

6. OTHER EFFECTS ON ENZYME ACTIVITY

- Ionic strength
- pH
- TEMPERATURE
- Shear
- Pressure (hydrostatic)
- Surface tension
- Chemicals (alcohol, urea, H₂O₂...)
- Light, sonication, ionising radiations

Reverzible

changes

Irreverzible



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Active side chains

Changes in activity of proteins are caused by changes of amino acid side chains.

Acidic: $-\text{COOH}$: Asp, Glu Basic: $-\text{NH}_2$: Lys, Arg
(and terminal $-\text{COOH}$ and $-\text{NH}_2$)

amide: $-\text{CO}-\text{NH}_2$: Asn, Gln

Polar: $-\text{OH}$: Ser, Thr $-\text{SH}$: Cys, $-\text{S}-\text{CH}_3$: Met

Imidazole: His Guanidin: Arg

H-bonds: $\text{C}=\text{O} \dots\dots \text{H}-\text{O}-$ $\text{C}=\text{O} \dots\dots \text{H}-\text{NH}-$



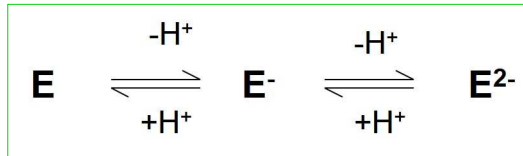
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Effect of pH

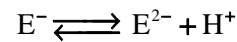
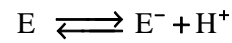
Proteins: + and – charged side chains ← their charge depends on dissociation ← determined by pH → it effects the active centre.

Recharge of enzyme:



Only E^- is active!

Ratio of active enzymes: $Y^- = \text{E}^- / \text{E}_0$



$$K_1 = \frac{\text{H}^+ \cdot \text{E}^-}{\text{E}}$$

$$K_2 = \frac{\text{H}^+ \cdot \text{E}^{2-}}{\text{E}^-}$$

$$\text{E}_0 = \text{E} + \text{E}^- + \text{E}^{2-}$$

Michaelis-féle pH függvények:

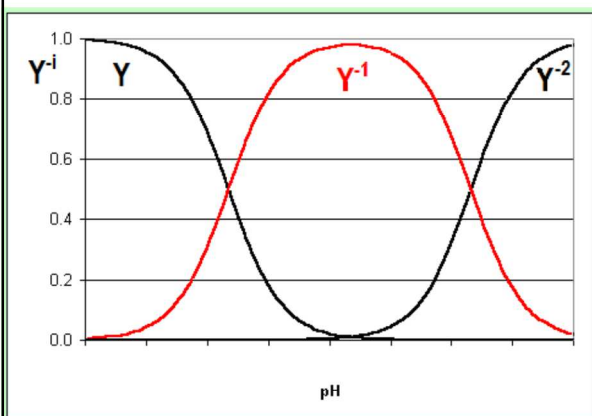
$$Y^- = \frac{1}{1 + \text{H}^+ / K_1 + K_2 / \text{H}^+}$$



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Effect of pH



$$Y^- = \frac{1}{1 + \text{H}^+ / K_1 + K_2 / \text{H}^+}$$

$$\text{H}^+_{\text{optimum}} = \sqrt{K_1 K_2}$$

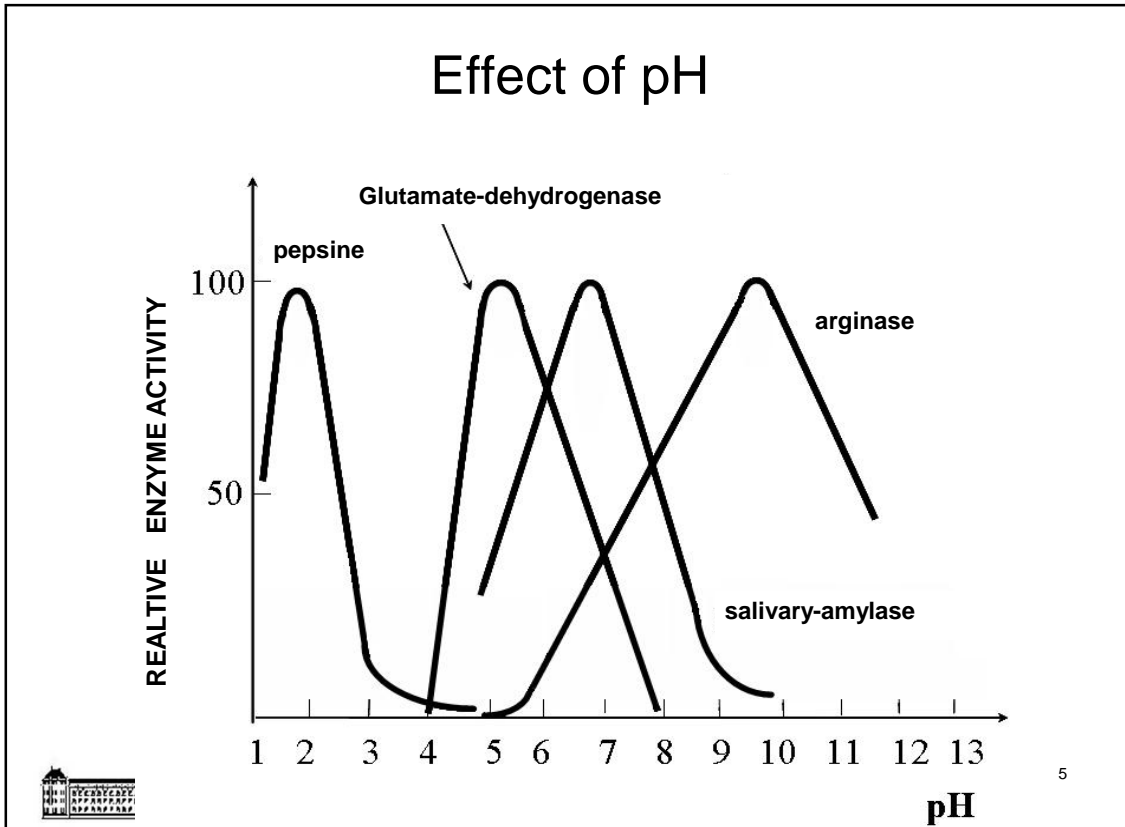
$$(\text{pH})_{\text{optimum}} = \frac{1}{2} (\text{p}K_1 + \text{p}K_2)$$

$$V_{\text{max}} = k_2 \text{E}_0 Y^- = k_2 \text{E}_0 \frac{1}{1 + \text{H}^+ / K_1 + K_2 / \text{H}^+}$$



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Effect of pH temperature

Double effect

- Increasing reaction rate
- decrease: denaturation
 - irreversible
 - reversible

↓
depends on treatment time, too!

$$\frac{dE_a}{dt} = -kE_a \longrightarrow E_a(t) = E_{a0}e^{-kt}$$


Effect of pH temperature

$$E_a \xrightleftharpoons{K_d} E_i \quad \frac{E_i}{E_a} = K_d = \exp\left(\frac{-\Delta G_d}{RT}\right) = \exp\left(\frac{-\Delta H_d}{RT}\right) \exp\left(\frac{\Delta S_d}{R}\right)$$

$S_d = \sim 900 \text{ KJ/mol.K}$
 $H_d = 280-310 \text{ KJ/mol}$

Large: sensitively reacts on small change
(one H-bond: 12,5-29,3 kJ/mol)

If: $V_{\max} = k_2(T)E_a$


$E_0 = E_a + E_i \rightarrow E_a = \frac{E_0}{1 + K_d}$

and $k_2(T) = \beta \left(\frac{k_B T}{h}\right) e^{\Delta S^*/R} \cdot e^{-E/RT}$

$$V_{\max} = \frac{\alpha T e^{-E/RT}}{1 + e^{\Delta S^*/R} \cdot e^{-\Delta H_d/RT}}$$

$\alpha = \text{combination of } (\beta, k_B, h, E_0, \Delta S^*)$

K_m also depends on T!



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