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Molecular motors: thermodynamics and the random walk

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The biochemical cycle of a molecular motor provides the essential link between its thermodynamics and kinetics. The thermodynamics of the cycle determine the motor's ability to perform mechanical work, whilst the kinetics of the cycle govern its stochastic behaviour. We concentrate here on tightly coupled, processive molecular motors, such as kinesin and myosin V, which hydrolyse one molecule of ATP per forward step. Thermodynamics require that, when such a motor pulls against a constant load f, the ratio of the forward and backward products of the rate constants for its cycle is $\exp[-(\Delta G + u_0 f)/kT]$, where $-\Delta G$ is the free energy available from ATP hydrolysis and u_0 is the motor's step size. A hypothetical one-state motor can therefore act as a chemically driven ratchet executing a biased random walk. Treating this random walk as a diffusion problem, we calculate the forward velocity v and the diffusion coefficient D and we find that its randomness parameter r is determined solely by thermodynamics. However, real molecular motors pass through several states at each attachment site. They satisfy a modified diffusion equation that follows directly from the rate equations for the biochemical cycle and their effective diffusion coefficient is reduced to $D - v^2\tau$, where τ is the time-constant for the motor to reach the steady state. Hence, the randomness of multistate motors is reduced compared with the one-state case and can be used for determining τ. Our analysis therefore demonstrates the intimate relationship between the biochemical cycle, the force–velocity relation and the random motion of molecular motors.

Keywords: molecular motors; thermodynamics; random walk; diffusion

1. INTRODUCTION

Biological molecular motors harness chemical free energy in order to perform mechanical work inside living cells. Important examples are the cytoskeletal motors kinesin, myosin V and cytoplasmic dynein, which are an essential part of the machinery for intracellular transport (Hirokawa 1998; Mehta *et al*. 1999; Hodge & Cope 2000; Kim & Endow 2000; Susalka *et al*. 2000). These remarkable microscopic engines fulfil a similar role to the internal-combustion heat engines that we use for everyday transport at the macroscopic level. Moreover, like heat engines, molecular motors are cyclic machines: heat engines perform mechanical work as part of a thermodynamic cycle that transfers heat from a hot to a cold reservoir, whilst molecular motors are able to perform work as part of a biochemical cycle, the net result of which is a chemical reaction, such as ATP hydrolysis (Lymn & Taylor 1971). However, an essential difference between macroscopic and microscopic motors is that heat engines operate deterministically and can execute their cycle at a constant rate, whilst molecular motors operate stochastically and are therefore subject to random fluctuations. Hence, a macroscopic vehicle powered by a heat engine can move at a constant velocity, whilst a microscopic vesicle transported by a molecular motor inevitably executes a random walk (Berg 1993).

The theory of heat engines is founded upon thermodynamics. In this paper, we show that thermodynamics also provides a fundamental basis for understanding molecular motors. The biochemical cycle is of central importance because it provides the essential link between thermodynamics, which determines a motor's ability to perform useful work and kinetics, which give rise to its stochastic behaviour. We find that molecular motors do indeed undergo a random walk, but it is a 'biased' random walk (Berg 1993; Astumian 1997) that allows them, on average, to perform mechanical work. A key step in the analysis is constructing the diffusion equation that governs the biased random walk. We show here that the diffusion equation for a motor follows directly from the rate equations for its biochemical cycle.

Svoboda *et al*. (1994) developed a statistical theory for analysing the random motion observed in single-molecule experiments on molecular motors. However, their analysis is restricted to motors that only make forward transitions, whilst real molecular motors may flip-flop back and forth between states for at least part of their cycle. Our more general approach takes account of both forward and backward transitions, and it demonstrates the intimate relationship between the biochemical cycle, the force–velocity relation and the random motion of molecular motors.

2. TIGHT COUPLING AND PROCESSIVE MOLECULAR MOTORS

Figure 1*a* shows a greatly simplified three-state model for the myosin V motor (Rief *et al*. 2000; Walker *et al*. 2000). Individual heads of this two-headed molecule are believed to undergo a cycle that is similar to the cross-bridge cycle in muscle, consisting of attachment, a power stroke (Huxley 1969; Huxley & Simmons 1971) and detachment. The shaded head in state 1 has just released ADP following its power stroke. It then binds ATP and detaches from actin, forming state 2. This allows the

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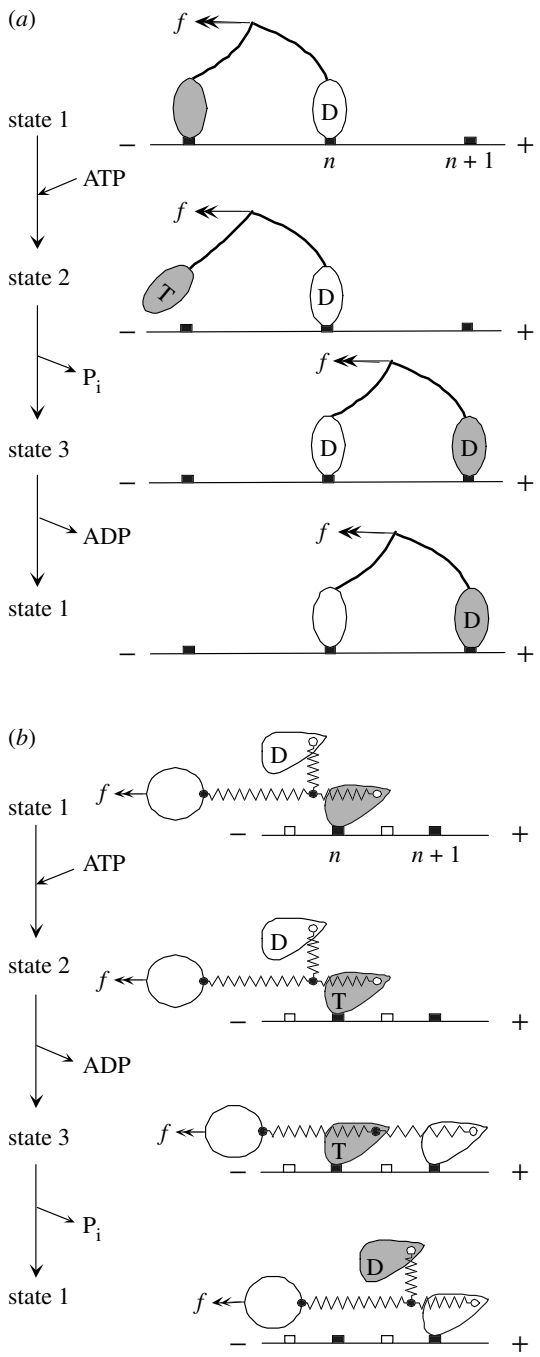

Figure 1. Simplified three-state cycles for tightly coupled, processive molecular motors. (*a*) Two-headed myosin V (Rief *et al*. 2000). (*b*) Dimeric kinesin (Thomas *et al*. 2001). For more details, see text.

unshaded head to execute its power stroke and the shaded head then rapidly attaches and releases inorganic phosphate (P_i) to produce state 3. Having executed its power stroke, the unshaded head is able to release ADP, which is believed to be the rate-limiting step in the cycle (Rief *et al*. 2000). The cycle then repeats with the roles of the two heads interchanged. Myosin V molecules therefore move processively along actin filaments (Mehta *et al*. 1999; Rief *et al*. 2000; Walker *et al*. 2000), remaining attached for many hydrolysis cycles and hydrolysing one molecule of ATP for each forward step of *ca*. 38 nm. By contrast, muscle cross-bridges detach completely from actin at the end of each hydrolysis cycle (Thomas & Thornhill 1998) and do not move processively. The mechanical cycles of both motors are tightly coupled to ATP hydrolysis, but myosin V is classified as a tightly coupled, processive molecular motor.

In a similar vein, figure 1*b* shows a simplified three-state kinetic scheme for conventional kinesin, which is another important tightly coupled, processive molecular motor. In this motor, neck-linker docking (Rice *et al*. 1999), which is represented schematically here by the shortening of a spring (Thomas *et al*. 2001), has an analogous role to the myosin power stroke (Vale & Milligan 2000). As with myosin V, the kinesin dimer moves processively, using alternating heads in order to proceed in 'hand-over-hand' fashion along a microtubule (Hackney 1994; Duke & Leibler 1996; Astumian & Derenyi 1999; Hancock & Howard 1999) and hydrolysing one molecule of ATP for each 8 nm step (Howard *et al*. 1989; Block *et al*. 1990; Hackney 1995; Hua *et al*. 1997; Schnitzer & Block 1997; Coy *et al*. 1999).

The molecular motors in figure 1 hydrolyse ATP and the net reaction may be written (in simplified form) as

$$\mathrm{ATP} \rightarrow \mathrm{ADP} + \mathrm{P_i}. \tag{2.1}$$

An important property of tightly coupled, processive molecular motors is that the tight coupling of mechanical stepping to ATP hydrolysis means that their average stepping velocity v is directly related to their average hydrolysis rate R by

$$v = u_0 R, \tag{2.2}$$

where u_0 is the fixed step size for the motor (*ca*. 38 nm for myosin V and 8 nm for kinesin). This direct relation between the stepping velocity and hydrolysis rate for tightly coupled, processive motors makes their analysis particularly straightforward. However, equation (2.2) does not apply to muscle cross-bridges, because they even hydrolyse ATP under 'isometric' conditions when $v = 0$. We therefore focus specifically in this paper on the behaviour of tightly coupled, processive molecular motors, the force–velocity relation of which may be determined analytically from the rate constants for the biochemical cycle.

3. KINETICS AND THERMODYNAMICS

Figure 2*a* shows a general conceptual kinetic scheme for a tightly coupled, processive molecular motor, which we suppose to be subjected to a constant load f. If the motor starts in state 1 at site n, then it must pass through states $1-N$ before it can make the transition from state N at that site to state 1 at the adjacent site $n+1$. For the purposes of illustration, we suppose that this cycle of events hydrolyses a single molecule of ATP, as in the kinesin and myosin V motors. As indicated in figure 2*a* and following Schnitzer *et al*. (2000), we denote the rate constants for the forward transitions by $k_1(f)$, $k_2(f)$, $k_3(f), \ldots, k_N(f)$, whilst the backward rate constants are denoted by $k_{-1}(f)$, $k_{-2}(f)$, $k_{-3}(f), \ldots, k_{-N}(f)$.

The load dependence of individual rate constants $k_i(f)$ is determined by the molecular motor's inner workings. However, irrespective of how the motor actually works, thermodynamics impose a strict constraint on the ratio

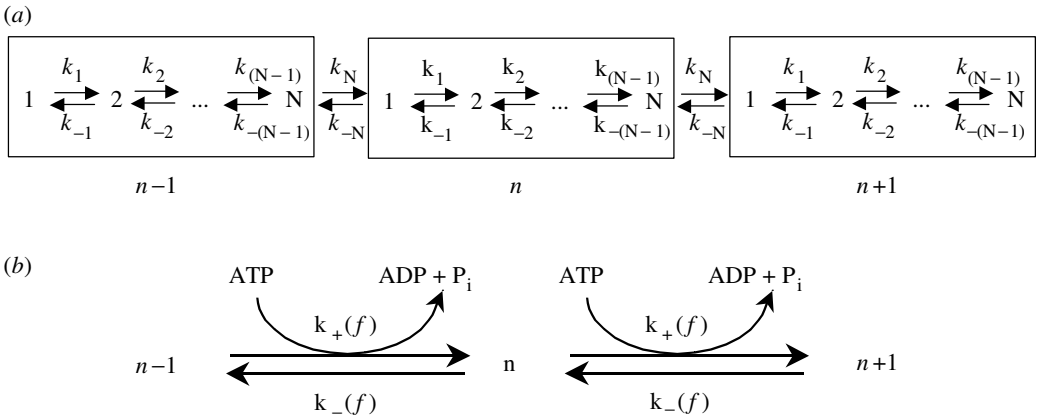

Figure 2. Kinetic schemes for a tightly coupled motor that hydrolyses one molecule of ATP in moving forwards from site n to $n+1$ while pulling against a constant load f. (*a*) A general N-state motor. (*b*) An idealized one-state motor.

$[k_1(f)k_2(f)k_3(f),\ldots,k_N(f)]/[k_{-1}(f)k_{-2}(f)k_{-3}(f),\ldots,k_{-N}(f)]$ of the products of the forward and backward rate constants for the motor's hydrolysis cycle. In order to see how this arises, we start by applying the law of mass action to the hydrolysis reaction in equation (1), which is catalysed by the forward motion of the molecular motor. Hence, we require that

$$[k_1(f)k_2(f)k_3(f),\ldots,k_N(f)]/[k_{-1}(f)k_{-2}(f)k_{-3}(f),\ldots,k_{-N}(f)] \propto [\mathrm{ATP}]/([\mathrm{ADP}][\mathrm{P_i}]), \quad (3.3)$$

which assumes that the solution of ATP, ADP and inorganic phosphate may be treated as being ideal. We also implicitly assume here that the reaction takes place at neutral pH and with a free-energy increase of ΔG per molecule of ATP, which is given by

$$\Delta G = \Delta G_0' + kT\ln([\mathrm{ADP}][\mathrm{P_i}]/[\mathrm{ATP}]), \quad (3.4)$$

where $\Delta G_0'$ is the standard free-energy increase per molecule for the hydrolysis reaction at pH 7, T is the absolute temperature and k is Boltzmann's constant. (Note that ΔG is therefore negative for a reaction that proceeds spontaneously.) It then follows that

$$[k_1(f)k_2(f)k_3(f),\ldots,k_N(f)]/[k_{-1}(f)k_{-2}(f)k_{-3}(f),\ldots,k_{-N}(f)] = A\exp(-\Delta G/kT). \quad (3.5)$$

In order to determine the pre-factor A, we invoke Einstein's principle of detailed balance, according to which the forward and backward rates for each transition in the cycle must be equal when the cycle is in thermodynamic equilibrium. The products of the rate constants on the left-hand side of equation (3.5) are therefore equal at equilibrium. As the load f is pulled forwards by a distance u_0 for each cycle of the tightly coupled motor, the system of load plus motor is in thermodynamic equilibrium when

$$u_0 f = -\Delta G. \quad (3.6)$$

Hence, $A = \exp(-u_0 f/kT)$ and it follows that the rate constants for the cycle must satisfy the thermodynamic relation

$$[k_1(f)k_2(f)k_3(f),\ldots,k_N(f)]/[k_{-1}(f)k_{-2}(f)k_{-3}(f),\ldots,k_{-N}(f)] = \exp[-(\Delta G + u_0 f)/kT]. \quad (3.7)$$

This equation expresses the fundamental link between kinetics and thermodynamics for a processive motor of step size u_0, the stepping of which is tightly coupled to its hydrolysis cycle. In essence, it arises because each full step of the motor hydrolyses one molecule of ATP and performs mechanical work $u_0 f$. Whatever the complex internal workings of the motor may be, the ratio of the forward and backward products of the rate constants for the cycle as a whole is always given by equation (3.7) and it can only depend on ΔG and $u_0 f$.

An alternative way of deriving equation (3.7) is to consider the thermodynamics of each individual transition in figure 2*a*. Hill (1974) essentially applied this approach to muscle cross-bridges and for a tightly coupled, processive motor we may write the relation for the forward and backward rate constants as

$$k_i(f)/k_{-i}(f) = [\exp[-(\Delta G_{Ai} + \Delta G_{Mi} + \Delta W_i)/kT], \quad (3.8)$$

where ΔG_{Ai} and ΔG_{Mi} are the respective increases in free energy for the ATP and for the motor in transition i, whilst ΔW_i is the work done by the motor on the load in that transition. It follows that the exponent on the right-hand side of equation (3.7) is the sum of the exponents on the right-hand side of equation (3.8) for all the transitions in a complete cycle. The sum of ΔG_{Ai} is just the overall ΔG for ATP hydrolysis, whilst the sum of ΔW_i is the work done per step $u_0 f$. However, because the motor works in a cycle, there is no overall change in its free energy (or, indeed, of any other thermodynamic function of state), so the sum of ΔG_{Mi} is zero. Hence, the overall relation in equation (3.7) only depends on ΔG and $u_0 f$ and is quite independent of the internal workings of the motor.

An important corollary of equation (3.7) is that any theoretical model for a tightly coupled, processive molecular motor must satisfy this relation in order to ensure consistency with thermodynamics. Hence, if a model does not satisfy equation (3.7), it ostensibly violates the second law of thermodynamics, indicating that it is either incorrect or incomplete. As we discuss elsewhere (Thomas *et al.* 2001), a recent theoretical model for kinesin (Schnitzer *et al.* 2000) appears to fall into this category.

Equation (3.7) only applies to a tightly coupled, processive motor pulling against a constant load f, so we must assume that the motor stays permanently attached

to its track. For example, we neglect dissociation from tubulin for the kinesin motor, as this is a very slow process (Howard *et al*. 1989; Block *et al*. 1990; Svoboda *et al*. 1993; Vale *et al*. 1996). Clearly, we could not apply a constant load to individual cross-bridges in muscle, because detachment from actin (after binding ATP) is an integral part of the cross-bridge cycle. In that case, the thermodynamic relation for the rate constants is established under isometric conditions when no work is performed on the load (Thomas & Thornhill 1998). Cross-bridges therefore obey the laws of thermodynamics (Thomas & Thornhill 2000), but we cannot calculate their force–velocity relation analytically. By contrast, in the following sections we show that the thermodynamic relation in equation (3.7) has a central role in determining both the force–velocity relation and the random diffusive motion of tightly coupled, processive molecular motors.

4. THE BIASED RANDOM WALK

The simplest model to which we can apply the thermodynamic relation in equation (3.7) is the one-state motor that is shown schematically in figure 2*b*. This model subsumes all of the complexity of a real motor's biochemical cycle into a single transition, which represents both the ATP hydrolysis and the stepping of the motor from attachment site n to the adjacent site $n+1$. Although this idealized model is an enormous over-simplification of how real motors work, it nevertheless illustrates some important general behaviour of tightly coupled, processive molecular motors.

If we denote the forward rate constant by $k_+(f)$ and the backward rate constant by $k_-(f)$, then the thermodynamic relation in equation (3.7) applied to the one-state motor requires that

$$k_+(f)/k_-(f) = \exp[-(\Delta G + u_0 f)/kT]. \tag{4.9}$$

The forward and backward transitions occur at random and in general $k_+(f) \neq k_-(f)$. Hence, the motor executes a 'biased' random walk along its track (Berg 1993; Astumian 1997). Note however that $k_+(f) = k_-(f)$ when $u_0 f = -\Delta G$, which is the condition in equation (3.6) for thermodynamic equilibrium. In this special case, the motor's random walk is unbiased, its average velocity is zero and (on average) it does no work on the load. The motor's random walk must of course be unbiased at equilibrium for consistency with the second law of thermodynamics (Feynman 1963; Astumian 1997), which precludes it from doing work when it is at thermal equilibrium.

In general, if we consider an ensemble of one-state motors, each of which is subjected to a constant load f, then their average forward velocity is

$$v = u_0[k_+(f) - k_-(f)]. \tag{4.10}$$

As we are dealing with an ergodic thermal system, this equation also determines the average velocity for an individual, tightly coupled, processive motor executing a biased random walk (provided of course that we neglect dissociation of the motor from its track). It is interesting to note that equations (4.9) and (4.10) are similar to general formulae for determining the rate of a chemical reaction in the linear regime of non-equilibrium thermodynamics (Kondepudi & Prigogine 1998). We show below that these equations can also be applied to the multistate kinetic scheme in figure 2*a*. Here, we consider three idealized, simple cases of a one-state motor that are of particular interest.

(a) *Case A*

Suppose that the rate constant $k_-(f)$ for backward transitions in equation (4.9) is independent of the load, in which case

$$k_+(f) = k_- \exp[-(\Delta G + u_0 f)/kT], \tag{4.11}$$

where k_- is a constant. The average forward velocity from equations (4.10) and (4.11) is then given by

$$v = u_0 k_- \{\exp[-(\Delta G + u_0 f)/kT] - 1\}. \tag{4.12}$$

Figure 3*a* shows the general form of the motor's force–velocity relation, in this case when $\Delta G = -10\,\mathrm{kJ\,M^{-1}}$ and $k_- = 1\,\mathrm{s^{-1}}$. It has essentially the same form as Feynman's (1963) thermally driven ratchet, the possible application of which to muscle cross-bridges was considered by Vale & Oosawa (1990). Astumian (1997) also discussed several different types of ratchet and in his terminology one might best describe the tightly coupled, processive molecular motor as a 'chemically driven ratchet', as it is the negative ΔG from ATP hydrolysis that drives the motor spontaneously forwards when $u_0 f < -\Delta G$.

(b) *Case B*

The second special case of a biased random walk occurs when the forward rate constant $k_+(f)$ is independent of the load. In this case, instead of equation (4.11) we have

$$k_-(f) = k_+ \exp[(\Delta G + u_0 f)/kT], \tag{4.13}$$

where k_+ is a constant. The average stepping velocity is then given by

$$v = u_0 k_+ \{1 - \exp[(\Delta G + u_0 f)/kT]\}. \tag{4.14}$$

Figure 3*b* shows the force–velocity relation that is produced by this equation when $\Delta G = -10\,\mathrm{kJ\,M^{-1}}$ and $k_+ = 1\,\mathrm{s^{-1}}$. This motor also moves forwards spontaneously when $u_0 f < -\Delta G$, but this forward motion is very slow compared with the rapid backward stepping that occurs when $u_0 f > -\Delta G$. A motor with this type of force–velocity relation would therefore produce rapid ATP synthesis when $u_0 f > -\Delta G$. The motor may be regarded as a chemically driven ratchet, but its polarity is reversed compared to case A. Note however that both motors are driven spontaneously forwards by coupling ATP hydrolysis to their forward steps.

(c) *Case C*

The third special case of a biased random walk is intermediate between cases A and B. Suppose that $k_+(f) = k_+ \exp(-u_0 f/2kT)$, where k_+ is a constant, in which case figure 3*c* shows the force–velocity relation when $\Delta G = -10\,\mathrm{kJ\,M^{-1}}$ and $k_+ = 1\,\mathrm{s^{-1}}$. As in cases A and B, the stepping velocity is zero when $u_0 f = -\Delta G$, but in this case there is no asymmetry between the forward and

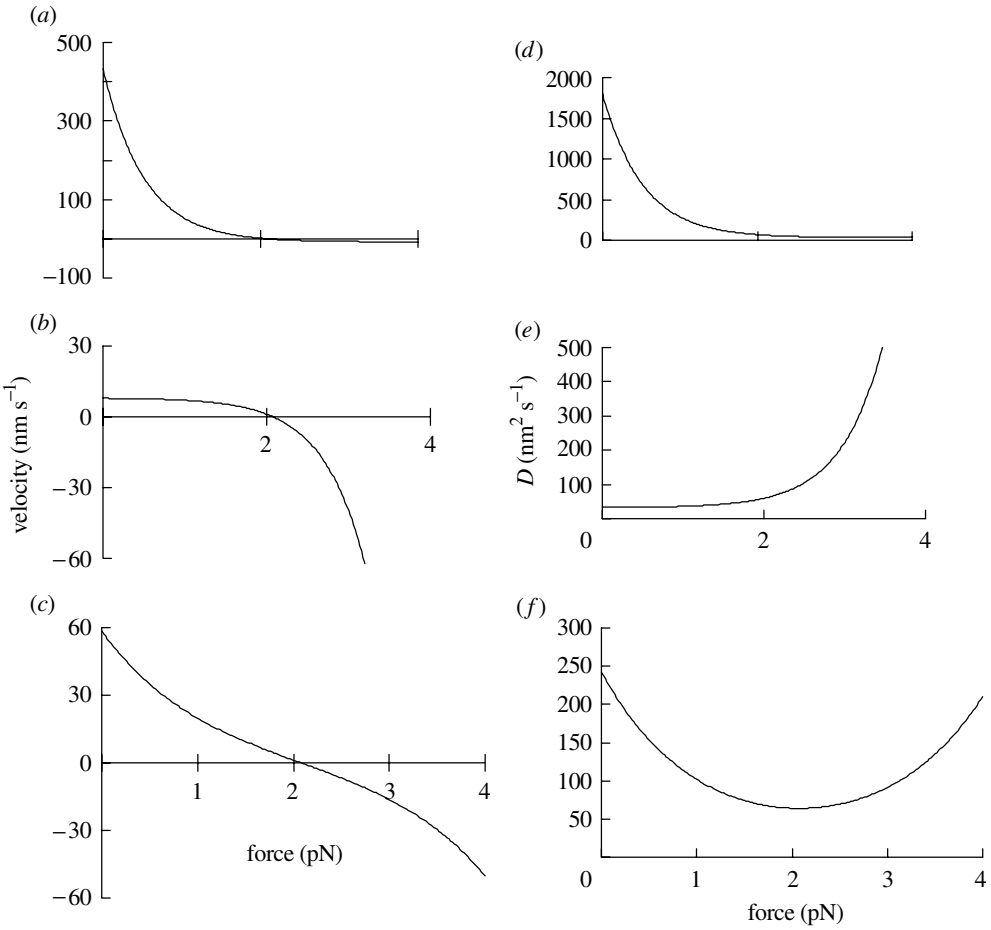

Figure 3. Ratchet behaviour of one-state motors with step size $u_0 = 8$ nm driven by $\Delta G = -10\,\mathrm{kJ\,M^{-1}}$. (*a–c*) The force–velocity relations for cases A, B and C, as discussed in the text. (*d–f*) The corresponding variations in the diffusion coefficient D.

backward stepping. Hence, the motor in figure 3*c* does not behave as a ratchet.

Case C demonstrates that the mechanical asymmetry that produces ratchet behaviour is not in itself an essential prerequisite for the operation of a molecular motor. The key feature of a motor is the preferential coupling of hydrolysis (or some other reaction) to its forward steps, thereby leading to the thermodynamic relation in equation (3.7). This makes forward steps more probable than backward steps when $u_0 f < -\Delta G$. Nonetheless, one should recognize that the ratchets in figure 3*a*,*b* represent the limiting cases for a tightly coupled, processive motor, where either k_- or k_+ is constant. The force–velocity relations for real multistate motors will in general lie somewhere between these two idealized extremes.

5. HEAT AND WORK

One might suppose that a one-state motor performs mechanical work $u_0 f$ per forward step by using energy supplied directly from ATP. Certainly, free energy from ATP hydrolysis is required in order to make $k_+(f) > k_-(f)$ when f lies between zero and $-\Delta G/u_0$ in equation (4.9). This biases the random walk so that the motor moves forwards spontaneously, thereby allowing it to perform mechanical work $u_0 f < -\Delta G$, in agreement with the second law of thermodynamics. However, as the motor works in a cycle, there is no change in its internal energy for a complete cycle. Hence, the first law of thermodynamics (which is applied, strictly speaking, to an ensemble of motors at steady state) requires that the work done per cycle $u_0 f$ equals the heat that the motor absorbs from the surroundings.

From a microscopic viewpoint, heat absorption causes transitions between different states (Mandl 1971). The motor absorbs heat in order to conduct the thermally activated transitions that perform mechanical work on the load, such as head attachment and detachment or the power stroke (or neck-linker docking) in figure 1. The details of this process depend on the inner workings of the particular molecular motor but, as with the thermodynamic relation in equation (3.7), the thermodynamic result is quite general. For the cycle as a whole, the work done by a motor at steady state is equal to the heat that it absorbs from the surroundings. This result applies to all molecular motors, including muscle cross-bridges (Thomas & Thornhill 2000).

Heat absorption by the motor decreases the entropy of the surroundings by $u_0 f/T$ per step, whilst there can be no change in the entropy of the motor itself for a complete cycle. Hence, this process cannot occur spontaneously on its own without violating the second law of thermodynamics. It is essential that the forward stepping of the motor is linked to a spontaneous process such as ATP hydrolysis that releases free energy ($-\Delta G$). The entropy of the system is then increased by the entropy of reaction

(ΔS) together with the entropy increase due to the release of heat ($-\Delta H$) to the surroundings, where ΔH is the enthalpy of the reaction. The net result is that the increase in entropy of the system as a whole, i.e. $\Delta S-\Delta H/T-u_0 f/T$, is positive, which is equivalent to the condition that the motor can perform mechanical work, i.e. $u_0 f < -\Delta G$ (as $\Delta G=\Delta H-T\Delta S$).

ATP hydrolysis is an exothermic reaction with a negative ΔH that contributes to the reaction's negative ΔG. Hence, one might be tempted to regard the exothermic reaction itself as the source of heat that drives the motor. However, as the motor is held isothermally at temperature T by virtue of its intimate thermal contact with the surrounding fluid, the heat of the reaction cannot be given to the motor alone. Furthermore, as discussed by Thomas & Thornhill (2000), the motor could in principle still perform work even if the hydrolysis reaction were endothermic (that is, if $\Delta H > 0$), provided that ΔG was negative. (One can achieve $\Delta G < 0$ by reducing [ADP] and [P_i] so that the entropy increase (ΔS) for the reaction is large, in which case the law of mass action drives the reaction forwards irrespective of ΔH.)

Hence, although ATP hydrolysis provides the free energy for driving the motor forwards, the immediate source of energy (or enthalpy) in order for the motor to perform work is the heat absorbed from the surroundings. This heat is provided by the thermal fluctuations that produce thermally activated motion of the moving parts of the molecular motor. In this respect, the chemically driven ratchet in case A above is similar to an idealized 'Brownian' or 'thermal' ratchet (Astumian 1997). However, one must recognize that both the negative ΔG from the hydrolysis and the heat absorbed from the surroundings are required for driving the ratchet.

6. RANDOMNESS AND DIFFUSION

We can analyse the biased random walk of the one-state motor in figure 2*b* in much greater detail by formulating it as a diffusion problem (Berg 1993). We start with the rate equation for the probability p_n of finding the motor at site n, i.e.

$$\mathrm{d}p_n/\mathrm{d}t = k_+(f)p_{n-1} - k_-(f)p_n + k_-(f)p_{n+1} - k_+(f)p_n. \tag{6.15}$$

Now we may approximate the occupation probability p_n by $u_0 p(x, t)$, where the probability density $p(x, t)$ is a function of position x and time t that is assumed to vary very slowly with x on the scale of the site separation u_0. Hence, p_{n+1} and p_{n-1} in equation (6.15) are given approximately by

$$p_{n\pm1}(t) = u_0 p(x, t) \pm u_0^2 \partial p/\partial x + 0.5u_0^3 \partial^2 p/\partial x^2. \tag{6.16}$$

It follows that the rate equation, i.e. equation (6.15), may be rewritten as

$$\partial p/\partial t = 0.5u_0^2[k_+(f) + k_-(f)]\partial^2 p/\partial x^2 - u_0[k_+(f) - k_-(f)]\partial p/\partial x = D\partial^2 p/\partial x^2 - v\partial p/\partial x. \tag{6.17}$$

This partial differential equation describes the diffusion and drift of a one-state motor executing a biased random walk along its track (Berg 1993). The average stepping velocity v agrees with the steady-state result in equation (4.10), whilst the diffusion coefficient D is

$$D = 0.5u_0^2[k_+(f) + k_-(f)]. \tag{6.18}$$

It is straightforward to show from equations (3.6), (4.9), (4.10) and (6.18) that at thermodynamic equilibrium the diffusion coefficient obeys the Einstein relation $D=\mu kT$, where the drift mobility $\mu=-\mathrm{d}v/\mathrm{d}f$. Note however that the Einstein relation only applies at the equilibrium load determined by equation (3.6). More generally, equations (4.9), (4.10) and (6.18) imply that v and D are related by

$$v = -(2D/u_0)\tanh[(\Delta G + u_0 f)/2kT]. \tag{6.19}$$

Figure 3*d–f* illustrates how the diffusion coefficient D depends on the load for the three idealized one-state motors with the force–velocity relations in figure 3*a–c*, respectively. The decrease in D with load in figure 3*d* is due to the decrease of $k_+(f)$ in equation (4.11), whilst the increase in D with load in figure 3*e* is due to the increase of $k_-(f)$ in equation (4.13). The behaviour of D in figure 3*f* is intermediate between these two extremes and, as with the force–velocity relation in figure 3*c*, it is symmetrical about the equilibrium load, where $u_0 f = -\Delta G$.

One way of solving the diffusion equation, i.e. equation (6.17), for the probability density $p(x, t)$ is to look for solutions of the form $\exp[i(kx-\omega t)]$. In that case, replacing $\partial/\partial t$ by $-i\omega$, $\partial/\partial x$ by ik and $\partial^2/\partial x^2$ by $-k^2$, we find that the angular frequency ω and the wavenumber k must satisfy the dispersion relation

$$\omega = kv - iDk^2. \tag{6.20}$$

If the motor starts at $x=0$ at $t=0$, then the initial probability density $p(x, 0)=\delta(x)$. With this initial condition, the Fourier transform of $p(x, t)$ together with equation (6.20) produces the standard solution

$$p(x, t) = [1/\sqrt{(4\pi Dt)}]\exp[-(x - vt)^2/4Dt]. \tag{6.21}$$

Figure 4*a* shows the typical Gaussian spatial variation in $p(x, t)$ at different times. On average, the motor tends to move forwards and its average position at time t is $\langle x\rangle = vt$, whilst the random diffusive motion produces a variance in its position that is given by $\langle \delta x^2\rangle = 2Dt$, where the angular brackets denote an ensemble average at time t. One can combine these two quantities into a 'randomness parameter' r (Svoboda *et al.* 1994), which for a motor with step size u_0 is defined as

$$r = \langle \delta x^2\rangle/u_0\langle x\rangle = 2D/u_0|v|, \tag{6.22}$$

where we have written $|v|$ in order to allow for the possibility that the velocity v may be negative. Equations (6.19) and (6.22) imply that the randomness for a one-state motor is

$$r = |\mathrm{cotanh}[(\Delta G + u_0 f)/2kT]|. \tag{6.23}$$

Hence, the randomness of this simple one-state model is determined solely by the thermodynamic parameters ΔG and $u_0 f$. Figure 4*b* shows how r varies with load for those motors the behaviour of which is shown in figure 3. Although the variations of v and D with load for these three motors are quite different, their randomness is exactly the same. Note that r tends to infinity as the

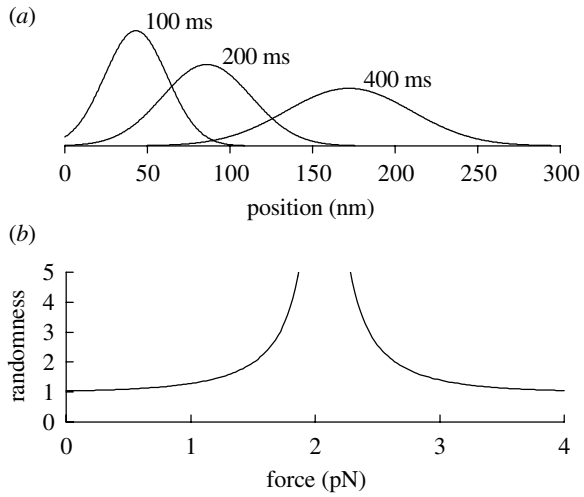

Figure 4. Diffusion and randomness for a one-state motor. (*a*) Variation in the probability density $p(x, t)$ at $t=100$, 200 and 400 ms with position x for the motor in figure 3*a*, when $f=0$ after starting at $x=0$. (*b*) Randomness r against load f for a one-state motor according to equation (6.23) when $\Delta G=-10\,\mathrm{kJ\,M^{-1}}$.

motor approaches the equilibrium load where $u_0 f=-\Delta G$, since the average stepping velocity v in equation (6.22) is zero at equilibrium. When backward transitions are neglected, $k_-(f)=0$ and we then find that $r=1$, which is the expected result for a so-called 'Poisson' motor (Svoboda *et al*. 1994). However, the inclusion of backward transitions in general produces $r>1$.

7. TWO-STATE MOTORS

A two-state motor has two states per attachment site, so we denote the occupation probabilities at site n by $p_{1,n}$ and $p_{2,n}$. The rate equations are

$$\mathrm{d}p_{1,n}/\mathrm{d}t = k_2 p_{2,n-1} - k_{-2} p_{1,n} + k_{-1} p_{2,n} - k_1 p_{1,n} \tag{7.24}$$

and

$$\mathrm{d}p_{2,n}/\mathrm{d}t = k_1 p_{1,n} - k_{-1} p_{2,n} + k_{-2} p_{1,n+1} - k_2 p_{2,n}, \tag{7.25}$$

where k_1 and k_{-1} are the rate constants for transitions between states at the same site and k_2 and k_{-2} are the rate constants for intersite transitions. (The rate constants generally depend on the applied load f, but, in order to simplify the notation, we have not explicitly shown this here.) In the continuum approximation, we use the probability densities $p_1(x, t)$ and $p_2(x, t)$ for the two states. It follows from equations (6.16), (7.24) and (7.25) that they satisfy the partial differential equations

$$\begin{aligned}\partial p_1/\partial t = {} & 0.5u_0^2 k_2 \partial^2 p_2/\partial x^2 - u_0 k_2 \partial p_2/\partial x \\ & + (k_{-1}+k_2)p_2 - (k_1+k_{-2})p_1,\end{aligned} \tag{7.26}$$

and

$$\begin{aligned}\partial p_2/\partial t = {} & 0.5u_0^2 k_{-2} \partial^2 p_1/\partial x^2 + u_0 k_{-2} \partial p_1/\partial x \\ & + (k_1+k_{-2})p_1 - (k_{-1}+k_2)p_2.\end{aligned} \tag{7.27}$$

Neglecting derivatives above second order, we can combine these two coupled equations into a single differential equation, i.e.

$$\partial p/\partial t = D\partial^2 p/\partial x^2 - v\partial p/\partial x - \tau\partial^2 p/\partial t^2, \tag{7.28}$$

which is the diffusion equation for the two-state motor. The characteristic time τ here is

$$\tau = 1/(k_1 + k_{-1} + k_2 + k_{-2}), \tag{7.29}$$

whilst the velocity v and diffusion coefficient D from equations (7.26)–(7.28) are

$$\begin{aligned}v &= u_0[k_+(f) - k_-(f)] \\ &= u_0 k_+(f)\{1 - \exp[(\Delta G + u_0 f)/kT]\}\end{aligned} \tag{7.30}$$

and

$$\begin{aligned}D &= 0.5u_0^2[k_+(f) + k_-(f)] \\ &= 0.5u_0^2 k_+(f)\{1 + \exp[(\Delta G + u_0 f)/kT]\},\end{aligned} \tag{7.31}$$

where the effective forward and backward rate constants $k_+(f)$ and $k_-(f)$ are

$$k_+(f) = k_1 k_2/(k_1 + k_{-1} + k_2 + k_{-2}), \tag{7.32}$$

and

$$k_-(f) = k_{-2} k_{-1}/(k_1 + k_{-1} + k_2 + k_{-2}). \tag{7.33}$$

Note that we have made use of the thermodynamic relation for the two-state motor in equations (7.30) and (7.31), i.e.

$$k_+(f)/k_-(f) = k_1 k_2/k_{-2} k_{-1} = \exp[-(\Delta G + u_0 f)/kT]. \tag{7.34}$$

The velocity (see equation (7.30)) for the two-state motor corresponds to equation (4.10) for the one-state motor, but with $k_+(f)$ now determined by equation (7.32). Similarly, the two-state diffusion coefficient D in equation (7.31) corresponds to the one-state relation in equation (6.18).

Equation (7.28), which is the two-state diffusion equation, is identical to the one-state equation, i.e. equation (6.17), for the biased random walk except for the additional $\tau\partial^2 p/\partial t^2$ term on the right-hand side. This very important extra term has a profound effect on the randomness and diffusion for the two-state motor. Consider the dispersion relation for equation (7.28), which, in contrast to equation (6.20), may be written as

$$\omega = kv - i(Dk^2 - \omega^2\tau). \tag{7.35}$$

In the limit where $k\to 0$, the two roots of this equation are given approximately by

$$\omega = kv - i(D - v^2\tau)k^2 \tag{7.36}$$

and

$$\omega = -i/\tau, \tag{7.37}$$

where we have kept the terms for $\mathrm{O}(k^2)$ in equation (7.36). Hence, equation (7.36) is the approximate dispersion relation for diffusive solutions for the two-state model in place of equation (6.20). It then follows that the effective diffusion coefficient for the two-state motor is

$$D_{\mathrm{eff}} = D - v^2\tau. \tag{7.38}$$

The $\tau\partial^2 p/\partial t^2$ term in equation (7.28) therefore reduces the diffusion coefficient by the amount $v^2\tau$. We discuss the

physical basis of equation (7.38) in electronic Appendix A and demonstrate that τ is the characteristic time for the stepping velocity v of the motor to reach the steady state. Note that we require $D_{\rm eff} \geqslant 0$ in equation (7.38), otherwise equation (7.36) would produce an unphysical solution that grows exponentially with time.

It follows from equations (6.22), (7.30), (7.31) and (7.38) that the randomness for the two-state motor is

$$r = 2D_{\rm eff}/u_0|v| = |\mathrm{cotanh}[(\Delta G + u_0 f)/2kT]| - 2|v|\tau/u_0. \tag{7.39}$$

Therefore, r has been reduced by $2|v|\tau/u_0$ compared with the one-state case in equation (6.23). Indeed, equation (6.23) may be regarded as a special case of equation (7.39) when $\tau = 0$, in which case the modified diffusion equation, i.e. equation (7.28), reduces to equation (6.17). We show below that equation (7.39) also applies to more complex multistate motors.

Note that, in the above analysis, we have not explicitly taken account of the alternation of heads that is believed to occur in two-headed processive motors such as myosin V and kinesin, as illustrated in figure 1. The complete cycle for a two-headed motor consists of two steps, one for each head, resulting in the hydrolysis of two molecules of ATP. Electronic Appendix B describes how the kinetics should be modified in that case. We show there that, when the two heads are identical (as in myosin V and conventional kinesin), the cycle may indeed be considered to be a single step for one of the heads, hydrolysing a single molecule of ATP.

8. MULTISTATE MOTORS

The complexity of real molecular motors generally requires us to go beyond the simple one-state or two-state models. As an example of a multistate motor, we consider a three-state motor, as shown for the simplified models of myosin V and kinesin in figure 1*a*,*b*. As discussed by Thomas *et al*. (2001) for kinesin, the stepping velocity v may be derived most simply from the steady-state hydrolysis rate R in equation (2.2). For an ensemble of such motors, each of which is subjected to a constant load f, the average hydrolysis rate per motor at steady state is

$$\begin{aligned} R &= k_1(f)P_1 - k_{-1}(f)P_2 = k_2(f)P_2 - k_{-2}(f)P_3 \\ &= k_3(f)P_3 - k_{-3}(f)P_1, \end{aligned} \tag{8.40}$$

where P_1, P_2 and P_3 are the probabilities of finding motors in states 1, 2 and 3 in the ensemble (irrespective of their attachment site), which satisfy the normalization condition $P_1 + P_2 + P_3 = 1$. The steady-state solution in equation (8.40) can be used for establishing the Michaelis–Menten relation for the effect of the concentration of ATP on the stepping velocity of a three-state motor (Astumian & Derenyi 1999; Thomas *et al*. 2001).

In order to analyse the diffusion of this motor, we start from the rate equations for the occupation probabilities $p_{1,n}$, $p_{2,n}$ and $p_{3,n}$ for finding the motor in states 1, 2 or 3 at site n:

$$\mathrm{d}p_{1,n}/\mathrm{d}t = k_3 p_{3,n-1} - k_{-3}p_{1,n} + k_{-1}p_{2,n} - k_1 p_{1,n}, \tag{8.41}$$

$$\mathrm{d}p_{2,n}/\mathrm{d}t = k_1 p_{1,n} - k_{-1}p_{2,n} + k_{-2}p_{3,n} - k_2 p_{2,n}, \tag{8.42}$$

and

$$\mathrm{d}p_{3,n}/\mathrm{d}t = k_2 p_{2,n} - k_{-2}p_{3,n} + k_{-3}p_{1,n+1} - k_3 p_{3,n}. \tag{8.43}$$

Hence, the corresponding differential equations for the probability densities p_1, p_2 and p_3 are

$$\begin{aligned} \partial p_1/\partial t = {} & k_3(p_3 - u_0\partial p_3/\partial x + 0.5u_0^2\partial^2 p_3/\partial x^2) \\ & - (k_1 + k_{-3})p_1 + k_{-1}p_2, \end{aligned} \tag{8.44}$$

$$\partial p_2/\partial t = k_1 p_1 - (k_{-1} + k_2)p_2 + k_{-2}p_3, \tag{8.45}$$

and

$$\begin{aligned} \partial p_3/\partial t = {} & k_{-3}(p_1 + u_0\partial p_1/\partial x + 0.5u_0^2\partial^2 p_1/\partial x^2) \\ & + k_2 p_2 - (k_{-2} + k_3)p_3. \end{aligned} \tag{8.46}$$

One can combine these three equations into a single partial differential equation, which (neglecting derivatives above second order) is of exactly the same form as equation (7.28), which is the modified diffusion equation. Alternatively, as shown in electronic Appendix C, one can look algebraically for exponential solutions to equations (8.44)–(8.46) that satisfy the dispersion relation in equation (7.36). Equations analogous to equations (8.44)–(8.46) can also be derived for even more complex N-state motors that follow the general kinetic scheme in figure 2*a*. Combining the N partial differential equations leads to equation (7.28), provided that derivatives above second order are neglected. The algebra becomes rather complicated as N increases but it can be tackled with software such as MATHEMATICA (Wolfram Research, Champain, IL, USA).

The key parameters that emerge from the analysis of any tightly coupled, processive motor are the effective forward rate constant $k_+(f)$, as in equation (7.34) and the time-constant τ. The former determines v and D through equations (7.30) and (7.31), whilst the latter is required together with v and D in order to determine $D_{\rm eff}$ in equation (7.38) and, hence, r in equation (7.39). Table 1 summarizes the crucial parameters $k_+(f)$ and τ for different models of molecular motors, ranging from the simple one-state model to a four-state model. Every state that we introduce into the model adds two more rate constants, the values of which may be difficult to determine from limited experimental data. Hence, some approximations are generally necessary in order to keep the number of states in the model to a minimum, which reduces the mathematical complexity and allows us to estimate rate constants from experimental data.

The velocity v and time-constant τ for a motor determine its randomness parameter r through equation (7.39). When ADP and P_i are nominally absent from the experimental saline solution, ΔG is very large and negative, and the thermodynamic term on the right-hand side of equation (7.39) is then essentially 1. Under these conditions,

$$r = 1 - 2|v|\tau/u_0. \tag{8.47}$$

Thus, we expect to find $r \leqslant 1$ for a tightly coupled, processive motor when $[\mathrm{ADP}] = [\mathrm{P_i}] = 0$. In practice, we may use equation (8.47) together with experimental measurements of the randomness r and the stepping

Table 1. Expressions for the effective forward rate constant $k_+(f)$ and time-constant τ for the tightly coupled, processive N-state motors in figure 2 with $N=1$, 2, 3 or 4.

N	$k_+(f)$	τ
1	$k_+(f)$	0
2	$\dfrac{k_1k_2}{k_1+k_{-1}+k_2+k_{-2}}$	$\dfrac{1}{k_1+k_{-1}+k_2+k_{-2}}$
3	$k_1k_2k_3/\{k_1(k_2+k_{-2}+k_3)+k_{-3}(k_{-1}+k_2+k_{-2})+k_{-1}(k_{-2}+k_3)+k_2k_3\}$	$(k_1+k_{-1}+k_2+k_{-2}+k_3+k_{-3})/\{k_1(k_2+k_{-2}+k_3)+k_{-3}(k_{-1}+k_2+k_{-2})+k_{-1}(k_{-2}+k_3)+k_2k_3\}$
4	$k_1k_2k_3k_4/[k_1k_2k_4+k_1k_2k_{-3}+k_{-1}k_{-3}k_{-4}+k_2k_{-3}k_{-4}+k_1k_4k_{-2}+k_{-1}k_4k_{-2}+k_1k_{-3}k_2+k_{-1}k_{-3}k_{-2}+k_{-1}k_{-4}k_{-2}+k_{-3}k_{-4}k_{-2}+k_1k_2k_3+k_1k_4k_3+k_{-1}k_4k_3+k_2k_4k_3+k_{-1}k_{-4}k_3+k_2k_{-4}k_3]$	$[(k_1+k_{-4})(k_{-1}+k_2+k_{-2}+k_3+k_{-3}+k_4)+(k_{-1}+k_2)\times(k_{-2}+k_3+k_{-3}+k_4)+(k_{-2}+k_3)(k_{-3}+k_4)-k_1k_{-1}-k_2k_{-2}-k_3k_{-3}-k_4k_{-4}]/[k_1k_2k_4+k_1k_2k_{-3}+k_{-1}k_{-3}k_{-4}+k_2k_{-3}k_{-4}+k_1k_4k_{-2}+k_{-1}k_4k_{-2}+k_1k_{-3}k_2+k_{-1}k_{-3}k_{-2}+k_{-1}k_{-4}k_{-2}+k_{-3}k_{-4}k_{-2}+k_1k_2k_3+k_1k_4k_3+k_{-1}k_4k_3+k_2k_4k_3+k_{-1}k_{-4}k_3+k_2k_{-4}k_3]$

velocity v for determining the internal time-constant τ for a motor (Thomas *et al*. 2001).

9. CONCLUDING REMARKS

We have shown that the biochemical cycle provides a fundamental link between thermodynamics and kinetics for a molecular motor. The rate constants for a tightly coupled, processive motor, such as kinesin and myosin V, pulling against a constant load f must satisfy the thermodynamic relation in equation (3.7) in order to ensure consistency with thermodynamics. Furthermore, we have shown that equation (3.7) allows us to derive an expression of the form of equation (7.30) for the force–velocity relation for the motor.

The hypothetical one-state motor in figure 2*b* provides a simple illustration of the importance of the thermodynamic relation for tightly coupled, processive molecular motors. This motor executes a biased random walk (Berg 1993; Astumian 1997) that allows it to move forwards spontaneously, thereby performing mechanical work per step of $u_0f<-\Delta G$ in agreement with the second law of thermodynamics. Indeed, figure 3*a*,*b* shows that it can behave as an idealized chemically driven ratchet (Astumian 1997). The force–velocity relations for all tightly coupled, processive motors generally lie somewhere between these two extremes. However, figure 3*c* shows that the mechanical asymmetry inherent in a ratchet is not in fact an essential prerequisite for the operation of a molecular motor.

Application of the first law of thermodynamics to the one-state motor shows that the immediate source of energy that allows the motor to perform steady-state mechanical work is the heat absorbed from its surroundings. This heat is provided by the thermal fluctuations that produce thermally activated (loosely speaking 'Brownian') motion of the moving parts of the molecular motor. However, in order to avoid violating the second law of thermodynamics, it is essential that the forward stepping of the motor is linked to a spontaneous process such as ATP hydrolysis that releases free energy ($-\Delta G$). This is the key asymmetry in the motor that allows it to perform mechanical work $u_0f<-\Delta G$, whilst the decrease in the entropy of its surroundings ($=u_0f/T$) is offset by the entropy of reaction (ΔS) together with the entropy increase due to the release of heat ($-\Delta H$) to the surroundings.

The biased random walk executed by the one-state motor can be described using diffusion equation (6.17), which follows directly from rate equation (6.15) for the molecular motor. The diffusion equation determines both the stepping velocity v and the diffusion coefficient D. When these two quantities are combined into a randomness parameter r (Svoboda *et al*. 1994), we find that $r=|\mathrm{cotanh}[(\Delta G+u_0f)/2kT]|$, so the randomness for a one-state motor is determined purely by the thermodynamic quantities ΔG and u_0f. Hence, the three one-state motors with very different force–velocity relations shown in figure 3 all have exactly the same randomness. In the absence of backward steps (that is, when $k_-(f)=0$), we find that $r=1$, in agreement with the statistical theory of Svoboda *et al*. (1994) for a 'Poisson' motor.

The rate equations for two-state and multistate motors also directly give rise to a diffusion equation that governs the biased random walk that allows them to perform mechanical work. However, we find that their modified diffusion equation, i.e. equation (7.28), contains an important additional term that opposes diffusion and reduces their effective diffusion coefficient to $D-v^2\tau$. Here, the time-constant τ is the characteristic time for the stepping velocity of a motor to reach a steady state. The randomness of a multistate motor is therefore reduced compared with the one-state case and when [ADP] and [P_i] are negligible we find that $r=1-2|v|\tau/u_0$. Hence, the time-constant τ for a tightly coupled, processive molecular motor can be determined experimentally from measurements of its randomness and stepping velocity. This interpretation of randomness is rather different from that of Svoboda *et al*. (1994), whose statistical theory requires that the rate constants for all backward transitions are zero. Nonetheless, as shown in electronic Appendix C, our analysis agrees with theirs in that particular limit.

In conclusion, our thermodynamic approach to tightly coupled, processive molecular motors emphasizes the central importance of the biochemical cycle. Furthermore, it establishes general results that are independent of

the detailed inner workings of a motor (provided that it exhibits tight coupling and processivity). The theory may be applied not only to linear motors, such as kinesin and myosin V, but also to rotary motors, such as ATP synthase (Boyer 1997; Weber & Senior 1997; Yasuda *et al.* 2001). The ideas presented here may therefore help us to understand the general principles that underlie the operation of biological molecular motors.

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