect $k_2 \sim 10^8$ $M^{-1}$ $s^{-1}$, $\Delta G^\circ \sim -5$ kcal/mol, so $K = \exp[-\Delta G^\circ/KT] \sim 4 \times 10^3$ $M^{-1}$, and $k_{-2} \sim 2 \times 10^4$ $s^{-1}$. Thus $k_3$ should not be very much smaller than this.

The estimate $k_2 = 10^8$ $M^{-1}$ $s^{-1}$ arises from von Smoluchowski diffusion-controlled reaction rate theory, assuming reactive particles with diffusion coefficients $3.6 \times 10^{-8}$ cm$^2$/s (fiberless phage) and $1.3 \times 10^{-7}$ cm$^2$/s (fibers), a reaction radius of 8 Å, and no orientational constraints. This is probably a considerable overestimate of $k_2$. A smaller value of this rate constant, or a stronger binding of fiber to whisker, leads to even lower estimates of $k_{-2}$. Thus, even less stringent requirements are imposed on $k_3$ calculated in the next section.

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To estimate the diffusion-controlled rate of attachment of the proximal fiber tip to the baseplate site when the fiber is tethered by a whisker, let us assume that the baseplate site defines a cone of half-angle $\theta_0$ centered at the north pole of a spherical polar coordinate system whose center is the whisker-fiber tethering site. We simplify by ignoring the restrictions that other parts of the phage will impose on the fiber motion, and assume that the fiber tip can diffuse freely over the surface of the sphere, with rotational diffusion coefficient, $D_R$. Then the rotational diffusion equation,

$$\frac{\partial c(\theta, t)}{\partial t} = \frac{D_R}{\sin\theta}\frac{\partial}{\partial\theta}\left(\sin\theta\,\frac{\partial c(\theta, t)}{\partial\theta}\right), \tag{5}$$

must be solved subject to the initial condition,

$$c(\theta, 0) = C_0, \qquad \theta \geq \theta_0, \tag{6}$$

and the boundary condition

$$c(\theta_0, t) = 0 \tag{7}$$

Here $2\pi c(\theta,t)\sin\theta d\theta$ is the probability that the fiber will be found in $(\theta, \theta + d\theta)$ at time $t$.

Although $c(\theta,t)$ itself cannot be written in closed form, simple expressions for the moments of the distribution of reaction times,

$$\overline{t^n} \equiv -\frac{\int_{\theta_0}^{\pi}\sin\theta\int_0^{\infty} t^n\frac{\partial c(\theta, t)}{\partial t}\mathrm{d}t\,\mathrm{d}\theta}{\int_{\theta_0}^{\pi}\sin c(\theta, 0)\,\mathrm{d}\theta}, \tag{8}$$

are readily derived (Appendix). For the boundary conditions specified by Eqs. 6 and 7, the mean lifetime is

$$\bar{t} = -\frac{1}{D_R}\left(1 + \frac{2\,\ell n \sin(\theta_0/2)}{\cos^2(\theta_0/2)}\right), \tag{9}$$

and the variance is

$$\overline{t^2} - \bar{t}^2 = \frac{1}{D_R^2}\left[3 + \frac{4\,\ell n \sin(\theta_0/2)}{\cos^2(\theta_0/2)}\left(1 + \frac{\ell n \sin(\theta_0/2)}{\cos^2(\theta_0/2)}\cos\theta_0\right)\right]. \tag{10}$$

Fig. 2 shows a plot of $D_R\bar{t}$ against $\theta_0$. The relative variance $(\overline{t^2} - \bar{t}^2)/\bar{t}^2$ increases steadily from 1.00 at $\theta_0 = 0$ to 1.67 at $\theta_0 = \pi$.

Taking the fiber as a rigid rod, modeled by a prolate ellipsoid of semi-major axis $a = 690$ Å and semi-minor axis $b = 20$ Å, one finds from the Perrin equation,

$$D_R = kT/\zeta = 3kT[2\,\ell n(2a/b) - 1]/16\pi\eta a^3, \tag{11}$$

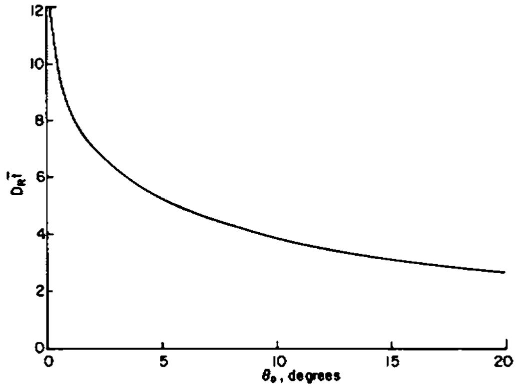

FIGURE 2 Dependence of mean lifetime $\bar{t}$, scaled by rotational diffusion coefficient $D_R$, on half-angle $\theta_0$ of reaction cone.

that at 20°C, $D_R = 5480\ \mathrm{s}^{-1}$. Thus the average rate constant,

$$\bar{k}_3 \equiv 1/\bar{t}, \tag{12}$$

for $\theta_0$ in the range 5–10° is on the order of $10^3\ \mathrm{s}^{-1}$. Specifically, with $\theta_0 = 5°$, it is $1.04 \times 10^3\ \mathrm{s}^{-1}$; and with $\theta_0 = 10°$, it is $1.40 \times 10^3\ \mathrm{s}^{-1}$.

## DISCUSSION

The foregoing calculations are obviously approximate in many ways. The mechanisms assumed in Eqs. 1 and 2 may well be oversimplified. The theoretical results for the translational bimolecular rate constant are used for a geometry quite different from that for which they were derived (8, 9). We have not ruled out that the step characterized by $k_2$ is intrinsically faster than that characterized by $k_1$, regardless of diffusion effects. The assumption of cylindrical symmetry in the rotational diffusion problem is clearly an oversimplification. The calculation of $D_R$ for the fiber ignores the kink in its middle, but since the kink angle appears in electron micrographs to be nearly constant at 156° (3), a straight rod is a good approximation. In addition, we must admit that alternative models for the acceleration are conceivable: for example, an attractive interaction (16) between fiber and whisker, which might extend along the entire length of the whisker.

However, we do not feel that these approximations are serious enough to invalidate the major points of this paper: that the whiskers may accelerate tail fiber attachment by speeding up the initial translational bimolecular interaction of the fibers with the fiberless phage particle; that this speeding up may simply be due to a larger acceptance angle, $\theta_0$, for whiskers relative to baseplate; and that subsequent rotational diffusion of the ends of whisker-tethered fibers to their baseplate attachment sites is probably not slow compared to fiber-whisker dissociation. In addition, we emphasize that no single rate constant can be defined for the diffusion-controlled reaction of a molecule confined to a spherical surface with a small patch (or another molecule) on that surface. The distribution of reaction half-lives has a relative standard deviation at least as large as the mean lifetime itself.

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## APPENDIX

We define the functions,

$$c_n(\theta) \equiv n \int_0^{\infty} t^{n-1}\, c(\theta, t)\, \mathrm{d}t$$

$$= - \int_0^{\infty} t^n \frac{\partial c(\theta, t)}{\partial t} \mathrm{d}t, \tag{A1}$$

multiply both sides of Eq. 13 by $t^{n-1}$, and integrate with respect to time over the entire interval $0 \leq t < \infty$:

$$-nc_{n-1}(\theta) = \frac{D_R}{\sin\theta} \frac{\mathrm{d}}{\mathrm{d}\theta}\left(\sin\theta \frac{\mathrm{d}c_n(\theta)}{\mathrm{d}\theta}\right), \qquad n \geq 1$$

$$c_0(\theta) \equiv c(\theta, 0). \tag{A2}$$

Eq. A2 can be solved for $c_n(\theta)$ to give the recursion formulae

$$c_n(\theta) = \frac{n}{D_R} \int_{\theta_0}^{\theta} \frac{\mathrm{d}\theta'}{\sin\theta'} \int_{\theta'}^{\pi} c_{n-1}(\theta'') \sin\theta''\, \mathrm{d}\theta''. \tag{A3}$$

The moments $\overline{t^n}$ are then obtained from Eqs. 16 and A1:

$$\overline{t^n} = \frac{\int_{\theta_0}^{\pi} c_n(\theta) \sin\theta \mathrm{d}\theta}{\int_{\theta_0}^{\pi} c_0(\theta) \sin\theta \mathrm{d}\theta}. \tag{A4}$$

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