

Microbial and Enzyme Kinetics

Enzyme Kinetics Tutorial 4a.

The activity of the enzyme fumarase which catalyses the reaction



is thought to be a function of the ionisation state of amino acid functional groups at the active site. In order to investigate this, a study of the effect of pH on V_{max} (expressed as a percentage of the peak value) for the reaction was carried out with the following results.

pH	4.5	5	5.5	6	6.5	7	7.5	8	8.5
V_{max} %	8	21	50	83	100	92	65	22	9

- Plot a graph of $\log v_{max}$ vs pH and determine the pK_a values of the ionising groups at the active site.
- Using the table below, suggest two amino acids which may be responsible for the for the pH dependence in this case.
- Explain how functional groups at the active site of an enzyme may result in a variation of activity with pH.

Amino Acid	Active Group	approx pK_a at 298K	Formula
Any	α -COOH	~2	
Any	α -NH ₂	~9	
Asp	β -COOH	~4	-CH ₂ COO ⁻
Glu	γ -COOH	~4	-CH ₂ CH ₂ COO ⁻
His	Imidazole	~7	-C ₃ H ₃ NH.NH ⁺
Lys	ϵ -NH ₂	~10	-CH ₂ (CH ₂) ₃ NH ₃ ⁺
Tyr	Phenolic OH	~10	-CH ₂ -Ph-OH