Coupling of Two Motor Proteins: A New Motor Can Move Faster

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We study the effect of a coupling between two motor domains in highly processive motor protein complexes. A simple stochastic discrete model, in which the two parts of the protein molecule interact through some energy potential, is presented. The exact analytical solutions for the dynamic properties of the combined motor species, such as the velocity and dispersion, are derived in terms of the properties of free individual motor domains and the interaction potential. It is shown that the coupling between the motor domains can create a more efficient motor protein that can move faster than individual particles. The results are applied to analyze the motion of RecBCD helicase molecules.

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Motor proteins are enzyme molecules that are responsible for generation of forces and molecular transport in biological systems [1,2]. They move along polar molecular tracks such as cytoskeletal filaments and DNA molecules, and the motion is powered by energy released from a hydrolysis of adenosine triposphate (ATP) molecules or related compounds. However, the mechanisms of the chemical energy transformation into the mechanical work are not fully understood [1].

Crystal structures of motor proteins reveal that they can be viewed as complex systems consisting of many domains [1,3]. It is assumed that the complexity of motor proteins appeared during the evolution as a way to perform simultaneously many functions. A good example is a RecBCD enzyme that belongs to a class of helicase motor proteins [4]. It processes DNA ends resulting from the double-strand breaks [3]. This protein unwinds the DNA molecule into two separate strands, and then it digests them by moving at the same time along the DNA [5–8]. RecBCD is a heterotrimer made of three proteins: RecB, RecC, and RecD [9]. Two subunits, RecB and RecD domains, have helicase activities, consume ATP, and act on $3'$- or $5'$-ended DNA strands, respectively [7,8,10]. Meanwhile, the third subunit (RecC) has no ATPase activity, and it functions as a clamp preventing the dissociation of the enzyme complex from the track [11]. Recent experiments provided data on DNA unwinding rates by RecBCD and the active subunits RecB and RecD at the single-molecule level [5,7,12,13]. Surprisingly, the speed of the protein complex is significantly faster than the unwinding rates of individual subunits [7]. These observations raise the general question important for all biological systems: How is the interaction between different subunits optimized to produce highly efficient multifunctional biological molecules? The work presented here aims to address this issue by developing a stochastic model of the motion of motor proteins consisting of two interacting subunits.

We assume that the motor protein complex consists of two particles, as shown in Fig. 1. Each subunit can move only along its own one-dimensional track that corresponds to the motion of RecB and RecD domains on the separate DNA strands. The position of the particle A on the upper lattice is given by the integer l, while m specifies the position of the lower domain B. Because of the link between the motor subunits only the limited number of the molecular configurations has to be considered. In the simplest description, we assume that 3 configurations are possible; i.e., $0 \leq |l - m| \leq 1$ (see Fig. 1). Our approach is related to the theoretical model of helicase motion proposed in Ref. [14], where the DNA unwinding is viewed as a result of interaction between the helicase and DNA fork, while we discuss the effect of the internal coupling of the subunits on the motion of proteins.

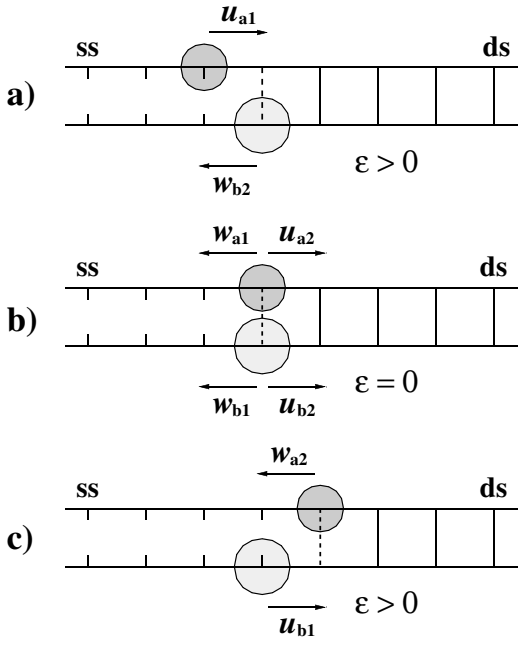

FIG. 1. Schematic view of a motor protein with two domains. Transition rates u_{ai} and w_{ai} with $i = 1, 2$ describe the motion of the domain A (small circles), while for the second particle B (large circles) the transitions rates are u_{bi} and w_{bi}. Only three configurations are allowed: (a) $(l-1, l)$ with the energy of interaction $\varepsilon > 0$, (b) (l, l) with $\varepsilon = 0$, and (c) $(l+1, l)$ with $\varepsilon > 0$.

The dynamics of the system can be described by a set of transition rates for domains A and B for discrete steps forward and backward along the corresponding tracks (Fig. 1). These rates depend not only on the type of the subunit but also on the specific configuration of the cluster. However, the dependence of rates on the DNA sequence is neglected. For the configurations $(l-1, l)$ the upper subunit A can only move forward with the rate u_{a1}, while the lower particle B can only hop backward with the rate w_{b2}: see Fig. 1(a). Similarly, in the configurations $(l+1, l)$ [Fig. 1(c)] the domain A can only go back with the rate w_{a2}, while the domain B can advance with the rate u_{b1}. In the configurations (l, l) [Fig. 1(b)] both particles can move forward and backward with the rates $u_{a2}(u_{b2})$ and $w_{a1}(w_{b1})$ for the upper and the lower subunit, respectively.

The interaction between the protein domains is specified by the parameter $\varepsilon \geq 0$ defined as the energy difference between the states $(l \pm 1, l)$ and (l, l), respectively (Fig. 1). We assume that the configuration (l, l) is energetically most favored, while the configurations $(l \pm 1, l)$ have a higher energy. It might be due to the internal stress and/or the work needed to break the bond between the bases in DNA. Then the detailed balance relations for the transition rates are

$$\frac{u_{j1}}{w_{j1}} = \frac{u_j}{w_j}\exp(+\varepsilon/k_BT), \quad \frac{u_{j2}}{w_{j2}} = \frac{u_j}{w_j}\exp(-\varepsilon/k_BT), \quad (1)$$

with $j = a$ or b. The rates u_j and w_j are the forward and backward transition rates for the subunit $A(j = a)$ and $B(j = b)$ in the case of $\varepsilon = 0$.

We introduce $P(l, m; t)$ as the probability to find the system in the configuration (l, m) at time t. It can be determined by solving of a set of independent master equations,

$$\frac{dP(l-1, l; t)}{dt} = u_{b2}P(l-1, l-1; t) + w_{a1}P(l, l; t) - (u_{a1} + w_{b2})P(l-1, l; t), \quad (2)$$

$$\frac{dP(l+1, l; t)}{dt} = u_{a2}P(l, l; t) + w_{b1}P(l+1, l+1; t) - (u_{b1} + w_{a2})P(l+1, l; t). \quad (3)$$

The corresponding equation for $P(l, l; t)$ is just a linear combination of two equations presented above, and therefore it is not considered. In addition, the probabilities satisfy the normalization condition,

$$\sum_{l=-\infty}^{+\infty} [P(l, l; t) + P(l-1, l; t) + P(l+1, l; t)] = 1, \quad (\text{all } t). \quad (4)$$

The solutions of the master equations can be found be summing over all integers $-\infty < l < +\infty$. Define new functions

$$P_0(t) = \sum_{l=-\infty}^{+\infty} P(l, l; t), \qquad P_1(t) = \sum_{l=-\infty}^{+\infty} P(l-1, l; t),$$
$$P_1'(t) = \sum_{l=-\infty}^{+\infty} P(l+1, l; t). \quad (5)$$

Then, using the conservation of probability, the steady-state distribution can be easily derived,

$$P_0 = 1/\Omega, \qquad P_1 = \beta/\Omega, \qquad P_1' = \alpha/\Omega, \quad (6)$$

where $\Omega = 1 + \alpha + \beta$, and α and β are given by

$$\alpha = \frac{u_{a2} + w_{b1}}{u_{b1} + w_{a2}}, \qquad \beta = \frac{u_{b2} + w_{a1}}{u_{a1} + w_{b2}}. \quad (7)$$

From the knowledge of the probability densities the dynamic properties of the motor complex, such as the mean velocity V and dispersion (effective diffusion constant) D, can be calculated [15]. The velocity is given by

$$V = \frac{1}{2}[(u_{a1} - w_{b2})P_1 + (u_{a2} + u_{b2} - w_{a1} - w_{b1})P_0 + (u_{b1} - w_{a2})P_1'], \quad (8)$$

which yields the following result:

$$V = \frac{1}{\Omega}(u_{a2} + u_{b2} - \alpha w_{a2} - \beta w_{b2}). \quad (9)$$

Similarly for the dispersion we obtain

$$D = \frac{1}{\Omega}\left[\frac{1}{2}(u_{a2} + u_{b2} + \alpha w_{a2} + \beta w_{b2}) - \frac{(V + w_{a2})(u_{a2} - \alpha V)}{u_{b1} + w_{a2}} - \frac{(V + w_{b2})(u_{b2} - \beta V)}{u_{a1} + w_{b2}}\right]. \quad (10)$$

If the motor domains are identical $(A = B)$, then the transition rates are related as

$$u_{a1} = u_{b1} = u_1, \qquad u_{a2} = u_{b2} = u_2, \qquad w_{a1} = w_{b1} = w_1, \qquad w_{a2} = w_{b2} = w_2, \quad (11)$$

and the expressions for the velocity and dispersion can be written in a much simpler form,

$$V = \frac{2(u_1u_2 - w_1w_2)}{u_1 + w_2 + 2(u_2 + w_1)}, \qquad D = \frac{u_1u_2 + w_1w_2 - V^2}{u_1 + w_2 + 2(u_2 + w_1)}. \quad (12)$$

Now consider the case of symmetric domains without interaction $(\varepsilon = 0)$. If the particles are allowed to move freely along its tracks, then their dynamic properties are

$$V_0 = u - w, \qquad D_0 = (u + w)/2, \quad (13)$$

with $u_1 = u_2 = u$ and $w_1 = w_2 = w$. However, the average velocity and dispersion of the protein cluster (without interaction) are different. From Eq. (12) we obtain

$$\frac{V(\varepsilon=0)}{V_0}=\frac{2}{3}, \qquad \frac{1}{3}\le\frac{D(\varepsilon=0)}{D_0}\le\frac{10}{27}. \tag{14}$$

These results show that in the case of $\varepsilon = 0$ the speed of the cluster is smaller than the rates of the free particles, as expected, although the fluctuations also decrease.

Much more interesting is the situation when the two motor domains interact ($\epsilon > 0$). Using the detailed balance conditions (1), the transition rates can be written as

$$u_{j1}=u_j\gamma^{1-\theta_{j1}}, \qquad w_{j1}=w_j\gamma^{-\theta_{j1}}, \qquad u_{j2}=u_j\gamma^{-\theta_{j2}}, \qquad w_{j2}=w_j\gamma^{1-\theta_{j2}}, \tag{15}$$

where $\gamma = \exp(\varepsilon/k_BT)$, and $j = a$ or b. The coefficients θ_{ji} determine how the interaction energy is distributed between the forward and backward transitions [1,14,15]. They are closely related to the load-distribution factors used in the single-particle models of motor proteins [15]. It is reasonable to approximate the distribution factors $0 \le \theta_{ji} \le 1$ as equal to each other since they describe the similar processes in the motion of individual motor domains. However, the situation of the state-dependent energy-distribution factors can also be considered.

For the motor protein complex with the symmetric domains ($A = B$) the effect of interactions can be analyzed by looking at the ratio of the cluster velocity to the velocity of the free noninteracting particles,

$$r_V=\frac{V}{V_0}=\frac{2\gamma^{1-\theta}}{2+\gamma}. \tag{16}$$

The dependence of the relative velocity on the interaction energy for different θ is shown in Fig. 2. The most interesting observation is that for small values of the energy-distribution factors ($\theta \le 0.23$) there is a range of interaction energies when the velocity of the protein's complex is *faster* than the velocities of the free particles. It contradicts the naive intuitive expectations, but it can be understood by considering again Eqs. (8) and (15). Large interaction energies increase the transitions rates u_{j1} and w_{j2} and lower the rates u_{j2} and w_{j1} for $j = a$ or b. At the same time the probabilities of nonvertical configurations P_1 and P_1' are exponentially decreasing functions of the interaction energy. Then each term in Eq. (8) has a maximum at some specific value of the interaction energy. Thus the dependence of the relative velocity on the interaction energy is a result of two opposing factors: the acceleration of forward rates is balanced by the decrease of the probabilities for nonvertical configurations, while the increasing probability of the vertical configuration is accompanied by the slowing down of forward transitions.

The expression for the relative dispersion of the protein cluster with symmetric domains ($A = B$) is given by

$$r_D=\frac{D}{D_0}=\frac{2\gamma^{1-\theta}}{2+\gamma}\left[1-\frac{2uw}{(u+w)^2}-\frac{4\gamma}{(2+\gamma)^2}\left(\frac{u-w}{u+w}\right)^2\right] =\frac{2\gamma^{1-\theta}}{2+\gamma}\,g(u,w;\gamma), \tag{17}$$

where it can be shown that $0.5 \le g(u, w; \gamma) < 1$. The interaction energy changes the dispersion in a similar way as the velocity; however, the effect is smaller. It can be seen from Fig. 3 where the ratio of the relative dispersion and velocity is plotted for different transition rates. For all values of the parameters the relative dispersion is always smaller than the relative velocity. It is interesting to note that there are situations when $r_V > 1$ and $r_D < 1$; i.e., the motor complex moves faster but fluctuates less than the free subunits, making it an extremely efficient motor protein.

We can now apply our model for the analysis of the motion of RecBCD helicases. It was shown experimentally that at 37 °C and 5 mM ATP the mutant RecBCD* (the domain RecD is nonfunctional) and the mutant RecB*CD (the domain RecB is nonfunctional) unwind the DNA with rates of 73 and 300 nucleotides/s, correspondingly [7]. Electron microscopy and biochemical assay data indicate

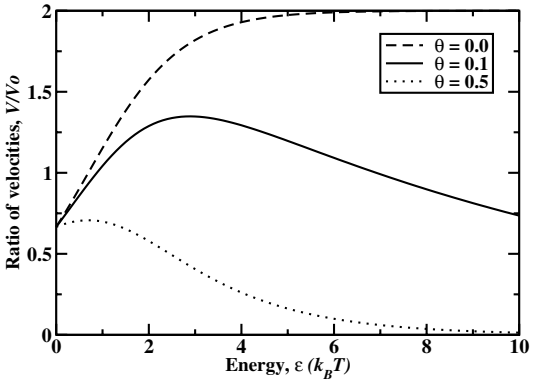

FIG. 2. The relative velocity for the complex motor protein with symmetric domains as a function of the interaction energy for different energy-distribution factors θ.

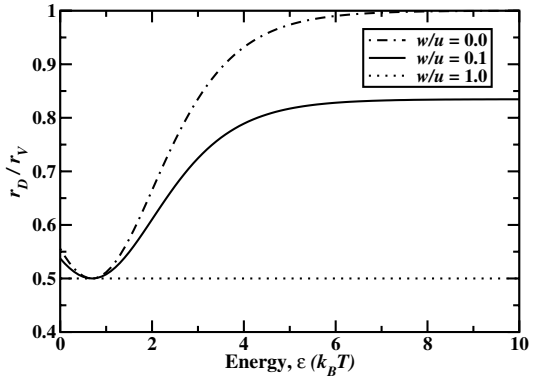

FIG. 3. The ratio of relative dispersion and velocity for the motor protein complex with symmetric domains as a function of the interaction energy for different forward and backward rates.

that the free helicases RecB and RecD move with the same speeds as the corresponding mutant RecBCD* and RecB*CD enzymes [16]. It can be argued that the backward rates w_{ji} are small [13], since the backward steps are rarely seen in the experiments. Then the average speed of DNA unwinding by the RecBCD complex can be approximated as

$$V \approx \frac{(u_a + u_b)\gamma^{1-\theta}}{\gamma + (u_a/u_b + u_b/u_a)}, \tag{18}$$

where $u_a = 73$ and $u_b = 300$ nucleotides/s are the velocities of the free RecB and RecD proteins. Assuming $\theta \approx 0$, we obtain that $V = 370$ nucleotides/s for the interaction energy $\varepsilon \simeq 6k_BT$, in agreement with experimentally observed values of the RecBCD velocity [7]. The predicted energy of interdomain interaction is very reasonable since it is larger than $2k_BT$ needed to break the bond between the base pair in DNA [4,14], but it is also smaller than the energy of a strong chemical bond. Note also that the maximal possible speed of the motor protein complex cannot exceed the sum of the velocities of the individual domains. Thus RecBCD is working with almost maximal possible efficiency.

Our theoretical model has been stimulated by the experimental observations on helicases. However, the effect of the interactions between the domains is general for many biological systems. Recent experiments on KIF3A/B kinesins, which are heterodimeric processive proteins involved in intracellular transport, suggest that the interaction between the motor domains is important for maximizing the performance of these enzymes [17]. By comparing the properties of mutant homodimeric KIF3A/A and KIF3B/B proteins with the wild-type molecules, it was shown that the independent sequential hand-over-hand model cannot explain the heterodimer velocity data. The theoretical model similar to the one discussed above can be developed to describe the coordination between motor heads via some interaction potential. We believe that taking into account the interdomain interaction is critical for a more realistic description of protein dynamics.

The presented theoretical approach neglects several features that might be important for understanding the mechanisms of biological transport. Our description of the protein dynamics and the biochemical transitions is rather oversimplified. For example, the existence of intermediate states is not taken into account [15]. We also assumed that the transition rates depend only on the configuration of the cluster and are independent of the specific nature of DNA bases because the sequence dependence is rather weak for helicases [4]. In addition, the effects of DNA elasticity and flexibility have not been considered.

In summary, the effect the interdomain coupling in the motor proteins has been discussed by developing a simple stochastic model. It was shown, using the explicit formulas for the velocity and dispersion, that the interaction between subunits in the enzyme might accelerate the speed of the cluster, as compared with the velocities of the free domains, without the significant increase in the fluctuations. This effect is due to the fact that the energy of interaction favors the compact vertical configurations, and it influences the forward and backward transitions differently. The asymmetry in energy-distribution factors results in a more efficient dynamics of the protein complex. Our theoretical method is used successfully to describe the dynamic properties of RecBCD helicases, and we argue that it is also relevant for other proteins. A possible mechanism of how the interdomain interaction makes the biological molecules more efficient is presented. In addition, our findings are closely related to a major result in the ratchet theory for continuous models of biological transport that the coupling between periodic potentials produces a drift [18].

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