Compare and contrast the reaction coordinate diagrams for chemical reactions and cytoskeletal force generators

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ABSTRACT Reaction coordinate diagrams are used to relate the free energy changes that occur during the progress of chemical processes to the rate and equilibrium constants of the process. Here I briefly review the application of these diagrams to the thermodynamics and kinetics of the generation of force and motion by cytoskeletal motors and polymer ratchets as they mediate intracellular transport, organelle dynamics, cell locomotion, and cell division. To provide a familiar biochemical context for discussing these subcellular force generators, I first review the application of reaction coordinate diagrams to the mechanisms of simple chemical and enzyme-catalyzed reactions. My description of reaction coordinate diagrams of motors and polymer ratchets is simplified relative to the rigorous biophysical treatment found in many of the references that I use and cite, but I hope that the essay provides a valuable qualitative representation of the physical chemical parameters that underlie the generation of force and motility at molecular scales. In any case, I have found that this approach represents a useful interdisciplinary framework for understanding, researching, and teaching the basic molecular mechanisms by which motors contribute to fundamental cell biological processes.

INTRODUCTION Cytoskeletal motors and polymer ratchets play key roles in intracellular transport and dynamics, cytoplasmic organization, cell locomotion, and cell division, processes that lie at the heart of molecular cell biology (Bagshaw, 1993; Inoue and Salmon, 1995; Bray, 2001; Howard, 2001; Pollard and Borisy, 2003; Vale, 2003; Ishikawa and Marshall, 2011; Green et al., 2012; McIntosh et al., 2012). In addition, "moving parts" analogous to motors and polymer ratchets are used by a broad range of macromolecular machines to carry out their function (Alberts and Miake-Lye, 1992; Block et al., 2007). Therefore to understand the fundamental problem of how molecules form living, moving, reproducing cells, we need to understand the underlying physical chemical principles by which motors and polymers generate force and motion.

Here I briefly discuss the application of reaction coordinate (also known as energy landscape, potential energy) diagrams to chemical reactions, enzyme-catalyzed reactions, polymerizing–depolymerizing polymer ratchets, and cytoskeletal motors, which I believe provides a convenient format for relating cellular processes that depend on the generation of force and motion to simpler chemical and biochemical reactions. An essay that I enjoyed reading in these pages many years ago (Mitchison, 1992) motivated me to write this essay, with the caveat that the author of that essay is an expert researcher in the field that he discussed, whereas I have not done significant research on my topic, even though I follow it with interest, as it provides a foundation for my lab's research. My essay follows the theme of a series of lectures that I developed over several years of teaching about biological motors and macromolecular machines to biochemistry and cell biology students, drawing from material that is available in various textbooks and reviews, but that has not to my knowledge been presented together in a simplified, readily accessible form.

SIMPLE CHEMICAL REACTIONS The basic features of a reaction coordinate diagram are conveniently illustrated with reference to a familiar S_N2 reaction as found in elementary organic chemistry texts (Figure 1; e.g.,
The reaction coordinate diagram for this reaction plots the changes in Gibbs free energy ($\Delta G$) during the conversion of reactants (R) to products (P; Figure 1B). It can be seen that P is of lower free energy than R, so this reaction is thermodynamically favorable, that is, it proceeds in the direction of P with the release of free energy ($\Delta G$ is negative). On the other hand, in order to react, R must acquire sufficient energy, the activation energy ($\Delta G^\ddagger$), to form the high-energy TS, which then has equal probability of relaxing into R or P. The magnitude of $\Delta G^\ddagger$ determines how kinetically favorable is the reaction: the higher its value, the smaller is the rate constant, $K$, and the slower does the reaction proceed.

Note that reaction coordinate diagrams do not display all possible pathways for a specific reaction, only the most probable one. For example, the diagram for the hypothetical dissociation reaction $\text{AB} \rightarrow \text{A} + \text{B}$ plots $\Delta G$ against the increasing bond length A—B for only one, most probable bond angle but omits additional pathways that exist for a large number of less probable bond angles. In addition, it should be noted that a TS is different from a reaction intermediate, such as the carbo-cation that forms during an $\text{S}_\text{n}1$ (first order) reaction, where R reacts via a first TS to form the relatively stable carbo-cation intermediate (I), which then reacts via a second TS to form P.

The hydrolysis of $\text{ATP} \rightarrow \text{ADP} + \text{P}_i$, which is more relevant to biological motors, occurs by a similar mechanism to the reaction of OH with methyl chloride, in that it involves the inline attack of $\gamma$-Pi by a nucleophilic OH or $\text{H}_2\text{O}$ (Figure 1D; Bagshaw, 1993, p. 65). It proceeds to equilibrium with the release of large amounts of free energy ($\Delta G = -50 \text{ kJ/mol or } -10^{-19} \text{ J/molecule under cellular conditions}$). The released energy can in principle be used to do work, for example, by generating force and motion. However, the large $\Delta G^\ddagger$ means that the uncatalyzed reaction proceeds to equilibrium very slowly (hours to days) in free solution and the released energy is dissipated as heat. Consequently, enzymes, that is, ATPases, such as the catalytic domains of cytoskeletal motors, are needed to make the reaction proceed at biologically useful rates (milliseconds to seconds) and to couple the released chemical free energy to mechanical work.

**ENZYME-CATALYZED REACTIONS**

The three-dimensional structure of each enzyme (be it a protein enzyme or a ribozyme) allows it to bind to a specific substrate (S) via multiple weak bonds and to undergo a conformational change, induced fit, in which mutual strain of E and S leads to formation of an E-TS complex in which TS has lower free energy ($\Delta G^\ddagger$) than free TS. Thus, as reflected in the reaction coordinate diagram (Figure 2), the enzyme-active site provides an environment in which the stabilization of TS is paid for by the released “binding energy” ($\Delta G_b$) as multiple weak bonds form. Here the binding energy “pays” for the lowering of the energy barrier, which enhances the rate constants for interconversion between S and P, with no effect on $K_{eq}$, but, as discussed later, a similar release of binding energy is used to generate pushing forces by polymer ratchets. Within the ES complex, substrate strain can also elevate the energy of the ground state, further facilitating TS formation. Thus enzymes massively enhance the rate at which equilibrium is attained; for example, catalase achieves a $10^{12}$-fold enhancement of the rate of $\text{H}_2\text{O}_2$ decomposition (Nelson and Cox, 2000, Chapter 8; Kuriyan et al., 2013, Chapter 16). Such stabilization of the TS by complementary fit between E and TS is a feature of the catalysis of ATP hydrolysis by the active site of the myosin-2 motor domain, for example. Here the inline attack of the tetrahedral $\gamma$-Pi of ATP by OH- leads to formation of the trigonal bipyramidal $\gamma$-Pi of the TS, which then makes multiple, stabilizing

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Suggs, 2002, Chapter 8). In the example shown, the hydroxide nucleophile attacks the tetrahedral carbon atom of methyl chloride from the opposite side to the chloride leaving group, forming a short-lived, high-energy trigonal bipyramidal transition state (TS) that “relaxes” into the stable products, tetrahedral methanol and $\text{Cl}^-$.
weak bonds to chemical groups brought into position by induced fit within the myosin-active site, thereby lowering the energy of the TS, leading to rate enhancement.

The situation is usually more complicated than the idealized case (Figure 2), as seen with reference to serine proteases (e.g., trypsin or chymotrypsin), which catalyze the hydrolysis of peptide bonds via mechanisms that are understood at atomic resolution (Figure 3; Williamson, 2012, Chapter 5). The enzyme’s polypeptide chain folds up to bring the three amino acids of the catalytic triad into position to form a charge relay system that enhances nucleophilic attack of the substrate carbonyl group by Ser-195 (Figure 3A). This leads to the conversion of the trigonal carbonyl C via transition state (TS1) to form a true tetrahedral intermediate (TI1). TI1, in turn abstracts a H⁺ from protonated His-57 to form, via a second TS2, the trigonal acyl-enzyme intermediate (AE) in which the NH₂-terminal segment of the substrate remains bound to Ser-195 and the COOH-terminal region is released (Figure 3B). A similar reaction involving two TS and a second TI releases the amino-terminal end of the cleaved polypeptide chain. Thus the reaction coordinate diagram (Figure 3C) reflects a pathway that proceeds via four transition states, two tetrahedral intermediates and a covalently bonded acyl enzyme intermediate. In this reaction, the overall ΔG is negative and the formation of the first TS has the highest ΔG⁺ and thus represents the rate-limiting step.

**POLYMER RATCHETS GENERATING “PUSHING” (COMPRESSIVE) FORCES**

Given that all subcellular processes are based on (sometimes very elaborate) chemical reactions, it is perhaps not surprising that
biophysicists and modelers have been able to extend the foregoing analysis of relatively simple chemical and biochemical reactions (Figures 1–3) to more complex cell biological processes such as intracellular transport, cell locomotion, and cell division (Figures 4–7). In the latter cases, to be discussed later, the molecular and quantitative details are generally less well understood than for the examples discussed previously; for example, the intermediates and transition states are not well defined, and thus the free energy profiles linking the initial and final states represent only intuitively appealing approximations of the true pathways. One interesting example is the polymer ratchet mechanism for leading-edge protrusion in cells that move by crawling over a surface. Most cell biology texts depict a crawling cell as undergoing cycles of leading-edge protrusion mediated by a zone of polymerizing actin, adhesion to the substratum, and actin-myosin–mediated trailing-edge retraction (Figure 4). Leading-edge protrusion has been extensively researched and is now understood to involve branched dendritic arrays of F-actin filaments in which polymerizing actin ratchets exert pushing forces that cause the cell membrane to protrude (Pollard and Borisy, 2003; Figure 5).

A simple way in which polymerizing actin filaments (or microtubules [MTs]) are proposed to generate pushing forces is via a thermal or Brownian ratchet mechanism (Feynman et al., 1963; Theriot, 2000; Howard, 2001; Mogilner and Oster, 2003; Phillips et al., 2009). In this model, the object being moved, for example, the cell membrane, undergoes random thermal motions at the growing plus end of the polymerizing filament. In the absence of the filament the object would simply undergo random thermal motion, that is, diffusion. However, the presence of the polymerizing filament serves to "rectify" these random motions, making movement directional. This is because, when such random motion produces a gap of sufficient size between the filament tip and the membrane, a subunit can
no ATP

\[ \Delta G_{\text{motor}} = \Delta G_{\text{motor}}^{\oplus} \quad \therefore K = K \quad \therefore 1\text{D random walk} \]

+ ATP

\[ \Delta G_{\text{motor}}^{\oplus} = \Delta G_{\text{ATPase}}^{\oplus} \quad \therefore K > K \quad \therefore \text{steps to } \oplus \text{end} \]

\[ \Delta G_{\oplus}^{\oplus} < \Delta G_{\oplus}^{\oplus} \therefore \quad \text{steps to end} \]

\[ x \]

\[ n-1 \quad \text{n} \quad \text{n+1} \]

FIGURE 6: Reaction coordinate diagram for a motor stepping along a polymer track whose plus (\(\oplus\)) end is oriented to the right. The reaction coordinate diagrams show the free energy change for a motor moving a distance \(X\) along a polymer track in the absence (top) and presence (center) of ATP fuel, which serves to bias random thermal motion in favor of vectorial movement. The cartoon (bottom) shows a kinesin motor stepping along a microtubule protofilament made of \(\alpha\beta\)-tubulin dimers with the \(\beta\)-tubulin facing the plus end of the microtubule. The motor starts at subunit \(n\) and can move \(\delta = 8\) nm in either the forward direction to subunit \(n + 1\) or backward to subunit \(n - 1\) in each discrete time step. In the absence of ATP (top) the activation energy for moving forward (\(\Delta G^{\oplus}\)) is equal to the activation energy for moving backward (\(\Delta G^{\ominus}\)), and hence the rate constants, that is, the probabilities of stepping forward or backward, are equal, and the motor diffuses randomly along the MT track executing a one-dimensional random walk where mean square displacement is given by \(\langle X^2 \rangle = 2Dt\), where \(D\) is the diffusion coefficient and \(t\) is elapsed time. This equation describes a Gaussian probability distribution of the motor’s position at a given time. ATP binding and hydrolysis by the kinesin motor domain releases free energy, which biases the random walk by tilting the energy landscape to the right, that is, toward the plus end of the MT (center). Now the activation energy (\(\Delta G^{\ominus}\)) for stepping forward from subunit \(n\) to \(n + 1\) is less than the activation energy for backstepping (\(\Delta G^{\oplus}\)), and because \(K = A \exp -\Delta G^{\ominus}/kT\), the rate constant \(K_{\text{on}}\) for stepping forward is greater than that for stepping backward \((K_\text{on})\). Hence the random walk driven by thermal energy is “rectified” or biased in favor of forward steps, and the kinesin motor steps persistently to the plus end of the MT, rendering backstepping unlikely. Here the motor’s mean-square displacement combines random diffusion with net drift, \(\langle X^2 \rangle = 2Dt + V^2t\), and so now the mean of the spreading Gaussian moves persistently toward the MT plus end with drift velocity, \(V\). Under the low-Reynolds number conditions that apply here, \(F = \mu V\), where \(F\) is force generated by the motor, \(\mu\) is the viscous drag coefficient, and \(V\) is the motor’s velocity.
GTP hydrolysis is stored as elastic energy within the polymer and can be released during depolymerization and used to pay for the “unbinding” of tubulin subunits as they depolymerize and also to exert pulling (tensile) forces on a load, for example, a chromosome, moving it a distance δ toward the spindle pole, so that work done is given by \( W = F \delta \). Thus the overall free energy change as the MT depolymerizes by one subunit and pulls the chromosome poleward is \( \Delta G = \Delta G_{\text{GTPase}} - (F \delta + \Delta G_{\text{unbinding}}) \).

FIGURE 7: A polymer ratchet generating “pulling” (tensile) forces. During mitosis, chromosomes are separated by two processes—anaphase A, during which chromosomes are pulled toward the spindle poles by depolymerizing MTs, and anaphase B, during which the spindle poles are moved apart, pulling the chromosomes along and further separating them (top left). In the “Pac-Man” component of anaphase A, the depolymerizing MT “pulls” on the kinetochore, but how a depolymerizing MT exerts a pulling force and maintains kinetochore attachment is an area of active investigation. In one model, the kinetochore attaches to a depolymerizing MT via multiple “fibrils,” each of which dynamically binds and releases the end of the shortening MT as the ensemble of fibrils maintains overall attachment (top right). The hydrolysis of GTP by tubulin within the MT polymer lattice releases free energy, which strains the lattice, elevating the free energy, so that now the free energy of the \( (n+1) \)-mer is greater than that of the \( n \)-mer and depolymerization is favored. The free energy released by GTP hydrolysis is stored as elastic energy within the polymer and can be released during depolymerization and used to pay for the “unbinding” of tubulin subunits as they depolymerize and also to exert pulling (tensile) forces on a load, for example, a chromosome, moving it a distance δ toward the spindle pole, so that work done is given by \( W = F \delta \). Thus the overall free energy change as the MT depolymerizes by one subunit and pulls the chromosome poleward is \( \Delta G = \Delta G_{\text{GTPase}} - (F \delta + \Delta G_{\text{unbinding}}) \).
extensively discussed, especially with reference to kinetochore MTs that shorten to pull chromosomes toward the mitotic spindle poles via a kinetochore-based “Pac-Man” mechanism (Inoue and Salmon, 1995; McIntosh et al., 2008). I have not found any published reaction coordinate diagrams for such a pulling MT polymer ratchet, but perhaps the one I put together for teaching purposes shown in Figure 7, or something similar, represents a reasonable starting point.

In this case, GTP-tubulin subunits in the “relaxed” conformation tend to polymerize via a reaction pathway that releases binding energy (\(\Delta G_{G}\)), that is, similar to the case for actin polymerization discussed earlier (Figure 4). In the spindle, such a growing MT could serve as pushing polymer ratchet that would apply a “polar ejection” force, \(F\), to push chromosome arms and possibly kinetochores a distance \(\delta\) per subunit added in a direction away from the spindle poles, so again the overall free energy drop is \(\Delta G = \Delta G_{B} - \delta F\). For this reaction to proceed in reverse, converting the MT to a “pulling” polymer ratchet requires a supply of free energy, which comes from the hydrolysis of bound GTP by the \(\beta\)-tubulin subunits within the polymer lattice (\(\Delta G_{GTPase} \approx 10^{-19}\) J/molecule; Figure 7). This converts tubulin to a strained, “tense” conformation in which an (n + 1)-mer is less stable and has a higher Gibbs free energy than a corresponding n-mer. Consequently the MT now has a tendency to depolymerize with release of free energy that can generate tensile force, \(F\), on a chromosome to pull it a distance \(\delta\) per subunit lost (Figure 7). Structurally at the kinetochore, the plus-ends of depolymerizing MTs tend to fray and curve downward toward their opposite minus ends located at the spindle poles, and it is proposed that ensembles of “kinetochore fibrils” that each dynamically attach to and release from the curving pfs are responsible for maintaining the attachment of the kinetochore, and hence the chromosome, to the end of the depolymerizing MT (McIntosh et al., 2008).

It is estimated that this mechanism can generate a force of tens of piconewtons per MT, far greater than the 1-pN force required to move the chromosome at rates observed against viscous drag, based on similar arguments to those discussed earlier for the kinesin motor. By comparison, the maximal force that an anaphase spindle is believed to exert on a chromosome (\(F_{\text{Max}} \approx 0.7\) nN) corresponds to \(-50\) pN/kMT for a kinetochore fiber consisting of 15 kMTs pulling the chromosome to the pole during anaphase A (Nicklas, 1983).

**SUMMARY**

In accord with reductionist principles (Crick, 1988), reaction coordinate diagrams provide a general picture of the physical chemical principles by which macromolecular machines contribute to the organization and function of living cells because they display in an idealized and simplified manner the thermodynamics and kinetics of enzymes, polymer ratchets, and motors. My description of reaction coordinate diagrams obviously oversimplifies the true mechanism compared with the more rigorous biophysical treatments that can be found in many of the cited references. On the other hand, the latter presentations rarely, if ever, seem to discuss how these diagrams (Figures 5–7) also apply to much simpler, more familiar chemical and biochemical reaction mechanisms (Figures 1–3). Conversely, the chemistry and biochemistry texts that I have used in class do not discuss the reaction coordinate diagrams of cytoskeletal motors and polymer ratchets. Even though my laboratory has not done significant work in the area of the biophysical mechanisms of cytoskeletal force generators, I have found these diagrams to be valuable both as an interdisciplinary tool for teaching biochemistry and cell biology majors and as a mechanistic foundation for our research on mitosis, intraflagellar transport/cilium biogenesis, and motor protein function. One significant advantage is their generality; that is, just as there exist a vast number of different organic reactions that conform to the reaction coordinate diagram for an \(S_n2\) reaction (Figure 1), it seems reasonable to believe that cytoskeletal motors, including kinesins, dyneins, and myosins, as well as other translocating motors, such as helicases moving along DNA, all conform to a reaction coordinate diagram of the type shown in Figure 6. On the other hand, such a general picture is obviously limited, and for each specific case it is necessary to dig deep into the detailed nuts and bolts to develop a precise quantitative and atomic resolution picture of its particular mechanism (Vale and Milligan, 2000).

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