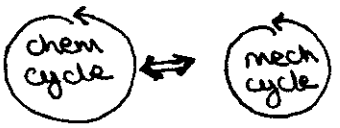
QB Lecture-3

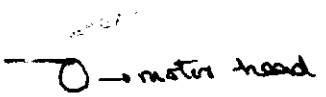
Jan 2007.

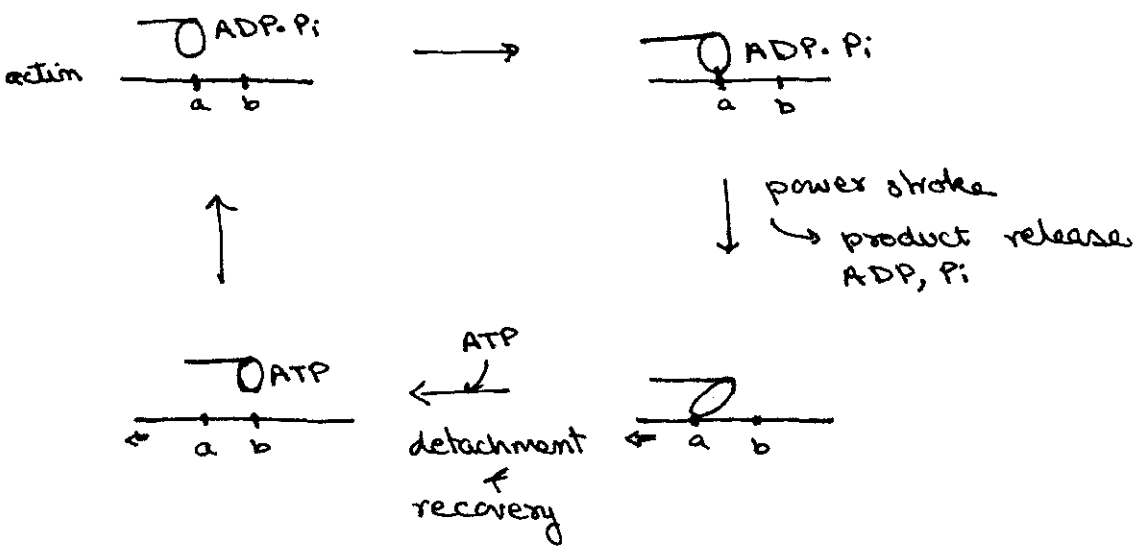
Molecular motors and mechanochemistry

The challenge in mechanochemistry is to connect the chemical & mechanical cycles of a motor in order to understand the transduction of chemical energy to mechanical work.



Smooth muscle myosin





- There are coupling rules between the chemical & mechanical cycle that prevent the motor from running backwards.

Landscape of a motor

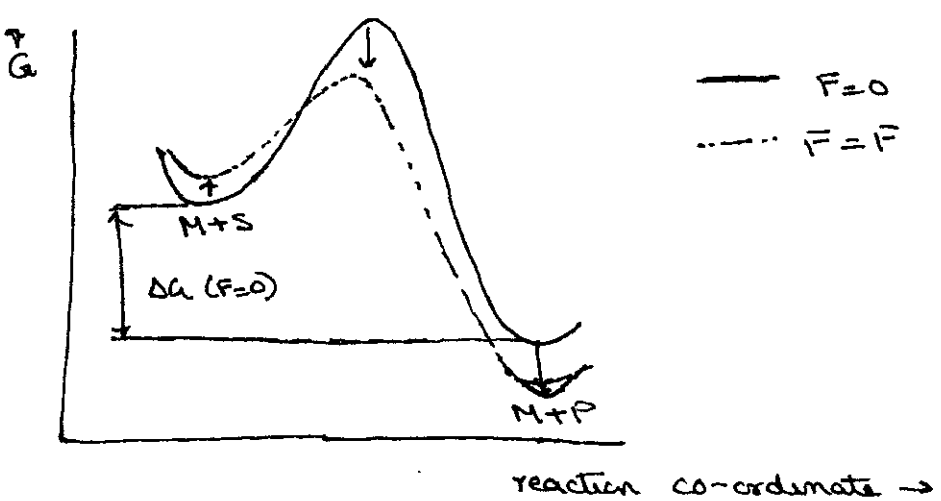
- motors are coupled to an energetically favorable reaction. Therefore motor reactions are energetically downhill.

- energy sources:

$$ATP/GTP \longrightarrow ADP/GDP + P_i \qquad \Delta G = 25kT \text{ or } 100pN\,nm$$

$$NTP \longrightarrow NP + PP_i$$

$$nH^+_{high} \longrightarrow nH^+_{low}$$



Mechanical properties of motors

- Step size : distance moved per catalytic cycle

- Stall force : - The force at which the velocity of the motor reduces to zero.
  Hence, this is the maximum force that a functional motor can generate

  - Stall force of a motor may have some relevance to its biological function.

    eg RNAP ($F_{stall} \approx 25$ pN) has to knock off obstacles encountered while travelling on DNA

    ϕ29 ($F_{stall} \sim 57$ pN) has a higher stall force presumably because of the high pressure build-up as it packages DNA inside the viral capsid.

- Mechanical efficiency $\Rightarrow$ $\eta = \frac{F \cdot \Delta x^{\ddagger}}{\Delta G} = \frac{\text{output work}}{\text{input energy}}$

  - Max efficiency is achieved at stall
  - $\eta < 100\%$ → energy dissipated as heat
    → work done on an orthogonal set

Mechanochemistry

- chemical reaction ⟶ force generation
- How does inhibitory or assisting force affect the output of the chemical reaction?
- Measurable outputs for a motor are quantities like velocity, stepping duration etc. Mechanochem is understood best by looking at the motor output at various chemical variables such as substrate concentrations, inhibitors etc & simultaneously at various forces
- A quick review of enzyme kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Under initial velocity conditions

$$v = \frac{V_{max}[S]}{[S] + K_m} \quad ; \quad K_m = \frac{k_{-1} + k_2}{k_1}$$

v = motor velocity

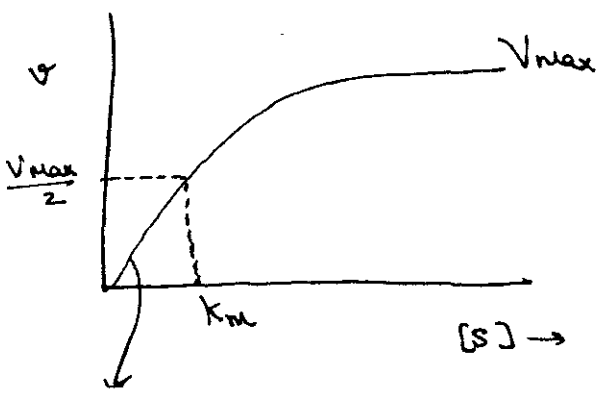
V_{max} = maximum velocity obtainable when substrate binding is not limiting
≡ k_{cat} for a style motor enzyme

$[S]$ = substrate concentration

K_m = $[S]$ at which $v = V_{max}/2$

At saturating substrate concentrations $v = V_{max}$

At $S \ll K_m$, $v = \frac{V_{max}[S]}{K_m}$



initial slope = $\frac{k_{cat}}{K_m}$ = effective 2nd order binding constant

Enzyme reactions are generally more complex than the one discussed earlier.

eg:-

$$E + S \rightleftharpoons ES1 \rightleftharpoons ES2 \longrightarrow EP1 \rightleftharpoons EP2 \rightleftharpoons E + P$$

V_{max} terms.

k_{cat}/K_m terms

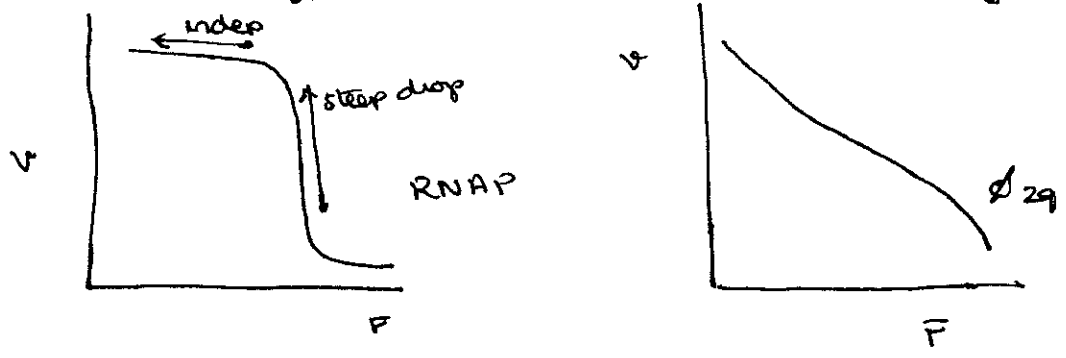
In general:-

k_{cat}/K_m includes all steps from substrate binding to the first irreversible step

V_{max} includes all steps other than substrate binding

Force-velocity curves & mechanism

eg: F-V curves at saturating substrate concentrations have different shapes for different enzymes



in RNAP, force dependent step is not rate limiting until stall

in ϕ29, force dependent step is rate limiting even at low forces.

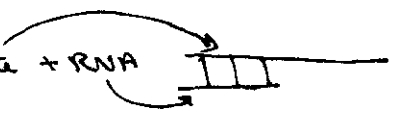
There are two major classes & models to describe motor mechanism

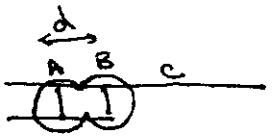
↳ Brownian ratchet

↳ Power stroke

① Brownian ratchet

In this model, the motor can thermally visit two adjacent binding sites on its track via brownian motion & this can be rectified by coupling it to another stochastic process which is energetically downhill such as ATP hydrolysis.

eg T7 RNAP  , DNA template + RNA 



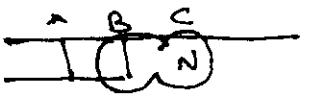
P-
(pre translocated state)

K(F)
k_1
k_{-1}

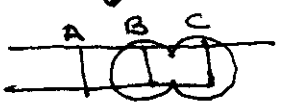
P+
(post translocated state)

(• rapid sliding btw P+ & P-)

1E NTP



↳ PPi, locked. no more sliding



In such a mechanism:

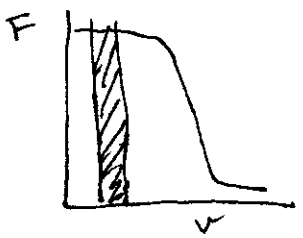
- chemical energy favors forward motion rather than doing the mechanical work directly on the motor
- rate of the reaction is proportional to the relative occupancies of P+ & P-

$$\frac{P+}{P-} = K \exp\left(-Fd/k_B T\right)$$

where d is the distance btw P- & P+

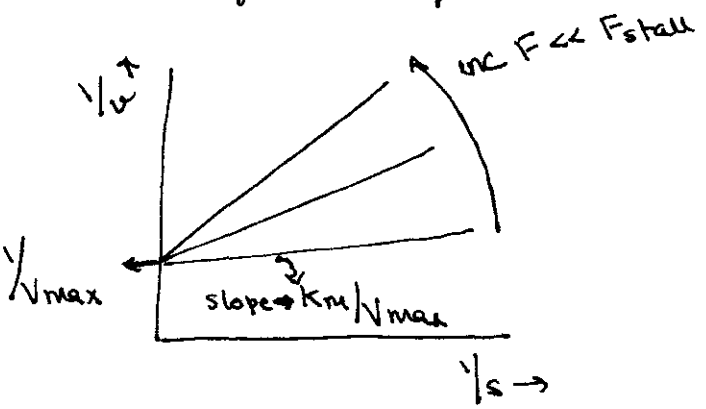
- The effect of force will be to push the k_1/k_{-1} equilibrium towards the "P-" state. This will result in a higher K_m at higher forces. In other words, more NTP will be required to reach V_{max}

Thomen et al, PRL 94, 128102 (2005).



experiments conducted away from stall force to look at inhibition of T7 RNAP by force at various substrate concentrations

Plot of $1/v$ vs $1/s$ at various forces



Plot resembles a simple competitive inhibition plot

- Vmax is the same at all forces
- kcat / Km decreases with increasing force
- Km increases with increasing force

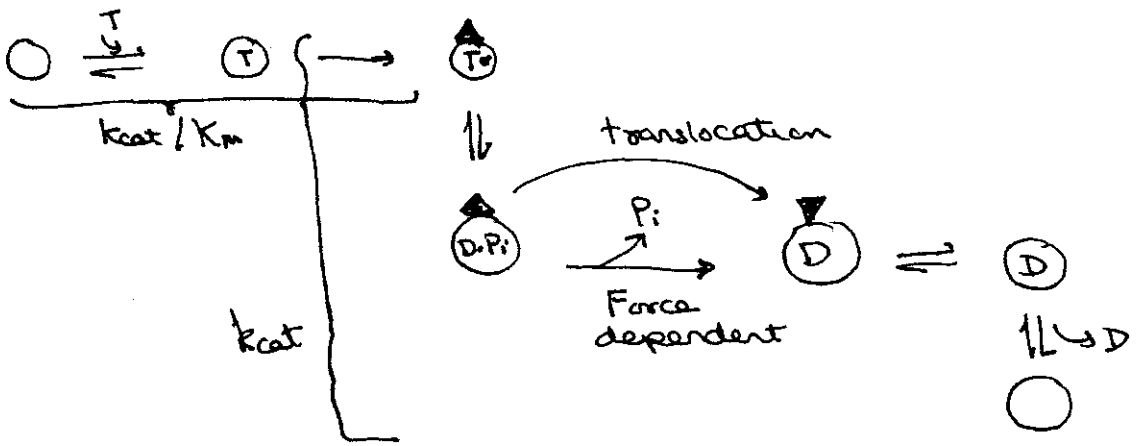
The data is fully consistent with a brownian ratchet model for T7 RNAP.

② Power stroke model (myosin, $\phi 29$)

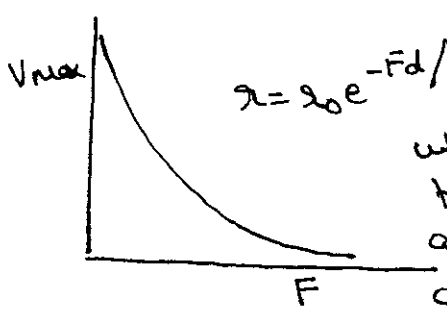
In this model, the chemical reaction is mechanically coupled to movement by some part of the molecule

eg $\phi 29$; Chemla, Y. et al Cell, Vol 122 (5), 683-692

minimal motor mechanism:- ○ motor
△ attachment to DNA



This model is consistent with the observation that Vmax decreases with force, where as kcat/Km is independent of force.



$r = r_0 e^{-Fd/k_BT}$

where d is the distance to the transition state & can be an indication of the size & the conformational change in the force dependent step.

Some points to keep in mind while looking at force-vel curves

- Not all cases can be clearly identified as brownian ratchet or power stroke.

- The dependance of Vmax & Vmax/km on force will be dictated by the details of the biochemical mechanism (ie first reversible step location, two-step versus one step substrate binding mechanisms etc). Hence interpretation of results depends on poor knowledge about the biochemical mechanism.

- Force could induce off-pathway inhibition introducing effects on Vmax & kcat/Km that have nothing to do with the on-pathway mechanism of the enzyme.

Reference :-

Bustamante et al Annual Rev Biochem 2004. 73 : 705-748