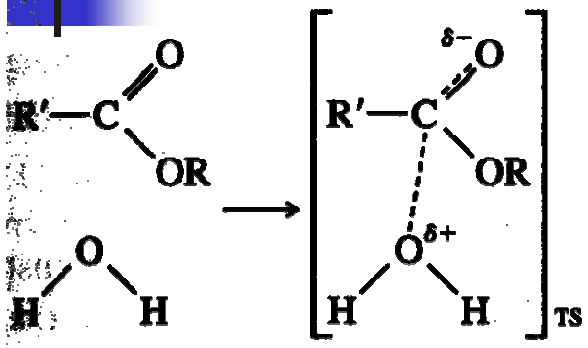
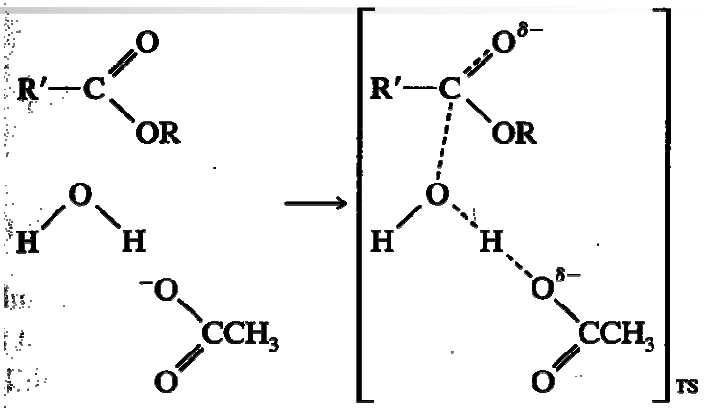
Mehanizmi dejstva enzima

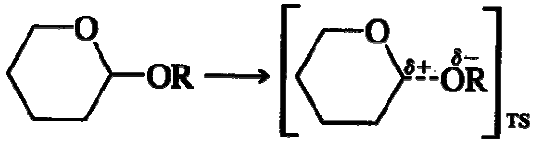
Himotripsin

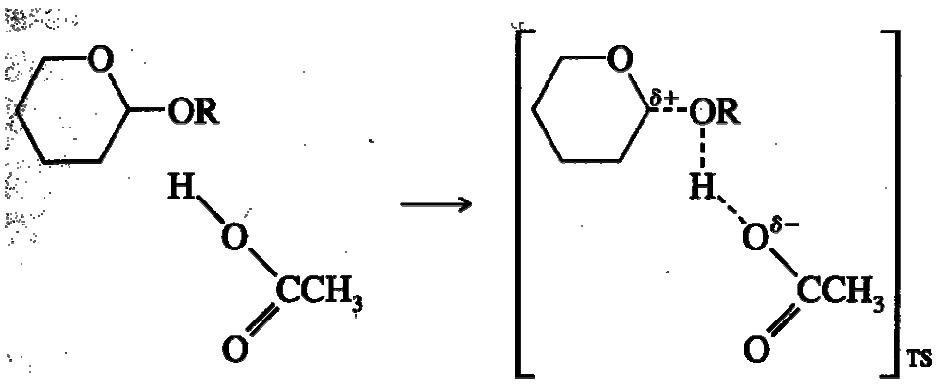
Principi katalize





General-base catalysis by acetate ion

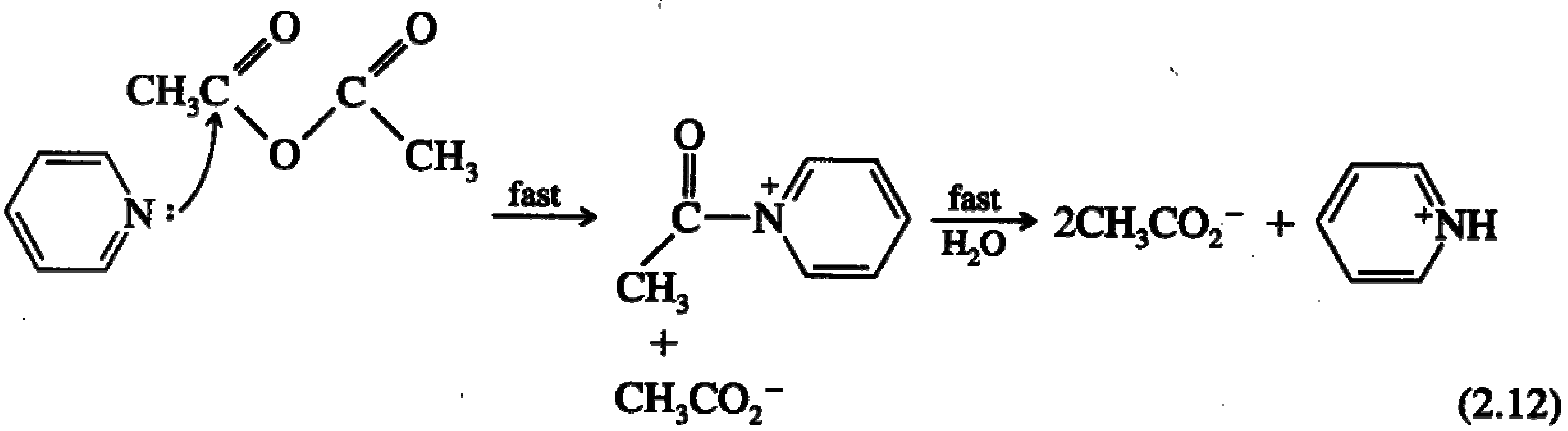




General-acid catalysis by acetic acid

- Specifična kiselo-bazna kataliza
- Elektrostatska kataliza
- Elektrofilna kataliza
- Nukleofilna kataliza (kovalentna kataliza)

Nukleofilna kataliza



(2.12)

Opšta kiselo-bazna kataliza

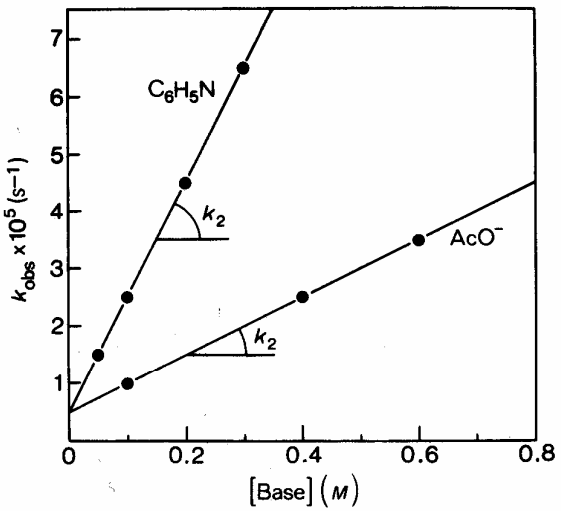


Figure 2.4 Determination of the rate constants for the general-base catalysis of the hydrolysis of ethyl dichloroacetate. The first-order rate constants for the hydrolysis are plotted against various concentrations of the base. The slope of the linear plot is the second-order rate constant (k_2). The intercept at zero buffer concentration is the "spontaneous" hydrolysis rate constant for the particular pH. A plot of the spontaneous rate constants against pH gives the rate constants for the H^+ and OH^- catalysis. It is seen that pyridine is a more effective catalyst than the weaker base acetate ion. [From W. P. Jencks and J. Carriuolo, *J. Am. Chem.* Soc. **83**, 1743 (1961).]

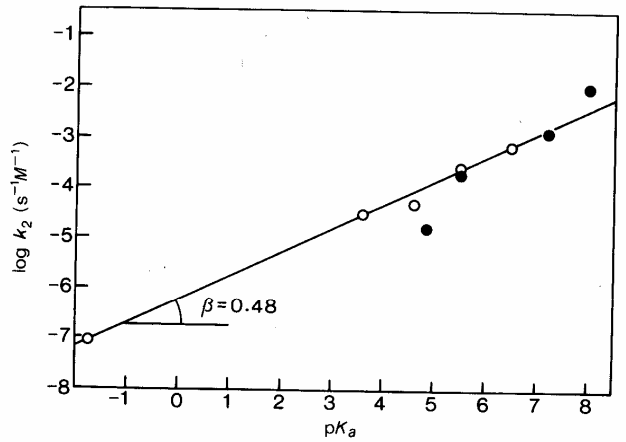


Figure 2.5 The Brønsted plot for the general-base catalysis of the hydrolysis of ethyl dichloroacetate. The logarithms of the second-order constants obtained from the plot of Figure 2.4 are plotted against the pK_a's of the conjugate acid of the catalytic base. The slope is the β value. Note that the points for amine bases (●) fall on the same line as those for oxyanion bases (○), showing that the catalysis depends primarily on the basic strength of the base and not on its chemical nature.

- $\log k_2 = A + \beta\ pK_a$ Brønsted-ova jednačina
- $\log k_2 = A - \alpha\ pK_a$
- Jonizaciono stanje katalizatora –odredjuje i efikasnost katalize...

Table 2.2 *Influence of β on general-base catalysis*

	(Rate in 1-*M* solution of base) ÷ (rate in water)	
β	$pK_a = 5$	$pK_a = 7$
0	1	1
0.3	2.9	8.6
0.5	44	427
0.7	951	2.4×10^4
0.85	9.7×10^3	4.9×10^5
1	1×10^5	1×10^7

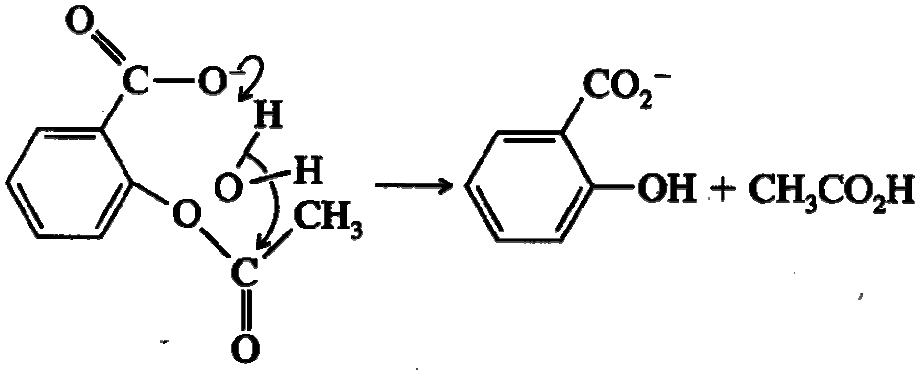
Table 2.3 *Influence of α and state of ionization on general-acid catalysis*

	(Rate in 1-*M* solution of acid) ÷ (rate in water)			(Rate in 1-*M* solution at pH 7) ÷ (rate in water)[a]		
α	$pK_a = 5$	$pK_a = 7$	$pK_a = 9$	$pK_a = 5$	$pK_a = 7$	$pK_a = 9$
0	1	1	1	1	1	1
0.3	31	8.6	2.9	1.3	4.8	2.9
0.5	4.3×10^3	427	44	42	214	43.2
0.7	6×10^5	2.4×10^4	951	6×10^3	1.2×10^4	940
0.85	2.5×10^7	4.9×10^5	9.7×10^3	2.4×10^5	2.4×10^5	9.7×10^3
1	1×10^9	1×10^7	1×10^5	1×10^7	5×10^6	9.9×10^4

[a] The rate of a 1-*M* solution of both the acid and the base forms compared with the uncatalyzed water reaction. It should be noted that the proton becomes an efficient catalyst at higher values of α. For $\alpha = 0.3, 0.5, 0.7, 0.85$, and 1.0, the reaction rate increases at pH 7 by factors of 1.0003, 2, 3×10^3, 1.3×10^6, and 5.5×10^8, respectively, thus swamping out the catalysis by other acids at the higher values.

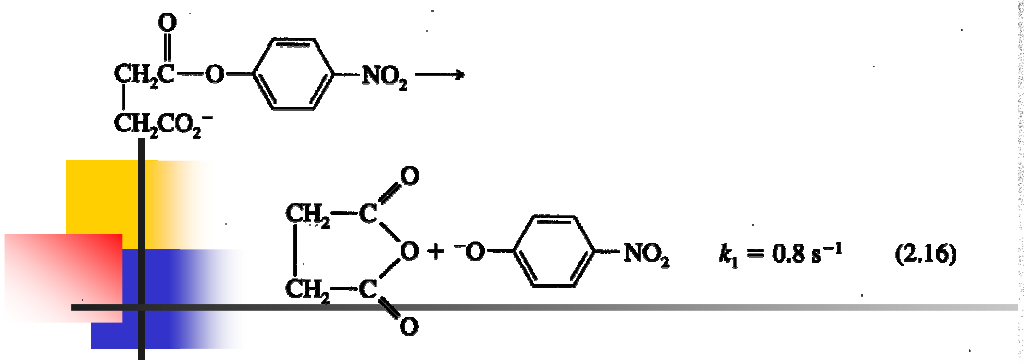
Intramolekularna kataliza

- Efektivna koncentracija grupe na enzimu: slučaj opšte bazne katalize



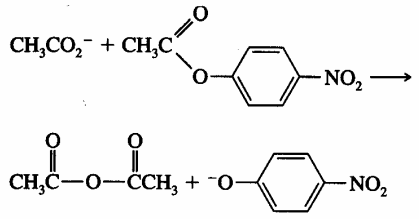
(2.15)

Hidroliza aspirina – efektivna koncentracija karboksilne grupe u aspirinu je 13 M



$k_1 = 0.8\ s^{-1}$ (2.16)

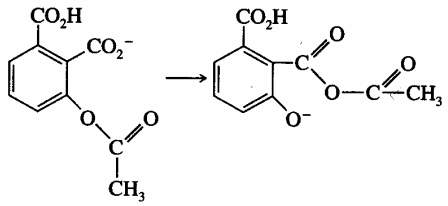
- Efektivna koncentracija nukleofila u intramolekularnoj katalizi



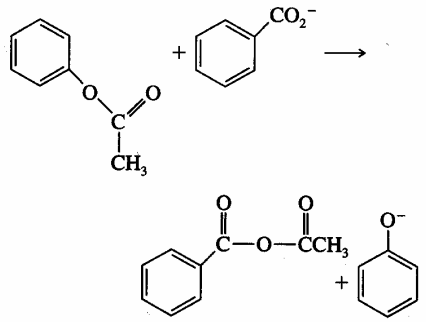
$k_2 = 4 \times 10^{-6}\ s^{-1}\ M^{-1}$ (2.17)

The effective concentration of $-CO_2^-$ is $k_1/k_2 = 2 \times 10^5\ M$.

2. *Rates of acyl transfer in aspirin derivatives*:[15]



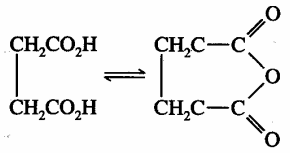
$k_1 \geq 0.02\ s^{-1}$ (2.18)



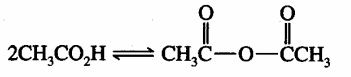
$k_2 \approx 10^{-10}\ s^{-1}\ M^{-1}$ (2.19)

The effective concentration of $-CO_2^-$ is $k_1/k_2 > 2 \times 10^7\ M$.

3. *Equilibria for acyl transfer in succinates*:[16,17]



$K_{eq} = 8 \times 10^{-7}$ (2.20)



$K_{eq} = 3 \times 10^{-12}\ M$ (2.21)

The effective concentration of $-CO_2H$ is $3 \times 10^5\ M$.

Entropija

- Translaciona, rotaciona i unutrašnja

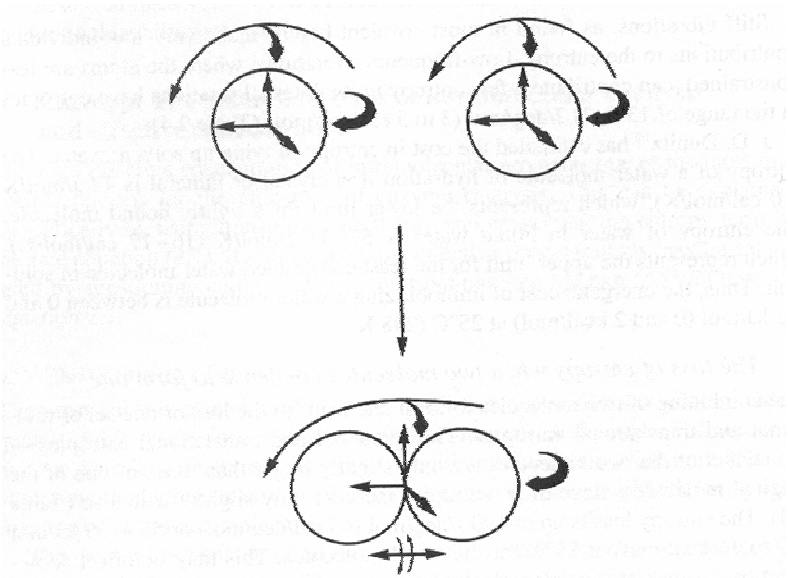
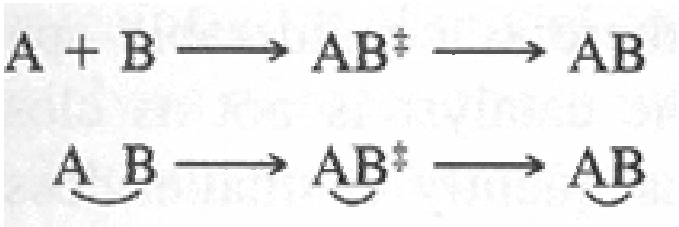


Figure 2.6 A free molecule has three degrees of translational entropy and three degrees of overall rotational entropy. When two molecules condense to form one, the resulting adduct has only three degrees of translational and three degrees of rotational entropy overall, a loss of three degrees of each. A compensating gain of internal vibrational and rotational entropy partly offsets this loss.



Elektrostatska kataliza

- $E = e_1 e_2 / Dr$
- Enzimi mogu da stabilizuju polarna prelazna stanja bolje od vode.
- 1. Dva ili tri fiksiranja dipola u enzimu mogu da stabilizuju naelektrisanje kao i voda.
- 2. Jonski par se može stabilizovati fiksiranim dipolima u enzimu efikasnije nego u vodi.
- **Enzimi koriste delove strukture da solvatizuju, a ne vodu.**

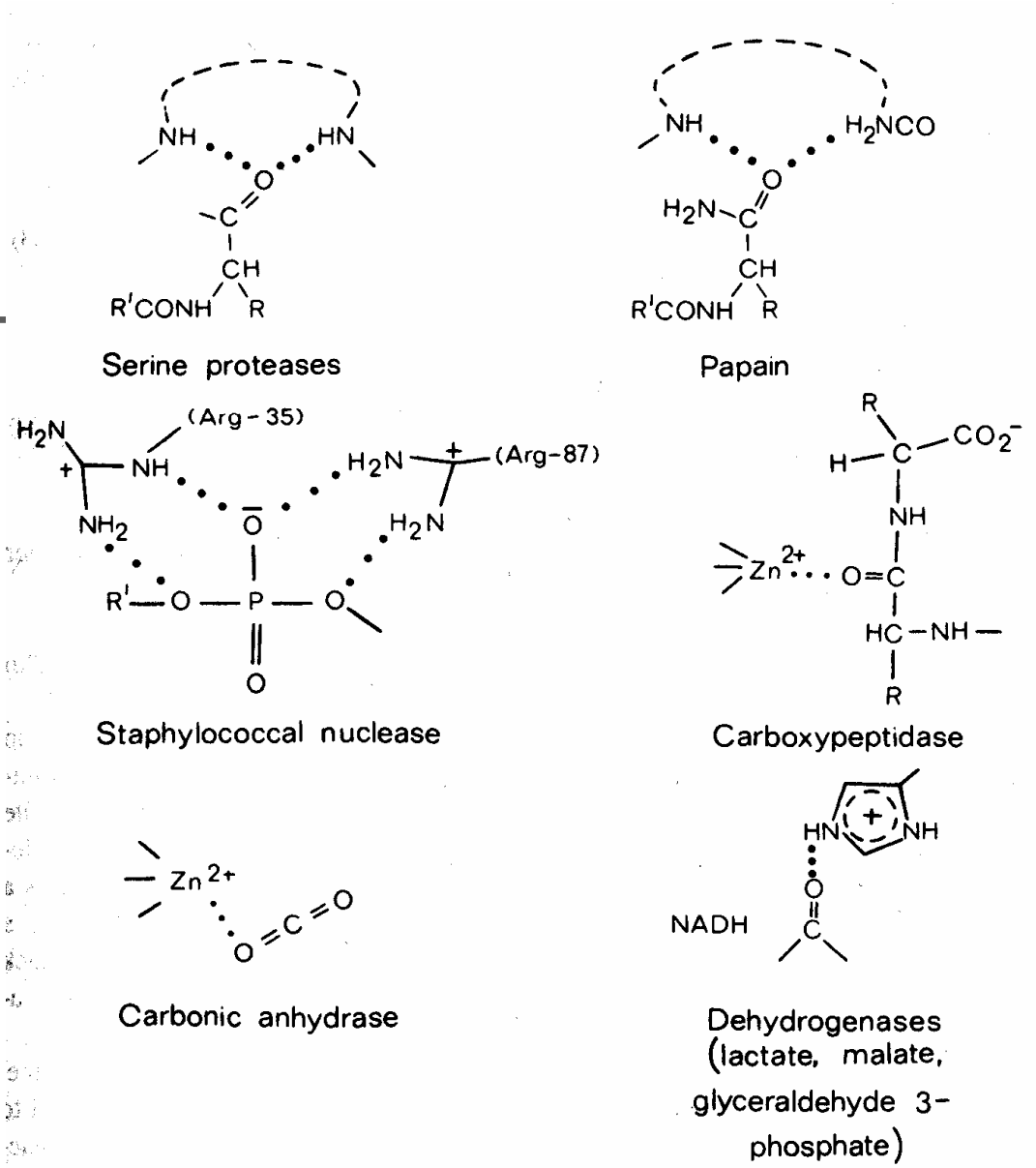
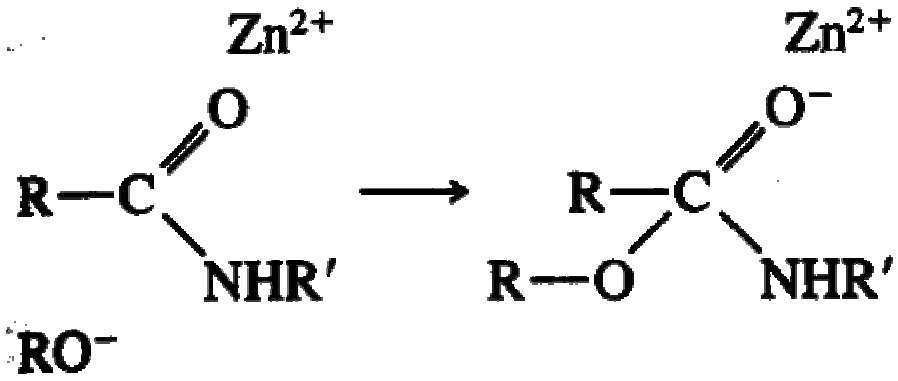


Figure 2.7 Solvation of substrates by enzymes.

Kataliza jonima metala

- a) Elektrofilna kataliza

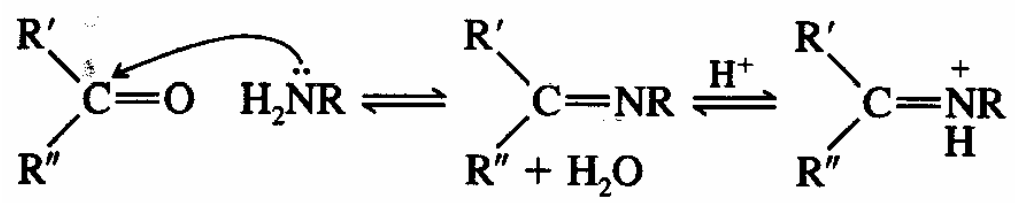
  - Karboksipeptidaza (polarizacija amida na nukleofilni napad i stabilizacija tetrehedralnog intermedijera).

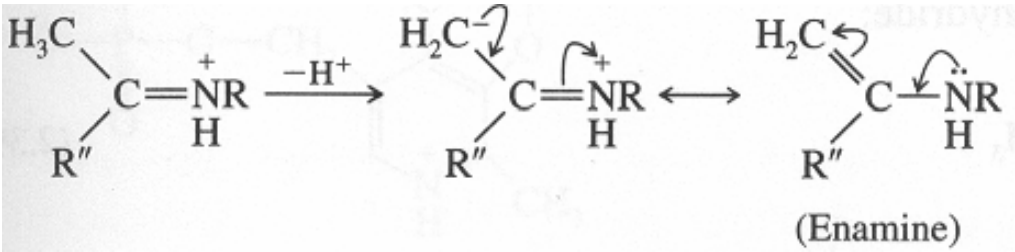


b) Izvor hidroksilnih jona na neutralnom pH (karboanhidraza)

Kovalentna kataliza

- Elektrofilna kataliza formiranjem Šifove baze

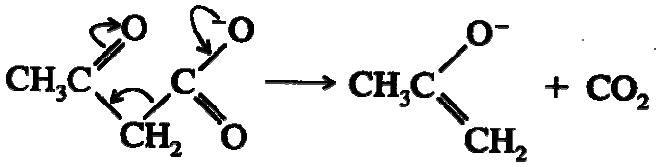


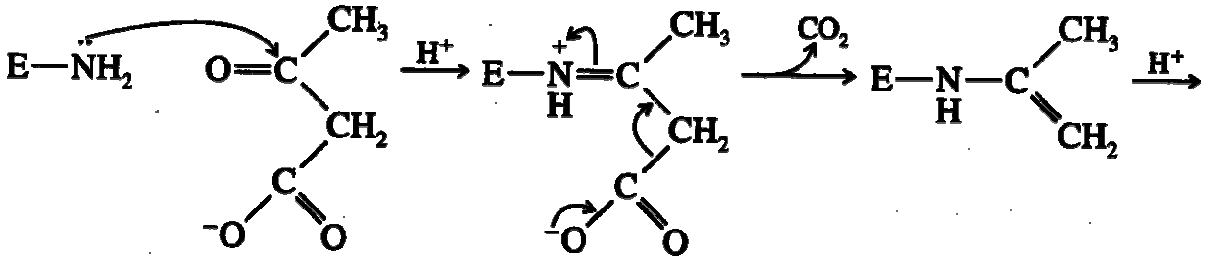


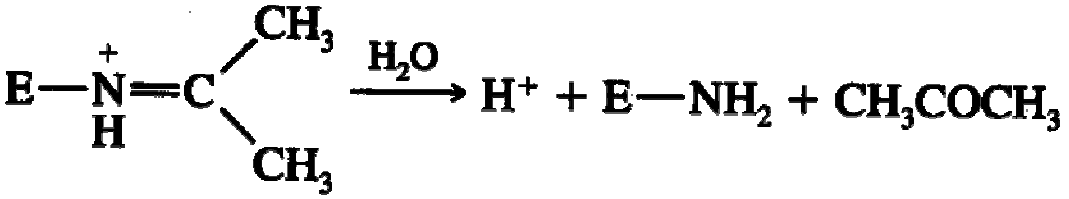
Metilenski ugljenik se aktivira i postaje nukleofilan.

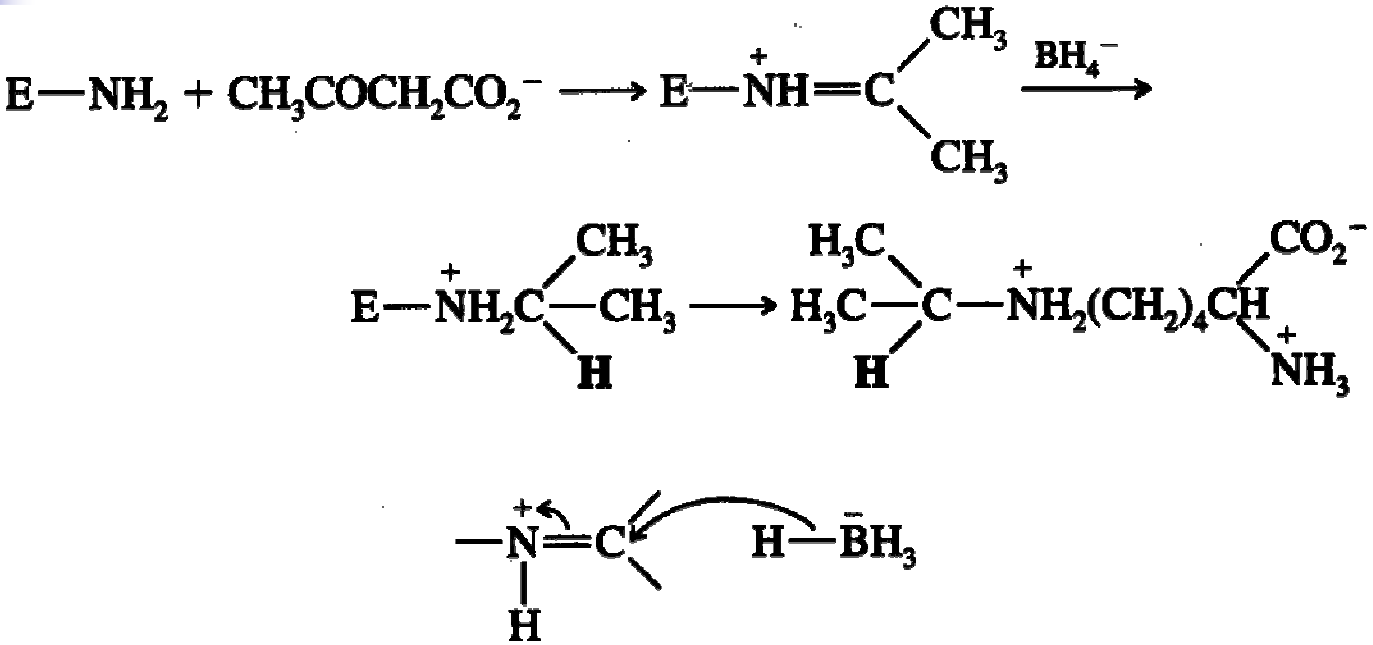
Karbonilni ugljenik se aktivira na nukleofilni napad zbog snažnog povlačenja elektrona ka protonovanom azotu.

Acetoacetat dekarboksilaza





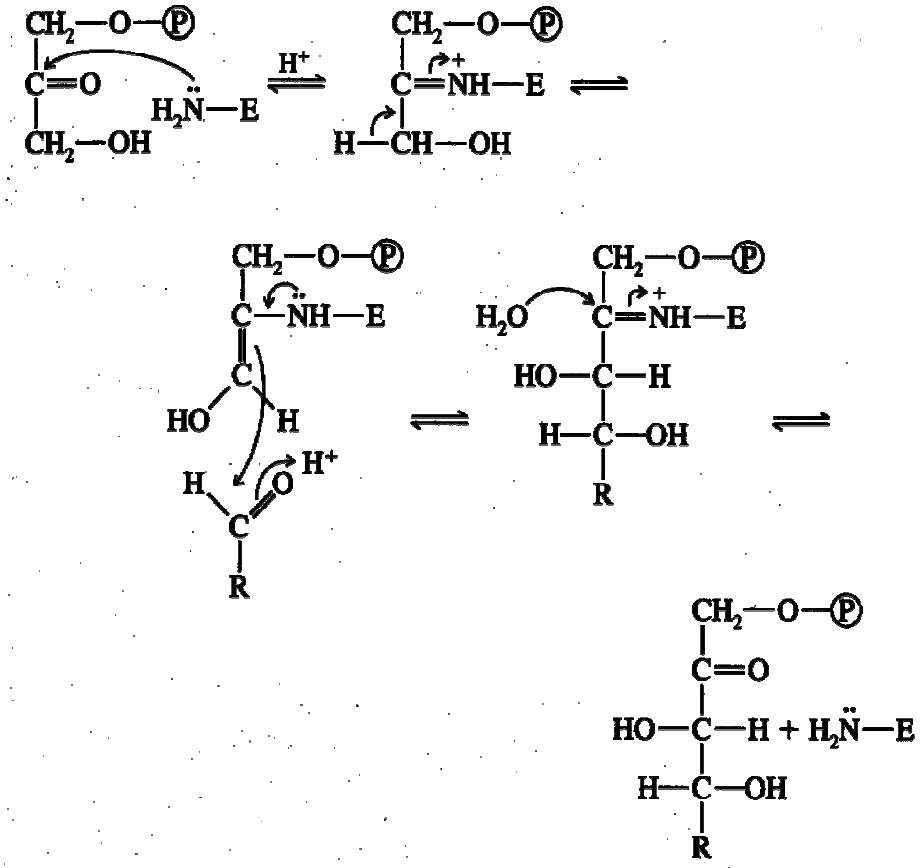




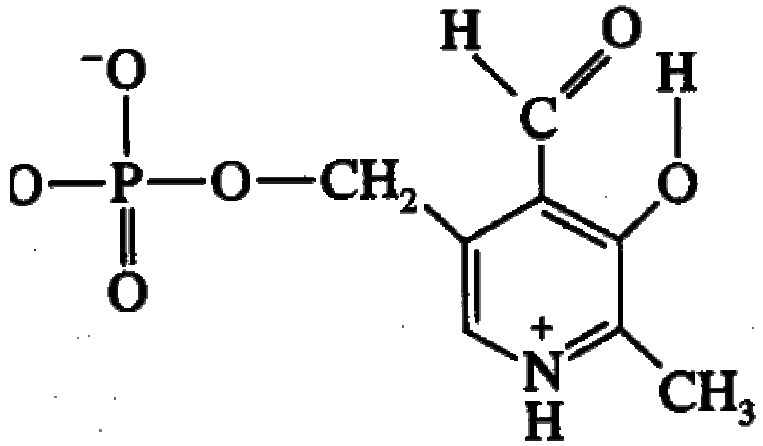
Izopropil lizin je detektovan u hidrolizatu enzima!

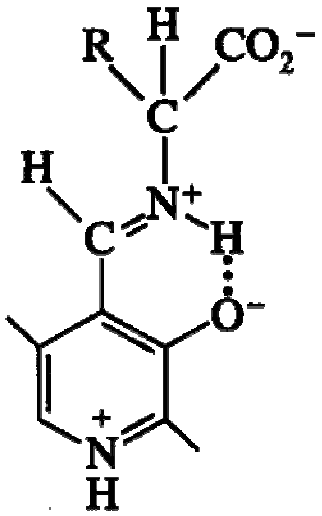
Aldolaza i transaldolaza

Katalizuju kondenzaciju aldola i povratnu reakciju uz formiranje Šifove baze (ugljenik je aktiviran u enaminu)

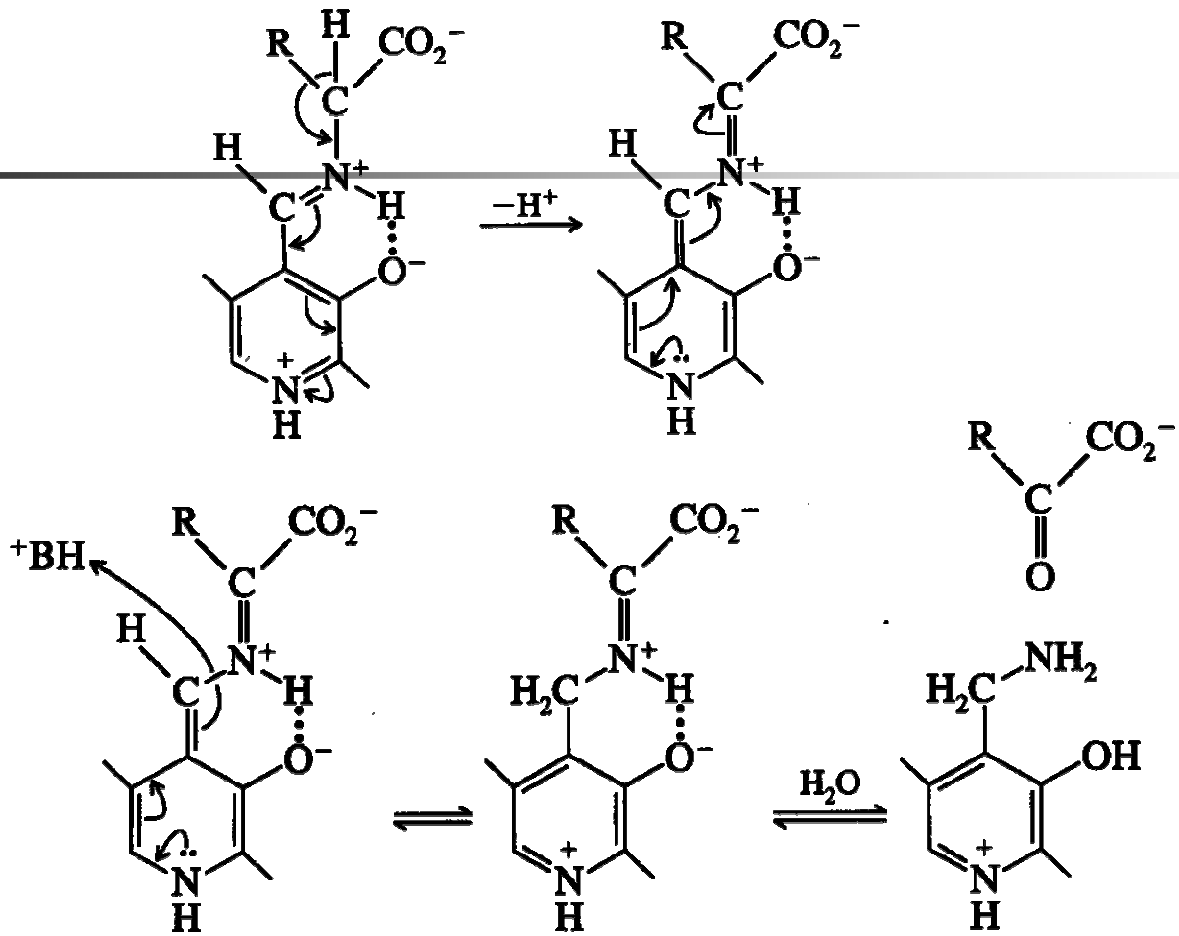


Piridoksal fosfat (elektrofilna kataliza)



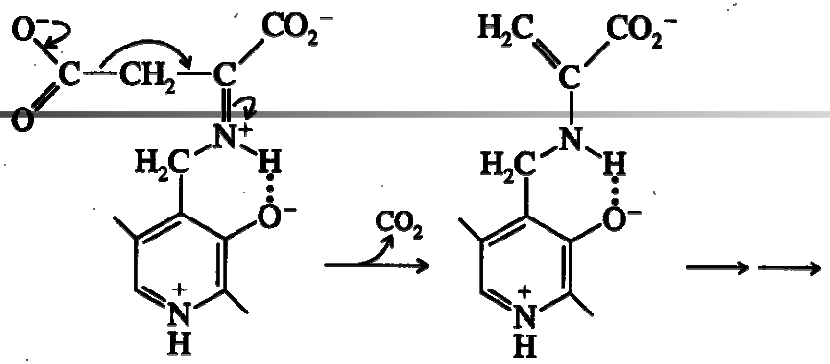


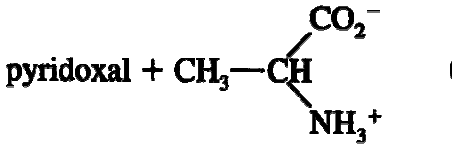
Uklanjanje α-vodonika: racemizacija ili transaminacija



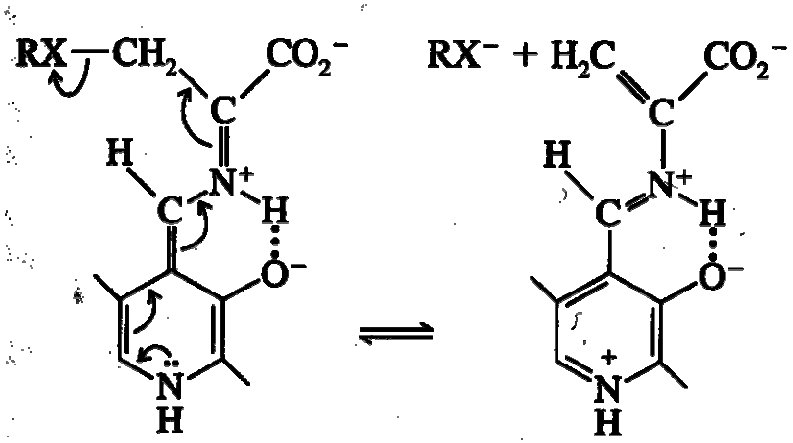
$$R'CH(NH_3^+)CO_2^- + R''COCO_2^- \rightleftharpoons R'COCO_2^- + R''CH(NH_3^+)CO_2^-$$

β-dekarboksilacija





Izmena bočnog lanca



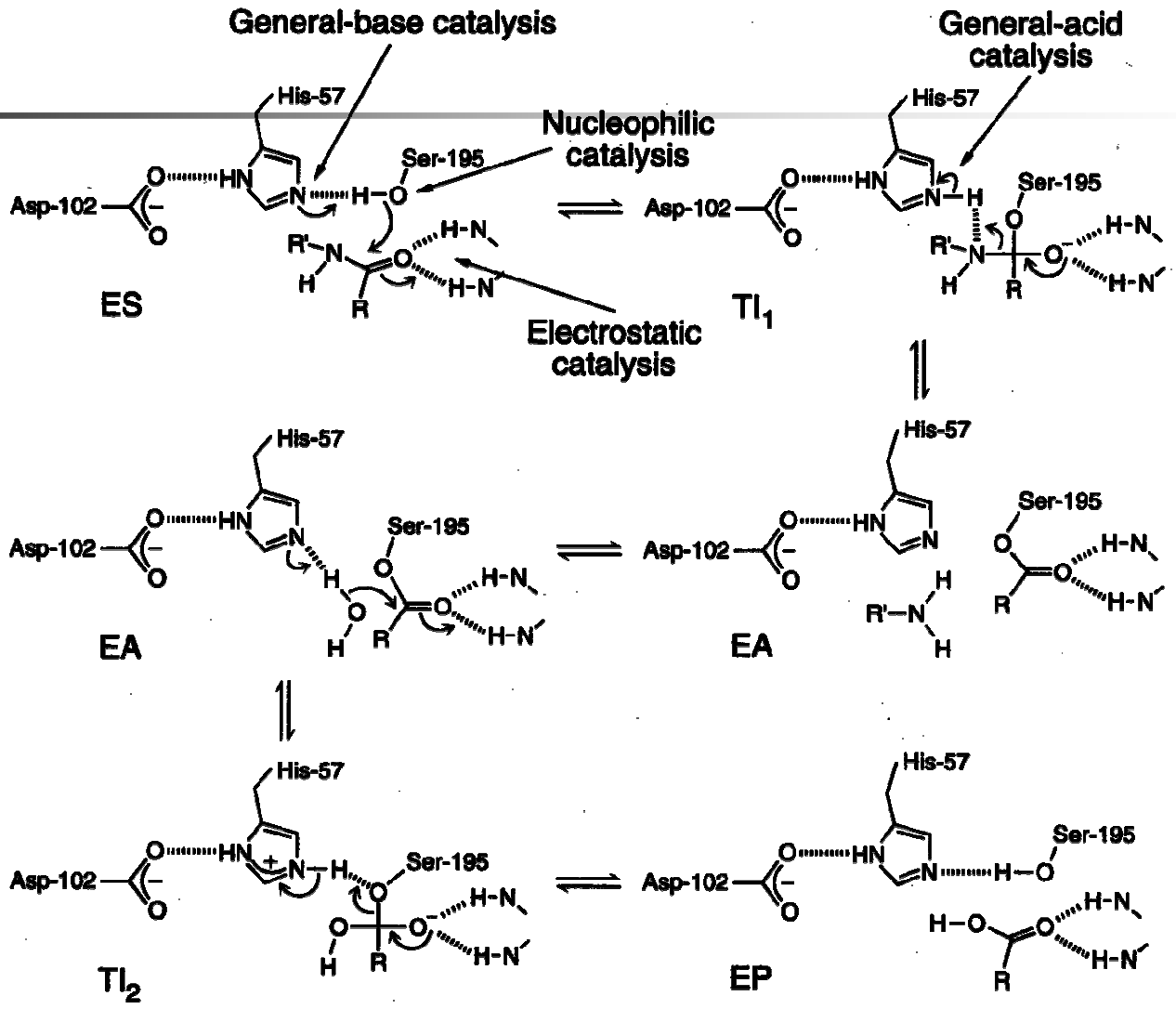
Nukleofilna kataliza

Table 2.5 *Nucleophilic groups in enzymes*

Nucleophile	Enzyme	Intermediate
—OH (serine)	Serine proteases	Acylenzyme
	Alkaline phosphatases, phosphoglucomutase	Phosphorylenzyme
—OH (threonine)[a]	Proteasome, amidases	Acylenzyme
OH^- (zinc-bound)	Carbonic anhydrase, liver alcohol dehydrogenase	—
—SH (cysteine)	Thiol proteases, glyceraldehyde 3-phosphate dehydrogenase	Acylenzyme
$—CO_2^-$ (aspartate)	ATPase (K^+/Na^+, Ca^{2+})	Phosphorylenzyme
$—NH_2$ (lysine)	Acetoacetate decarboxylase, aldolase, transaldolase, pyridoxal enzymes	Schiff base
	DNA ligase	Adenylenzyme (phosphoamide)
Imidazole (histidine)	Phosphoglycerate mutase, succinyl-CoA synthetase, nucleoside diphosphokinase, histone phosphokinase, acid phosphokinase	Phosphorylenzyme
—OH (tyrosine)	Glutamine synthetase	Adenylenzyme
	Topoisomerases	Nucleotidylenzyme (phosphotyrosine)

[a] The nucleophile resides in an N-terminal threonine. The general-base is the free N-terminal $—NH_2$ group.

Mehanizam dejstva himotripsina



Mehanizmi enzimima katalizovanih reakcija

- Redosled konverzije različitih kompleksa enzima kojima se S konvertuje u proizvod
- Brzine kojima se ovi kompleksi interkonvertuju
- Strukture
- Kinetičke studije
- X-ray kristalografija
- Protein inženjering
- Detekcija intermedijera
- Hemijsko obeležavanje i hemijske modifikacije bočnih ostataka u enzimima

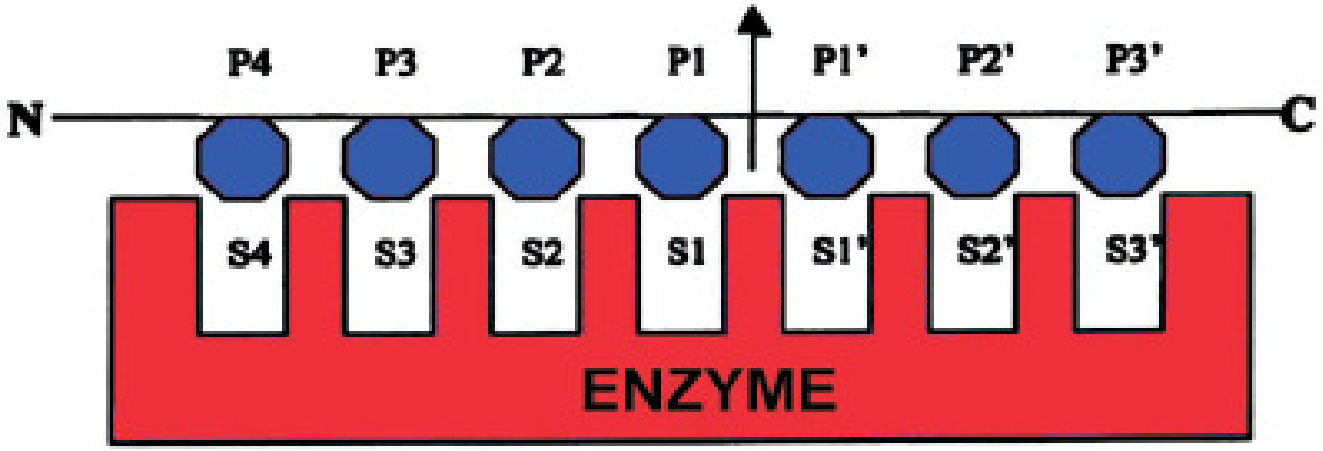


Figure 2.: Schechter and Berger nomenclature.

S4–S3' – substrate binding sites within the active site of the enzyme, P4–P3' – substrate residues that bind to S4–S3' sites, arrow indicates the scissile bond (Schechter & Berger, 1967).

Proteaze

- Serin proteaze
- Karboksil – aspartil (kisele) proteaze
- Tiol (cistein) proteaze
- Cink proteaze
- Monomerni enzimi, mase 15 – 35 kDa
- Adenovirusi (cistein proteaze), pikornavirusi (serin proteaze), retrovirusi (aspartil proteaze).
- (N-termunus) S1 – S1` veza se raskida

Familija serin proteaza - struktura

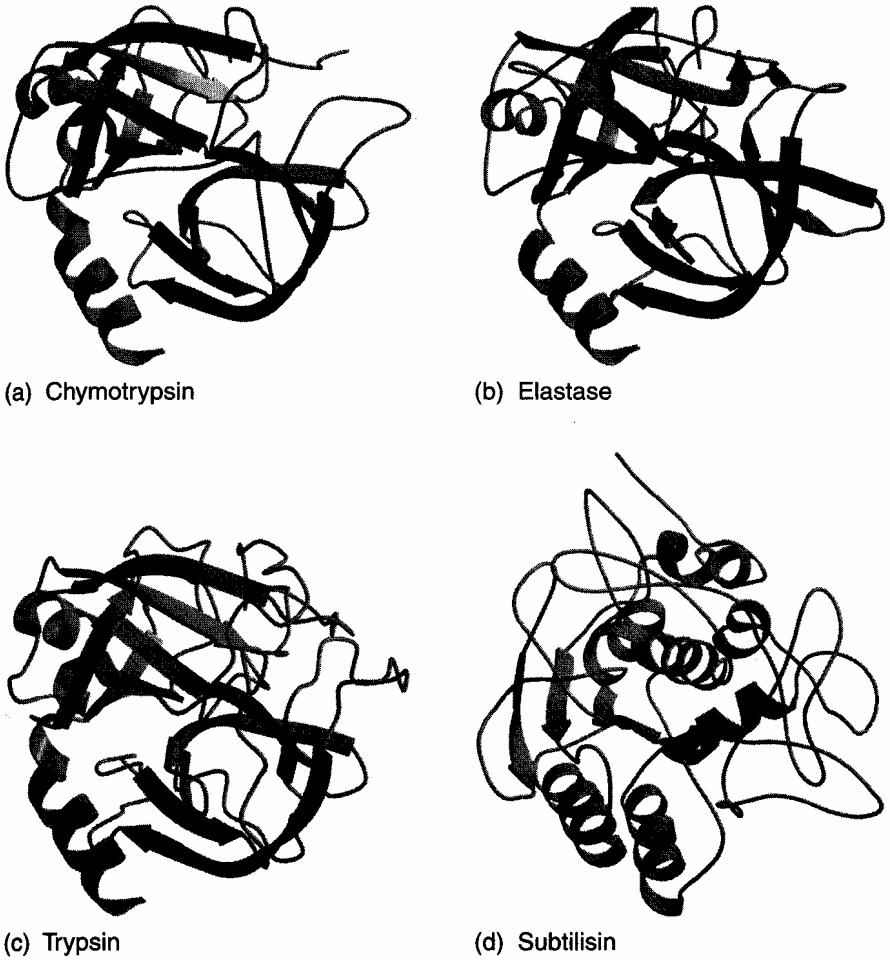
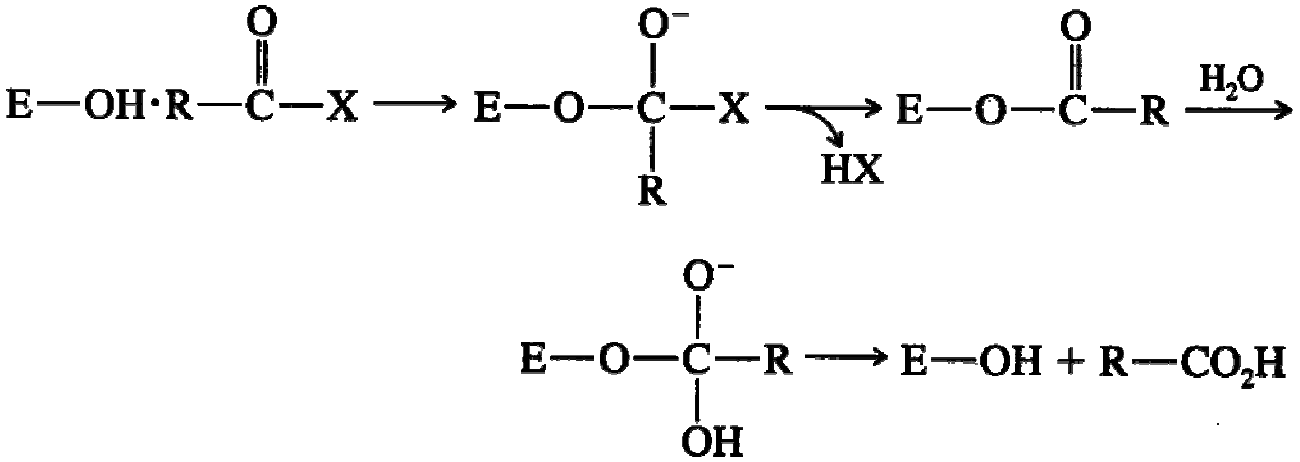


Figure 1.17 The polypeptide chains of (a) chymotrypsin, (b) elastase, (c) trypsin, and (d) subtilisin drawn in MolScript.

Serin proteaze

Dva domena, katalitička trijada Asp-102, His-57, Ser-195 se nalazi izmedju dva domena



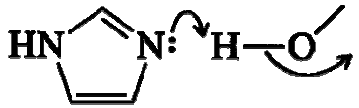
Formira se acil-enzim.

Nema direktnih dokaza za formiranje tetraedarskog intermedijera

Indirektni dokazi: x-ray Enz-Inh kompleksa

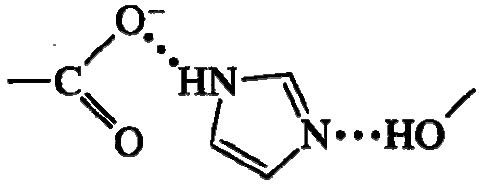
Katalitička trijada u serin proteazama

Katalitička trijada Asp-102, His-57, Ser-195



His jonizuje na pH 6.8

charge-relay system



pKa Asp neobično niska (2)

Protein inženjeringom potvrdjena uloga katalitičke trijade

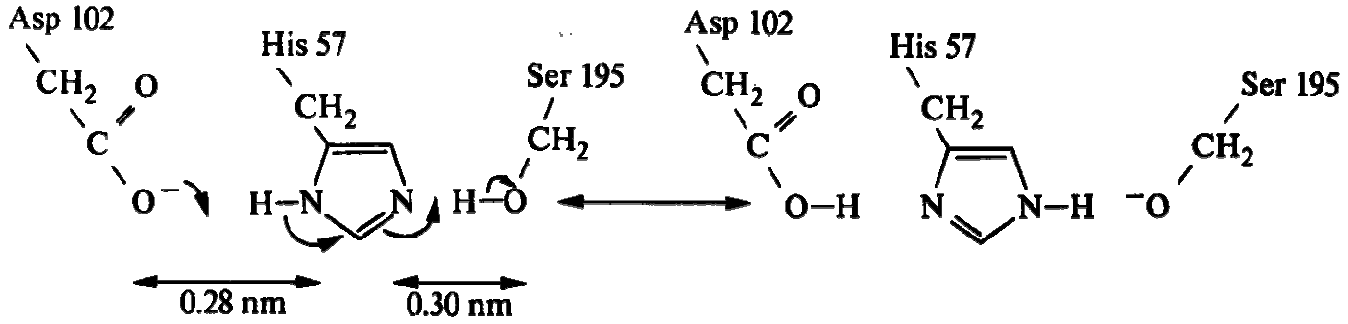


Fig. 5.14. The charge-relay system in chymotrypsin.[44]

Doprinos oksianjon vezivnog mesta katalizi

- Nakon mutageneze katalitičke trijade (Ala), kataliza je smanjena, ali i dalje 1000 puta veća od spontane hidrolize
- Komplementarnost aktivnog mesta enzima prelaznom stanju reakcije (vibraciona spektroskopija)

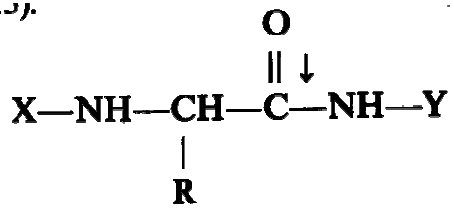
Table 16.2 *Structural requirements in the deacylation of acylchymotrypsins (at 25°C)*[a]

Acylchymotrypsin R— (RCO_2E)	k_{cat} (s^{-1}) (for deacylation)	k_{OH^-} ($s^{-1} M^{-1}$) (for hydrolysis of RCO_2CH_3)
CH_3—	0.01	0.19
$C_6H_5CH_2CH_2$—	0.178	0.15
$CH_2(NHCOCH_3)$—	0.12	2.48
L-$C_6H_5CH_2CH(NHCOCH_3)$—	111	1.94

[a] From A. Dupaix, J.-J. Bechet, and C. Roucous, *Biochem. Biophys. Res. Commun.* **41**, 464 (1970); I. V. Berezin, N. F. Kazanskaya, and A. A. Klyosov, *FEBS Lett.* **15**, 121 (1971) (see Table 7.3).

Deacilacija

Acilacija – dodatna mesta vezivanja S1 – S5 povećavaju k_{cat}, ali ne utiču na Km



Mehanizam dejstva himotripsina

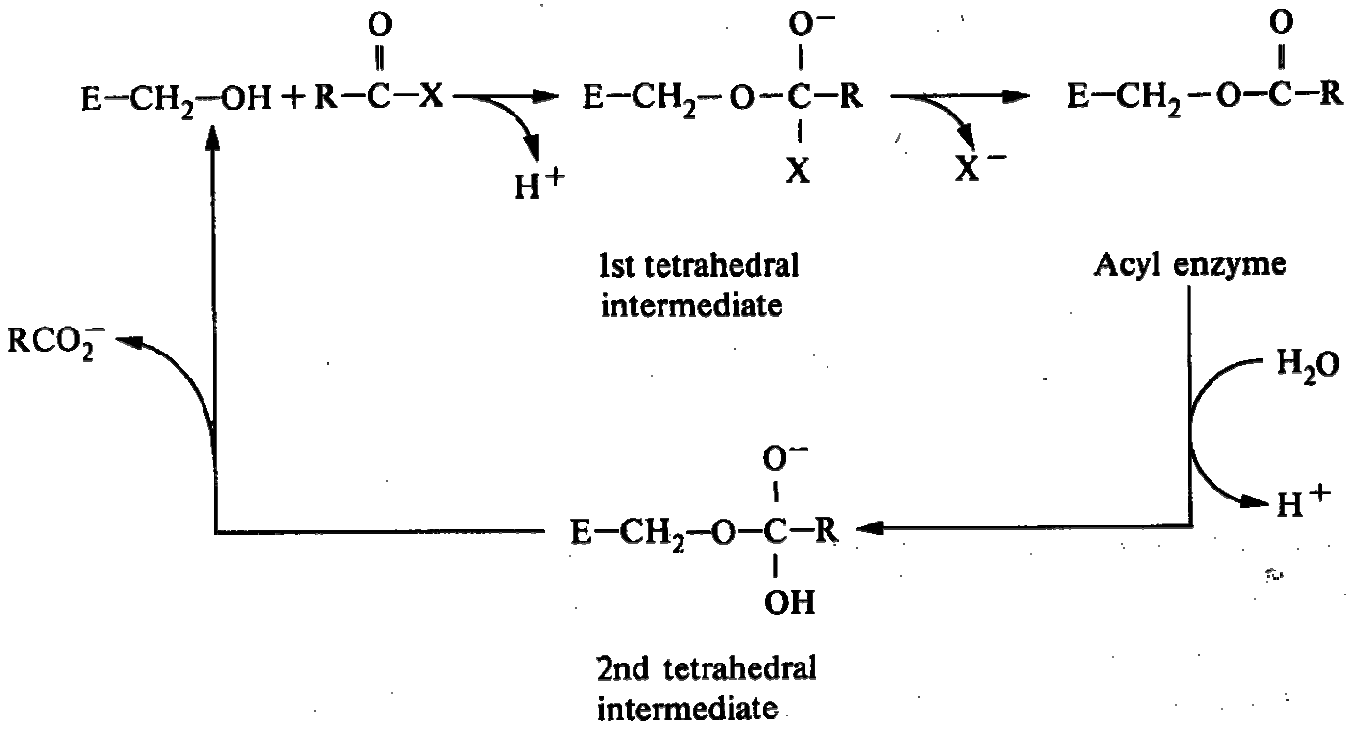
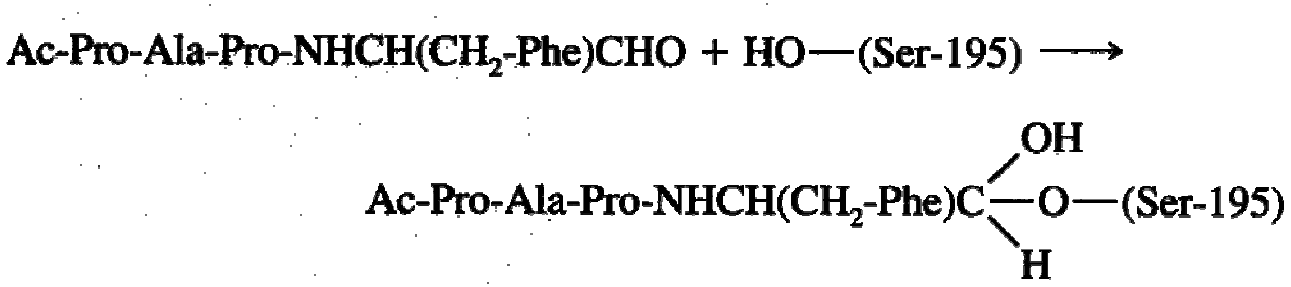


Fig. 5.17. The mechanism of action of chymotrypsin showing the tetrahedral intermediates involved in the formation and breakdown of acyl enzyme.

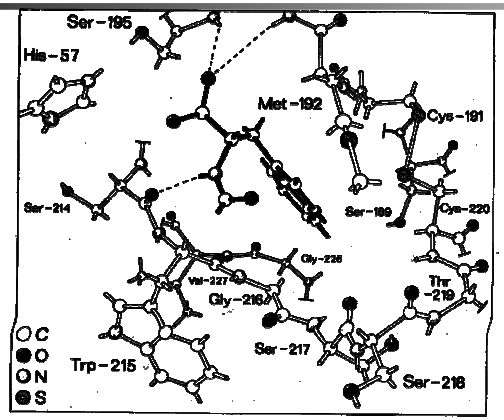
Serin proteaze – tetreadarski intermedijer

Intermedijer se ne nagomilava tokom reacije

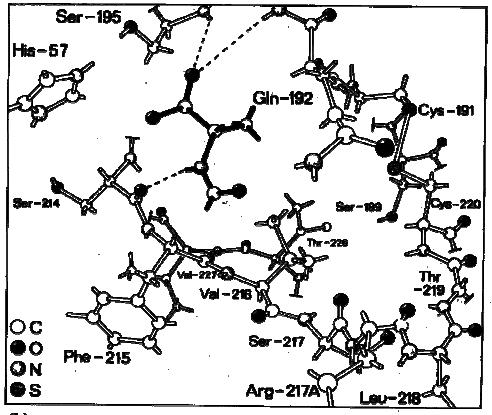
Dokazi za njegovo postojanje su samo indirektni



Himotripsin – struktura aktivnog mesta



(a)



(b)

Figure 1.18 Comparison of the binding pockets in (a) chymotrypsin, with *N*-formyl-L-tryptophan bound, and (b) elastase, with *N*-formyl-L-alanine bound. The binding pocket in trypsin is very similar to that in chymotrypsin, except that residue 189 is an aspartate to bind positively charged side chains. Note the hydrogen bonds between the substrate and the backbone of the enzyme.

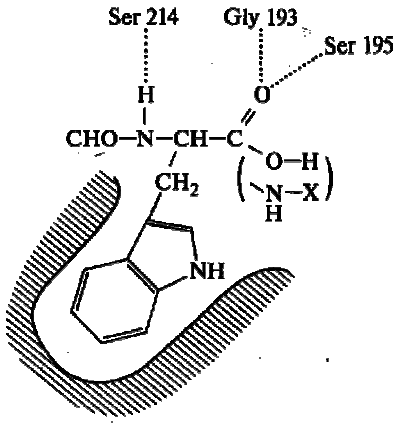


Fig. 5.15. Binding of the inhibitor N-formyl tryptophan to chymotrypsin. The structure of the substrate is indicated in brackets.

Himotripsin – neproduktivno vezivanje

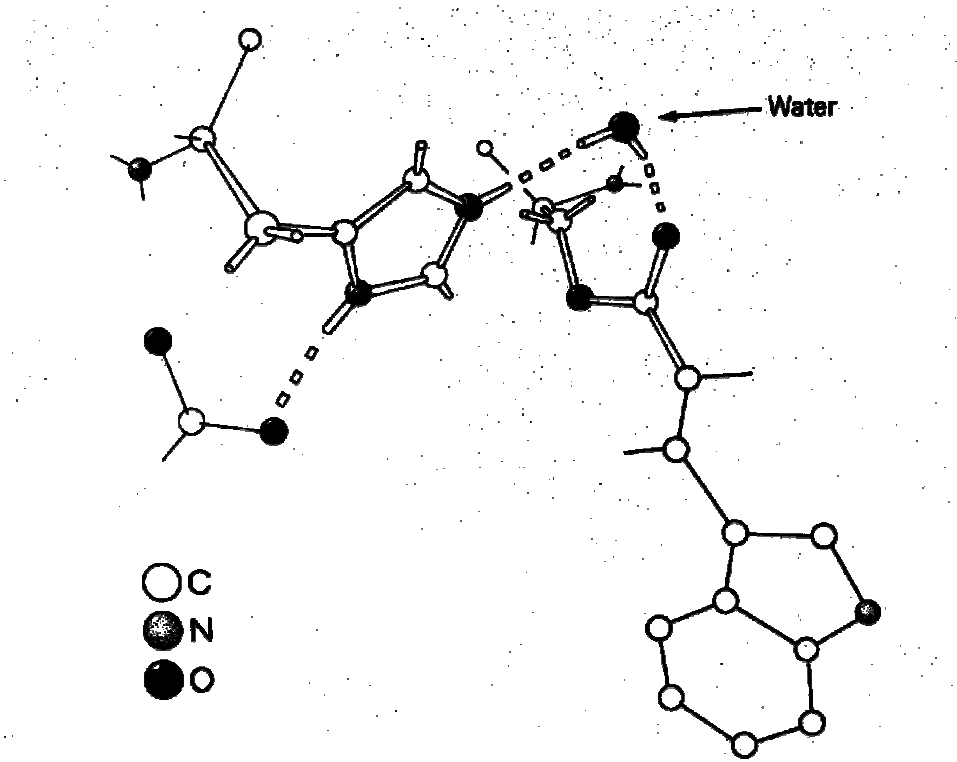
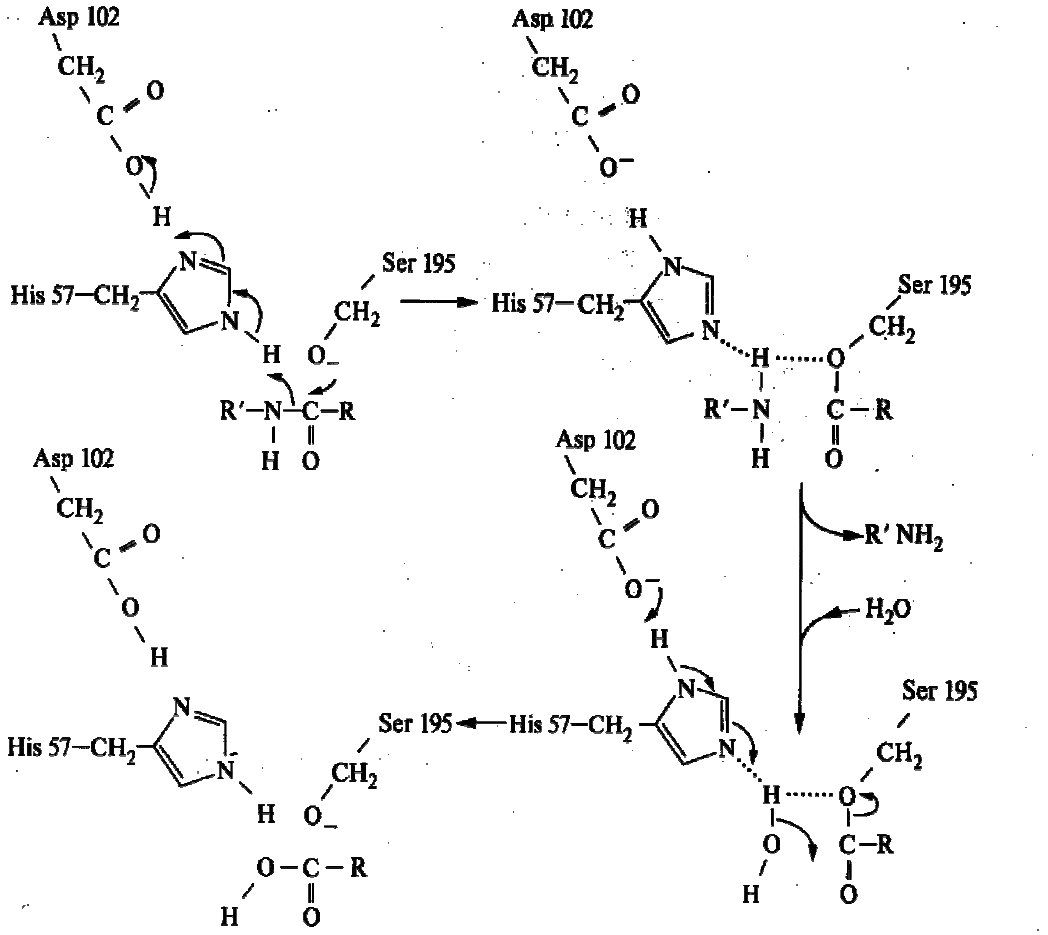


Figure 16.4 The crystal structure of indolylacryloyl-chymotrypsin. [From R. Henderson, *J. Molec. Biol.* **54**, 341 (1970).] Note that the carbonyl oxygen of this nonspecific acylenzyme is not bound between the NH groups of Ser-195 and Gly-193, but is nonproductively linked to His-57 by a hydrogen-bounded water molecule. This is the acylenzyme that was found to deacylate at the same rate in solution and in the crystal (Chapter 1). [G. L. Rossi and S. A. Bernhard, *J. Molec. Biol.* **49**, 85 (1970).]

Mehanizam dejstva himotripsina na amidnim supstratima



Hemijska modifikacija His-57

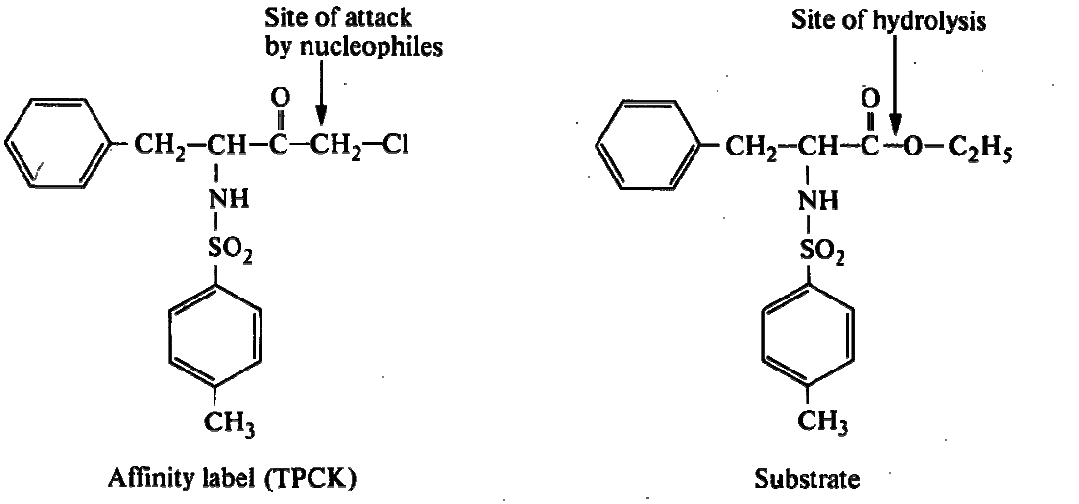


Fig. 5.18. The structure of the affinity label TPCK and its relationship to a substrate of chymotrypsin.

TPCK – N-p-toluensulfonil-L-fenilalanin hloromehtilketon modifikuje His-57 himotripsina, ali ne i tripsina

TLCK - N-p-toluensulfonil-L-lizin hlorometillketon modifikuje aktivno mesto tripsina, a neaktivan je na himotripsinu

Zimogeni – aktivacija himotripsina

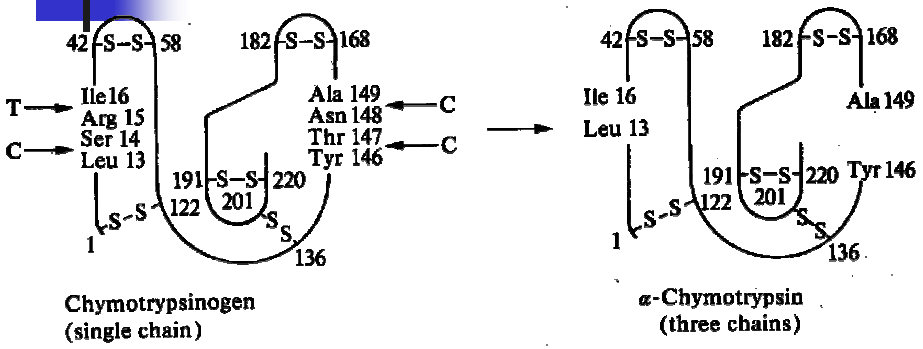


Fig. 5.13. Activation of chymotrypsinogen. T and C represent the actions of trypsin and chymotrypsin respectively.

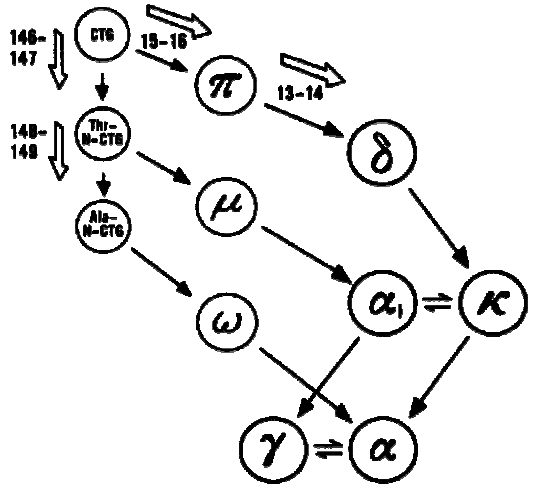


Figure 16.5 Activation of chymotrypsinogen (CTG). It was seen in Chapter 1, Figure 1.1, that CTG is susceptible to tryptic cleavage at the (Arg-15)—(Ile-16) bond, and to chymotryptic cleavages at (Tyr-146)—(Thr-147), (Ile-13)—(Ser-14), and (Asn-148)—(Ala-149) (the latter two because chymotrypsin has relatively broad specificity). In the rapid activation of chymotrypsinogen ([CTG]/[trypsin] ≈ 30), there is sufficient trypsin present to activate all the CTG before the accumulated chymotrypsin autolyzes or cleaves the CTG. The pathway of activation is CTG → π-chymotrypsin → δ-chymotrypsin → κ-chymotrypsin ($+\alpha_{1-}$) → α-chymotrypsin ($+\gamma$-). The κ and α_1 forms are two different conformational states of the same primary structure, as are the α and γ. During the slow activation ([CTG]/[trypsin] ≈ 10^4), the small fraction of trypsin activates the zymogen slowly, allowing the chymotrypsin that is initially formed to cleave the unactivated zymogen to form neochymotrypsinogens (N-CTG's). Alpha$_1$- and α-chymotrypsin are produced from the N-CTG's via the generation of μ- and ω-chymotrypsin by tryptic cleavages. [From S. K. Sharma and T. R. Hopkins, *Biochemistry* **18**, 1008 (1979); *Bioorganic Chem.* **10**, 357 (1981).]

Krucijalna uloga Ile-16: eksperiment dvostrukog obeležavanja

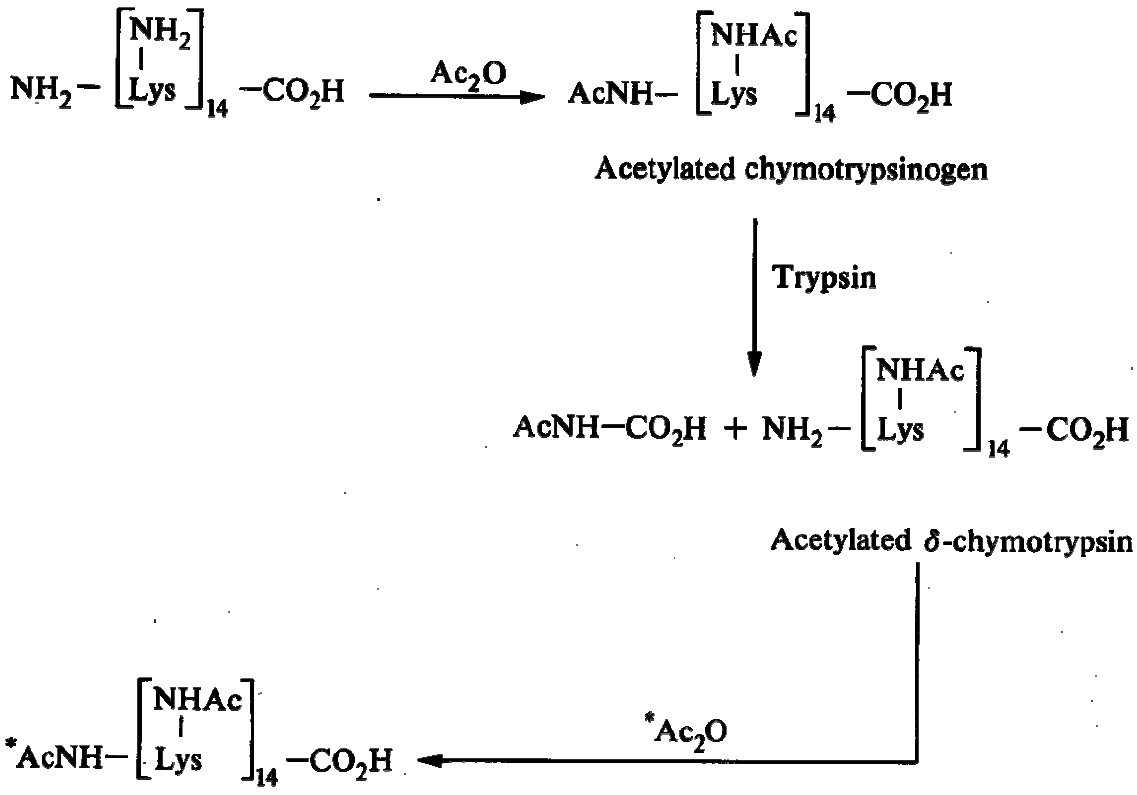


Fig. 5.19. Acetylation of the α-amino group of Ile 16 in chymotrypsin by a double-labelling experiment. Ac_2O is acetic anhydride.

Serin proteaze – specifičnost prema supstatima (struktura vezivnog džepa)

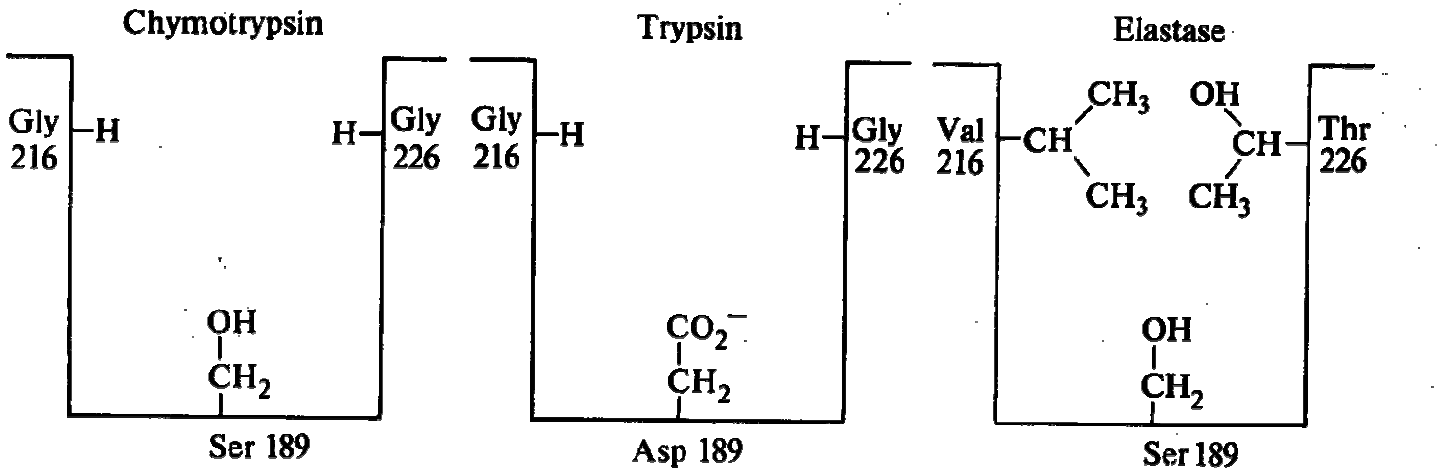
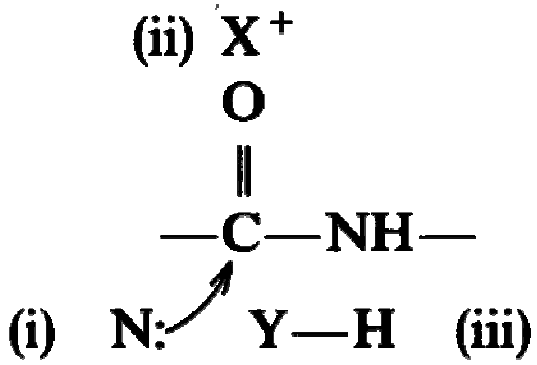


Fig. 5.16. Comparison of the substrate binding pockets of the serine proteinases chymotrypsin, trypsin, and elastase.

- Himotripsin: Ile-16 i Asp-194 formiraju vezivni džep gradeći jonski most u okruženju niske dielektrične konstante

Odnos mehanizma dejstva himotripsina i drugih proteinaza



- (i) nukleofil – napad na karbonilnu grupu + formiranje tetraedarskog intermedijera
- (ii) pozitivno naelektrisana vrsta (ili –NH- grupe koje grade vodoničnu vezu)– povećava susceptibinost karbonilne grupe na nukleofilni napad i stabilizuje tetraedarski intermedijer
- (iii) donor protona