Protein kinetics

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Outline

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General kinetics

$$a\mathrm{A} + b\mathrm{B} \rightarrow c\,\mathrm{C} + d\,\mathrm{D} \tag{1}$$

$$v = -\frac{1}{a}\frac{\mathrm{d}[\mathrm{A}]}{\mathrm{d}t} = -\frac{1}{b}\frac{\mathrm{d}[\mathrm{B}]}{\mathrm{d}t} = +\frac{1}{c}\frac{\mathrm{d}[\mathrm{C}]}{\mathrm{d}t} = +\frac{1}{d}\frac{\mathrm{d}[\mathrm{D}]}{\mathrm{d}t} = \frac{\mathrm{d}\xi}{\mathrm{d}t} \tag{2}$$

$$v = k[\mathrm{A}]^{\alpha}[\mathrm{B}]^{\beta} \tag{3}$$

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- Reaction velocity v is derivation of reaction extent ξ by time.
- Sign convention – reactants decrease, products increase.
- For elemental reactions $\alpha = a, \beta = b$, where α, β are partial reaction orders.
- This does NOT hold for more complex mechanisms.

Integrated rate equation

- Simplest interesting case

$$\mathrm{A} \rightarrow \mathrm{B} \tag{4}$$

$$\frac{\mathrm{d}[\mathrm{A}]}{\mathrm{d}t} = -k[\mathrm{A}] \tag{5}$$

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- Let's integrate:

$$\frac{\mathrm{d}[\mathrm{A}]}{[\mathrm{A}]} = -k\mathrm{d}t \tag{6}$$

$$\int \frac{1}{[\mathrm{A}]}\mathrm{d}[\mathrm{A}] = -k \int \mathrm{d}t \tag{7}$$

$$\ln[\mathrm{A}] - \ln[\mathrm{A}]_0 = -kt \tag{8}$$

$$[\mathrm{A}] = [\mathrm{A}]_0 \mathrm{e}^{-kt} \tag{9}$$

2nd order integrated rate equation

- Slightly more difficult case

$$\mathrm{A} \rightarrow \mathrm{B} \tag{10}$$

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- After integration:

$$\int \frac{1}{[\mathrm{A}]^2} \mathrm{d}[\mathrm{A}] = -k \int \mathrm{d}t \tag{12}$$

$$\frac{1}{[\mathrm{A}]} - \frac{1}{[\mathrm{A}]_0} = kt \tag{13}$$

Reaction half time

- First order – concentration independent:

$$\ln \frac{[\mathrm{A}]_0}{2} - \ln[\mathrm{A}]_0 = -kt_{1/2} \tag{14}$$

$$t_{1/2} = \frac{\ln 2}{k} \tag{15}$$

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- Second order – decreasing concentration prolongs the half time:

$$\frac{2}{[\mathrm{A}]_0} - \frac{1}{[\mathrm{A}]_0} = kt_{1/2} \tag{16}$$

$$t_{1/2} = \frac{1}{k[\mathrm{A}]_0} \tag{17}$$

Kinetics of equilibrium processes

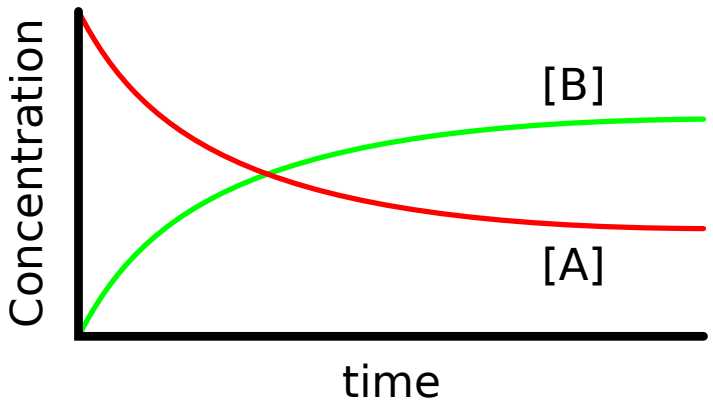
- Example of reversible reaction – isomerisation:

$$\mathrm{A} \overset{k}{\underset{k'}{\rightleftarrows}} \mathrm{B} \tag{18}$$

$$\frac{\mathrm{d}[\mathrm{A}]}{\mathrm{d}t} = -k[\mathrm{A}] + k'[\mathrm{B}] \tag{19}$$

$$\frac{\mathrm{d}[\mathrm{A}]}{\mathrm{d}t} = -(k+k')[\mathrm{A}] + k'[\mathrm{A}]_0, \quad \text{pokud } [\mathrm{B}]_0 = 0 \tag{20}$$

$$[\mathrm{A}] = \frac{k' + k - (\mathrm{k} + \mathrm{k}')\mathrm{t}}{k + k'}[\mathrm{A}]_0 \tag{21}$$



Convergence to equilibrium

- In equilibrium, velocities equalize.

$$v = v' \tag{22}$$

$$k[\mathrm{A}] = k'[\mathrm{B}] \tag{23}$$

$$\frac{[\mathrm{B}]}{[\mathrm{A}]} = \frac{k}{k'} = K_{\mathrm{eq}} \tag{24}$$

- Rate of relaxation to equilibrium can be studied by eg. ”T-jump”techniques.
  - fast change of temperature changes K_{eq}
  - system starts to relax – measurable signal changes
  - even very fast processes can be analyzed – orders of μs

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temperature very important factor
empirically $10\,^{\circ}\mathrm{C} \rightarrow 2 - 4\times$ acceleration

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Arrhenius equation – empiric

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$$\ln k = -\frac{E_A}{R} \cdot \frac{1}{T} + \ln A \tag{26}$$

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Eyring equation – derived from statistical thermodynamics

$$k = \frac{k_B T}{h} \cdot \exp\left(-\frac{\Delta G^{\ddagger}}{RT}\right) \tag{27}$$

$$\ln k = -\frac{\Delta G^{\ddagger}}{R} \cdot \frac{1}{T} + \ln T + \ln\left(\frac{k_B}{h}\right) \tag{28}$$

$$\ln k = -\frac{\Delta H^{\ddagger}}{R} \cdot \frac{1}{T} + \ln T + \frac{\Delta S^{\ddagger}}{R} + \ln\left(\frac{k_B}{h}\right) \tag{29}$$

Outline

Energetic barrier

- Conversion of substrate S to product P

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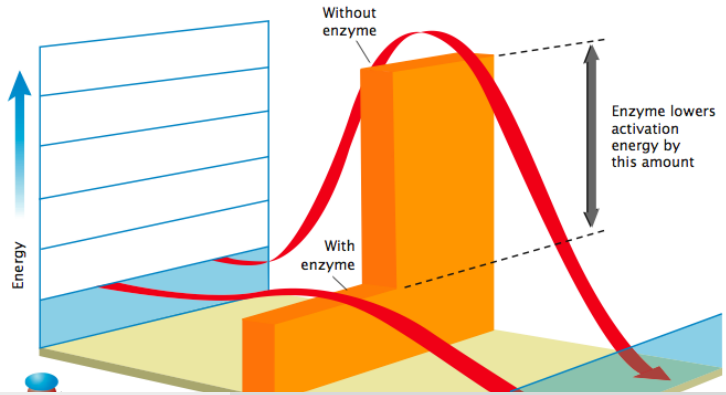
- Enzyme accelerates the reaction by „decrease“ of activation energy E_A
- In reality, it takes the reaction through different reaction coordinate

$$S \xrightarrow{E} P$$



Michaelis–Menten kinetics

Assumptions

$$\mathrm{S} + \mathrm{E} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{P} + \mathrm{E} \tag{30}$$

- Negligible amount of product – otherwise we must include reverse reaction and the analysis gets complex.
- Substrate exceeds the enzyme: $[\mathrm{S}]_0 \gg [\mathrm{E}]_0$
- Stationary state: $\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{ES}] = 0$

Michaelis–Menten kinetics

Derivation

$$v_0 = k_2[\mathrm{ES}] \tag{31}$$

$$\frac{\mathrm{d}[\mathrm{ES}]}{\mathrm{d}t} = k_1[\mathrm{E}][\mathrm{S}] - k_{-1}[\mathrm{ES}] - k_2[\mathrm{ES}] \tag{32}$$

$$\frac{\mathrm{d}[\mathrm{ES}]}{\mathrm{d}t} = k_1[\mathrm{E}]_0[\mathrm{S}] - [\mathrm{ES}]\left(k_1[\mathrm{S}] + k_{-1} + k_2\right) = 0 \tag{33}$$

$$[\mathrm{ES}] = \frac{k_1[\mathrm{E}]_0[\mathrm{S}]}{k_1[\mathrm{S}] + k_{-1} + k_2} \tag{34}$$

$$v_0 = \frac{k_2[\mathrm{E}]_0[\mathrm{S}]}{\frac{k_{-1}+k_2}{k_1} + [\mathrm{S}]} \tag{35}$$

$$V_{\mathrm{lim}} = k_2[\mathrm{E}]_0, \quad K_{\mathrm{M}} = \frac{k_{-1} + k_2}{k_1} \tag{36}$$

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- Initial velocity is proportional to enzyme concentration.
- Dependence of v_0 on $[\mathrm{S}]$ is hyperbolic, approaching limit velocity v_{lim}.

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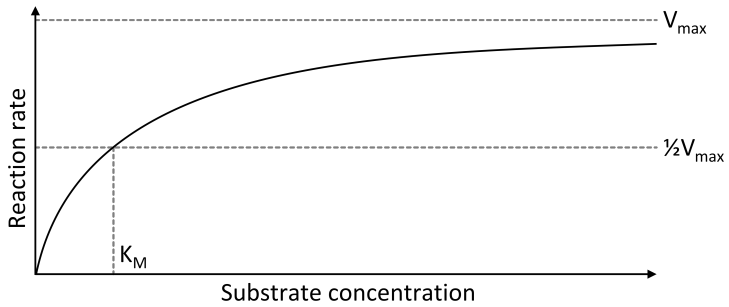
- K_{M} is independent of enzyme concentration $[\mathrm{E}]_0$.
- Turnover number – $k_{\mathrm{cat}} = k_2 = \frac{v_{\mathrm{lim}}}{[\mathrm{E}]_0}$



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irreversible – permanent deactivation of enzyme – e.g. by covalent bond

Outline

Kinetics of denaturation and renaturation

- Usual assumption of simple two-state process:

$$\mathrm{D} \underset{k'}{\overset{k}{\rightleftharpoons}} \mathrm{N} \tag{37}$$

- For kinetics of folding and unfolding:

$$A_t - A_\mathrm{R} = (A_\mathrm{N} - A_\mathrm{R})\,\mathrm{e}^{-(k+k')t} \tag{38}$$

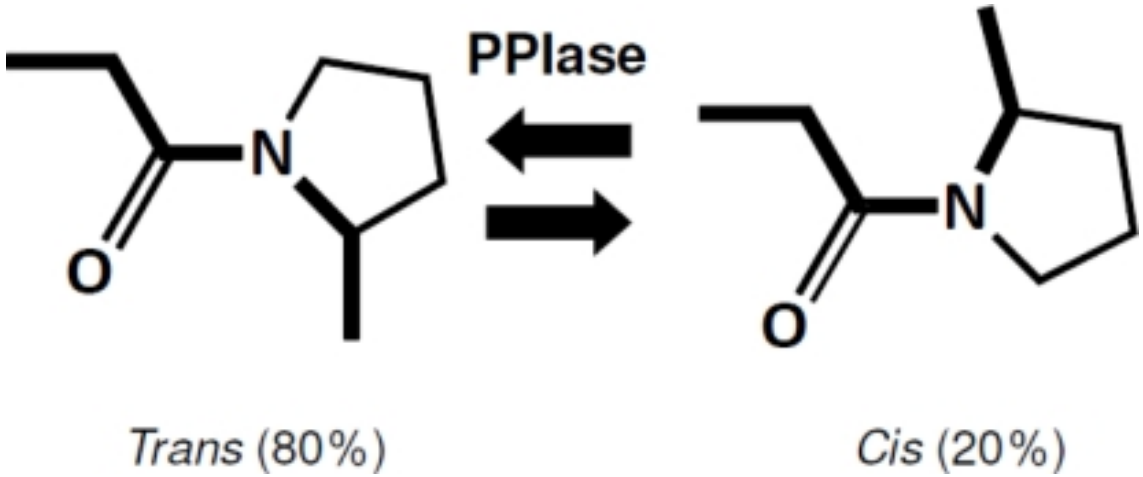
$$A_\mathrm{R} - A_t = (A_\mathrm{R} - A_\mathrm{D})\,\mathrm{e}^{-(k+k')t} \tag{39}$$

  where A denotes values of e.g. absorbation A_N for native, A_D for denatured state, A_R in equilibrium and A_t in time t

- Classical first order kinetics.

Isomerisation of proline

- Often cause of folding problems – isomerisation of proline peptidic bond
  - In oligopeptides, ca 10–30 % bonds of X-Pro in *cis* state
  - In proteins, only ca 7 % in *cis*
- Isomerisation slow – tens of seconds.
- Helper enzyme – *prolylisomerase*



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Oligomerisation

- Simplest and very often case – homodimerisation:

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$$\frac{\mathrm{d}[\mathrm{D}]}{\mathrm{d}t} = k_{\mathrm{on}}[\mathrm{M}]^2 - k_{\mathrm{off}}[\mathrm{D}] \tag{42}$$

$$\frac{\mathrm{d}[\mathrm{M}]}{\mathrm{d}t} = 2k_{\mathrm{off}}[\mathrm{D}] - 2k_{\mathrm{on}}[\mathrm{M}]^2 \tag{43}$$

Kinetics – exercise

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Alcoholic

- Grown man (80 kg) got 1.5 ‰ of alcohol in blood after drinking vodka.
- After several hours following concentrations were measured:

Time [h]	2	3.5	5	6
Alcohol concentration [‰]	1.24	1.05	0.86	0.73

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1. How much vodka did the man drink?
2. Calculate the order of reaction for alcohol degradation in human body and its rate constant.
3. How long after drinking will the man be able to drive a car without losing his driving license?

Alcoholic – solution

1. About 5 large shots ☺

   80 kg – ca 60 % of water = 48 kg of water – 1.50 ‰ = 72 g of alcohol – 40% vodka – ca 180 g of vodka. Be careful, alcohol is less dense than water ($\rho = 0.8\ \mathrm{g.cm^{-3}}$), therefore the volume of vodka was about 225 ml.

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   Rate constant $k = 0.13\ \text{‰}.\mathrm{h^{-1}}$

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3. About 11 hours after finishing vodka drinking – the blood concentration of alcohol drops below 0.1 ‰.

Enzymatic activity

- Initial substrate concentration – $10\ \mu\mathrm{mol.dm}^{-3}$
- Michaelis constant – $K_\mathrm{M} = 2\ \mathrm{mmol.dm}^{-3}$
- After 1 minute – 2 % of substrate converted to product.

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1. How much substrate was converted after 3 minutes?
2. What is the limiting velocity?
3. The limiting velocity will be achieved at $[\mathrm{S}]_0 = 0.2\ \mathrm{mol.dm^{-3}}$. How much substrate will convert in 3 minutes?

Enzymatic activity – solution

1. $5.6\ \%$, first order kinetics ($[\mathrm{S}] \ll K_\mathrm{M}$), $k = 0.02\ \mathrm{min}^{-1}$
2. $v_\mathrm{lim} = 40.2\ \mu\mathrm{mol.dm}^{-3}.\mathrm{min}^{-1}$
3. Concentration of product will be $120\ \mu\mathrm{mol.dm}^{-3}$, being $0.06\ \%$ of substrate.

References

- Zuckerman, Daniel M. *Statistical Physics of Biomolecules. An Introduction*
- Atkins, Peter; de Paula, Julio. *Physical Chemistry*
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