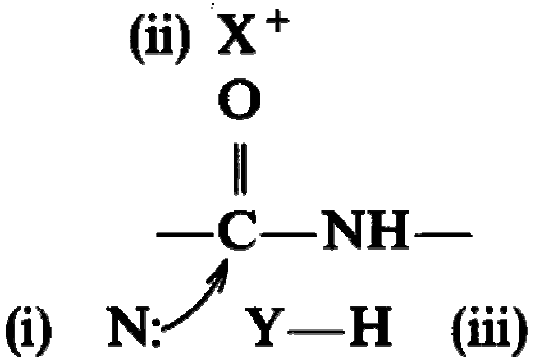
Mehanizmi dejstva odabranih enzima 2

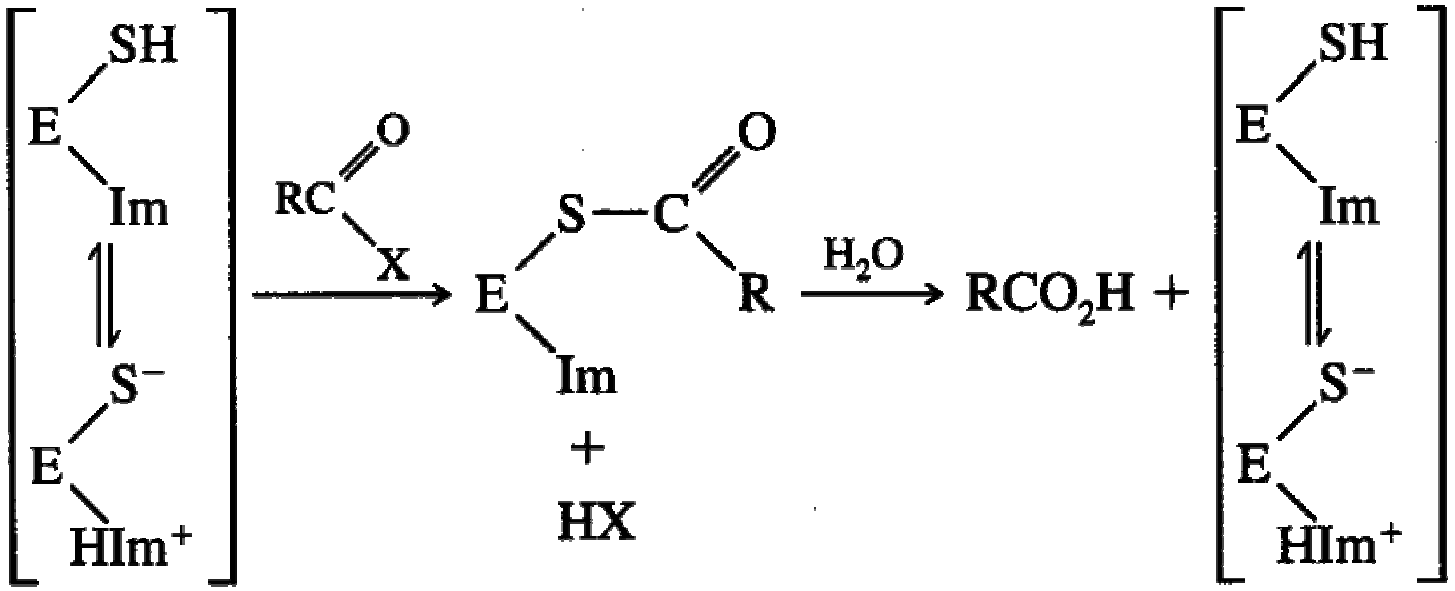
Odnos mehanizma dejstva himotripsina i drugih proteinaza



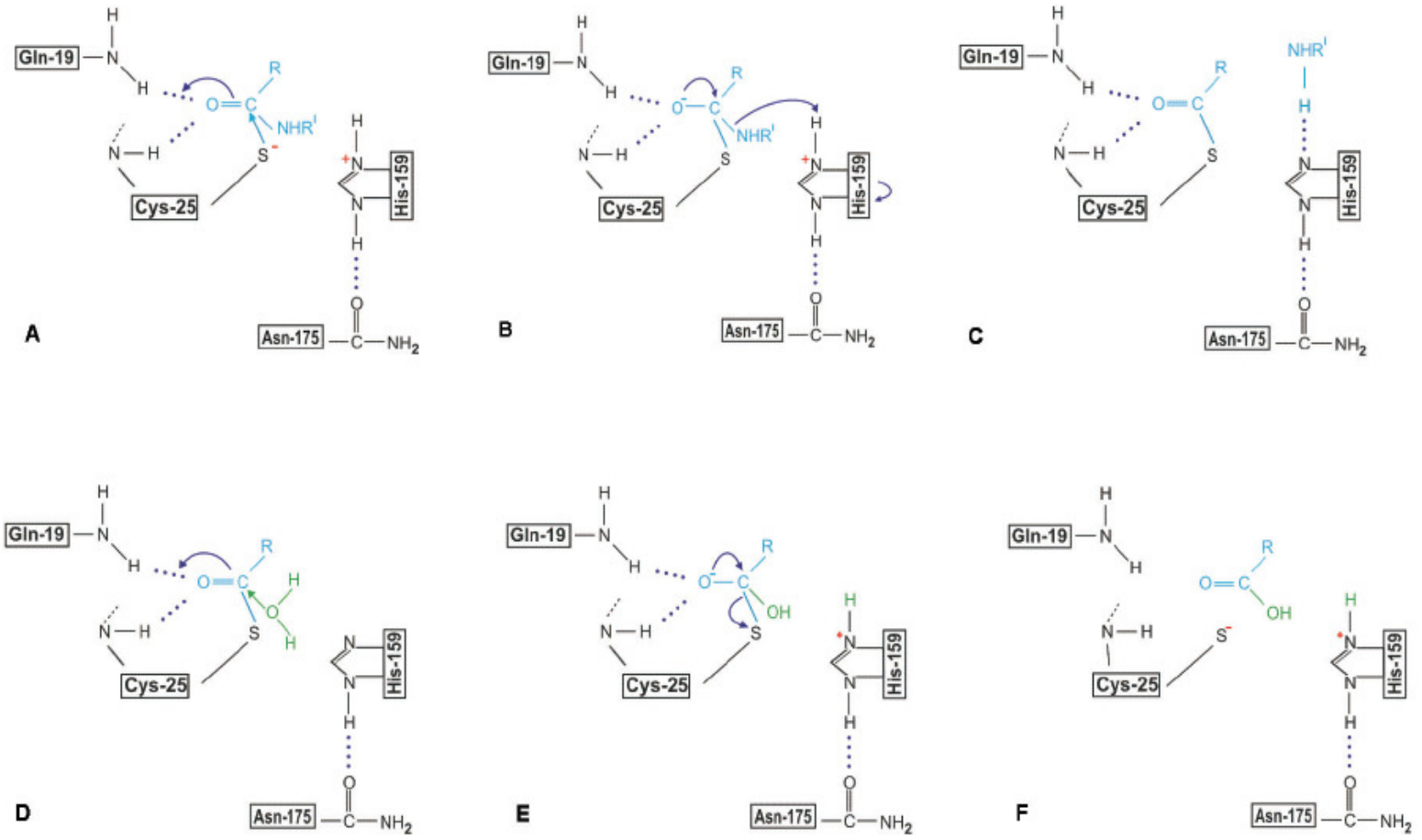
- (i) nukleofil – napad na karbonilnu grupu + formiranje tetraedarskog intermedijera
- (ii) pozitivno naelektrisana vrsta (ili –NH- grupe koje grade vodoničnu vezu)– povećava susceptibinost karbonilne grupe na nukleofilni napad i stabilizuje tetraedarski intermedijer
- (iii) donor protona

Cistein proteaze

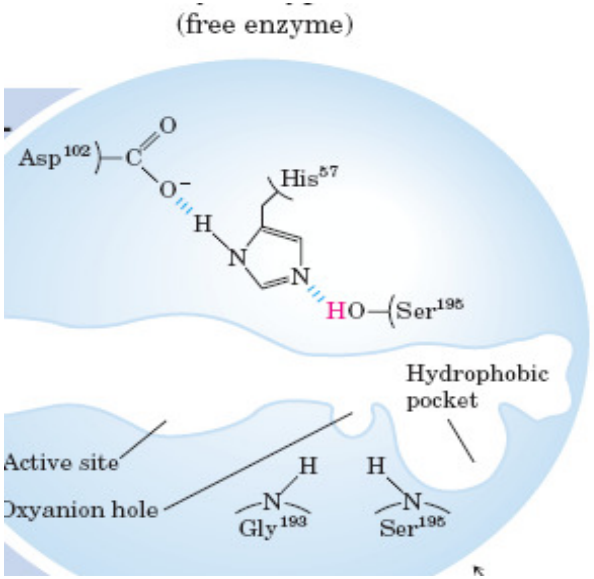
Papain, ficin, bromelain, aktinidin
Kalpain, klostripain, pikornavirus proteinaza, streptokokalna proteinaza, i IL-1-b-konvertujući enzim

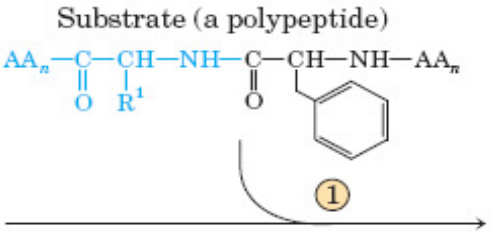


Mehanizam dejstva papaina

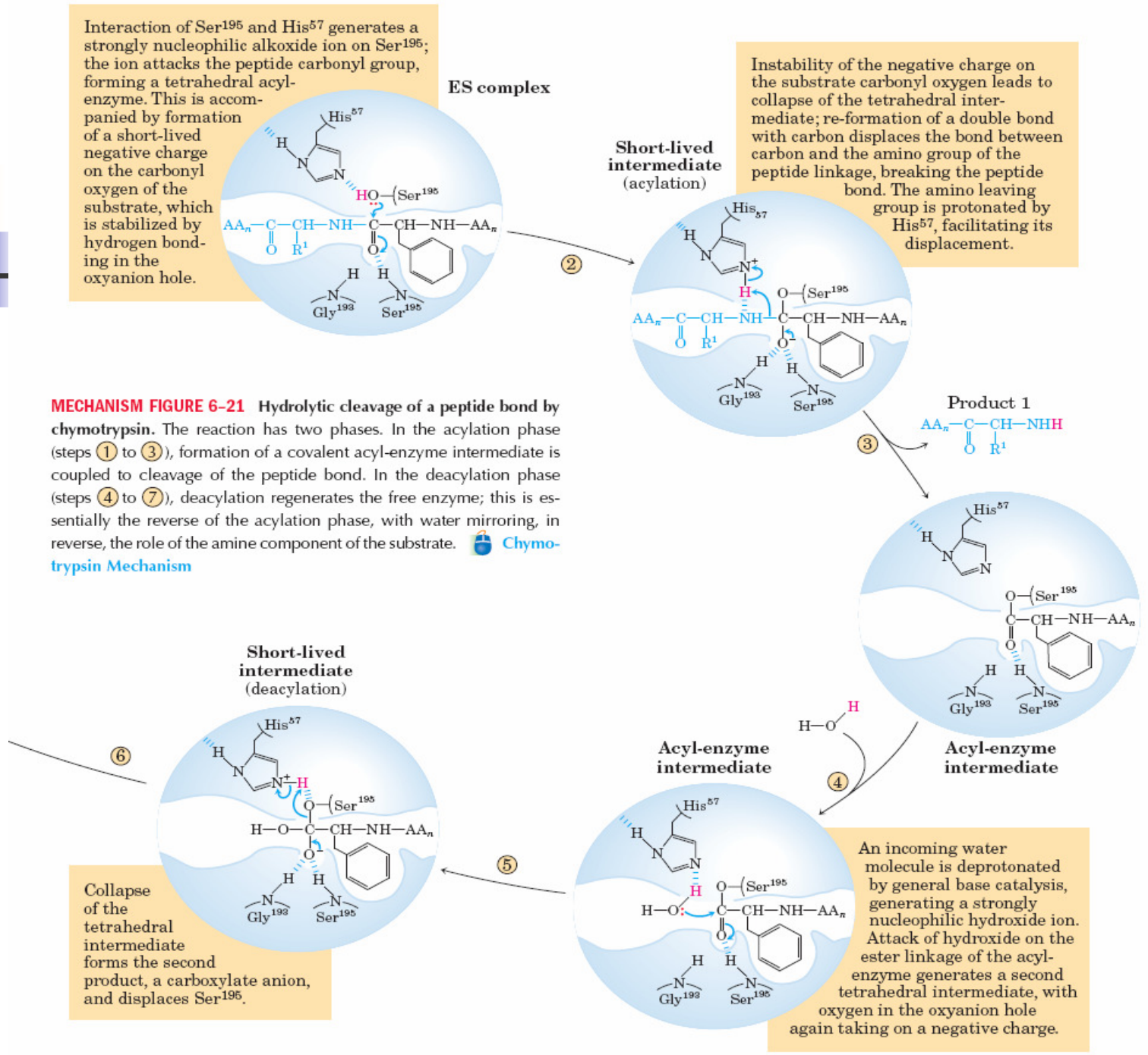


Himotripsin

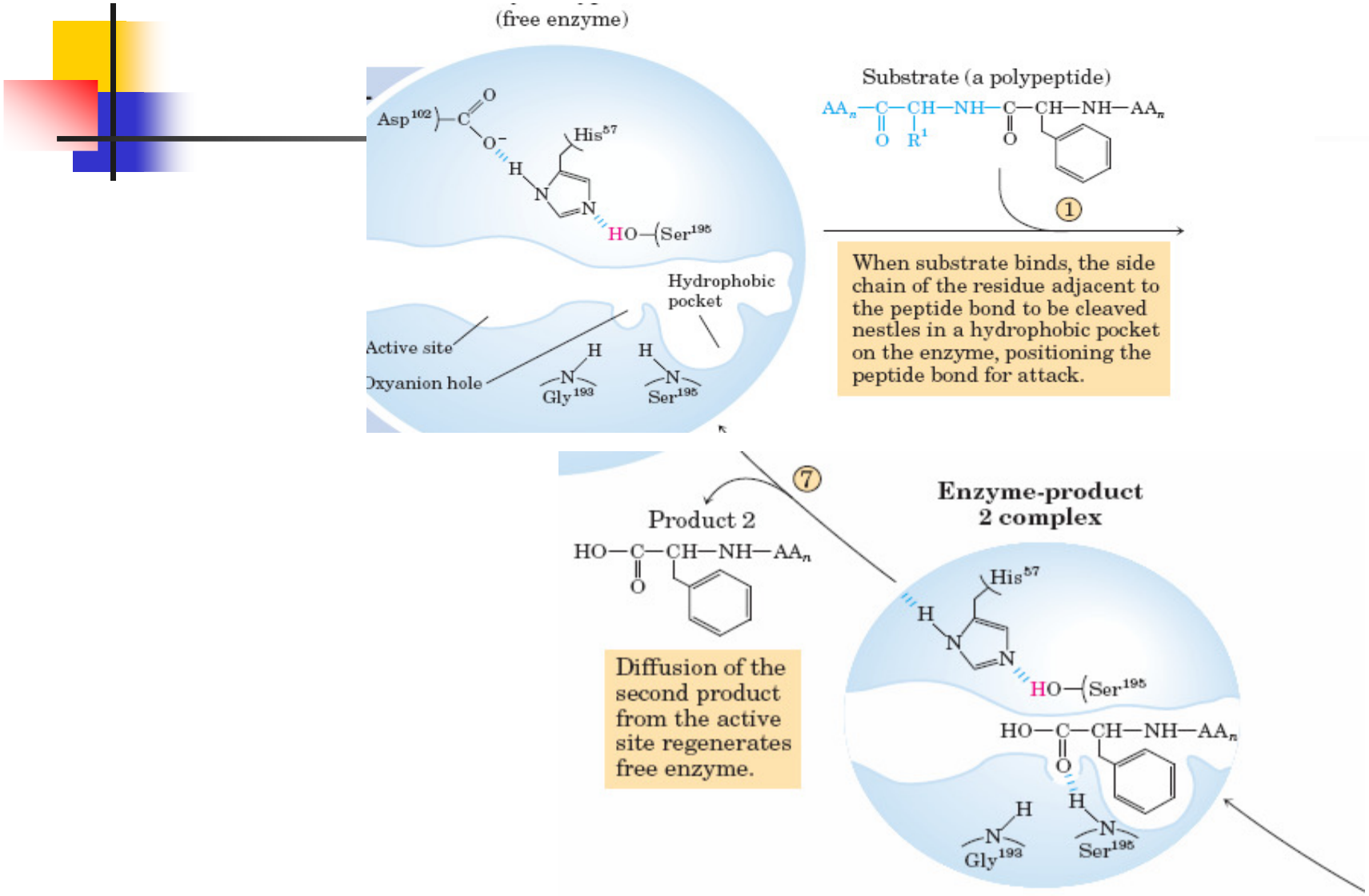




When substrate binds, the side chain of the residue adjacent to the peptide bond to be cleaved nestles in a hydrophobic pocket on the enzyme, positioning the peptide bond for attack.



MECHANISM FIGURE 6–21 **Hydrolytic cleavage of a peptide bond by chymotrypsin.** The reaction has two phases. In the acylation phase (steps 1 to 3), formation of a covalent acyl-enzyme intermediate is coupled to cleavage of the peptide bond. In the deacylation phase (steps 4 to 7), deacylation regenerates the free enzyme; this is essentially the reverse of the acylation phase, with water mirroring, in reverse, the role of the amine component of the substrate. Chymotrypsin Mechanism


(free enzyme)
Substrate (a polypeptide)
When substrate binds, the side chain of the residue adjacent to the peptide bond to be cleaved nestles in a hydrophobic pocket on the enzyme, positioning the peptide bond for attack.
Hydrophobic pocket
Active site
Oxyanion hole
Product 2
Diffusion of the second product from the active site regenerates free enzyme.
Enzyme-product 2 complex

Cink proteaze

- Pankreasna karboksipeptidaza A (1 atom Zn i Mr 34 kDa), egzopeptidaza, spec. Phe. Postoji kao u formi zimogena, (prokarboksipeptidaza) A koja se aktivira tripsinom, ali je takođe aktivna forma enzima.
- Karboksipeptidaza B, spec. Lys i Arg (Asp)
- Kolagenaza, angiotenzin-konvertujući enzim, termolizin, matriksin i leucin aminopeptidaza (heksamer).
- Jon cinka može da deluje kao elektrofil i polarizuje karbonilnu grupu, ali i kao izvor nukleofilnih hidroksilnih jona.

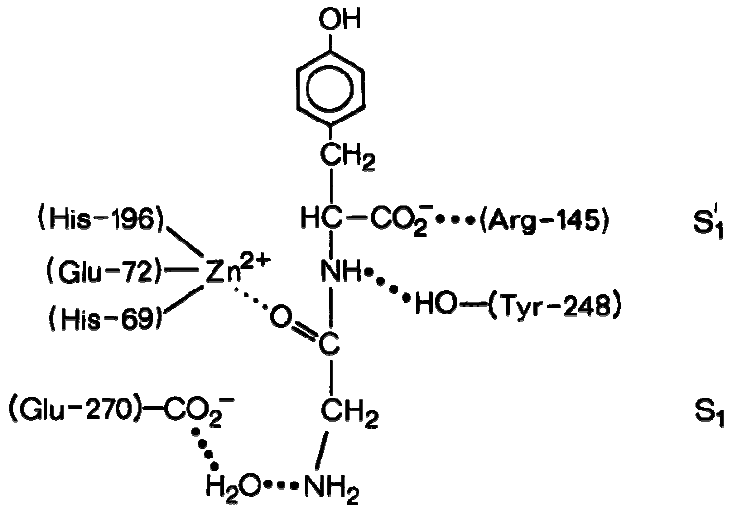


Figure 16.6 The postulated productively bound complex of carboxypeptidase A and a polypeptide substrate. [Courtesy of W. N. Lipscomb.]

Mutant Tyr248Phe ne pokazuje izmenjenu katalitičku aktivnost

Mehanizam dejstva karboksipeptidaze

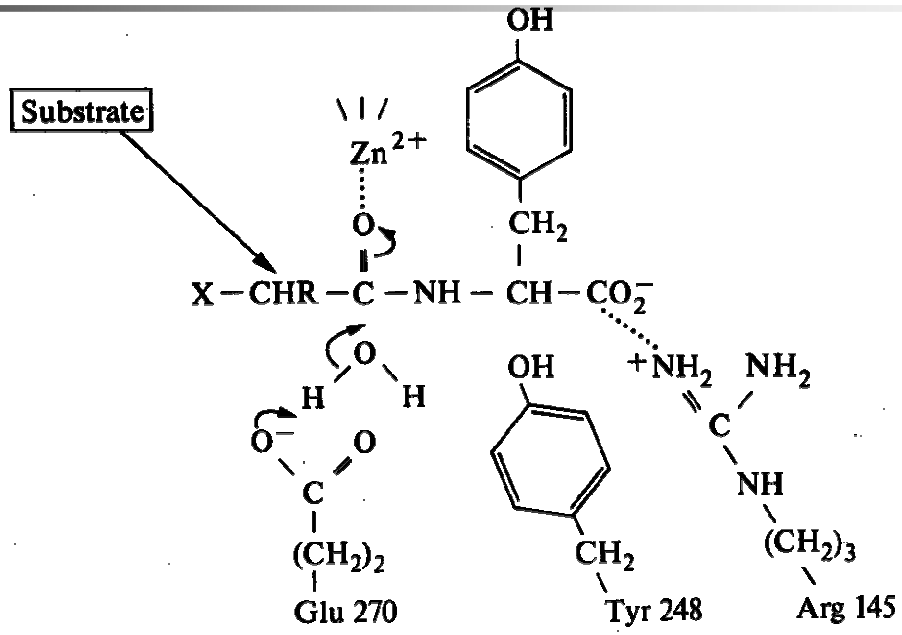
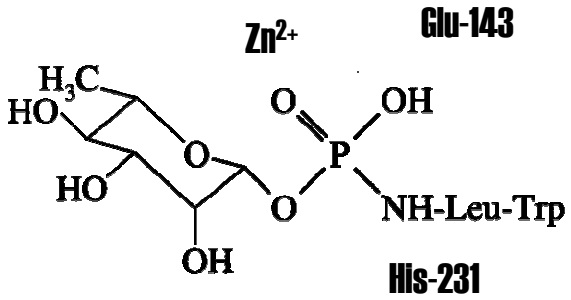


Fig. 5.21. Part of the proposed mechanism of action of carboxypeptidase.[56] An alternative mechanism involves direct attack of the side chain of Glu 270 on the carbonyl group of the substrate.

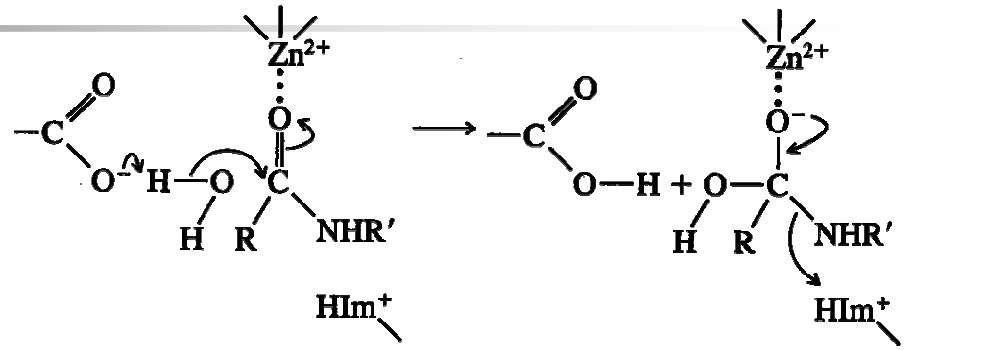
Termolizin: Glu 143 je lociran dublje u aktivnom mestu I vezan za molekul vode: Umesto Tyr-248, postoji His-231

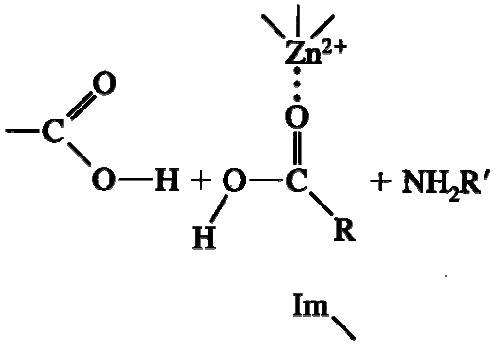
Endopeptidaza: širok usek umesto dubokog džepa egzopeptidaza za koji se vezuje supstrat.

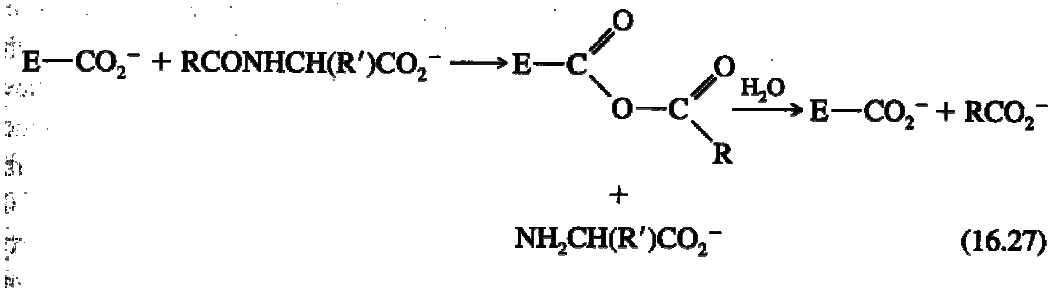
Struktura aktivnog mesta termolizina



Fosforamidon (Inh)







Glu-143 može da reaguje kao nukleofil i moguće je alkilovati α-hloroketonskim ireverzibilinim inhibitorima.

Karboksil (aspartil) proteaze

-Pepsin, katepsin D, himozin, renin

-Mr oko 35 kD

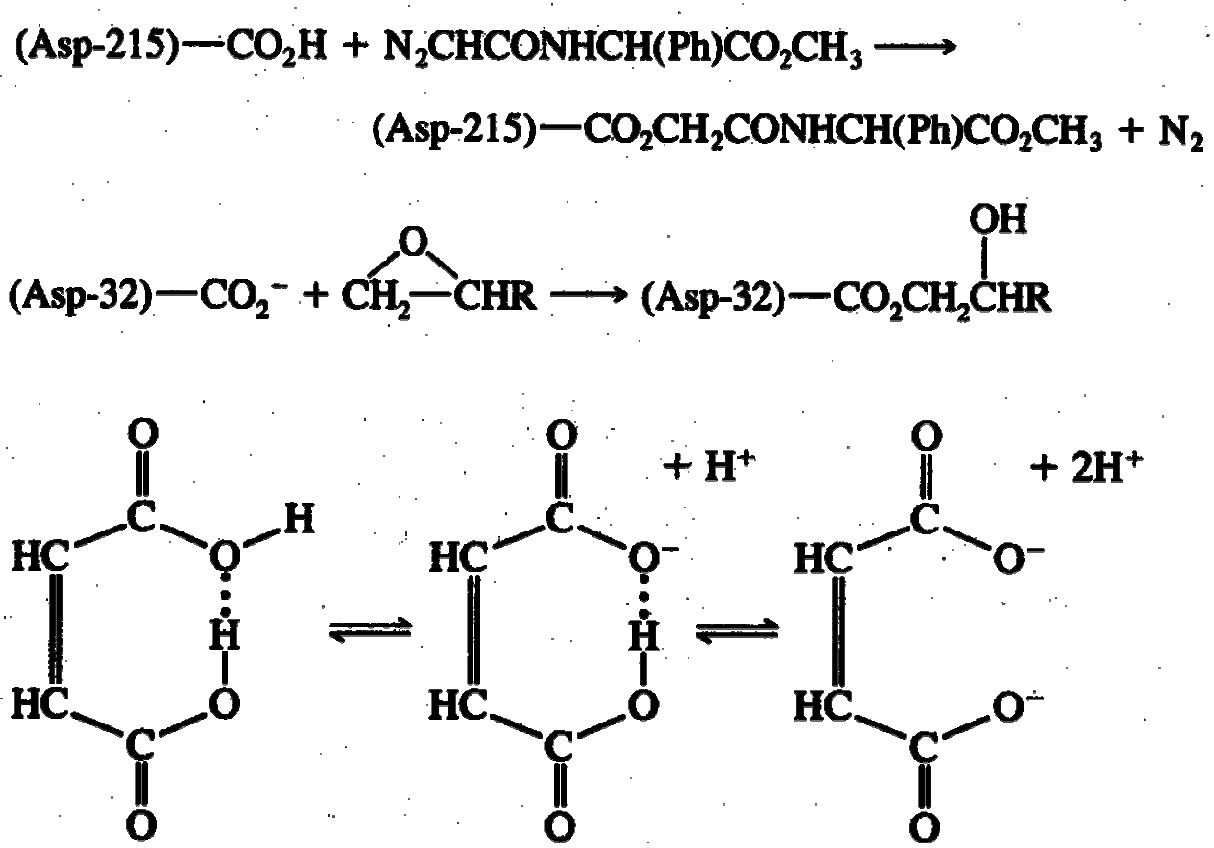
-Retrovirusi (HIV, target za AIDS terapiju)

-Ne postoji jednostavan hemijski model mehanizma dejstva ove familije enzima.

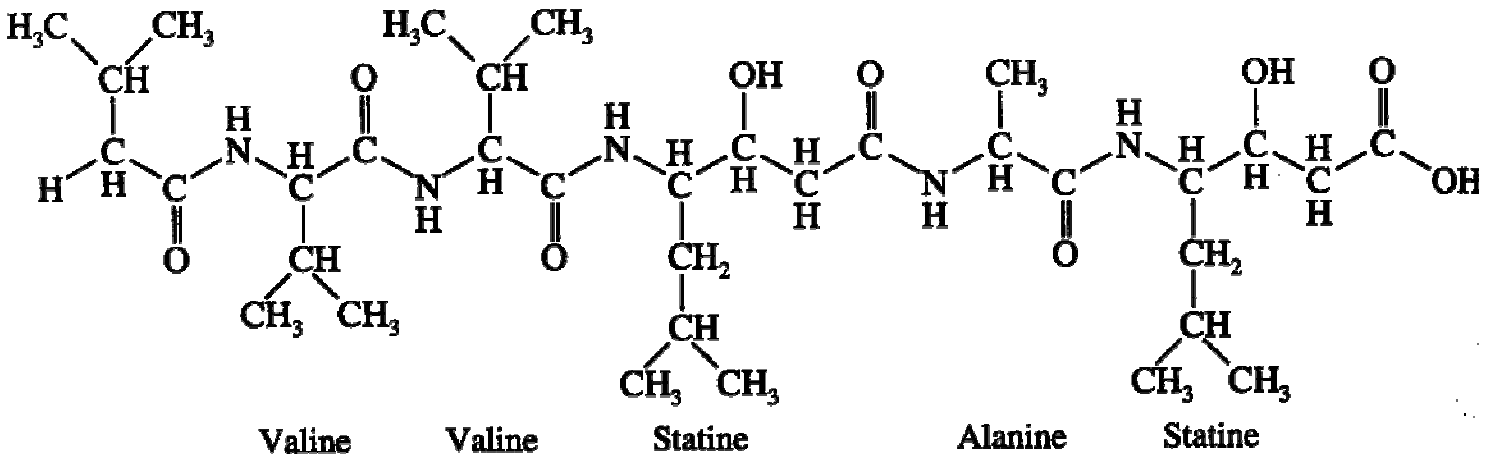
-Pepsin: Ser-68 fosforilovan, nema katalitičku funkciju

-Aktivno mesto može da vezuje i do 7 bočnih ostataka supstrata (pref. hidrofobne amino-kiseline)

Pepsin


(Asp-215)—CO2H + N2CHCONHCH(Ph)CO2CH3 ⟶
(Asp-215)—CO2CH2CONHCH(Ph)CO2CH3 + N2
(Asp-32)—CO2− + CH2—CHR ⟶ (Asp-32)—CO2CH2CHR
OH
+ H+
+ 2H+

Struktura inhibitora - pepstatina



Zimogen pepsina se aktivira intra (pH ispod 2) i intermolekulskom reakcijom

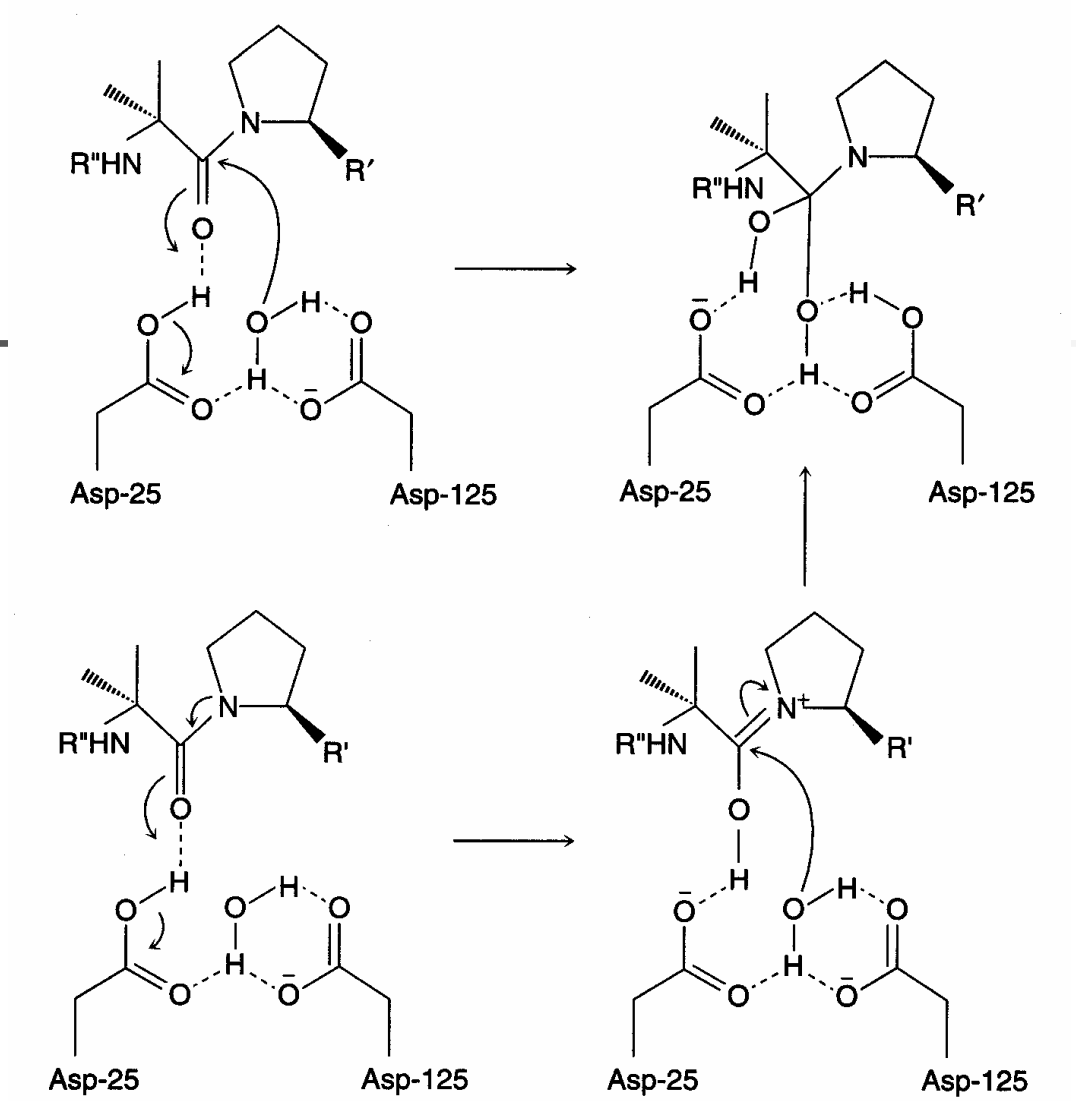
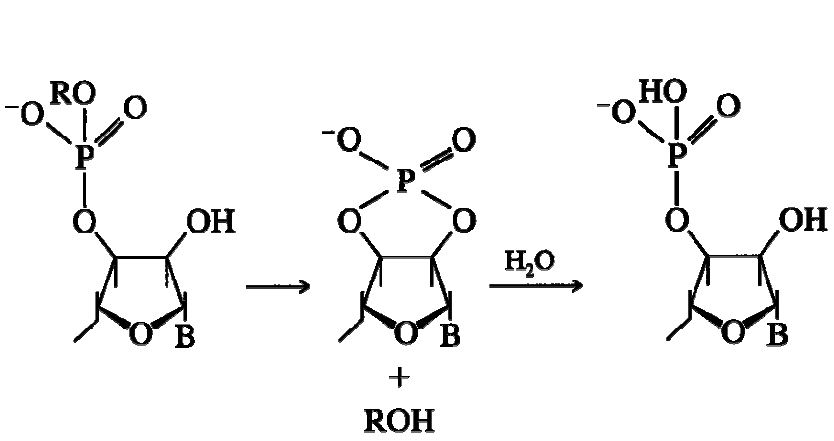
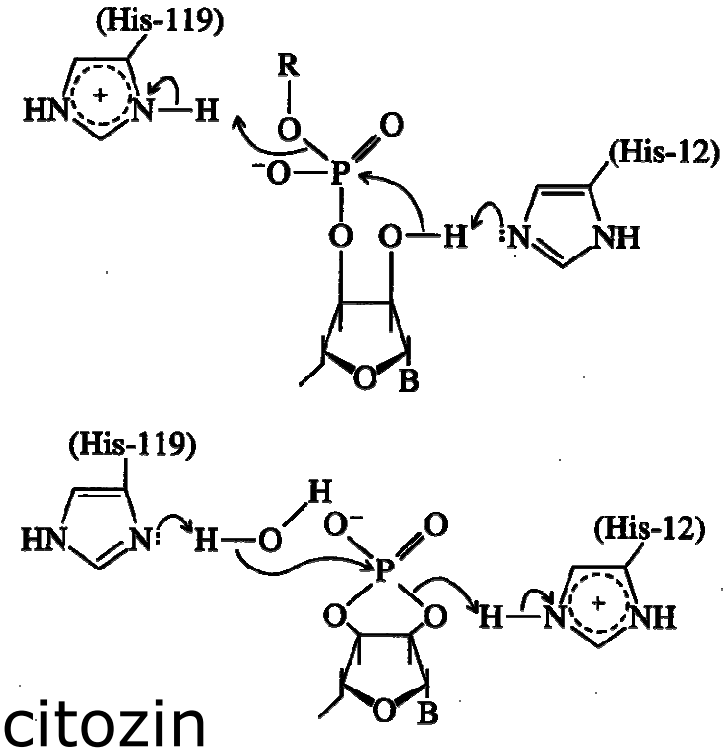


Figure 16.7 Mechanism of aspartyl proteases involving general acid-base catalysis and the formation of a protonated terahedral intermediate. *Bottom:* Proposal by T. J. Rodriguez. T. A. Angeles, and T. D. Meek, *Biochemistry* **32**, 12380 (1993), that the first step is peptide bond isomerization. This accounts for the observed *inverse* $^{15}N/^{14}N$ kinetic isotope effect, which implies that bonding with the N atom becomes stiffer in the transition state.

Ribonukleaza



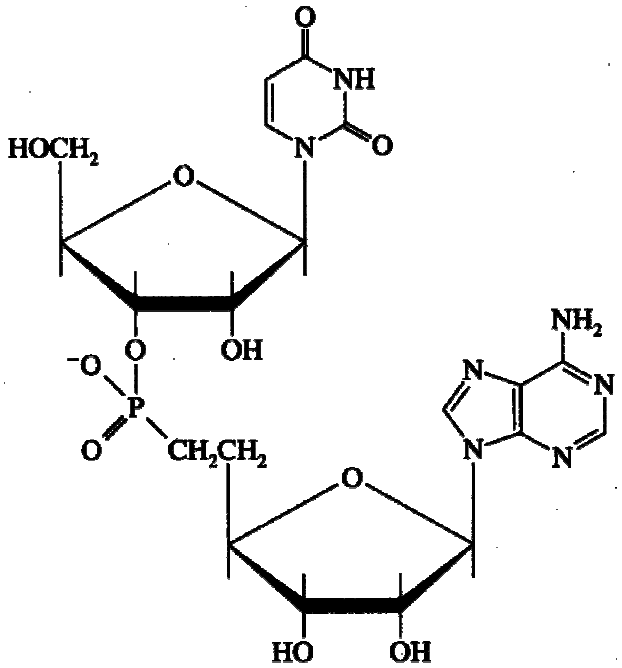


Baza u polozaju B: uracil ili citozin

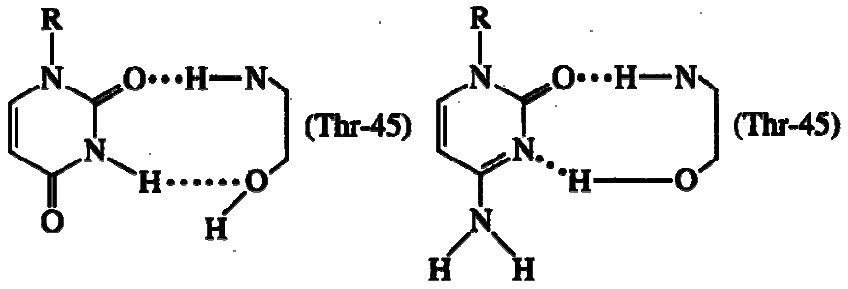
Ribonukleaza

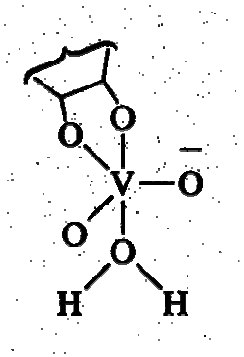
Strukture ribonukleaze A & njenih kompleksa

Stereospecifičnost prema pirimidinima (na 3' kraju)



Analog supstrata (UpcA) - 3D struktura





Uridin vanadat

Primarna struktura lizozima

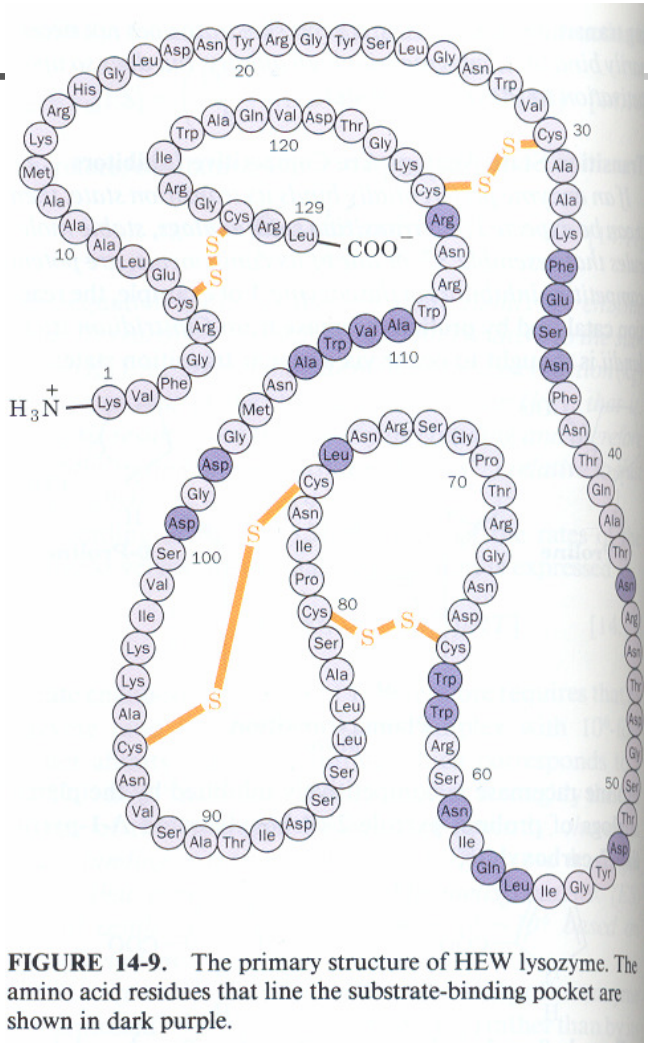


FIGURE 14-9. The primary structure of HEW lysozyme. The amino acid residues that line the substrate-binding pocket are shown in dark purple.

Struktura HEW lizozima

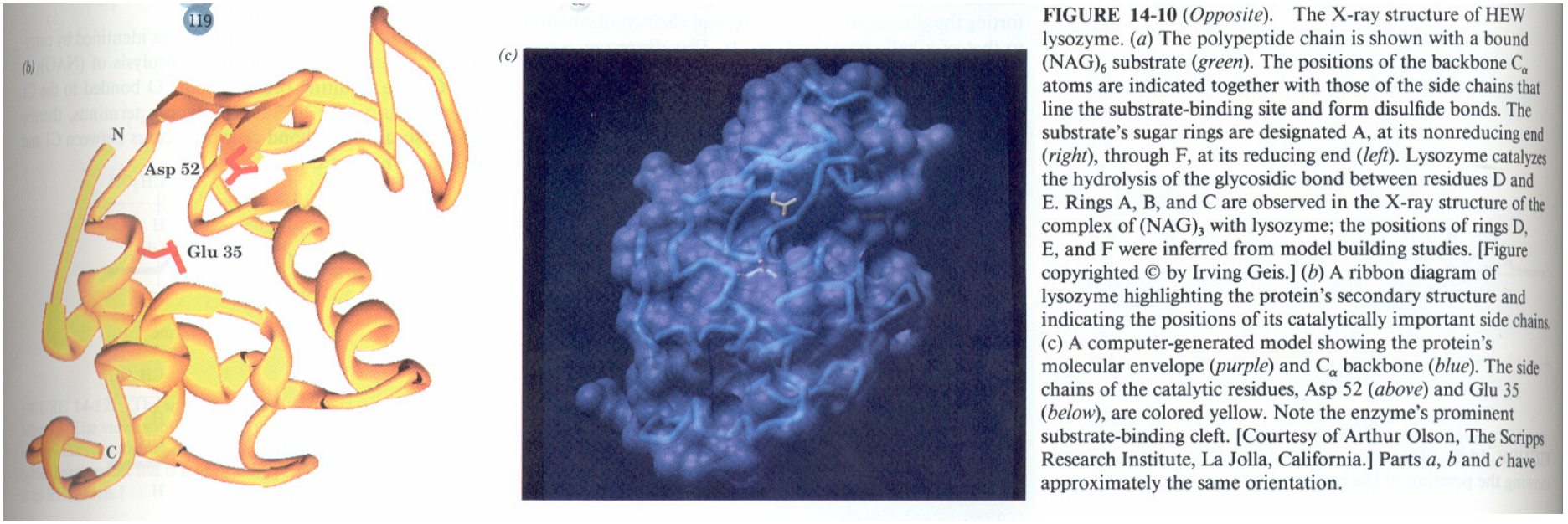


FIGURE 14-10 (*Opposite*). The X-ray structure of HEW lysozyme. (*a*) The polypeptide chain is shown with a bound $(NAG)_6$ substrate (*green*). The positions of the backbone C_α atoms are indicated together with those of the side chains that line the substrate-binding site and form disulfide bonds. The substrate's sugar rings are designated A, at its nonreducing end (*right*), through F, at its reducing end (*left*). Lysozyme catalyzes the hydrolysis of the glycosidic bond between residues D and E. Rings A, B, and C are observed in the X-ray structure of the complex of $(NAG)_3$ with lysozyme; the positions of rings D, E, and F were inferred from model building studies. [Figure copyrighted © by Irving Geis.] (*b*) A ribbon diagram of lysozyme highlighting the protein's secondary structure and indicating the positions of its catalytically important side chains. (c) A computer-generated model showing the protein's molecular envelope (*purple*) and C_α backbone (*blue*). The side chains of the catalytic residues, Asp 52 (*above*) and Glu 35 (*below*), are colored yellow. Note the enzyme's prominent substrate-binding cleft. [Courtesy of Arthur Olson, The Scripps Research Institute, La Jolla, California.] Parts *a*, *b* and *c* have approximately the same orientation.

Supstrat za lizozim

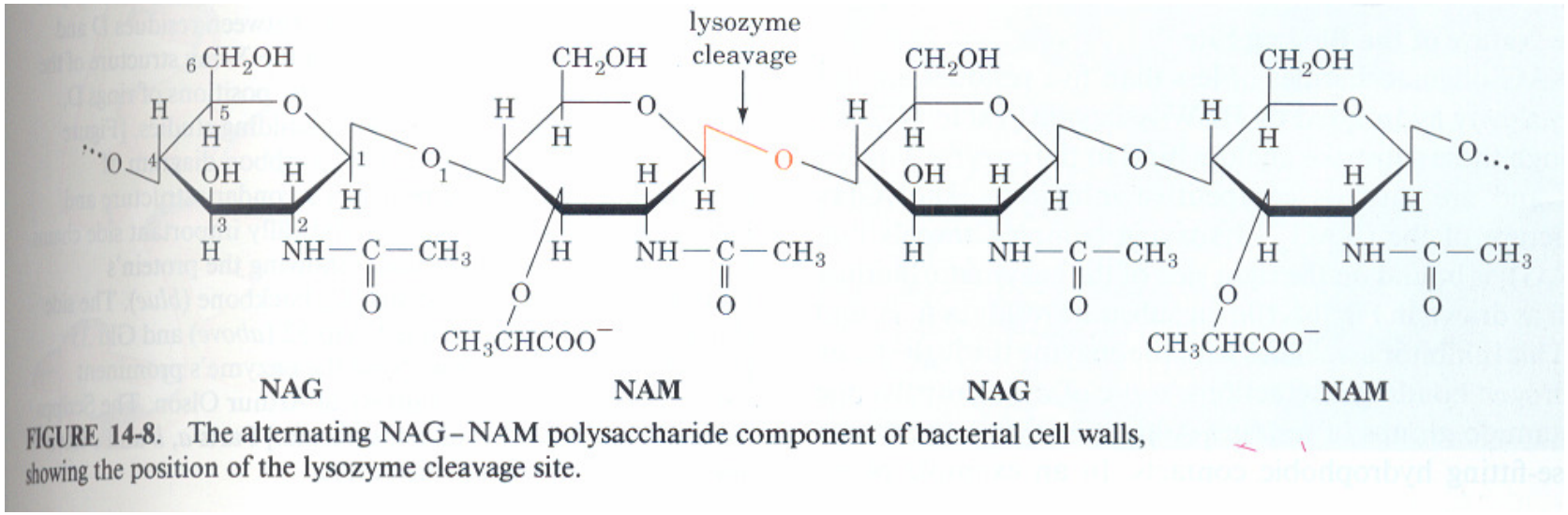
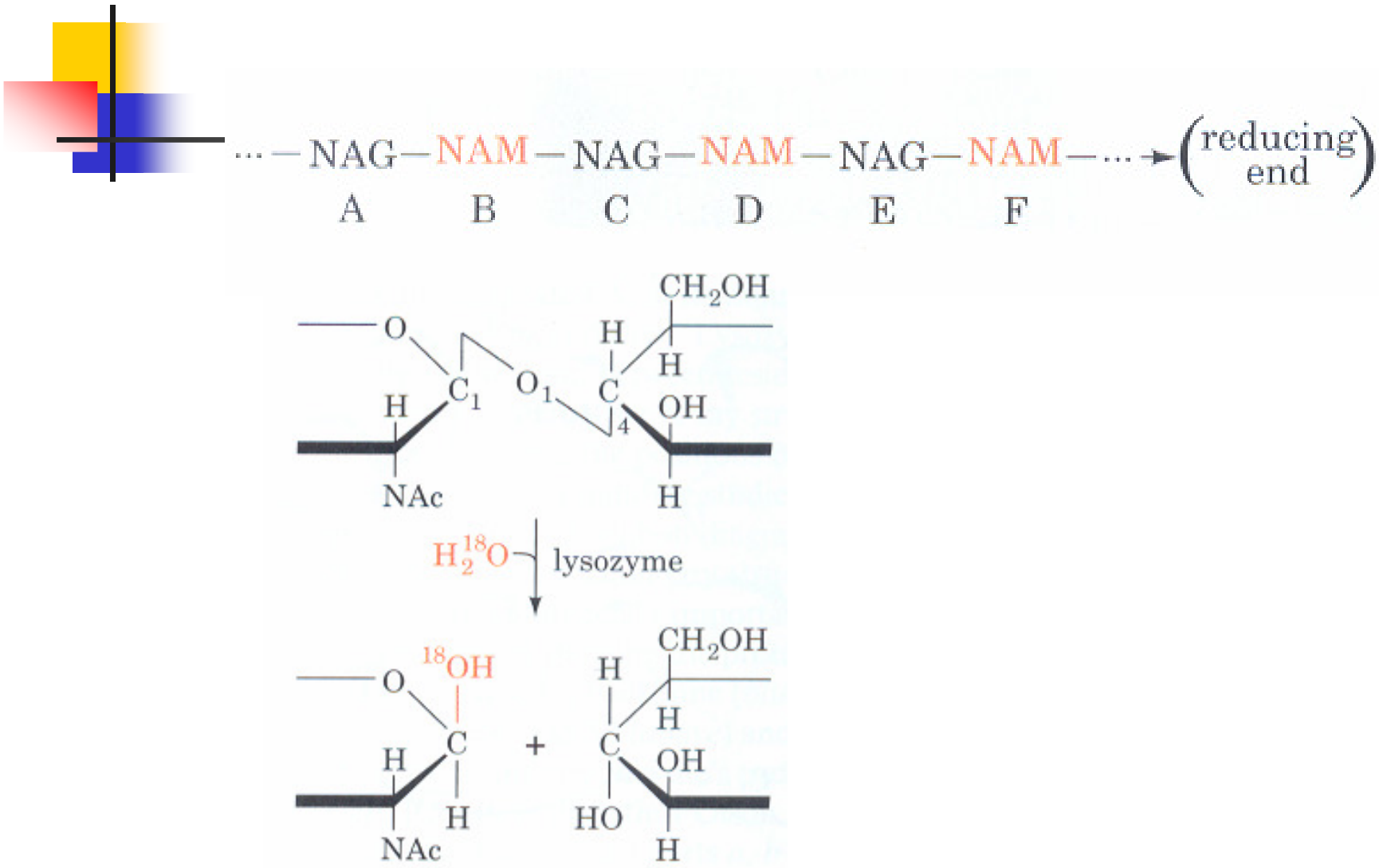


FIGURE 14-8. The alternating NAG–NAM polysaccharide component of bacterial cell walls, showing the position of the lysozyme cleavage site.

TABLE 14-2. RATES OF HEW LYSOZYME-CATALYZED HYDROLYSIS OF SELECTED OLIGOSACCHARIDE SUBSTRATE ANALOGS

Compound	$k_{cat}(s^{-1})$
$(NAG)_2$	2.5×10^{-8}
$(NAG)_3$	8.3×10^{-6}
$(NAG)_4$	6.6×10^{-5}
$(NAG)_5$	0.033
$(NAG)_6$	0.25
$(NAG - NAM)_3$	0.5

Source: Imoto, T., Johnson, L.N., North, A.C.T., Phillips, D.C., and Rupley, J.A., *in* Boyer, P.D. (Ed.), *The Enzymes* (3rd ed.), Vol. 7, *p.* 842, Academic Press (1972).


··· — NAG — NAM — NAG — NAM — NAG — NAM — ···
A B C D E F
reducing end
CH2OH
O
H
H
C1
O1
C
H
OH
4
NAc
H
H2 18O
lysozyme
18OH
O
C
H
H
NAc
+
CH2OH
H
H
C
OH
HO
H

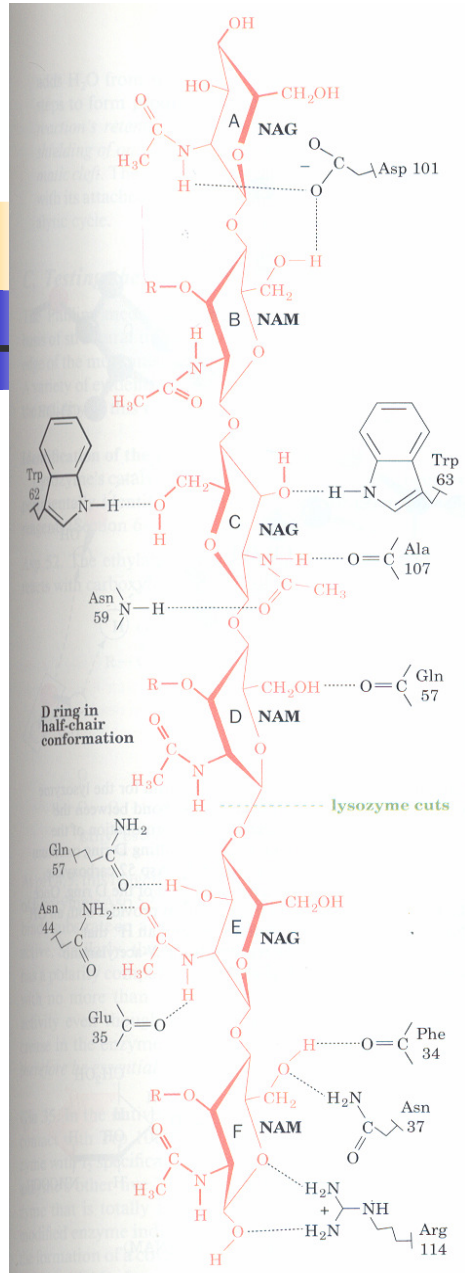


FIGURE 14-12. The interactions of lysozyme with its substrate. The view is into the binding cleft with the heavier edges of the rings facing the outside of the enzyme and the lighter ones against the bottom of the cleft. [Figure copyrighted © by Irving Geis.]

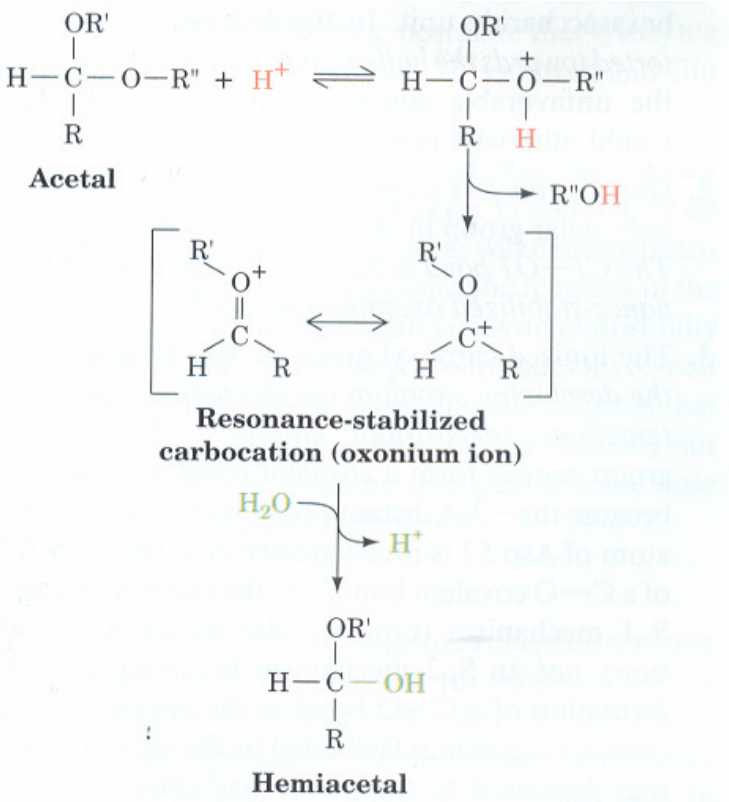


FIGURE 14-13. The mechanism of the nonenzymatic acid-catalyzed hydrolysis of an acetal to a hemiacetal. The reaction involves the protonation of one of the acetal's oxygen atoms followed by cleavage of its C—O bond to form an alcohol (R″OH) and a resonance-stabilized carbocation (oxonium ion). The addition of water to the oxonium ion forms the hemiacetal and regenerates the H^+ catalyst. Note that the oxonium ion's C, O, H, R, and R′ atoms all lie in the same plane.

Katalitički mehanizam

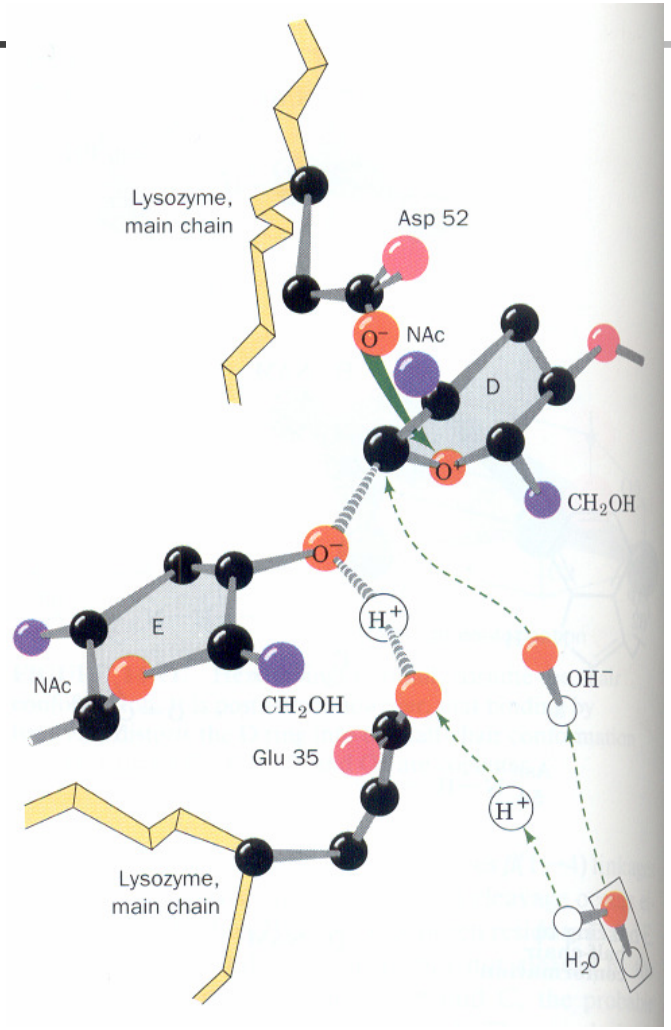


FIGURE 14-14. The Phillips mechanism for the lysozyme reaction. The cleavage of the glycosidic bond between the substrate D and E rings occurs through protonation of the bridge oxygen atom by Glu 35. The resulting D-ring oxonium ion is stabilized by the proximity of the Asp 52 carboxylate group and the enzyme-induced distortion of the D ring. Once the E ring is released, H_2O from solution provides both an OH^- that combines with the oxonium ion and an H^+ that reprotonates Glu 35. NAc represents the *N*-acetylamino substituent at C2 of each glucose ring.

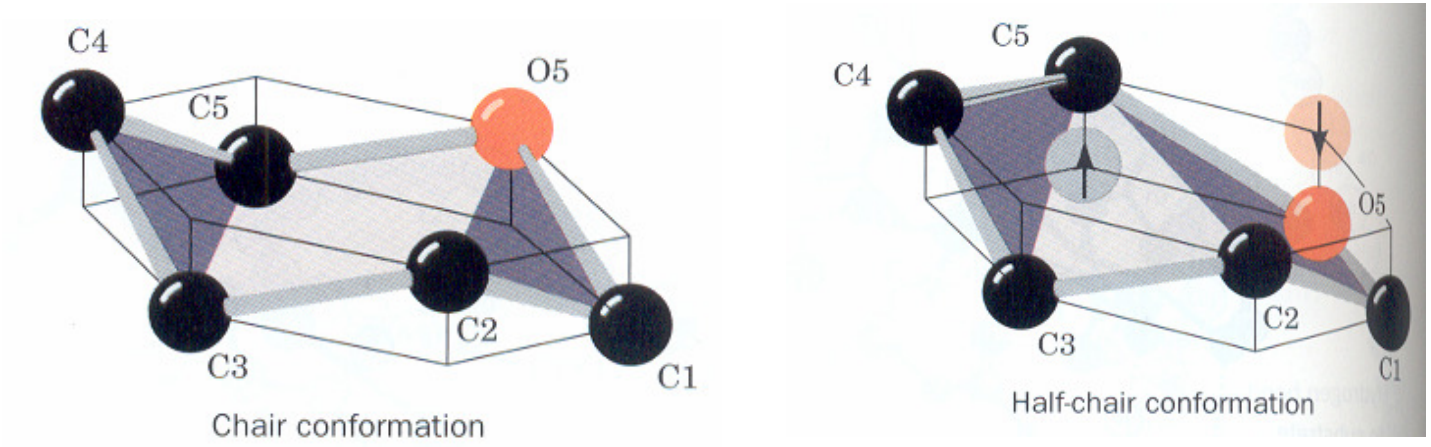


FIGURE 14-11. Hexose rings normally assume the chair conformation. It is postulated, however, that binding by lysozyme distorts the D ring into the half-chair conformation such that atoms C1, C2, C5, and O5 are coplanar.

Oksokarbenijum jon

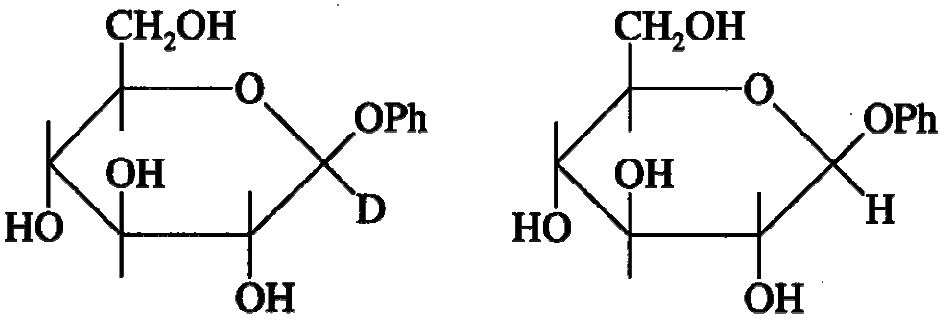


TABLE 14-3. BINDING FREE ENERGIES OF HEW LYSOZOME SUBSITES

Site	Bound Saccharide	Binding Free Energy ($kJ \cdot mol^{-1}$)
A	NAG	−7.5
B	NAM	−12.3
C	NAG	−23.8
D	**NAM**	**+12.1**
E	NAG	−7.1
F	NAM	−7.1

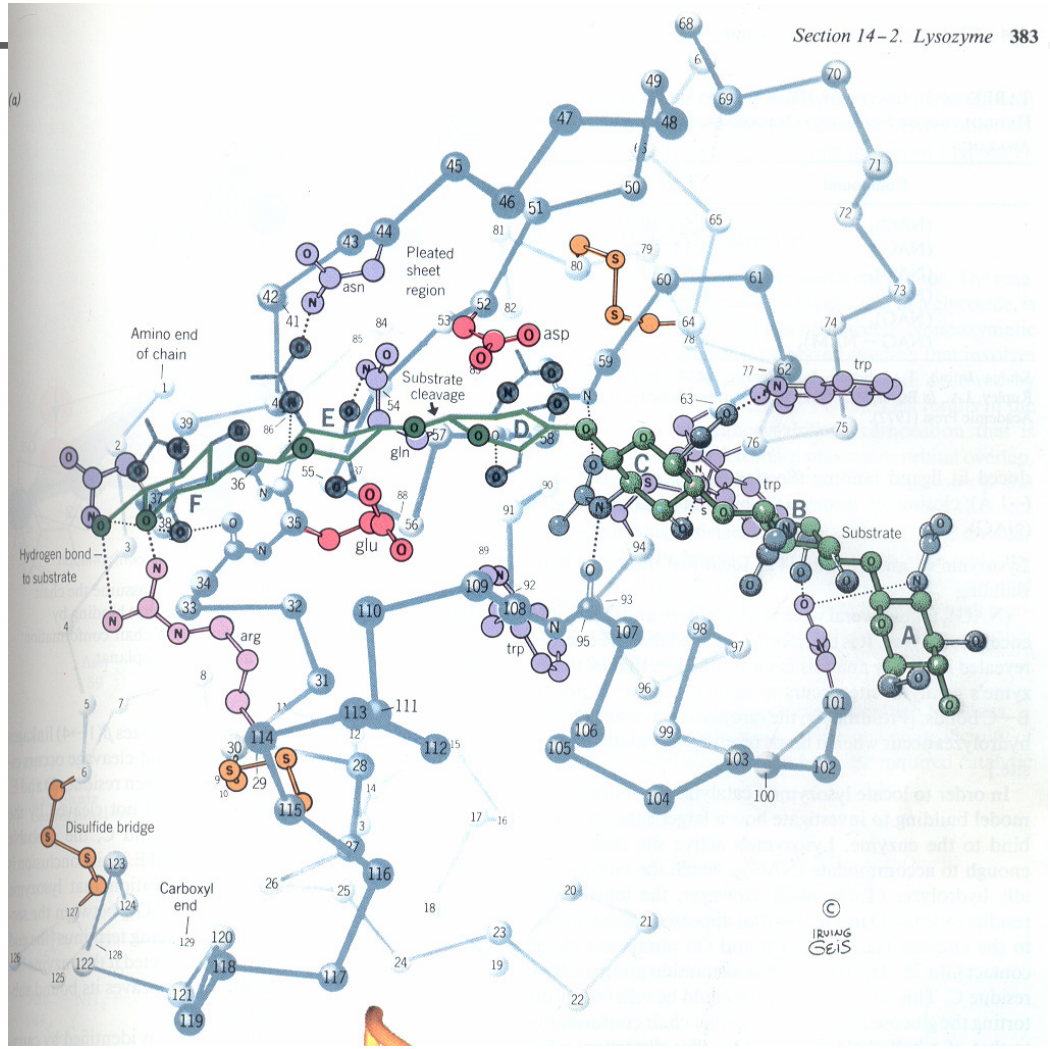
Source: Chipman, D.M. and Sharon, N., *Science* **165,** 459 (1969).

Table 16.4 *Binding energies of subsites in hen egg white lysozyme*[a]

Site	Residue binding[b]	Binding energy	
		kJ/mol	kcal/mol
A	NAG	−8	−2
B	NAG	−12	−3
	NAM	−16	−4
C	NAG	−20	−5
D	NAM	+12	+3
	NAG	0	0
E	NAG	−16	−4
F	NAG	−8	−2

[a] See also M. Schindler, Y. Assaf, N. Sharon, and D. M. Chipman, *Biochemistry* **16**, 423 (1977).
[b] NAG = *N*-acetylglucosamine; NAM = *N*-acetylmuramic acid.

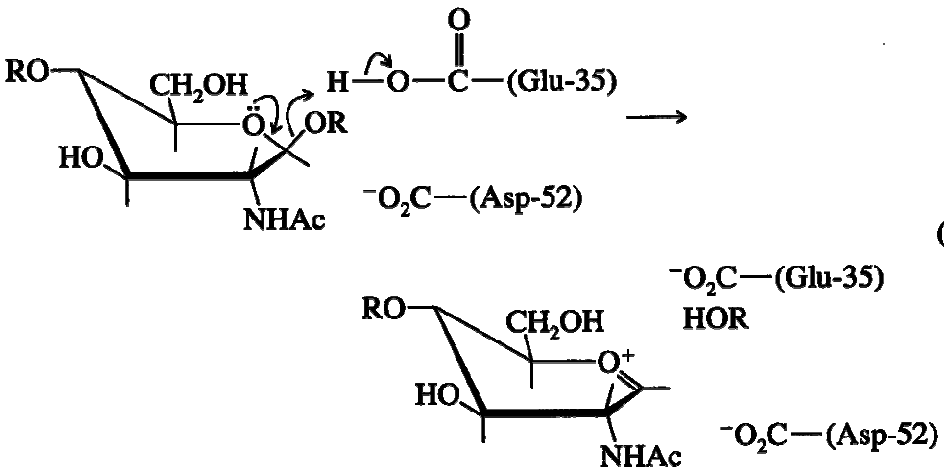
Struktura HEW lizozima sa vezanim supstratom



Lizozim

Postoji šest podmesta (A, B, C, D, E i F) za vezivanje glukopiranoznog prstena supstrata

- Veza koja se raskida (D/E) je blizu karboksilne grupe Asp 52
- Intermedijer je oksokarbenijum jon koji je stabilizovan Asp 52
- Alkohol se izdvaja nakon opšte kisele katalize Glu 35
- Šećerni ostatak u položaju D je u konformaciji polu –stolice
- Mali polisaharidi se vezuju za položaj A, B i C, izbegavajući napon u položaju D



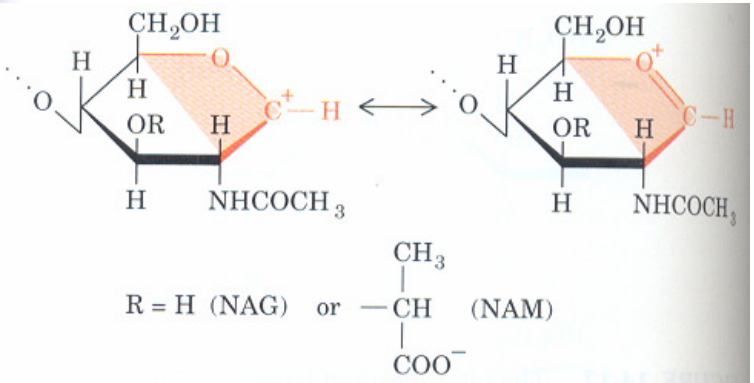
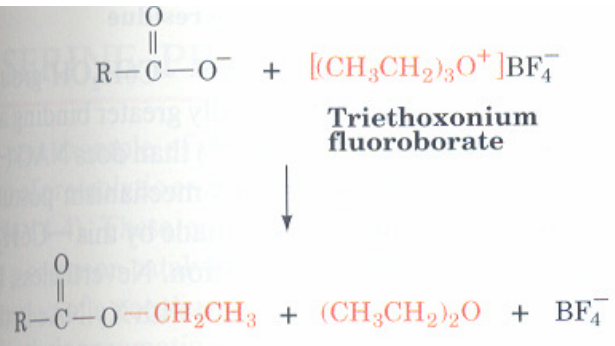


FIGURE 14-15. The oxonium ion transition state of the D ring in the lysozyme reaction is stabilized by resonance. This requires that atoms C1, C2, C5, and O5 be coplanar (*shading*); that is, the hexose ring must assume the half-chair conformation.



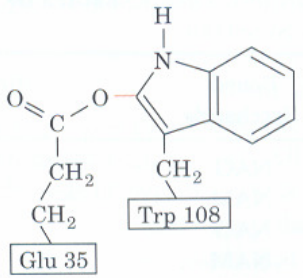


FIGURE 14-16. The I_2 oxidation of lysozyme results in the formation of a covalent bond between the side chains of Glu 35 and Trp 108.

Trietoksonijum fluoroborat reaguje sa Asp 52 i inaktivira enzim
Mutacija Asp52Asn inaktivira enzima

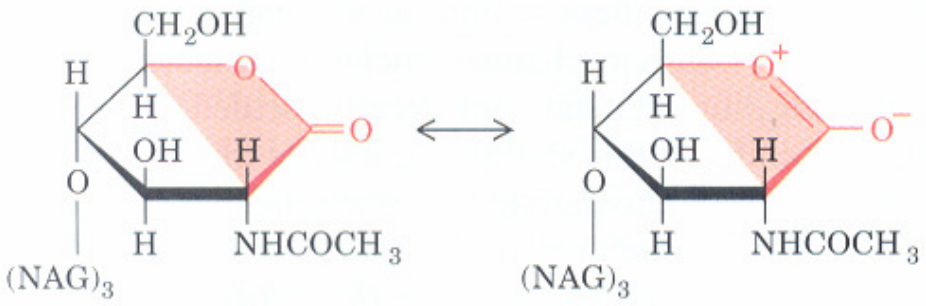
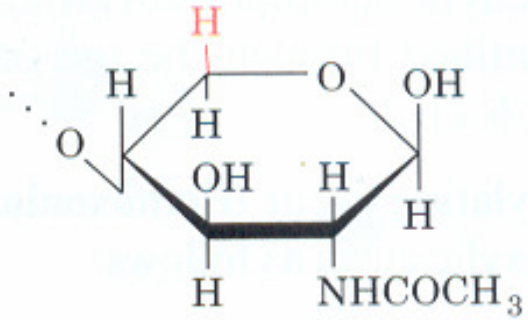
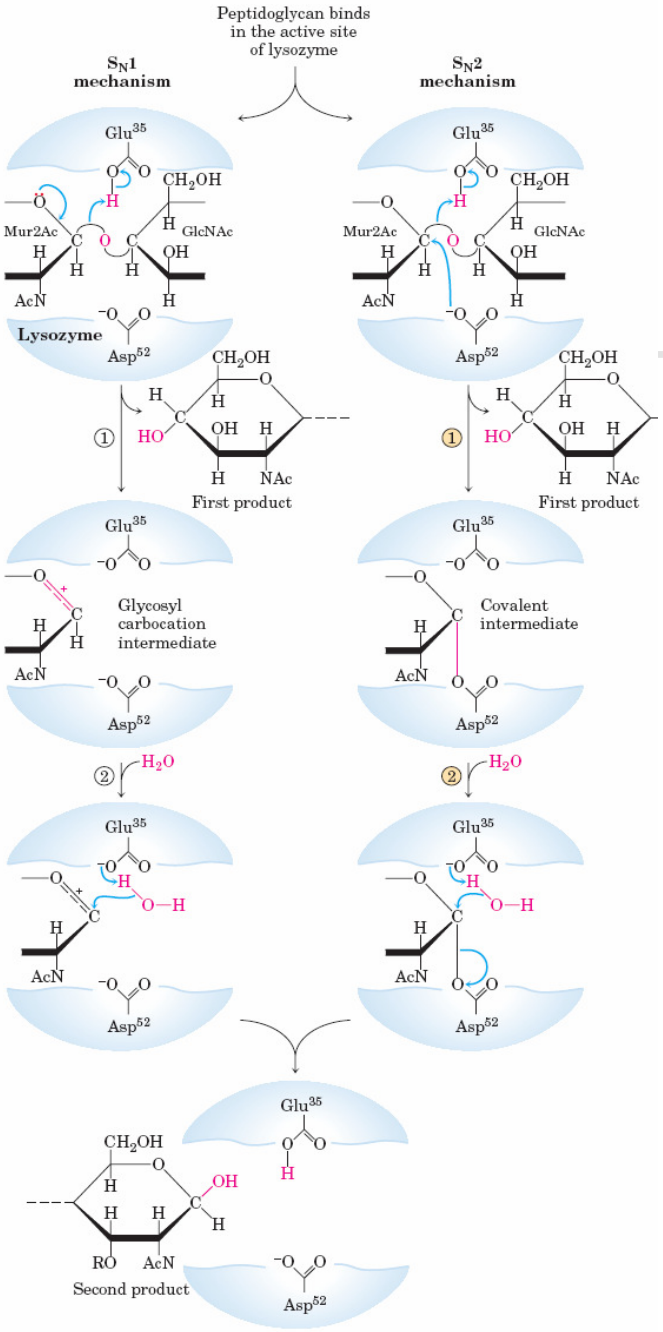


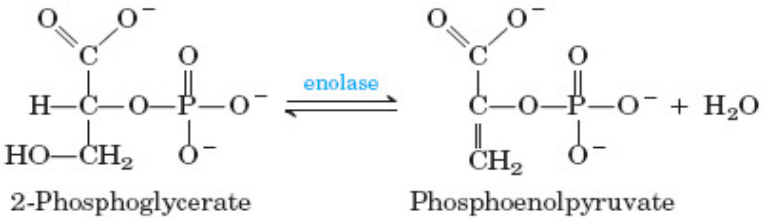
FIGURE 14-17. The δ-lactone analog of $(NAG)_4$. Its C1, O1, C2, C5, and O5 atoms are coplanar (*shading*) because of resonance as is the D ring in the transition state of the lysozyme reaction (compare with Fig. 14-15).

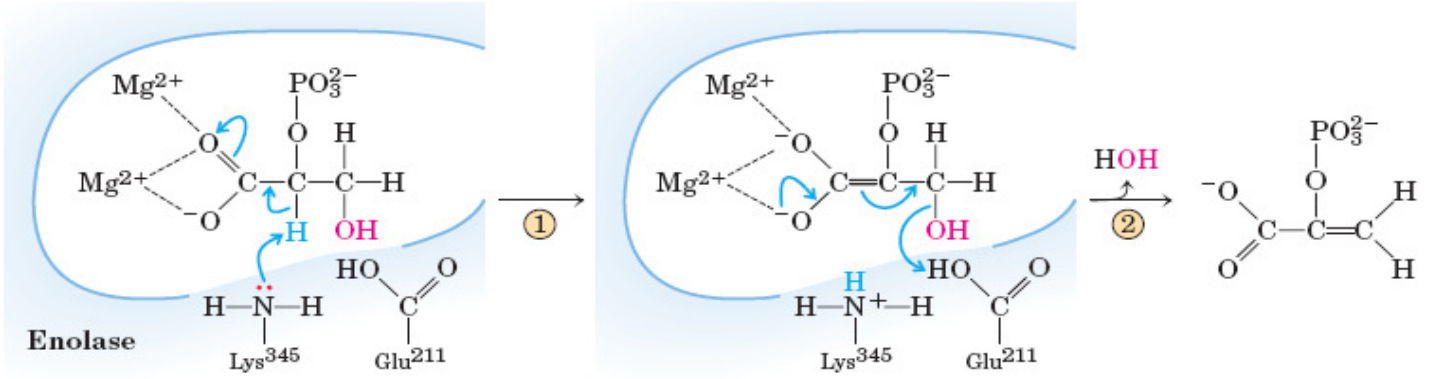


***N*-Acetylxylosamine residue**

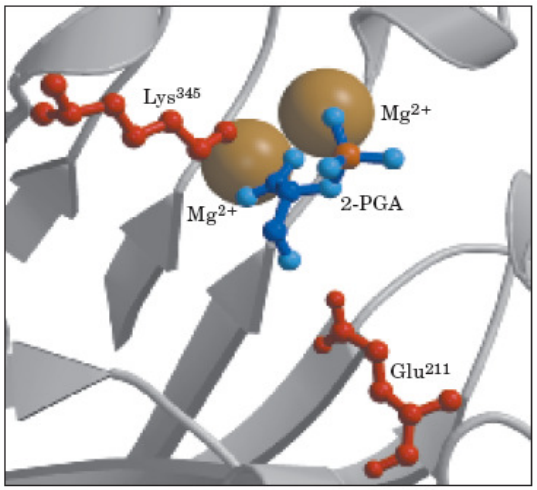

Peptidoglycan binds in the active site of lysozyme
S_N1 mechanism
S_N2 mechanism
Glu35
CH2OH
Mur2Ac
GlcNAc
AcN
Lysozyme
Asp52
First product
Glycosyl carbocation intermediate
Covalent intermediate
H2O
Second product
(a)

Enolaza





(a) 2-Phosphoglycerate bound to enzyme — Enolic intermediate — Phosphoenolpyruvate

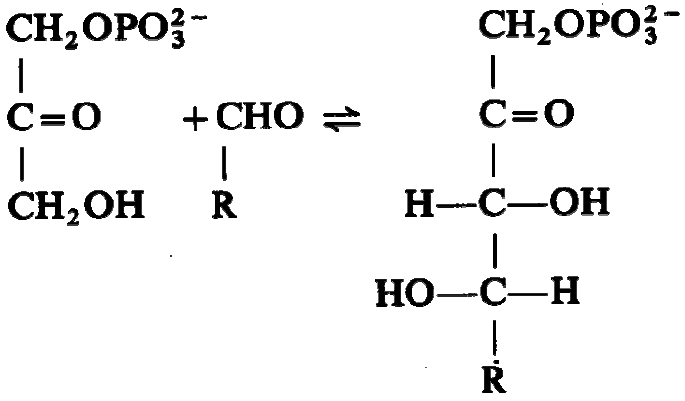


MECHANISM FIGURE 6–23 **Two-step reaction catalyzed by enolase.** **(a)** The mechanism by which enolase converts 2-phosphoglycerate (2-PGA) to phosphoenolpyruvate. The carboxyl group of 2-PGA is coordinated by two magnesium ions at the active site. A proton is abstracted in step ① by general base catalysis (Lys^{345}), and the resulting enolic intermediate is stabilized by the two Mg^{2+} ions. Elimination of the —OH in step ② is facilitated by general acid catalysis (Glu^{211}). **(b)** The substrate, 2-PGA, in relation to the Mg^{2+} ions, Lys^{345}, and Glu^{211} in the enolase active site. Hydrogen atoms are not shown. All the oxygen atoms of 2-PGA are light blue; phosphorus is orange (PDB ID 1ONE).

Aldolaza

5.5.3 Fructose-bisphosphate aldolase

Fructose-bisphosphate aldolase catalyses the following aldol condensation reaction



Izoforme FBP aldolaze

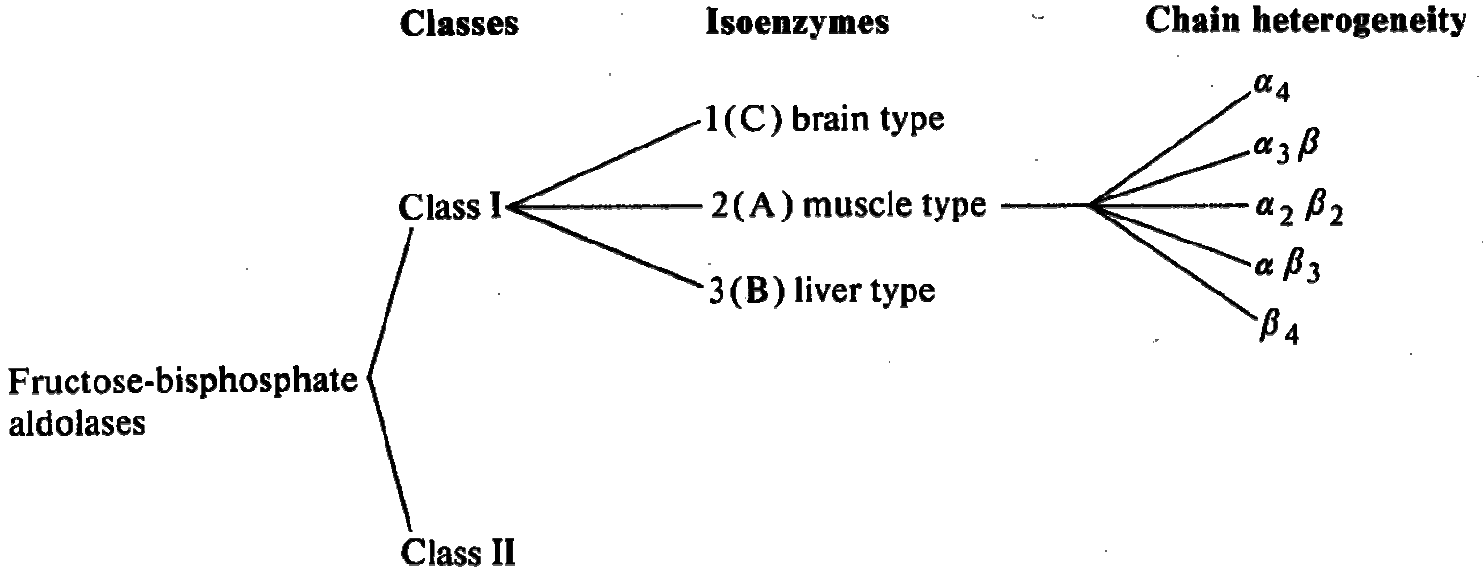


Fig. 5.29. The variety of forms in which fructose-bisphosphate aldolases occur.

Mehanizam dejstva FBP aldolaze

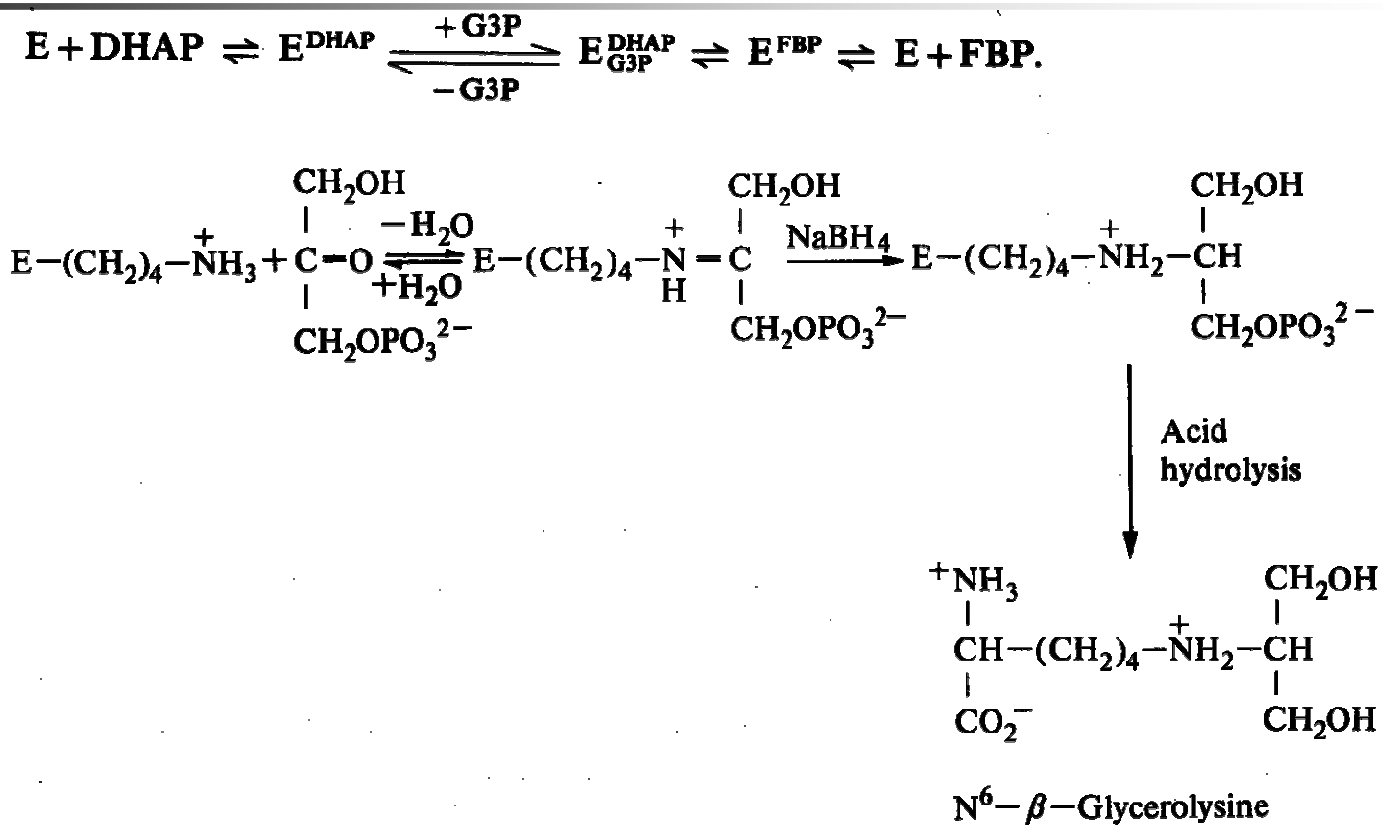


Fig. 5.30. Trapping of the Schiff base formed between fructose-bisphosphate aldolase and dihydroxyacetone phosphate.

Mehanizam dejstva FBP aldolaze

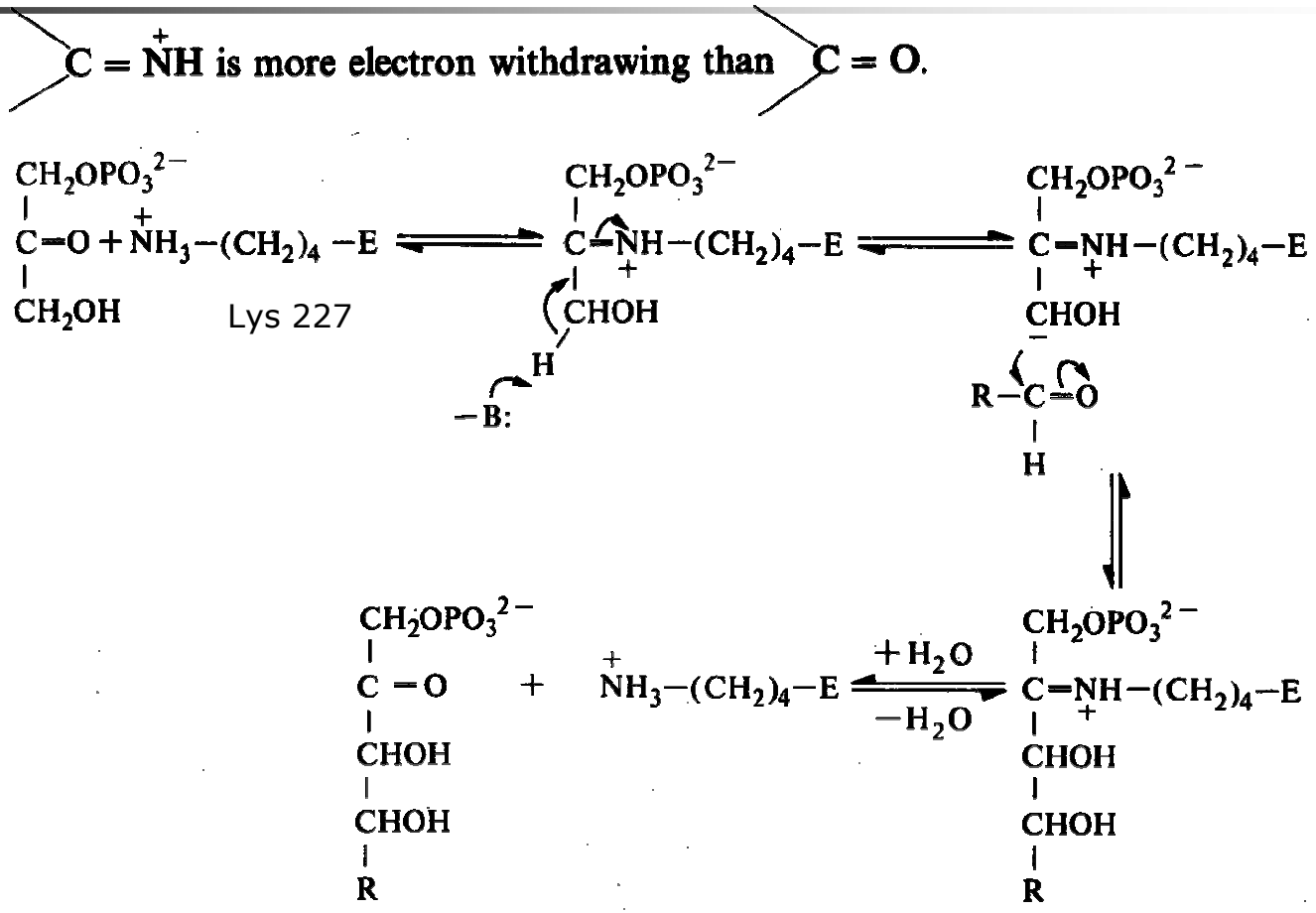


Fig. 5.31. The mechanism of fructose-bisphosphate aldolase proceeding via a protonated Schiff base.

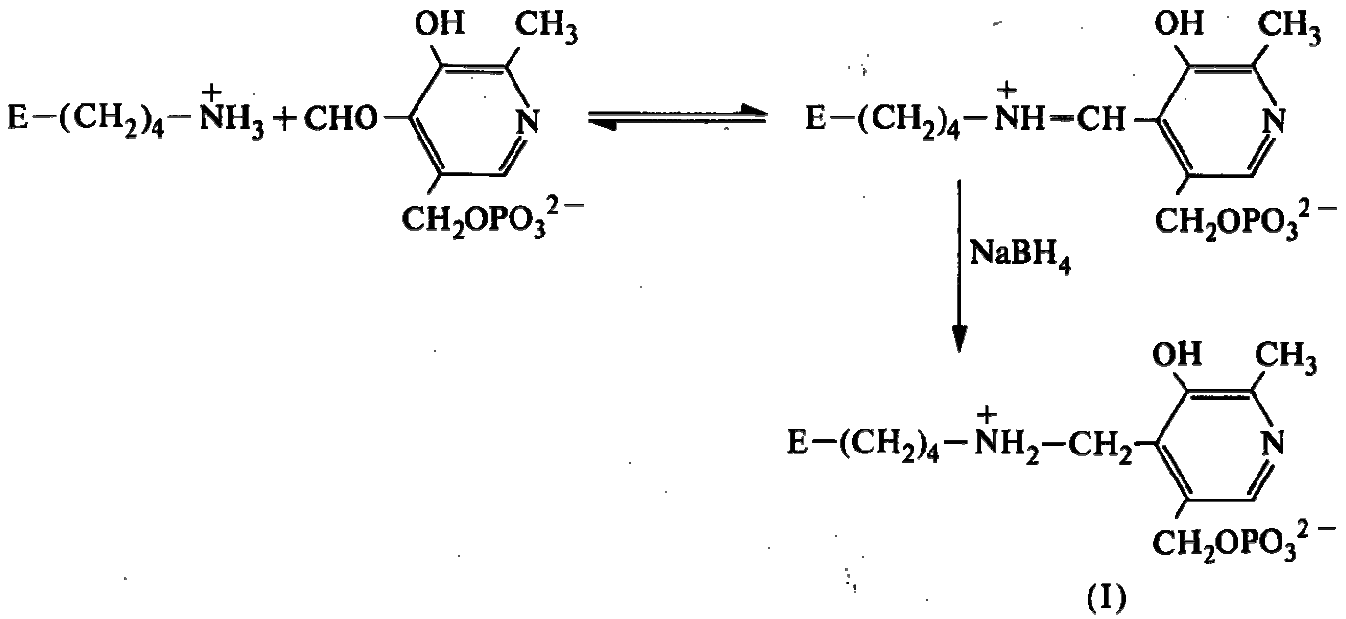


Fig. 5.32. Formation of a Schiff base by reaction of fructose-bisphosphate aldolase with pyridoxal phosphate.

Hemijske modifikacije histidina

His 359: transfer protona

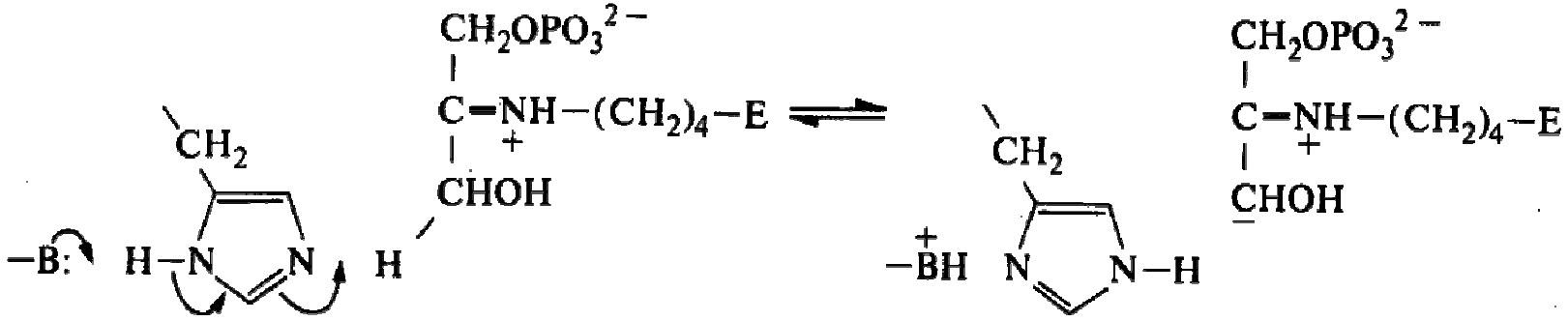


Fig. 5.33. Possible role of a histidine side chain in proton transfer reactions in the fructose-bisphosphate aldolase–dihydroxyacetone phosphate complex.

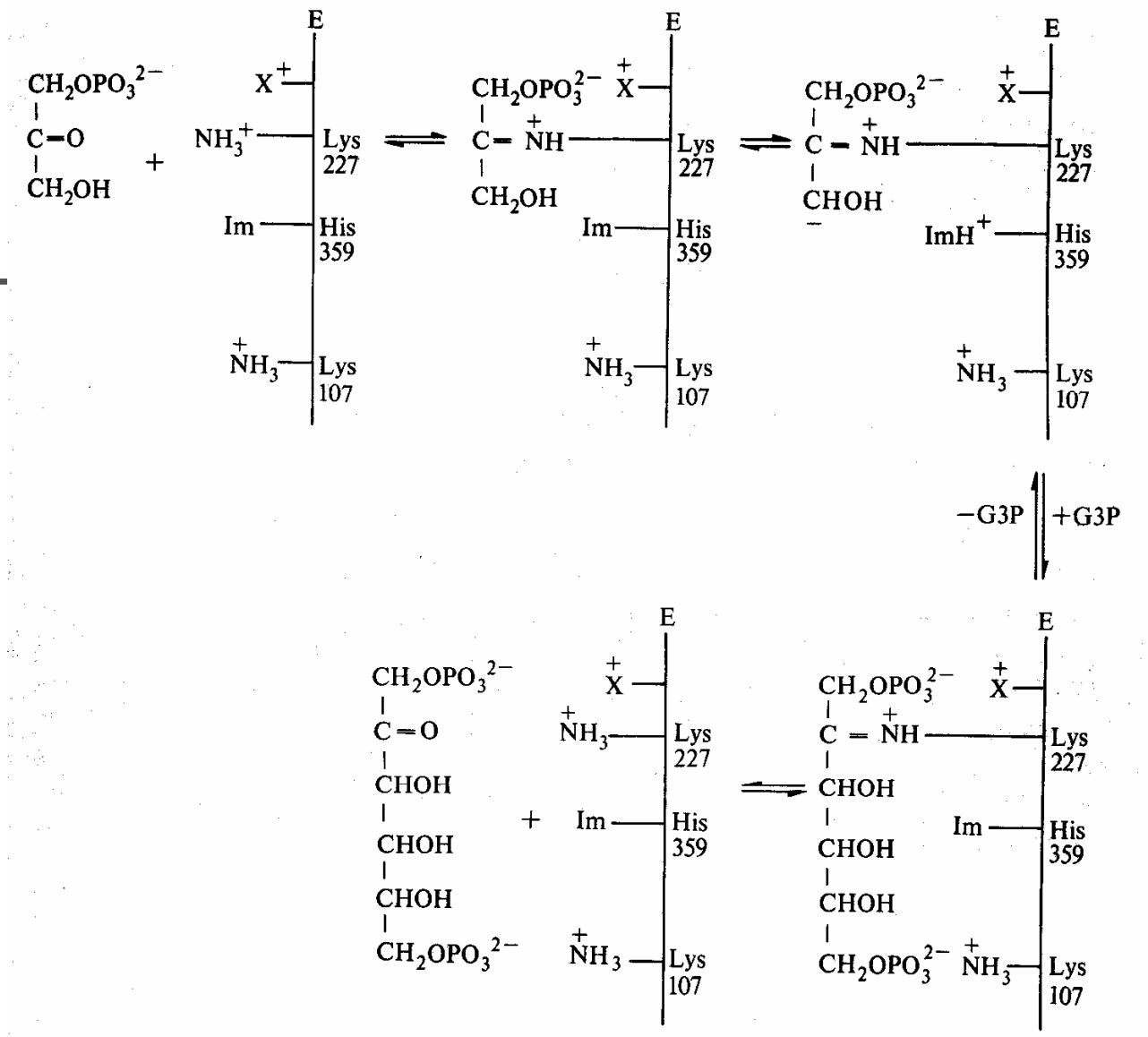
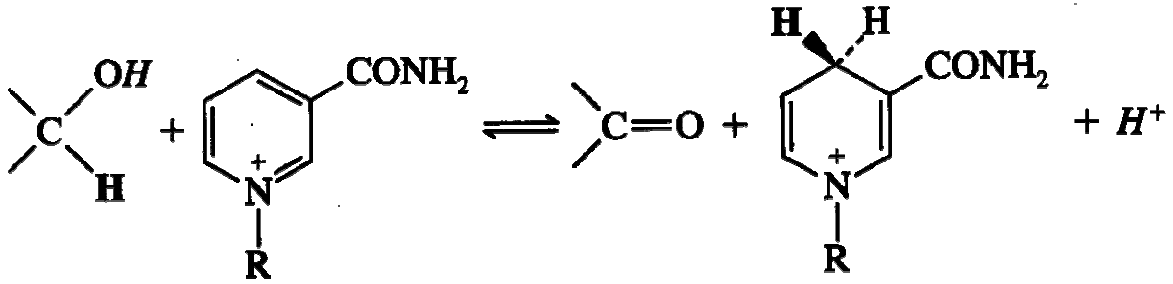


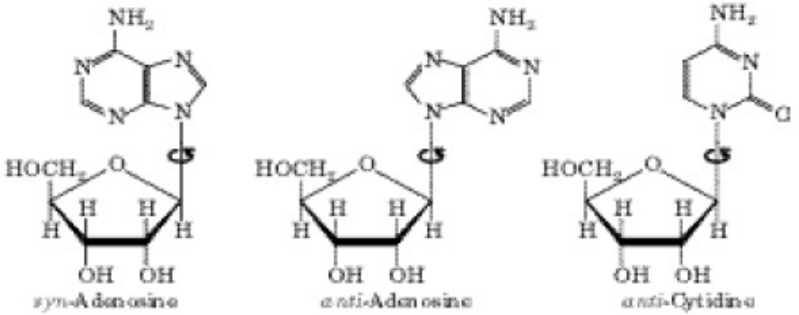
Fig. 5.34. The outline mechanism of fructose-bisphosphate aldolase. For the sake of clarity details of the side chains have been omitted, and Im is used as an abbreviation for imidazole. G3P is D-glyceraldehyde 3-phosphate.

Dehidrogenaza



NAD⁺ je vezan u **anti** konformaciji oko glikozidne veze u **klasi A**, dok je u **klasi B** vezan u **syn** konformaciji

Klasa A katalizuje redukciju reaktivnijih karbonila.



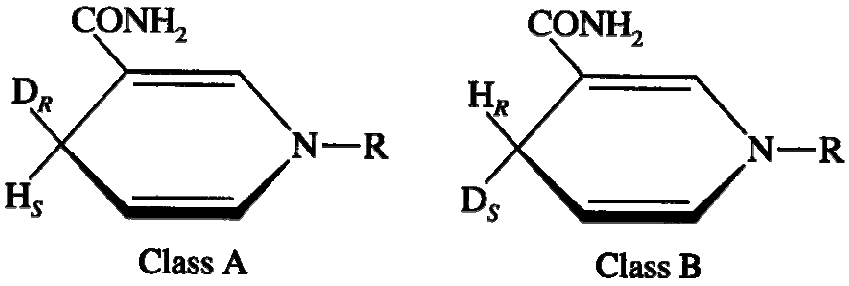


Table 16.1 *The coenzyme specificity of some dehydrogenases*

Dehydrogenase	Coenzyme required	Stereospecificity class
Glutamate	NAD^+ or $NADP^+$	B
Glucose 6-phosphate	$NADP^+$	B
3-Glycerol phosphate	NAD^+	B
Glyceraldehyde 3-phosphate	NAD^+	B
Malate (soluble)	NAD^+	A
Alcohol	NAD^+	A
Lactate	NAD^+	A
Isocitrate	$NADP^+$	A

Struktura NAD^+ vezujućeg mesta u dehidrogenazama

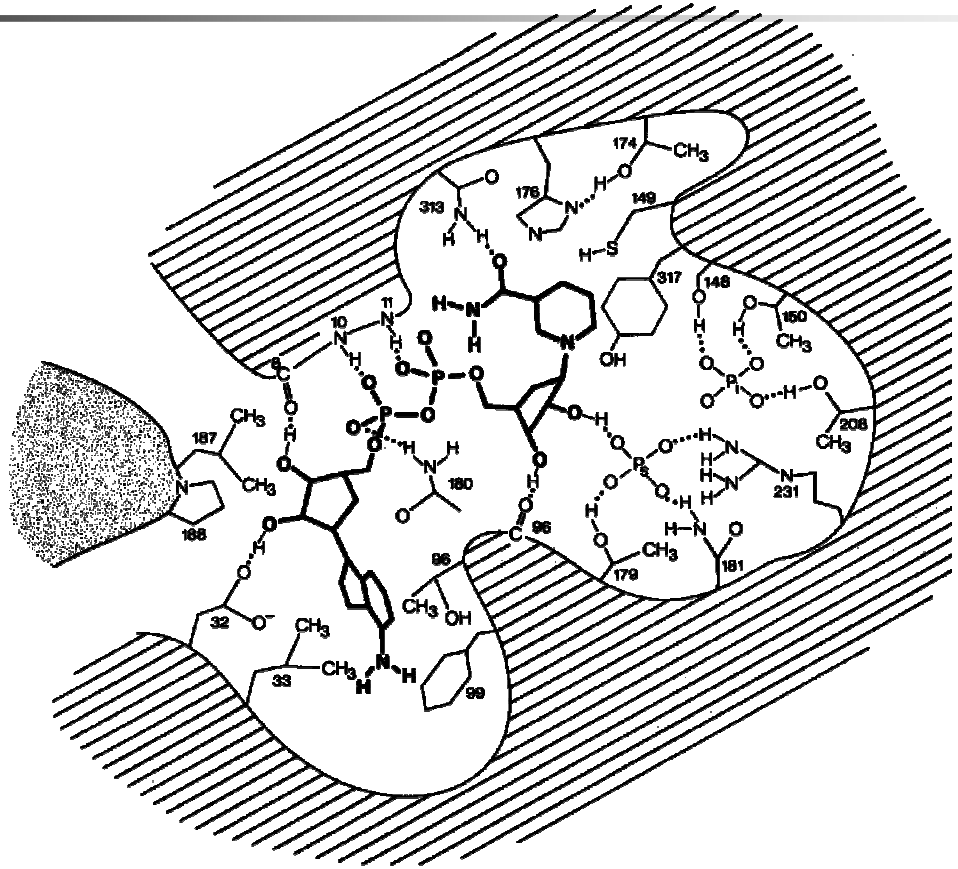


Figure 16.1 The binding of NAD^+ to glyceraldehyde 3-phosphate dehydrogenas from *Bacillus stearothermophilus*. [From G. Biesecker, J. I. Harris, J. C. Thierry, J. E. Wa and A. J. Wonacott, *Nature, Lond.* **266**, 328 (1977).]

Interakcije u vezivanju NAD+ za vezujuće mesto u dehidrogenazama

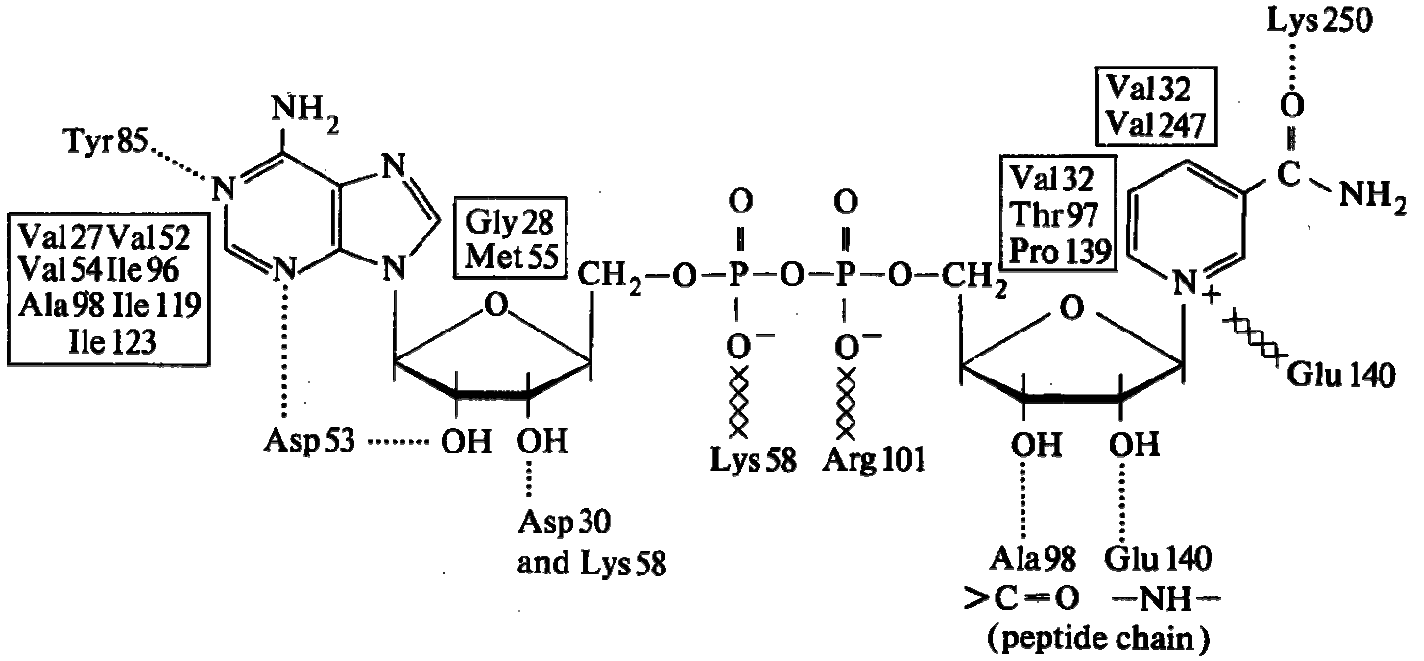
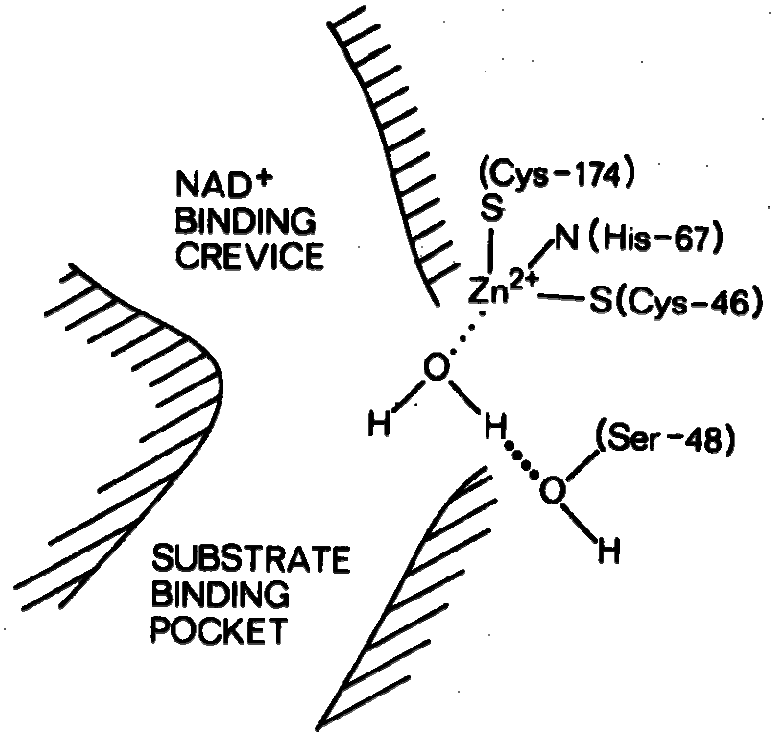


Fig. 5.39. Some of the interactions involved in the binding of NAD^+ to lactate dehydrogenase.[84] The dotted lines represent hydrogen bonds, the crosses electrostatic interactions and the boxes hydrophobic interactions.

Struktura aktivnog mesta alkohol dehidrogenaze

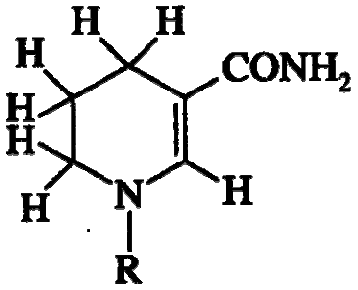
- Metaloenzimi (Zn)
- Struktura horse liver alkohol dehidrogenaze: simetričan dimer, Mr jedne podjedinice 40 kDa, vezuje 1 NAD^+ i dva jona Zn. Dve forme: E i S (EE, ES, SS izozimi)
- Struktura alkohol dehidrogenaze iz kvasca: tetramer, Mr 145 kDa, svaki lanac vezuje jedan NAD^+ i jedan jon Zn^{2+}.



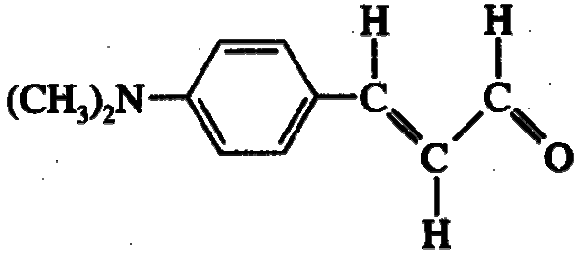
16.2 Sketch of the active site of horse liver alcohol dehydrogenase.

Apoenzim ima funkcionalnu grupu sa pKa 9.2, dok je u holoenzimu ova grupa sa pKa 7.6

Struktura ES (ternarnog) kompleksa

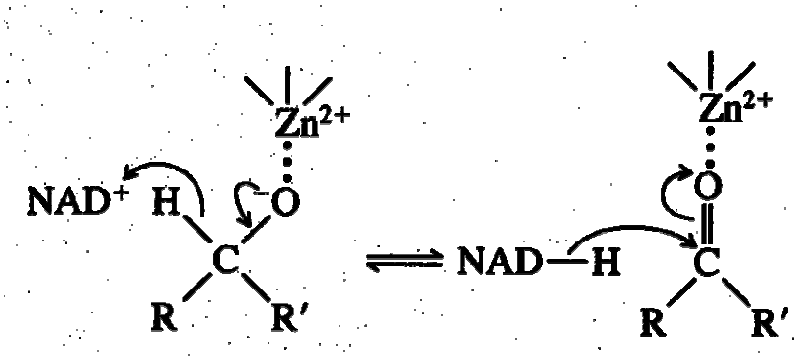


H_2NADH

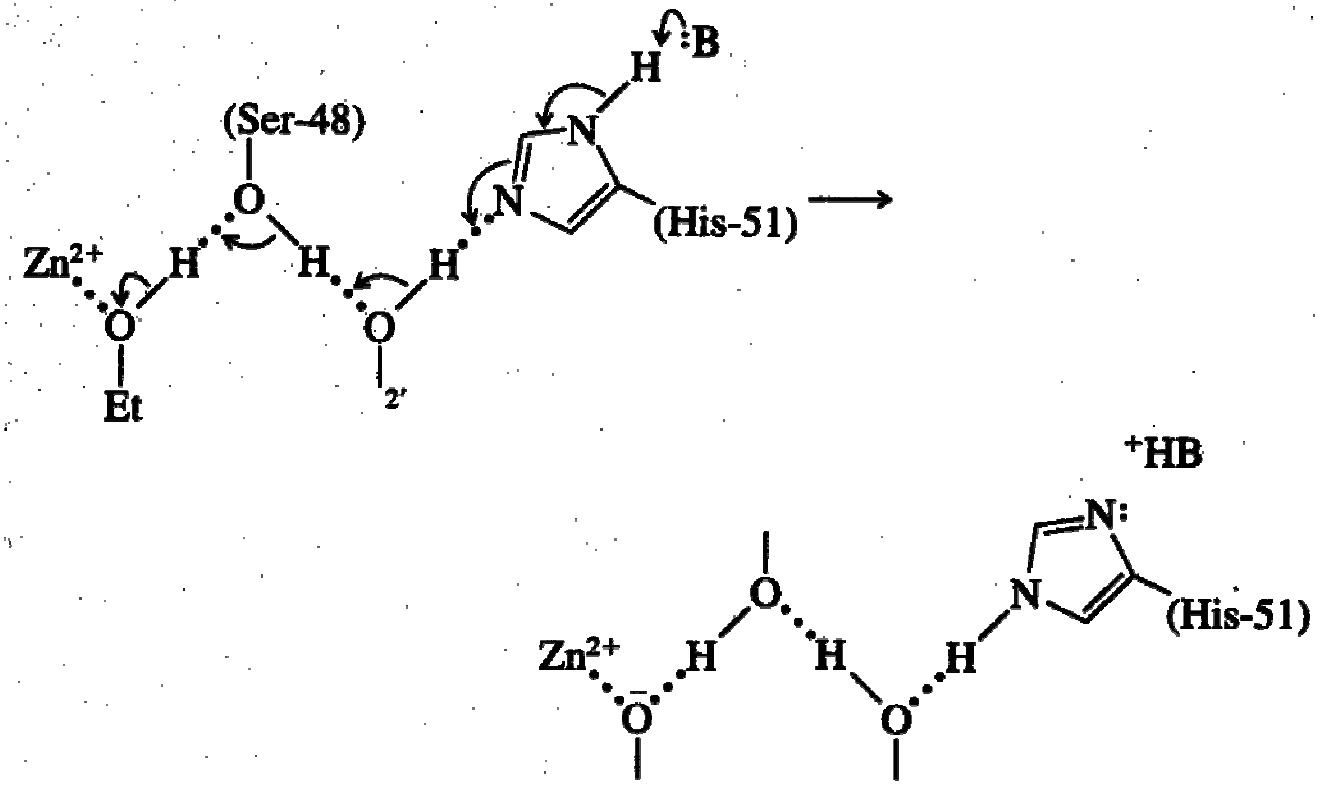


DACA
(chromophoric)

Jonizaciono stanje alkohola? Da li se vezuje kao alkoholatni anjon?



Sistem za transfer protona



Kinetički mehanizam

Uredjeni mehanizam

Konformaciona promena po vezivanju koenzima (kinetičkim metodama je detektovana izoemerizacija enzim-koenzim kompleksa u drugi kompleks)

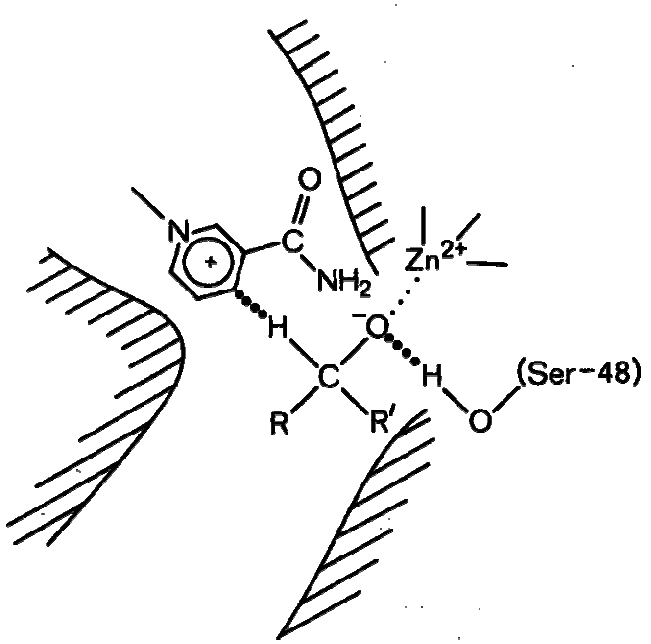


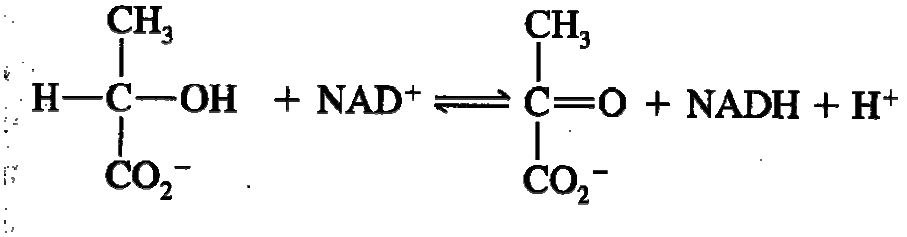
Figure 16.3 A proposed model for the productively bound ternary complex of horse liver alcohol dehydrogenase. It is suggested that the ionized alcohol displaces the zinc-bound water molecule shown in Figure 16.2. [Courtesy of C.-I. Brändén.]

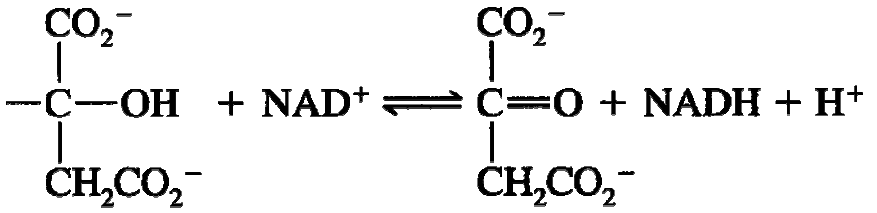
Promena specifičnosti prema supstratu u alkohol dehidrogenazama protein inženjeringom

- Horse liver enzim: Ser-48, Leu-57 i Phe-93
- Dehidrogenaza iz kvasca: Thr-48, Trp-57 I Trp-94
- Jako reaktivan prema EtOH i slabo reaktivan prema haksanolu.
- Mutant Trp94Ala: aktivnost prema heksanolu je povećana 6 puta, a prema EtOH je smanjena 350 puta.
- Mutant Trp54Leu bolje katalizuje dugolančane alkohole i alkohole koji se granaju na položaju 4.

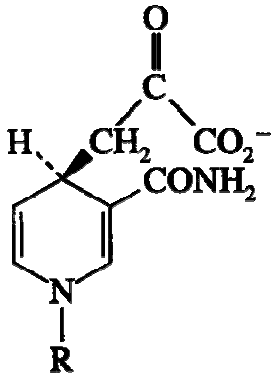
L-laktat i L-malat dehidrogenaze

- LDH – tetramer Mr 140 kD, M i H podjedinice.
  - Molekul je simetričan i podjedinice su strukturno ekvivalentne.

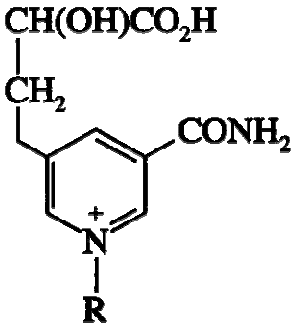




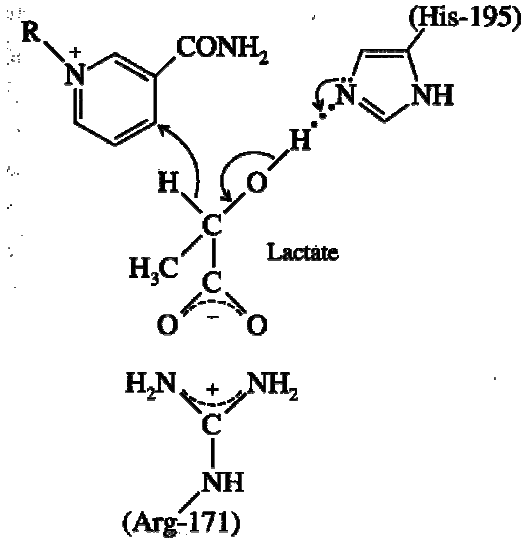
Struktura kompleksa ES



NAD-pyruvate



S-Lac-NAD$^+$



His-195 orijentiše supstrat i funkcioniše kao kiselo-bazni katalizator, uklanjajući proton sa laktata.

Arg171 Lys mutacija slabi vezivanje supstrata za 5 kcal/mol.

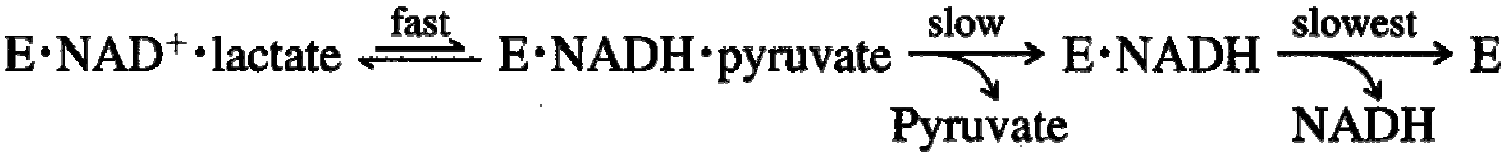
Kinetički mehanizam LDH

Uredjen mehanizam

Po vezivanju koenzima dolazi do konformacione promene

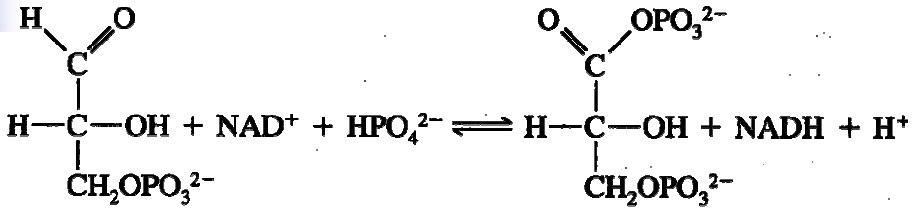
Neke od DH su alosterno regulisane

His-195 je reaktivan prema DEPC (pKa 6.7)

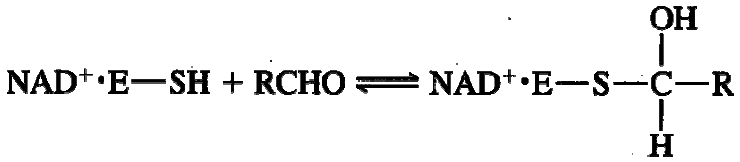


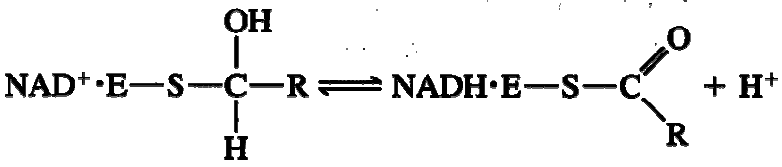
Mutacija Gln102Arg konvertuje laktat dehidrogenazu u malat dehidrogenazu

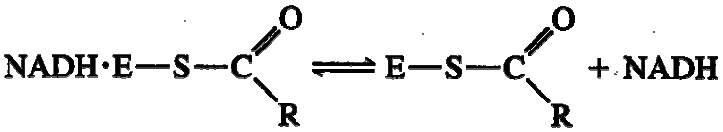
Gliceraldehid 3-fosfat dehidrogenaza

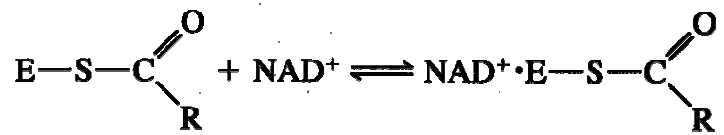


Tetramer, Mr 150 kDa







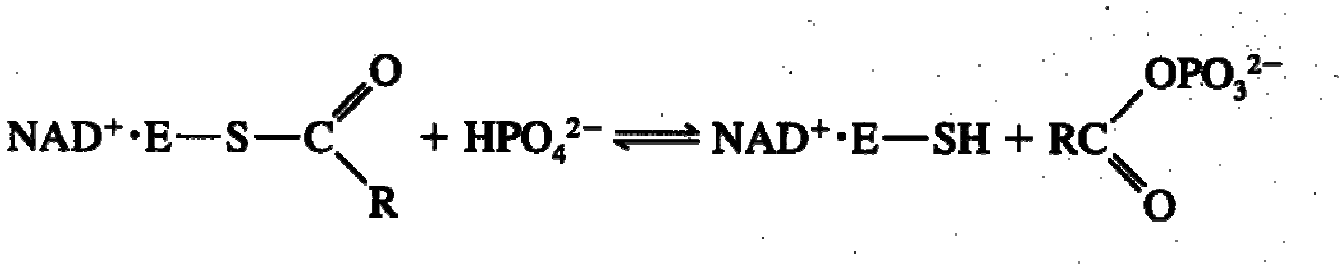


Mehanizam:

1. Formiranje hemitioacetala
2. Dehidrogenacija nastale alkoholne grupe (formiranje tioestra)
3. NAD^+ zamenjuje NADH (sledeći korak ide sporo ukoliko je NADH vezan za enzim)

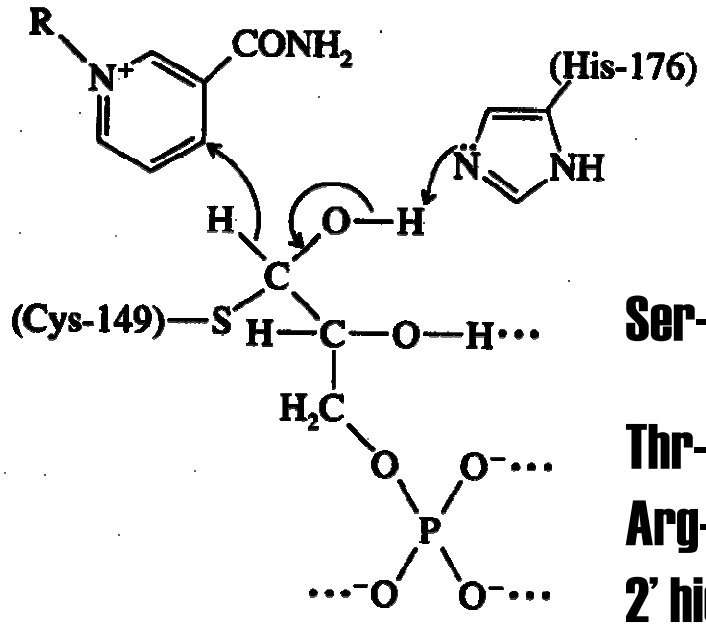
Gliceraldehid 3-fosfat dehidrogenaza

4. Formiranje acilfosfata



Otpuštanje NADH (u 3. koraku) odredjuje ukupnu brzinu reakcije

Struktura aktivnog mesta



Ser-148

Thr-179

Arg-231

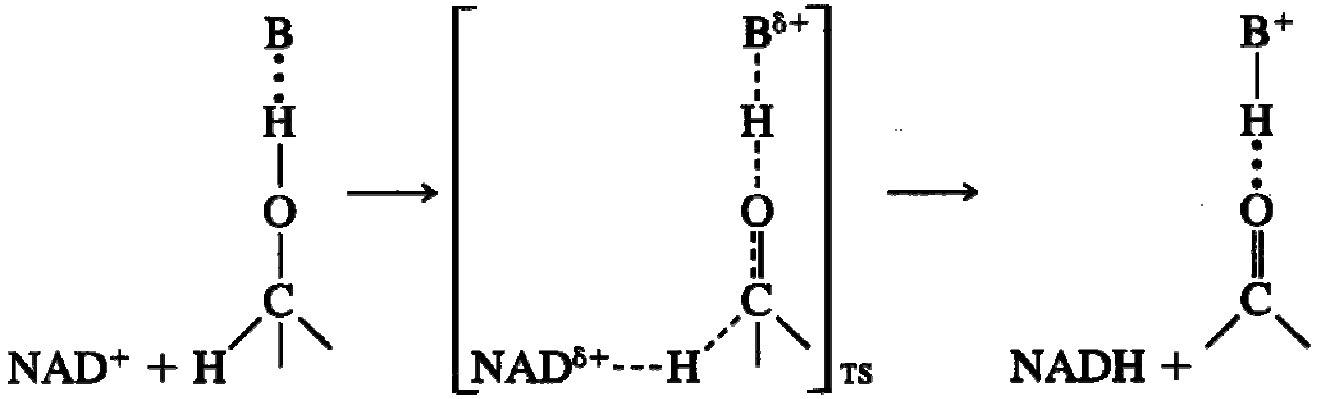
2' hidroksil riboze (NAD_+)

Vezivanje NAD_+ je kooperativno

Vezivanje giceraldehid 3-fosfata je nezavisno za sve podjedinice

Holoenzim i apoenzim imaju različite konformacije (indukovano slaganje)

Dehidrogenaze – generalizacija mehanizma dejstva



Redukovani S se preferencijalno vezuje za baznu formu katalizatora
pKa baze ternarnog kompleksa (E-NAD$^+$-alkohol) je niže od pKa baze ternarnog kompleka E-NADH-RCHO
Postoje i stereospecifični zahtevi prema supstratu – enantiomeri supstrata se ne vezuju produktivno
NADH se vezuje čvršće od NAD$^+$