Transport Effects on the Kinetics of Protein-Surface Binding

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ABSTRACT A detailed model is presented for protein binding to active surfaces, with application to the binding of avidin molecules to a biotin-functionalized fiber optic sensor in experiments reported by S. Zhao and W. M. Reichert (American Chemical Society Symposium Series 493, 1992). Kinetic data for binding in solution are used to assign an intrinsic catalytic rate coefficient k to the biotin-avidin pair, deconvoluted from transport and electrostatic factors via application of coagulation theory. This intrinsic chemical constant is built into a reaction-diffusion analysis of surface binding where activity is restricted to localized sites (representing immobilized biotin molecules). The analysis leads to an effective catalytic rate coefficient k_{eff} characterizing the active surface. Thereafter, solution of the transport problem describing absorption of avidin molecules by the macroscopic sensor surface leads to predictions of the avidin flux, which are found to be in good agreement with the experimental data. The analysis suggests the following conclusions. 1) Translational diffusion limitations are negligible for avidin-biotin binding in solution owing to the small (kinetically limiting) value $k = 0.00045$ m/s. 2) The sparse distribution of biotin molecules and the presence of a repulsive hydration force produce an effective surface-average catalytic rate coefficient k_{eff} of order 10^{-7} m/s, much smaller than k. 3) Avidin binding to the fiber optic sensor occurs in an intermediate regime where the rate is influenced by both kinetics and diffusion.

INTRODUCTION

The binding of ligands and enzymes to proteins, cells, and other active surfaces is central to chemoreception (Berg and Purcell, 1977; DeLisi and Wiegel, 1981; Northrup, 1988; Shoup and Szabo, 1982; Wiegel, 1983; Zwanzig, 1990; Zwanzig and Szabo, 1991), repressor-operator association (Berg and Blomberg, 1976, 1977, 1978; Richter and Eigen, 1974), and the operation of biosensors (Zhao and Reichert, 1992a,b). Theoretical work in the biophysics literature has addressed the interplay between transport, electrostatics, and site-specific kinetics in determining the net rate of association, as reviewed exhaustively, e.g., by Noyes (1961), Calef and Deutch (1983), Berg and von Hippel (1985), Keizer (1987), and Wu and Nitsche (1995). There have also been direct experimental observations of the rates of protein binding to active surfaces in the form of ligand-functionalized substrates (Schmitt and Knoll, 1991; Ahlers et al., 1992; Schmidt et al., 1992; Zhao and Reichert, 1992a,b; Muller et al., 1993; Spinke et al., 1993). The avidin-biotin association reaction is ideal for a comparison between experimentally determined and theoretically predicted association rates, owing to the availability of relevant kinetic data obtained under a variety of different conditions. In particular, 1) the kinetics for avidin binding to soluble biotin are known (Green, 1975), 2) the kinetics for avidin binding to surface-immobilized biotin have been determined (Zhao and Reichert, 1992a,b), and 3) the three-dimensional structure of avidin is well characterized (Livnah et al., 1993).

In the kinetic measurements of streptavidin or avidin binding to immobilized biotin, the most relevant studies have focused on their specific adsorption to planar lipid monolayers containing biotin conjugated to double-chained surfactants (Zhao and Reichert, 1992a,b; Schmitt and Knoll, 1991; Schmidt et al., 1992). In the latter studies, the surface density of biotin surfactant was controlled by adjusting its mol fraction in the lipid mixture. Either the rate of fluorescently labeled protein adsorption, determined by the time dependence of fluorescence increase at the membrane surface, or the time-dependent change in the mass at the surface was determined as a function of the bulk protein concentration. The square root time dependence of streptavidin binding to immobilized biotin (Schmitt and Knoll, 1991) indicated that the latter reaction was diffusion controlled.

The purpose of this paper is twofold. First, we develop in the next section a detailed and general model for protein binding to surfaces with multiple localized active sites, with full accounting for hydrodynamic wall effects and energetic interactions with the surface. The analysis has elements in common with, e.g., the investigations of Berg and Purcell (1977), Shoup and Szabo (1982), Wiegel (1983), Northrup (1988), Zwanzig (1990), and Lucas et al. (1994) in addressing reaction with multiple surface sites; with, e.g., Chou and Zhou (1982), Shoup and Szabo (1982), Allison et al. (1985), Northrup et al. (1986, 1987), Sharp et al. (1987), Nambi et al. (1991), Luty et al. (1993) and Zhou (1993) in incorporating intermolecular forces; and with, e.g., Friedman (1966), Honig et al. (1971), Deutch and Felderhof (1973), McCammon et al. (1975), Wolynes and Deutch (1976), Wolynes and McCammon (1977), Northrup and Hynes (1979), Zientara et al. (1982), Northrup et al. (1984), and Kim and Zukoski

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(1990) in including hydrodynamic interactions. Part of its novelty lies in combining all these elements with rigor, and in applying them to a real system. Second, we embed the analysis within a broader three-part theory in the section titled Binding of Avidin to a Biotin-Functionalized Surface to calculate rates of avidin absorption by a biotinylated fiber optic sensor, and compare the results with experimentally determined avidin fluxes reported by Zhao and Reichert (1992a).

FORMULATION OF THE MODEL PROBLEM

The physical system addressed, depicted in Fig. 1, involves the Brownian motion of spherical solutes of radius a_A and bulk diffusivity D_∞ toward an active planar surface at $z = 0$,

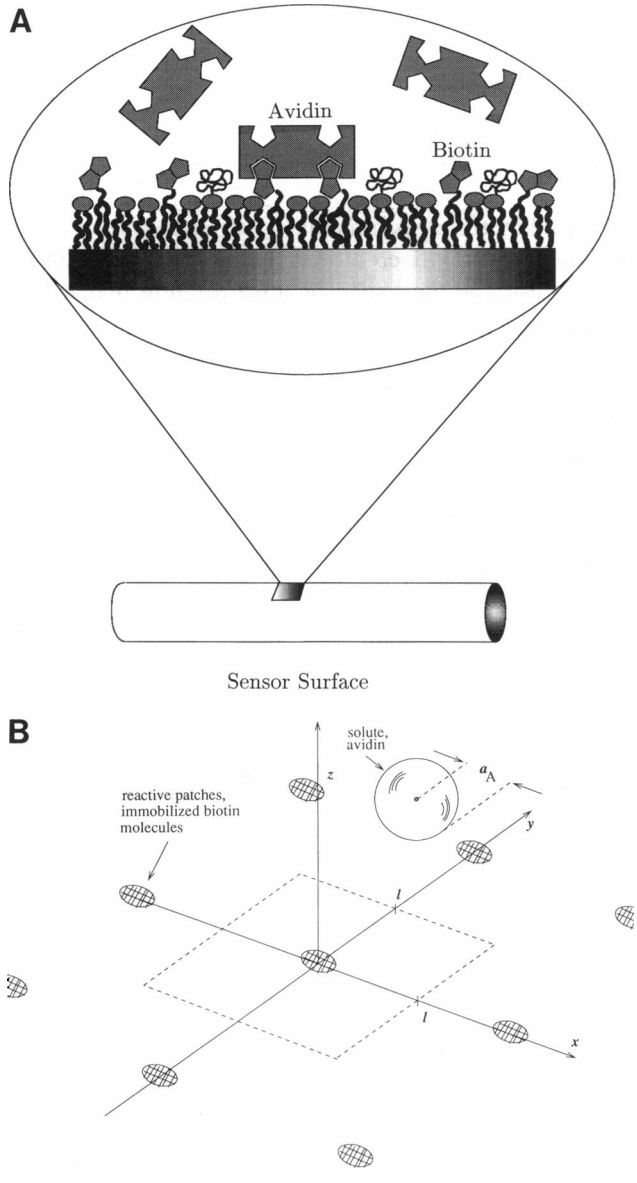

FIGURE 1 Definition sketch. (*a*) The actual physical situation. (*b*) The model problem.

as described by the transport equation (cf. Brenner, 1980, pp. 94–96)

$$\nabla \cdot [e^{-E}\mathbf{D} \cdot \nabla(e^{E} C)] = 0 \tag{1}$$

governing the solute concentration $C(x, y, z)$ in dilute solution. The parallel and perpendicular components $d_\parallel$ and $d_\perp$ of the dimensionless diffusion dyadic

$$D_\infty^{-1}\,\mathbf{D} \equiv \mathbf{d} = (\mathbf{ii} + \mathbf{jj})d_\parallel + \mathbf{kk}d_\perp \tag{2}$$

are unequal and depend upon the vertical distance z between the solute center and the surface owing to hydrodynamic wall effects. Numerical values are calculable from the hydrodynamic tabulations and formulas given by Brenner (1961), Goldman et al. (1967), and Falade and Brenner (1988). Only at distances large compared with the solute radius do both asymptotically approach unity. The function $E = \Psi(x, y, z)/k_BT$ represents the potential Ψ for any energetic interactions between solutes and the surface rendered dimensionless with k_BT; it decays to zero as $z \to \infty$.

Binding to the surface is described by a boundary condition that equates the normal flux with a rate of reaction that is first-order in the local solute concentration, viz.,

$$\mathbf{k} \cdot e^{-E}\mathbf{D} \cdot \nabla(e^{E}C) = k_0F(x, y)C \qquad at \quad z = a_A. \tag{3}$$

The catalytic rate coefficient $k_0F(x,y)$ can depend strongly upon the position of the solute on the surface. We shall take $F(x, y)$ to have multiple peaks centered at the nodes of a square lattice with period $2l$, corresponding to a periodic array of localized reactive sites. Only when a solute touching the wall is sufficiently close to the center of a site does it react at an appreciable rate determined by the magnitude of k_0. As a reasonable but arbitrary kinetic model, we are content to utilize the Gaussian functional form $F(x, y) = f(x/l, y/l)$ with

$$f(x/l, y/l) = \exp\{-[(x/l)^2 + (y/l)^2]/2w^2\} \tag{4}$$

as a representation of site-specific reaction in the zeroth unit cell $-l < x < l$, $-l < y < l$. The product wl represents a measure of the dimensional peak width. In consequence of the periodicity of F, the concentration distribution $C(x, y, z)$ is also periodic, i.e.,

$$C(x + 2l, y, z) = C(x, y + 2l, z) = C(x, y, z) \qquad \text{for all} \quad x, y, z. \tag{5}$$

At large distances from the surface, the transport process reduces to one-dimensional diffusion toward the plane $z = 0$. Thus, C must asymptotically approach a linear variation, viz.,

$$\partial C/\partial z \sim \beta \qquad \text{as} \quad z \to \infty. \tag{6}$$

Equating the flows through a square patch at very large

z with the total rate of consumption occurring in the surface unit cell over which it lies leads to the mass conservation constraint

$$4l^2D_\infty \beta = k_0 \int_{-1}^{1}\int_{-1}^{1} F(x, y)\, C(x, y, a_A)\, dx\, dy. \quad (7)$$

We shall write the concentration distribution in the form

$$C(x, y, z) = \beta l\left[\alpha + \int_0^\zeta ds/d_\perp + u(\xi, \eta, \zeta)\right] \quad (8)$$

in recognition of the fact that lateral variations u in the concentration, arising from chemical heterogeneity of the surface, decay to zero at large elevations, leaving a one-dimensional distribution ($\alpha + \int ds/d_\perp$) corresponding to a z-directed Brownian motion with variable diffusivity $d_\perp$. Here

$$\xi = x/l, \quad \eta = y/l, \quad \zeta = (z - a_A)/l \quad (9)$$

represent dimensionless position coordinates. Absence of the potential E from the one-dimensional distribution in Eq. 8 reflects the tacit assumption that E decays to zero more quickly than z^{-1} as $z \to \infty$. The governing equations then express themselves as

$$\nabla_\xi \cdot [e^{-E}\mathbf{d} \cdot \nabla(e^E u)] = -\nabla_\xi \cdot \left\{ e^{-E}\mathbf{d} \cdot \nabla_\xi\left[e^E\left(\alpha + \int_0^\zeta ds/d_\perp\right)\right]\right\}, \quad (10)$$

$$\zeta > 0,$$

$$\mathbf{k} \cdot e^{-E}\mathbf{d} \cdot \nabla_\xi(e^E u) = -\mathbf{k} \cdot e^{-E}\mathbf{d} \cdot \nabla_\xi\left[e^E\left(\alpha + \int_0^\zeta ds/d_\perp\right)\right] + \kappa f(\xi, \eta)(\alpha + u), \quad (11)$$

$$\zeta = 0,$$

$$u \to 0, \zeta \to \infty, \quad (12)$$

$$u(\xi + 2, \eta, \zeta) = u(\xi, \eta + 2, \zeta) = u(\xi, \eta, \zeta)\ \forall\ \xi, \eta, \zeta, \quad (13)$$

with

$$\frac{\kappa}{4}\int_{-1}^{1}\int_{-1}^{1} f(\xi, \eta)\, u(\xi, \eta, 0)\, d\xi\, d\eta + \left[\frac{\kappa}{4}\int_{-1}^{1}\int_{-1}^{1} f(\xi, \eta) d\xi\, d\eta\right]\alpha = 1. \quad (14)$$

The quantity

$$\kappa = k_0 l/D_\infty \quad (15)$$

denotes a Damköhler number based upon the kinetic peak height k_0 and the bulk solute diffusivity D_∞; it represents a ratio of reaction (binding) to diffusion rates.

It can be shown via calculus of variations (Carrier and Pearson, 1976, Chapter 10) that solving Eqs. (10–11) is equivalent to minimizing the functional

$$\begin{aligned} I = &\int_0^1\int_0^1\int_0^\infty \nabla_\xi(e^E u) \cdot e^{-E}\mathbf{d} \cdot \nabla_\xi(e^E u) d\xi\, d\eta\, d\zeta \\ &+ 2\int_0^1\int_0^1\int_0^\infty \nabla_\xi(e^E u) \cdot \left[\mathbf{d} \cdot (\nabla_\xi E)\left(\alpha + \int_0^\zeta ds/d_\perp\right) + \mathbf{d} \cdot \mathbf{k}(1/d_\perp)\right] d\xi\, d\eta\, d\zeta \\ &+ \kappa\int_0^1\int_0^1 f(\xi, \eta) e^{E(\xi, \eta, 0)}\,[u(\xi, \eta, 0)]^2 d\xi\, d\eta \\ &+ 2\kappa\alpha\int_0^1\int_0^1 f(\xi, \eta) e^{E(\xi, \eta, 0)}\, u(\xi, \eta, 0)\, d\xi\, d\eta; \end{aligned} \quad (16)$$

cf. Phillips et al. (1989, 1990) in connection with variational formulations for diffusion problems, albeit without reaction. Integration is restricted to one-quarter $0 \le \xi \le 1$, $0 \le \eta \le 1$ of the (ξ, η) unit cell by symmetry. In actual calculations, the semi-infinite interval $0 \le \zeta < \infty$ is truncated at a finite value ζ_{max} where u is assumed to vanish.

A numerical approximation for u is developed by expanding u in a finite series

$$u(\xi, \eta, \zeta) = \sum_{m=0}^{N}\sum_{n=0}^{N}\sum_{p=1}^{P} c_{mnp}\Phi_{mnp}(\xi, \eta, \zeta), \quad (17)$$

$$\Phi_{mnp}(\xi, \eta, \zeta) = \cos(m\pi\xi)\cos(n\pi y)\sin[p\pi(\zeta - \zeta_{max})/2\zeta_{max}]. \quad (18)$$

Minimization of I subject to the constraint imposed by Eq. 14 then leads to the linear system

$$\sum_{mnp} A_{m'n'p',mnp}\, c_{mnp} + A_{m'n'p',\alpha}\,\alpha = B_{m'n'p'}\ \forall\ m'n'p', \quad (19)$$

$$\sum_{mnp} A_{\alpha,mnp}\, c_{mnp} + A_{\alpha,\alpha}\,\alpha = B_\alpha$$

for the coefficients c_{mnp} and the constant α, where

$$\begin{aligned} A_{m'n'p',mnp} = &\int_0^1\int_0^1\int_0^{\zeta_{max}} \nabla_\xi(e^E\Phi_{m'n'p'}) \cdot e^{-E}\mathbf{d} \cdot \nabla_\xi(e^E\Phi_{mnp})\, d\xi\, d\eta\, d\zeta \\ &+ \kappa\int_0^1\int_0^1 f(\xi, \eta) e^{E(\xi, \eta, 0)}\,\Phi_{m'n'p'}(\xi, \eta, 0)\,\Phi_{mnp}(\xi, \eta, 0)\, d\xi d\eta, \end{aligned} \quad (20)$$

$$A_{m'n'p',\alpha} = \int_0^1 \int_0^1 \int_0^{\zeta_{max}} \nabla_\xi(e^E \Phi_{m'n'p'}) \cdot \mathbf{d} \cdot (\nabla_\xi E)\, d\xi\, d\eta\, d\zeta + \kappa \int_0^1 \int_0^1 f(\xi, \eta) e^{E(\xi, \eta, 0)}\, \Phi_{m'n'p'}(\xi, \eta, 0)\, d\xi\, d\eta, \quad (21)$$

$$A_{\alpha,mnp} = \kappa \int_0^1 \int_0^1 f(\xi, \eta)\, \Phi_{mnp}(\xi, \eta, 0)\, d\xi\, d\eta, \quad (22)$$

$$A_{\alpha,\alpha} = \kappa \int_0^1 \int_0^1 f(\xi, \eta) d\xi\, d\eta, \quad (23)$$

$$B_{m'n'p'} = -\int_0^1 \int_0^1 \int_0^{\zeta_{max}} \nabla_\xi(e^E \Phi_{m'n'p'}) \cdot \mathbf{d} \cdot (\nabla_\xi E) \left(\int_0^\zeta ds/d_\perp \right) d\xi\, d\eta\, d\zeta + \int_0^1 \int_0^1 e^{E(\xi, \eta, 0)} \Phi_{m'n'p'}(\xi, \eta, 0)\, d\xi\, d\eta, \quad (24)$$

$$B_\alpha = 1. \quad (25)$$

Integrations are effected via Gaussian quadrature with refinement near $\xi = 0$, $\eta = 0$, and near $\zeta = 0$ to accurately incorporate structure associated with localized reactivity and near-surface interactions. Final calculations truncate the vertical domain at $\zeta_{max} = 1$ owing to the rapid decay of u with ζ, and utilize a number of modes given by $M = N = P = 8$. A typical concentration profile is shown in Fig. 2, demonstrating localized depletion near the reactive site on the surface.

The content of the solution to the full problem, which incorporates spatial heterogeneity of the catalytic rate coefficient and energetic interactions between the solute and the reactive surface, can be summarized in the form of an effective catalytic rate coefficient k_{eff} defined by the following statement: at large distances from the surface the actual concentration field (and flux) matches the concentration field for *noninteracting* ($E \equiv 0$) solutes approaching a surface having *uniform* activity given by the coefficient k_{eff}. The transport problem defining k_{eff} is simply

$$(d/dz)(d_\perp\, dC/dz) = 0 \quad \text{for} \quad z > 0, \quad (26)$$

$$dC/dz \sim \beta \quad \text{as} \quad z \to \infty, \quad (27)$$

$$D_\infty d_\perp\, dC/dz = k_{eff} C \quad \text{at} \quad z = a_A, \quad (28)$$

for which the solution is

$$C = \text{const.} + \beta l \int_0^\zeta ds/d_\perp \text{ with const.} = D_\infty \beta / k_{eff}, \quad (29)$$

where $\zeta = (z - a_A)/l$ as before. Since $u \to 0$ as $\zeta \to \infty$, this function reproduces the large-z behavior of the true concentration field (Eq. 8) provided that const. $= \beta l \alpha$, or

$$k_{eff} = D_\infty / l\alpha. \quad (30)$$

BINDING OF AVIDIN TO A BIOTIN-FUNCTIONALIZED SURFACE

Based on the preceding analysis, we develop here a theoretical description of the binding of avidin molecules to a biotin-functionalized fiber optic sensor reported by Zhao and Reichert (1992a). The analysis proceeds in three stages. First, coagulation theory is used to deconvolute kinetics from molecular size, transport and electrostatic factors and to assign to the avidin-biotin pair an intrinsic catalytic rate coefficient matched with the rate of binding in solution. Second, this coefficient is built into the analysis of the preceding section to determine the effective surface reactivity characterizing a surface with localized biotin sinks. Third, the effective surface reactivity is introduced into a macroscopic diffusion-reaction analysis of avidin binding to the fiber optic sensor.

Homogeneous kinetics of biotin-avidin binding

Based on established structural information (Green, 1975; Zhao and Reichert, 1992a; Livnah et al., 1993) and an experimentally reported diffusivity (Green, 1975, p. 96), biotin and avidin are described approximately in terms of the spherical entities shown in Fig. 3. Biotin (B) is regarded as a rigid sphere of radius 3 Å. Avidin (A) is modeled as a spherical steric shell of radius 39 Å surrounding a rigid hydrodynamic core of radius 36 Å owing to the considerable irregularity of its surface. The 36 Å core radius represents the Stokes-Einstein radius of avidin, and the 39 Å steric shell radius is roughly consistent with its molecular volume. This simple model for molecular surface roughness (cf. Smart and Leighton, 1989) is used to obviate the lubrication singularity, which technically precludes intermolecular contact (Wolynes and Deutch, 1976; Kim and Zukoski, 1990). It is analogous to the utilization of slip boundary conditions (Wolynes and Deutch, 1976; Northrup et al., 1984). Thus, the respective steric and hydrodynamic core radii are assigned the values

$$a_A = 39 \text{ Å}, \; a_{A,core} = 36 \text{ Å}, \; a_B = a_{B,core} = 3 \text{ Å}. \quad (31)$$

At pH values near neutral, avidin is assumed to behave as if it possesses a uniform surface charge density amounting to a total of eight positive elementary charges ($+8\, e_c$). This value derives from a summation over all charged amino acids (see Green, 1975, p. 90) assuming full ionization at neutral pH. Because of the latter assumption, this net charge likely overestimates the electrostatic enhancement of binding rates. Biotin is assumed to behave like a single negative point charge ($-e_c$) because of an ionized carboxyl group.

The theory of coagulation (Smoluchowski, 1917; Collins and Kimball, 1949; van den Ven, 1989, pp. 347–352; Kim and Zukoski, 1990) furnishes an expression for the rate of binding in solution having the expected second-order form

$$-d[B]/dt = -d[A]/dt = k'[A][B] \quad (32)$$

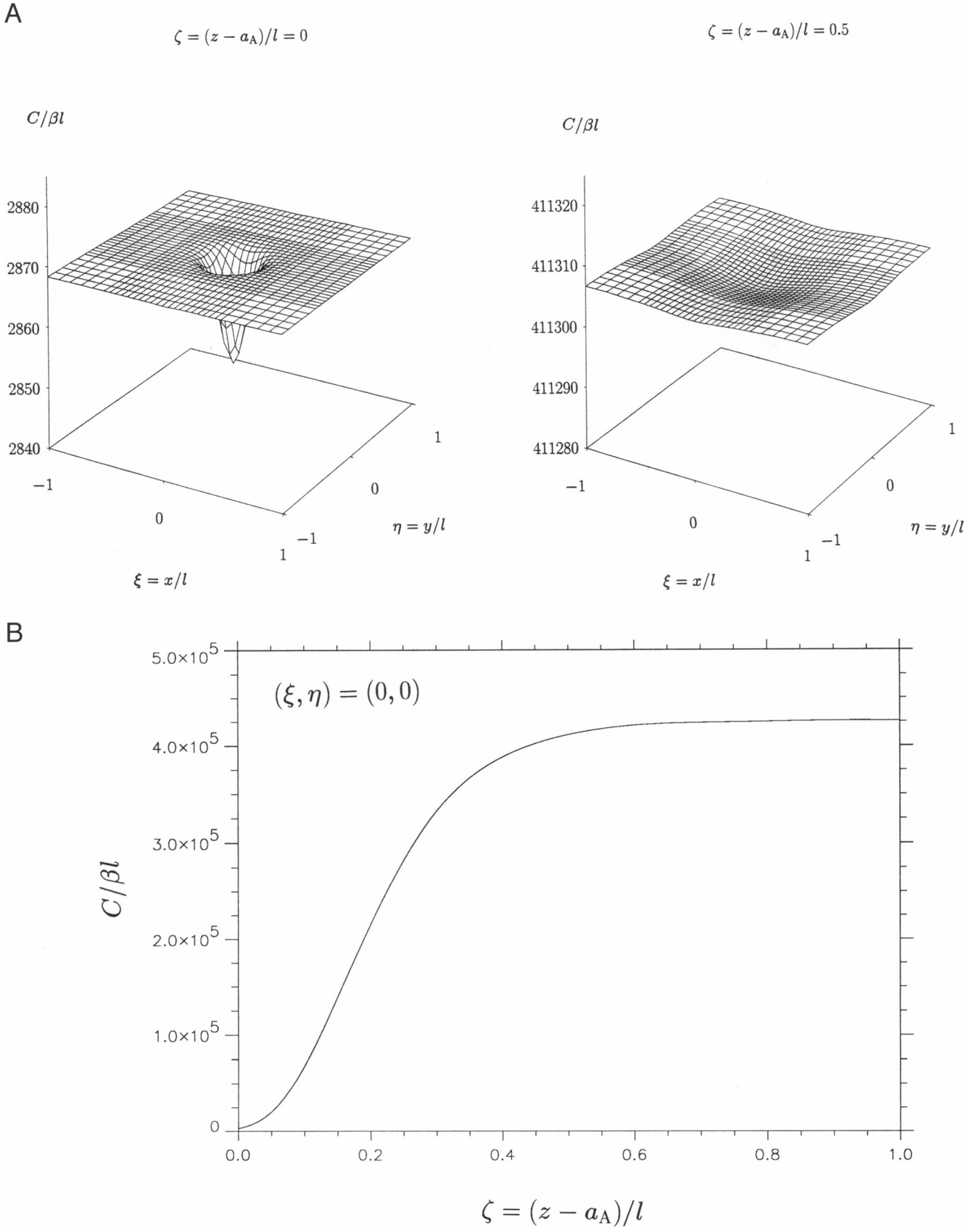

FIGURE 2 Concentration profiles: the dependence of solute concentration upon position, (*a*) C as a function of x and y at the wall and at a higher elevation, (*b*) C as a function of z at $(x,y) = (0,0)$ corresponding to the center of the active site in the zeroth unit cell. Calculations correspond to the fourth line of Table 1, i.e., $l = 30.0$ Å.

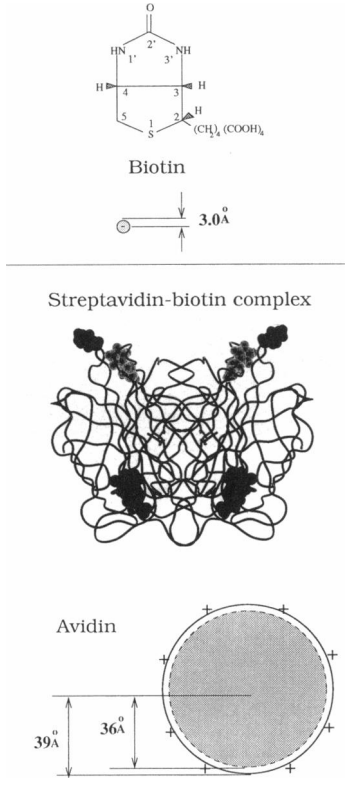

FIGURE 3 Structures and simple hydrodynamic models for biotin and avidin.

where the rate constant k' represents a convolution of geometrical, transport, electrostatic, and kinetic factors. Binding actually occurs at any one of four independent localized sites on the avidin molecule (Green, 1975). However, there is theoretical evidence to suggest that molecules with several active sites can reasonably be characterized in terms of uniformly reactive entities (cf. Berg and Purcell, 1977; DeLisi and Wiegel, 1981; Northrup, 1988; Zwanzig, 1990). We therefore consider the chemical interaction to be nonspecific, quantified by a uniform catalytic rate coefficient k (with dimensions of velocity), which incorporates both the intrinsic kinetics and any operative molecular reorientation effects. Adaptation of existing coagulation theory (Smoluchowski, 1917; Collins and Kimball, 1949; van den Ven, 1989, pp. 347–352; Kim and Zukoski, 1990) then leads to the following expression relating k and k':

$$k' = \frac{4\pi D_{rel}(a_A + a_B)N_{Avo}}{D_{rel}/k(a_A + a_B)e^{-E(1)} + \int_1^\infty d\rho/\rho^2 G(\rho)e^{-E(\rho)}}. \quad (33)$$

Here

$$D_{rel} = \frac{k_B T}{6\pi\mu}\left(\frac{1}{a_{A,core}} + \frac{1}{a_{B,core}}\right) \quad (34)$$

represents the diffusivity for relative diffusion of noninteracting spheres, and N_{Avo} represents Avogadro's number. $G(\rho)$ denotes the hydrodynamic hindrance factor for relative radial motion as a function of the dimensionless center-to-center distance $\rho = r/a_A + a_B$) (defined such that $\rho = 1$ at contact). It is approximated by the far-field asymptotic expansion (Batchelor, 1976)

$$G = 1 - \frac{6\lambda}{(1+\lambda)^2\underline{\rho}} + \frac{8\lambda(1+\lambda^2)}{(1+\lambda)^4\underline{\rho}^3} - \frac{60\lambda(1+\lambda^3)}{(1+\lambda)^5\underline{\rho}^4} + O(\underline{\rho}^{-6}), \quad (35)$$

where

$$\lambda = \frac{a_{B,core}}{a_{A,core}} = 0.0833\ldots \quad \text{and} \quad \underline{\rho} = \frac{2r}{a_{A,core} + a_{B,core}} = 2\left(\frac{a_A + a_B}{a_{A,core} + a_{B,core}}\right)\rho = (2.153\ldots)\rho. \quad (36)$$

$E(\rho)$ denotes the potential energy for ionically screened avidin-biotin electrostatic interaction in units of $k_B T$, given by the solution of the linearized Poisson-Boltzmann equation as the following (cf. Israelachvili (1992), Chapter 12):

$$E(\rho) = \frac{-8e_c^2}{4\pi\epsilon k_B T}\left[\left(1 + \frac{a_A}{\phi}\right)\left(a_A + a_B\right)\rho\right]^{-1} \exp\left[-\frac{(a_A + a_B)\rho - a_A}{\phi}\right]. \quad (37)$$

The experimentally reported rate constant k' is 7×10^4 m^3/mol·s (Green, 1975, p. 103), and in the absence of information we assume this value to correspond to the rate at physiological ionic strength, equivalent to 0.15 molar NaCl, so that $\phi = 7.8$ Å (Israelachvili, 1992, p. 238). (The value of k' is based on measured exchange rates of soluble biotin with that bound to the protein. Since the latter exchange is likely attenuated by the slow dissociation, the true forward binding rate would be greater.) In any case $k' = 7 \times 10^4$ m^3/mol·s yields $k = 0.00045$ m/s according to Eq. 33 with the physicochemical inputs embodied in Eqs. 34–37.

It is worth making a few observations regarding the physical implications of the parameter values estimated here. First, by numerical quadrature it is found that the integral in Eq. 33 excluding the electrostatic factor $e^{-E(\rho)}$ has the value $\int_1^\infty d\rho/\rho^2 G(\rho) = 1.14$. This indicates that the effect of hydrodynamic interactions would be moderate, reducing the association rate by roughly 12 % relative to noninteracting solutes, if there were no electrostatic attraction ($E(\rho) \equiv 0$) and if binding were diffusion limited ($k \to \infty$). The fact that the diffusion term $\int_1^\infty d\rho/\rho^2 G(\rho)e^{-E(\rho)} = 1.12$ is small compared with the kinetic term $D_{rel}/k(a_A + a_B)\,e^{-E(1)} = 358$ in the denominator of Eq. 33 means that avidin-biotin binding is kinetically (not diffusionally) limited in the context of our homogeneously reactive model. Thus, all transport factors (diffusivities, hydrodynamic interactions) have a negligible quantitative influence on the binding rate. This is consistent with the extraction of the intrinsic rates from exchange data (Green, 1975, p. 103), where the exchange kinetics will influence the apparent rate. Primarily through the Boltzmann

factor $e^{-E(1)} = e^{+0.154}$ in this kinetic term, inclusion of the electrostatic attraction decreases the calculated value of k by ~14%.

Characterization of the biotin-functionalized surface

The preceding theory for association in solution has separated the catalytic rate coefficient k from molecular sizes, translational diffusion, and nonspecific electrostatic forces. However, according to our simple homogeneous sphere-sphere model, k still represents a convolution of intrinsic site-specific kinetics with rotational diffusion, site-specific (steering) forces prompting molecular alignment, and steric factors associated with departures from the spherical shape. Application of the value of k estimated above to a biotinylated surface is strictly valid only if all these physical factors are the same in the solution and surface environments. In fact, they are not. First, rotation of avidin is hindered to a greater extent by a planar surface than by a single isolated biotin molecule, although hydrodynamic wall effects on rotation are quite weak (see Goldman et al., 1967). Second, biotin molecules are affixed quite rigidly to the surface, whereas they rotate freely in solution. If all immobilized biotins have their reactive faces pointed upward, then surface reaction is enhanced relative to reaction in bulk solution, where the rate represents an orientation average over reactive and nonreactive approaches. This difference would be mitigated by steering forces that prealign biotin molecules in solution. If there is a distribution over bound biotin orientations, then the estimate of k applies only insofar as the statistical orientational distribution on the surface is equivalent to the dynamic orientation average in solution. This, however, is unlikely due to the short (12 Å) tether used in the experiments. Third, steric effects associated with the somewhat nonspherical shape of avidin would have a quantitative influence on the rate, probably impeding rotation and reducing it somewhat. In the absence of definitive experimental and theoretical information regarding these various factors, we shall be content to utilize the catalytic rate coefficient k computed above from binding data in solution as a rough estimate for the peak height k_0 characterizing the intrinsic kinetics of avidin-biotin binding when biotin is immobilized at the surface. We postulate a tolerance (or effective radius of the binding site associated with each bound biotin) of order 3 Å, the radius of a biotin molecule. This means that the avidin-surface contact point must lie within O(3 Å) of the center of a biotin site for binding to occur with appreciable probability. These physical assumptions are introduced into the analysis of the Formulation of the Model Problem section by setting $k_0 = 0.00045$ m/s and $wl = 3$ Å. The latter value is probably an overestimate.

Judging from the small electrostatic effect in 0.15 M salt solution discussed above, it is reasonable to neglect direct electrostatic forces between avidin and the surface in the much more concentrated (0.5 M) NaCl solutions employed by Zhao and Reichert (1992a). However, there is another phenomenon that very likely gives rise to an interaction force not operative in solution, namely the presence of weakly adsorbed, hydrated ions. Hydrated counterions bind weakly to charged surface groups at elevated ionic strengths, which reduces the effective electrostatic surface potential, and the binding of these ions gives rise to a steric "hydration" or "protrusion" barrier at the membrane surface that can extend up to 30 Å from the interface (Pashley, 1981; Israelachvili and Pashley, 1982). The increased repulsive potential at the surface of this Stern layer is of order k_BT, as estimated from direct force measurements (Israelachvili and Pashley, 1982), a sizable barrier that could significantly reduce the measured rate of avidin binding to biotin membranes. It is worth noting that ionic strength-dependent hydration significantly attenuates the adhesion measured between streptavidin and membrane-bound biotin-derivatized membranes. Hydration repulsion reduces the strength of surface binding–or the number of successful binding events–at the membrane surface by 50% (Leckband et al., 1994; D. E. Leckband, unpublished observations). The NaCl concentrations used in the experiments by Zhao and Reichert (1992a) are well above the "critical hydration concentration" for sodium ions (Pashley, 1981). Since the avidin structure and biotin-binding mechanism are nearly identical to those of streptavidin (Green, 1975) and the same biotin-lipid was used in both the force and kinetic measurements, the evidence for a repulsive hydration force from direct force measurements likely carries over to the present system. Such an effective repulsion has also been observed in antibody systems (Ebato et al., 1994). We model this effect simply by postulating a repulsive potential with $O(k_BT)$ magnitude and 3 Å decay length (Israelachvili and Pashley, 1982; Schiby and Ruckenstein, 1983) given by

$$E = E_0 \exp[(z - a_A)/3\text{ Å}] = E_0 \exp(l\zeta/3\text{Å}), \tag{38}$$

$$E_0 = \text{an } O(1) \text{ number} \approx 5.$$

One unit of k_BT corresponds roughly to a single ion-surface potential. Thus, a repulsive potential of order 5 k_BT implies that an avidin binding event requires the desorption of several hydrated ions from the surface, a reasonable supposition in view of the comparatively large size of the avidin molecule.

To calculate the requisite hindered diffusion coefficients $d_\parallel$ and $d_\perp$, we appropriately combine Stokes resistance coefficients given by Brenner (1961) and Goldman et al. (1967). Values of $d_\parallel$ are obtained by interpolation in the table of Goldman et al. (1967) supplemented with far field and lubrication asymptotes at large and small z, respectively. Values of $d_\perp$ are obtained via numerical summation of the pertinent series formula (Brenner, 1961). The dimensionless z-coordinate utilized in the papers cited is the vertical distance from the center of the hydrodynamic core to the wall divided by the core radius, i.e., $z/a_{A,core} = (l\zeta + a_A)/a_{A,core}$.

Assuming a square lattice of sites, the various surface densities of biotin targets considered by Zhao and Reichert (1992a) can be assigned a dimensional unit cell length l. With

TABLE 1 Calculated surface-average rate coefficients characterizing avidin binding to the biotin-functionalized surfaces of Zhao and Reichert (1992a) at different biotin coverages

Biotin target density 10^{15} molecules/m^2	l Å	k_{eff} 10^{-7} m/s	$k_{eff}L/D_\infty$
9.27	51.9	0.149	0.368
15.9	39.7	0.268	0.662
22.2	33.6	0.378	0.936
27.8	30.0	0.475	1.175
35.2	26.7	0.602	1.489
42.7	24.2	0.731	1.807
55.6	21.2	0.954	2.360

We elected to explain theoretically the data reported in Zhao and Reichert's (1992a) original paper on this subject. Further experiments have been reported (Zhao and Reichert, 1992b).

the preceding kinetic, intermolecular force and hydrodynamic inputs, the theory discussed in the Formulation of the Model Problem section then leads to numerical values of k_{eff} presented in Table 1.

Rate of binding to the fiber optic sensor

The absorbing surface manufactured by Zhao and Reichert (1992a) consists of an exposed section of a glass fiber having length $2L = 3$ mm and diameter $2R = 600$ μm. As the chemical heterogeneity of the surface occurs on a length scale much smaller than L, the reactive surface can be regarded as locally flat, so that our estimate of k_{eff} derived for a planar surface applies to very good approximation. The preceding detailed considerations of hydrodynamic wall effects notwithstanding, it is asymptotically correct here to consider avidin molecules to have a constant diffusivity equal to the bulk value $D_\infty = k_B T/6\pi\mu a_{A,core} \cong 6.1 \times 10^{-7}$ cm^2/s owing to the negligible molecular size compared with the dimensions of the glass fiber.

The macroscopic diffusion problem of calculating the rate at which a reactive cylinder absorbs avidin molecules from a solution with bulk concentration $C_{bulk} = 8.99 \times 10^{20}$ molecules/m^3 (Zhao and Reichert, 1992a) is solved approximately via slender body theory, according to which the (axisymmetric) avidin concentration field $C(r,z)$ in the surrounding quiescent fluid is given by a line distribution of point-sinks at the axis:

$$C(r, z) = C_{bulk} + \int_{-L}^{L} \frac{F(s)\, ds}{4\pi\sqrt{r^2 + (z - s)^2}}. \quad (39)$$

The sink density F is approximated by a truncated series of the form

$$F(s) = \sum_{j=0}^{J} A_j (s/L)^{2j} \quad (40)$$

(cf. Cox (1970), p. 793), in which the coefficients are obtained numerically via least-squares minimization of the error in the boundary condition

$$D_\infty \partial C/\partial r = k_{eff} C \quad (41)$$

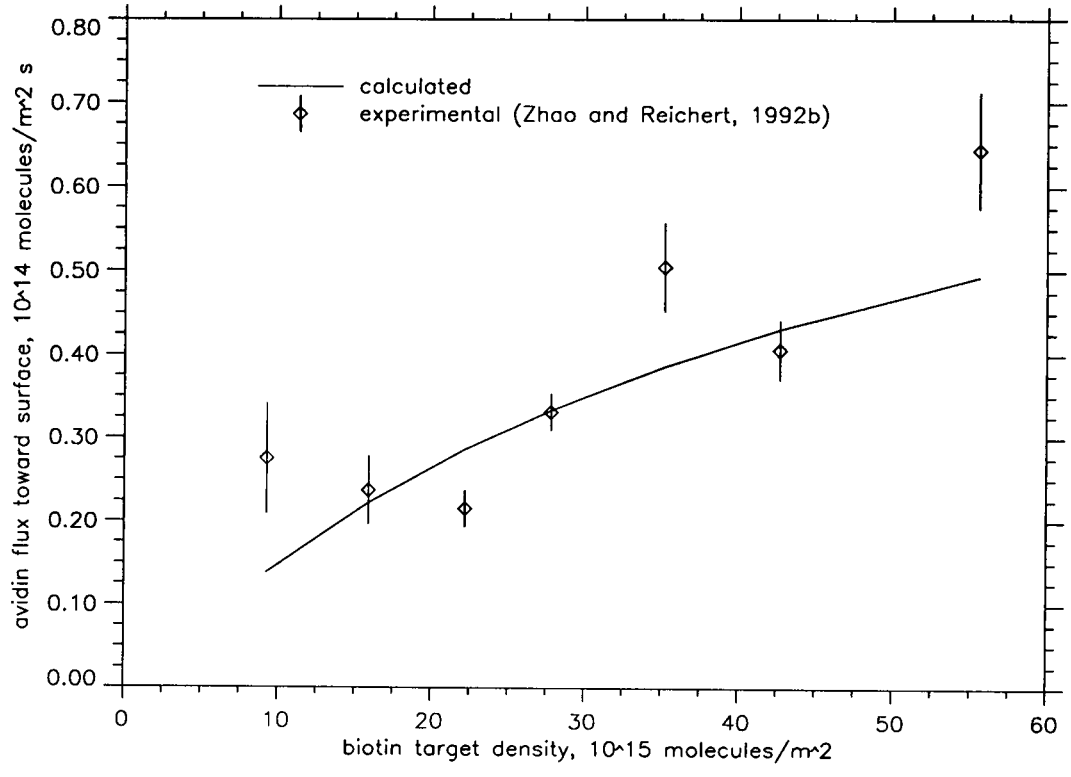

FIGURE 4 Comparison of theoretically calculated fluxes of avidin toward the fiber-optic sensor (*solid curve*) with measurements reported by Zhao and Reichert (1992a) (*symbols with error bars*).

summed over points evenly distributed between $z = 0$ and $z = L$. This procedure leads to an approximation for the net absorption rate, given by

$$\text{Rate} = -D_\infty L C_{\text{bulk}} \sum_{j=0}^{J} A_j/(j + 1/2) \qquad (42)$$

asymptotically correct to within a relative error of order R/L. The avidin flux is obtained by dividing this rate by the lateral surface area of the fiber, $4\pi RL$.

Fig. 4 compares the calculated and measured avidin fluxes (in molecules/m^{2}s) at the various biotin coverages considered by Zhao and Reichert (1992a). (The experimental avidin fluxes are obtained by multiplying the surface target densities listed in the second column of their Table I, p. 130 by the time constants listed in the fourth column. This procedure applies only to target densities smaller than 33×10^{11} molecules/cm^2. For larger target densities (last three lines of table; Zhao and Reichert, 1992a) the avidin flux must be based on this maximum value.)

DISCUSSION

The agreement between theoretically predicted and experimentally measured avidin fluxes (Fig. 4) is good. It is worth noting that the presence of a repulsive potential of order $5\,k_BT$ (attributed here to desorption of several hydrated ions on the biotinylated surface per avidin binding event) is needed to explain the data. Since some of the parameters entering the calculations are only estimable to within an $O(1)$ factor (in particular the biotin site size $wl = 3$ Å and the steric barrier height $E_0 = 5$ in units of k_BT), the numerical values were adjusted somewhat in view of the experimental data. Nevertheless, the fact that all parameters have orders of magnitude that are physically reasonable suggests that the overall theory is intrinsically sound and offers an accurate description of the underlying physics.

The calculations, as reconciled with experiment, suggest the following conclusions. First, translational diffusional limitations are negligible for avidin-biotin binding in solution because the diffusion term $\int_1^\infty d\rho/\rho^2 \text{Ge}^{-E} = 1.12$ is negligible compared with the kinetic term $D_{\text{rel}}/k(a_A + a_B)e^{-E(1)} = 358$ in the denominator of Eq. 33. Second, the sparse distribution of bound biotin molecules and the steric repulsive force attributed to hydration repulsion collectively reduce the effective rate coefficient k_{eff} for avidin binding to the biotinylated surface to a value ($O(10^{-7}$ m/s)) well below that of the intrinsic rate coefficient $k = 0.00045$ for avidin-biotin binding in solution. Direct electrostatic effects would seem to be unimportant at the high ionic strength conditions employed by Zhao and Reichert (1992a). Third, avidin binding to the macroscopic fiber optic sensor occurs in an intermediate regime where both diffusion and kinetics significantly affect the rate. This last fact may be deduced from the $O(1)$ magnitudes of the macroscopic Damköhler numbers $k_{\text{eff}}L/D_\infty$ included in Table 1, which indicate roughly comparable rates of binding and diffusion to the sensor surface.

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