Theoretical calculation methods for kinesin in fast axonal transport

(muscle formalism/differential equations/Monte Carlo simulation)

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ABSTRACT The method of making Monte Carlo calculations of the velocity of fast axonal transport is described and applied in a relatively simple case. These illustrative calculations are supplemented by a differential equation solution of the same problem, valid as an asymptotic limit. The latter treatment is closely related to the theory of muscle contraction.

This paper concludes a series of three (1, 2) on the theoretical treatment of the presumed role of kinesin in fast axonal transport. Necessary biochemical details are not yet available; hence a realistic kinetic diagram cannot be used. Consequently, this paper is devoted to methods that can be applied once sufficient biochemical details are available. We introduce and illustrate these methods by means of a simple two-state ATPase cycle that was, in fact, used for a similar purpose in relation to the theory of muscle contraction (3, 4). We shall make explicit calculations based on this simple model. Similar calculations can then be carried out in the future with the present calculations as a guide, using a more realistic kinetic diagram.

We begin with a differential equation approach that is a slight generalization of analogous calculations for the muscle contraction problem. This type of calculation is useful for perspective and as a kind of asymptotic limit (see below), but real calculations require the Monte Carlo method. The latter involve considerable computer time; we were fortunate to be able to use the National Cancer Institute Cray. The computations reported here are practical only on a supercomputer.

The Differential Equation Method

It is assumed that the reader is familiar with the general background about kinesin theory in refs. 1 and 2. We adopt the model introduced on pp. 306–311 of ref. 3 (for a single actin site) and the generalization presented on pp. 118–125 of ref. 4 (for an array of equivalent actin sites). One substantive change, compared to the muscle model (3, 4), is that a given kinesin site on the moving vesicle may be empty (i.e., no kinesin bound). One notational change here is that x (Fig. 1) locates the kinesin site relative to the arbitrarily assigned m = 0 microtubule site. Thus x increases to the right in Fig. 1; the velocity of motion of the vesicle relative to the microtubule is \( v \) (positive to the right). Part of the kinetic diagram for a particular kinesin site (as in Fig. 1) is shown in Fig. 2. The state E refers to the empty kinesin site; U is the occupied (by kinesin) but unattached (to a microtubule site) state. In fact, Fig. 1 illustrates state U. The binding rate constant \( \alpha \) is pseudo-first-order. The off rate constant \( \alpha' \) is first order. There is only one biochemical attached state (in this model), but attachment of a given kinesin may occur (with different probabilities) on a number of different microtubule sites \( m \). The function \( f(x) \) is the first-order attachment rate constant for a given kinesin with site at \( x \) (Fig. 1) onto the \( m = 0 \) microtubule site. The rate constant for attachment of this same kinesin onto, say, the \( m = 2 \) site is \( f(x - 2) \), as indicated in Fig. 2.

The detachment rate constant from \( m = 0 \), when the kinesin site is at \( x \), is \( g(x) \). (This is not the inverse of \( f \); the inverse of \( f \) is \( f' \) and the inverse of \( g \) is \( g' \); both \( f' \) and \( g' \) are considered to be negligible.) The detachment rate constant from, say, \( m = -2 \) when the kinesin site is at \( x \) is \( g(x + 2) \), as shown in Fig. 2. One attachment–detachment cycle \( f, g \) is accompanied by the hydrolysis of one molecule of ATP. The functions \( f(x) \) and \( g(x) \) chosen for this example (3, 4) are:

\[
\begin{align*}
\text{for } x = -2d & : & m = -2 & \quad f(x) = 3k e^{-x^2/2\sigma^2} \\
\text{for } x = -\delta & : & m = 0 & \quad g(x) = \kappa(0.15 + e^{3.5x/\delta}).
\end{align*}
\]

These functions are illustrated in Fig. 3, with \( \kappa = 0.175 \text{ ms}^{-1} \) and \( \sigma = 60 \AA \); they are used in the calculations below. Other parameters used in the calculations are \( \alpha = 0.05 \text{ ms}^{-1} \), \( \alpha' = 0.005 \text{ ms}^{-1} \), and \( \delta = 80 \AA \).

Also included in Fig. 3 is the force function (3) \( F(x) = -Kx \), where \( K = kT/\sigma^2 \). This is the force exerted on the vesicle by the kinesin at \( x \) when it is attached to \( m = 0 \). In Fig. 1 this force would be negative. If this kinesin is attached, say, to \( m = 1 \), the force is \( -K(x - \delta) \) (positive in Fig. 1).

Our object, in this muscle-like (4) calculation, is to find the mean force \( \bar{F} \) on the vesicle contributed by one kinesin site (usually occupied by a kinesin) when the vesicle is moving with a constant velocity \( v \). Implicit in the method of calculation of \( \bar{F} \) (below) and in the constancy of \( v \) is the assumption that the random assortment of \( n_K \) kinesin sites on the vesicle

![Fig. 1. Schematic representation of a kinesin molecule (K) bound on a kinesin site (a) that is on a vesicle adjacent to a microtubule strand. The strand has attachment sites \( m = 0, \pm 1, \cdots \).](image1)

![Fig. 2. Kinetic diagram for a single kinesin site at x, with some rate constants shown. The complete diagram would have \( f(x - m\delta) \) into and \( g(x - m\delta) \) out of each value of \( m, m = 0, \pm 1, \cdots \).](image2)
that can interact with microtubule sites (on several microtubule strands) is uniformly distributed (2) with respect to the microtubule-site interval, say, $x = 0$ to $x = \delta$. The actual value of $n_k$ is perhaps of order 10 but the constant velocity and uniform distribution assumptions that we make here imply that $n_k$ is very large (as in the muscle problem). Hence this is an asymptotic kind of calculation when applied to kinesin.

We consider the mean properties of a particular kinesin site (Fig. 1), moving with velocity $v$ and with diagram as in Fig. 2. Let $p_E(x)$ or $p_U(x)$ be the respective probabilities that the site is in state E or state U when the site is at $x$. Let $p(x)$ be the probability that the kinesin site is occupied and that the kinesin is attached to microtubule site $m = 0$. The probability of attachment to site $m$ is then $p(x - m\delta)$. The functions $p_E(x)$ and $p_U(x)$ are periodic in $x$ (period $\delta$) but $p(x)$ is not periodic. The kinetic differential equations are then (4)

\begin{align}
\nu \frac{dp_E}{dx} &= \alpha' p_U - \alpha p_E \\
\nu \frac{dp_U}{dx} &= \alpha p_E - \alpha' p_U + \sum_m p(x - m\delta)g(x - m\delta) \\
&- p_U\sum_m f(x - m\delta) \\
\nu \frac{dp}{dx} &= f(x)p - g(x)p,
\end{align}

where $m = 0, \pm 1, \cdots$. At each $x$, the normalization condition is

\begin{equation}
\sum_m p_E(x) + p_U(x) + \sum_m p(x - m\delta) = 1. \tag{6}
\end{equation}

Eq. 4 is redundant in view of Eq. 6. Given the solution of Eqs. 3, 5, and 6, for an assigned value of $v$, the mean force exerted by one kinesin site is calculated from (3, 4)

\begin{equation}
\bar{F} = \frac{k}{\delta} \int_0^\delta \sum_m (x - m\delta)p(x - m\delta)dx. \tag{7}
\end{equation}

The uniform distribution of microtubule sites, mentioned above, appears explicitly here in the given form of the integral. The integrand in Eq. 7 is periodic in $x$. The function $\bar{F}(v)$ is the so-called force–velocity curve.

Eqs. 3, 5, and 6 were solved numerically by an iteration procedure, with the parameters already mentioned, for several values of $v$. When $v = 0$ an analytical solution can be obtained easily. The curve $\bar{F}(v)$ is given in Fig. 4. For $v = 14$ Å ms$^{-1}$ ($v_{\text{max}} = 14.65$), $p(x)$ is shown in Fig. 5. Incidentally, $v = 10$ Å ms$^{-1} = 1$ μm s$^{-1}$. The functions $p_{E}(x), p_{U}(x), \Sigma_m p(x - m\delta) = \Sigma_m$ are periodic in $x$, but with small amplitudes (the limits in the case $v = 14$ are 0.02497–0.02487 for $p_{E}$, 0.260–0.238 for $p_{U}$, and 0.737–0.715 for $\Sigma_m$). The mean values (over $\delta$) are $\bar{p}_E = 0.0249$, $\bar{p}_U = 0.2492$, and $\Sigma_m = 0.7259$. $\Sigma_m(x)$ is the probability of attachment on any microtubule site; $p(x)$ is this probability for $m = 0$ only (or for any one particular microtubule site at the same $x$, since all sites are equivalent).
As already explained (1, 2), in this kind of calculation the velocity of motion of the vesicle is determined by a friction coefficient $\zeta$ and the relation $nK_F(v) = \zeta v$. In Fig. 4, we arbitrarily choose $v = 14$. The use of the calculated value (Eq. 7) $F_0 \delta x^2/\kT = 59.479 \text{Å}^2$ at $v = 14$, $nK = 10$, and a temperature of 25°C gives $\zeta = 6.07 \times 10^{-3} \text{g s}^{-1}$, a reasonable value (2).

The Monte Carlo Method

The vesicle has $nK$ "active" (2) kinesin sites, each of which can interact with one of several strands of the microtubule. These sites are assumed to be equivalent except for their locations relative to the nearest-neighbor microtubule strand period $\delta$. We start with some particular distribution of these locations within $\delta$ and this relative distribution is maintained throughout the simulation (see below) as the vesicle moves. A random distribution in $\delta$ (selected by random numbers) is most realistic but for theoretical purposes we have also used in some calculations a distribution evenly spaced throughout $\delta$ and also a sharp distribution (all kinesin sites have the same $x$). In general, kinesin site 1 is located at $x_1 = x$, between 0 and $\delta = 80 \text{Å}$; site 2, at $x_2 = x + \Delta x_2$; site 3, at $x_3 = x + \Delta x_3$; etc., where $0 \leq \Delta x_i \leq \Delta x_3, \cdots$. That is, the sites are ordered and $\Delta x_1 = 0$. Thus site $i$ ($i = 1, \cdots, nK$) is at $x_i$ and if $x_1 = x = 0$, all other $x_i < 80$. Physically, the $nK$ kinesin sites interact with many different (equivalent) microtubule inter-site intervals $\delta$, on several strands, but mathematically we consider all $nK$ sites to be adjacent to each other, as described above.

The vesicle moves in the course of the simulation (see below), so $x$ changes. However, all the $\Delta x_i$ (relative positions) remain constant. If the $i$th site is in an attached state, attachment of this kinesin might be at any $m_i = 0, 1, \cdots$. Merely to simplify the stored arithmetic, whenever $x_i = x$ passes the value 80 Å ($v$ positive), before the next transition all $x_i$ are reduced in value by 80 Å and all $m_i$ (attached) are reduced by 1. Similarly, whenever $x_i = x$ passes the value 0 Å ($v$ negative), all $x_i$ are increased by 80 Å and all $m_i$ (attached) are increased by 1. These computational changes have no physical significance because all microtubule sites and intervals are equivalent.

Each of the $nK$ kinesin sites has its own $x_i$, $m_i$, and kinetic transition diagram, as in Fig. 2 (all sites have the same $\alpha$, $\alpha'$, $f$, $g$). However, the kinesin sites are not independent of each other because they all (when attached) make contributions to $v$, as described below, and because all $x_i$ change together ($\Delta x_i$ constant) as the vesicle moves. Thus all $nK$ sites must be treated as a single kinetic system in the course of the simulation: system transitions are followed one at a time, and any such transition might occur in any one of the $nK$ diagrams.

Transitions are instantaneous, but the vesicle moves between transitions. Unlike the differential equation treatment above, where the friction coefficient $\zeta$ enters the problem only through the auxiliary relation $nK_F = \zeta$, here $\zeta$ is involved in the details of the motion. At any point in the simulation, $\zeta = nK$ kinesin sites are occupied and attached. Let us use the subscript $j$ to refer to these attached kinesin sites. Then the instantaneous total force exerted on the vesicle is

$$F_t = -K \sum_{j=1}^{nK} (x_j - m_j \delta). \quad [8]$$

The site labeling here will usually be different than in $1 \leq i \leq nK$. The value of $F_t$ will jump discontinuously at every attachment ($f$) or detachment ($g$) transition. Also, it will change smoothly as the vesicle moves ($x$ changes) between transitions. $F_t$ remains constant at $\alpha$ and $\alpha'$ transitions. In every case we assume that the instantaneous velocity of the vesicle follows the instantaneous total force without any delay (2), so that $v = F_t/\zeta$.

After each transition, $v$ is calculated from

$$v = \frac{K \sum_{j=1}^{nK} (x_j - m_j \delta)}{\zeta} = \frac{K \sum_i x_i}{\zeta}. \quad [9]$$

Note that $\Sigma_i$ is not the same quantity as $\Sigma_m$ above. If we introduce $K = \kT/\sigma^2$ and

$$\zeta = nK_F \frac{59.479 \kT nK}{14 \sigma^2} \quad [10]$$

from the differential equation calculation at $v = 14 \text{Å ms}^{-1}$, Eq. 9 becomes.
\[ v = -18.830 \frac{\Sigma}{nK} \, \text{Å} \text{ms}^{-1}. \] \[ \text{[11]} \]

This is the practical expression used: \( F_i \) is bypassed and \( v \) is calculated directly from \( \Sigma \).

Between any two transitions in the simulation, the equation of motion of the vesicle is, from Eq. 8,

\[ v = \frac{dx}{dt} = F_i = \frac{nKx}{\xi} - \frac{K}{\xi} \sum_{j=1}^{nK} (\Delta x_j - m_j \delta). \] \[ \text{[12]} \]

The summation term is a constant. Hence it is easy to solve the differential equation in \( x \) and to obtain \( x(t) \) and \( v(t) \) explicitly, after introducing the initial values. The velocity, whether positive or negative, decays exponentially toward zero between transitions, according to \( e^{-nKo_t} \). The decay constant can be written (Eq. 11)

\[ nK/\xi = 18.830(n/n_K) \, \text{ms}^{-1}. \] \[ \text{[13]} \]

To anticipate, we mention that, when \( n_K = 10 \), the mean value of \( n/n_K \) is 0.7451 and the mean time \( t \) between transitions is 0.2228 ms. Hence, for \( n_K = 10 \), a typical fractional decay in \( v \), between transitions, is from 1 to \( e^{-nKo_t} = 0.0439 \), which is considerable. Incidentally, this jumpiness in \( v \) is not inconsistent with an apparently smooth video motion because intervals of \( \approx 20 \) ms are short enough to produce visual smoothness. Here 90 transitions would be averaged, visually, in 20 ms. A more realistic biochemical model, (1, 2) would have about 4 transitions per kinesin molecule per 80 Å attachment-detachment cycle. With a typical velocity of 20 Å/ms\(^{-1}\), 10 kinesin molecules would undergo about 200 transitions in 20 ms.

We consider next the selection of the time \( t \) between transitions, as determined by a random number \( R \) (uniformly distributed between 0 and 1). Immediately after any transition (call this \( t = 0 \)), all \( x_i \) are known and hence all rate constants (in the \( n_K \) diagram) are known. Let \( r(0) \) be the sum of the rate constants for all possible transitions in the \( n_K \) diagram, at \( t = 0 \). The possible transition or transitions in a given diagram depend on the particular state of that kinesin site at the time. As time passes, we know \( x(t) \) from Eq. 12 and hence we know how all the rate constants change with time. Thus we have \( r(t) \). If \( r \) is a constant (as in most problems), the relation between \( R \) and \( t \) is simply \( R = e^{-r \tau} \). But with \( r \) a function of \( t \), the generalization is easily seen to be

\[ R = \frac{r(t)}{r(0)} \exp \left[ -\int_0^t r(\tau)d\tau \right]. \] \[ \text{[14]} \]

For each randomly selected \( R \) (i.e., following each transition), \( t \) must be found from Eq. 14 by numerical integration. This is the most time-consuming part of the program. Another random number then selects the actual transition at \( t = \tau \); the probability of each possible transition is proportional to the value of its rate constant at \( t = \tau \).

The main quantity of interest in the calculation is the mean velocity \( \bar{v} \). This is found most simply by dividing the cumulated distance traveled between transitions [from each \( x(t) \)] by the cumulated time \( \tau \) between transitions (the sum of \( \tau \) values). An alternative computation of \( \bar{v} \), which provides a check, is

\[ \bar{v} = \frac{1}{\tau} \sum_{n=1}^{N} \int_0^{\tau_n} v_m(t) dt, \] \[ \text{[15]} \]

where \( N \) is the number of transitions in the simulation, \( v_m(t) \) is the velocity (see Eq. 12) after the \( m \)th transition, and \( \tau_n \) is the time between transitions \( m \) and \( m + 1 \). Replacing the integrand above by \( v_m^2 \) gives \( \bar{v}^2 \). Thus we can calculate \( \bar{v}^2 = v^2 - \bar{v}^2 \), the variance in \( v \).

Other quantities of interest that can be calculated are \( \bar{v} \) the mean time per transition (\( t = \tau/N \)); the fraction of time that the \( n_K \) kinesin sites spend in states E, U, and attached; and the Monte Carlo version of the function \( p(x) \) introduced in the preceding section. Also, it is instructive to examine distance, time, and initial and final velocities for successive intervals between individual transitions.

**Monte Carlo Results**

We assume (2) that a realistic value of \( n_K = 10 \) sites, distributed randomly in the interval \( \delta = 80 \) Å. Using \( \xi \) in Eqs. 10 and 11, we expected (2) that we would obtain \( \bar{v} = 14 \) Åms\(^{-1}\), after averaging \( \bar{v} \) over a number of random distributions. Instead we found \( \bar{v} = 11.49 \) (Experimental values are about twice as large as this; the discrepancy is not surprising in view of the simplicity and arbitrariness of the kinetic diagram). We then realized that the differential equation approach is based on a perfectly uniform distribution of sites and, more important, on an absolutely constant \( v \). In the Monte Carlo calculations with \( n_K = 10 \), \( v \) decays very considerably (see Eq. 13) between transitions; \( v \) is anything but constant. However, in the mathematically interesting but physically unrealistic limit of a uniformly spaced large \( n_K \), using Eq. 11, we would expect \(\bar{v} \rightarrow 14 \) because the decay in \( v \) between transitions approaches zero as \( n_K \rightarrow \infty \) (see below).

With this introduction, we proceed now to summarize our Monte Carlo results.

Most simulations comprised 100,000 transitions, but the range used was 20,000 to 10\(^6\), after a discard of 2000. Eq. 11 was used in all cases. For \( n_K = 10 \), three kinds of distribution were tried: random, uniform spacing (i.e., every 8 Å), and (for contrast) all kinesin sites at the same \( x \). The results are summarized in Table 1. With \( n_K = 10 \) and with the numbers of transitions used, the effect of the type of distribution on the results in the table is not clear (see below, however). The mean values quoted following Eq. 13 and in the paragraph above are based on the random and uniform distributions (900,000 transitions). The large values of \( \sigma_v^2 \) for \( n_K = 10 \) are a consequence of the relatively large jumps in \( v \) at each transition and of the large decrease (0.0439 on average, as already mentioned) in \( v \) between transitions. The values of \( \bar{v}/n_K \), should be compared with \( \Sigma_m = 0.7259 \) from the differential equation solution at \( v = 14 \) (see above). The dashed curve for \( p(x) \) in Fig. 5 is from one \( n_K = 10 \) run of 200,000 transitions with a random distribution.

The normalization relations for \( p(x) \) are

\[ \int_{-\infty}^{\infty} p(x)dx = \Sigma_m \delta = \bar{v}/n_K. \] \[ \text{[16]} \]

Because \( \bar{v} = 11.49 \) (with \( n_K = 10 \)) is significantly less than \( v = 14 \), we carried out a number of simulations (uniform and "same \( x \)" distributions) with \( n_K = 160 \) and \( n_K = 800 \), using Eq. 11. These are of mathematical interest primarily. The
results are included in Table 1. It appears that $v = 14$ is approached at large $n_K$, as expected. (However, $\bar{v}$ fluctuates from one run to another more than do the other quantities in the table.) Also, note that $\bar{n}/n_K$, for $n_K = 800$ (uniform distribution) is very close to $\bar{x}_m = 0.7259$ from the $v = 14$ differential equation solution (in fact, the value of $\bar{n}/0.2492$, is the same from the two calculations). Similarly, Fig. 5 shows that $p(x)$ for $n_K = 800$ (one run of 500,000 transitions, uniform distribution) is close to the differential equation $p(x)$. Small values of $\sigma^2$ for $n_K = 800$ arise because the jumps in $v$ at each transition are relatively small when $n_K$ is large and because there is only a very small mean decrease in $v$ between transitions in this case. For the uniform distributions in Table 1, the values of $e^{-nK/\zeta}$ for $n_K = 160$ and 800 are 0.8392 and 0.9653, respectively, compared to 0.0439 for $n_K = 10$. Because $\bar{n}/n_K$ and $n_K\bar{t}$ are approximately independent of $n_K$ (Table 1), the exponent $n_K\bar{t}/\zeta$ (see also Eq. 13) varies with $n_K$ approximately as $1/n_K$.

Unlike $\bar{v}$, fluctuations in $\sigma^2$ from run to run are quite small so that there is virtually no doubt that the difference between 30.52 and 25.51 shown in Table 1, for $n_K = 800$, is significant. One would in fact expect the “same $x$” value of $\sigma^2$ to be larger, according to the following argument. In the limit $n_K \to \infty$, the uniform distribution case would become equivalent to Eqs. 3–7, with $v$ a parameter. The auxiliary relation $n_K\bar{F}(v) = \zeta \bar{F}$ then determines the actual vesicle velocity, as explained above. In this limit, then, $v$ is constant and $\sigma^2 = 0$. However, in the case of a large collection of kinesin sites all with the same $x$, differential equations can again be used but the vesicle velocity would no longer be independent of $x$; that is, $v$ would be periodic in $x$. Thus $v$ does not enter the differential equations simply as a constant parameter; $v$ must be introduced along with $\zeta$ in essentially the same fashion as in the Monte Carlo calculations. Thus in Eqs. 3–5, $v$ is replaced by

$$\frac{dx}{dt} = \frac{n_K\bar{F}(x)}{\zeta} = -\frac{n_K}{\zeta} \sum_m (x - m\delta)p(x - m\delta)$$  \[17\]

$$= -18.830 \sum_m (x - m\delta)p(x - m\delta).$$  \[18\]

In eq. 18 we have used $n_K/\zeta$ from Eq. 10. The differential equations 3–5 are now much more complicated. Because $dx/dt$ is periodic in $x$, $\sigma^2 > 0$. Thus, in the limit $n_K \to \infty$, $\sigma^2$ (same-$x$ distribution) $> \sigma^2$ (uniform distribution) $= 0$.

A careful master equation analysis shows that the use of Eq. 17 as described actually involves an approximation, but this does not affect the qualitative argument.

Another difference between a uniform distribution and a same-$x$ distribution for $n_K = 800$ is shown in Fig. 5. The circles apply to the former case and the dotted fragment to the latter (200,000 transitions). The unusual shape of $p(x)$ in the same-$x$ case is also seen at $n_K = 160$ and at $n_K = 10$. However, superimposed on this shape (in the neighborhood of the shoulder) are slight damped oscillations (period 10 Å) at $n_K = 10$ and much stronger, but still damped, oscillations at $n_K = 10$. We would like to thank Drs. Kai-Li Ting, Robert Jernigan, and Jeff Saxe for helping us with the Cray computer.