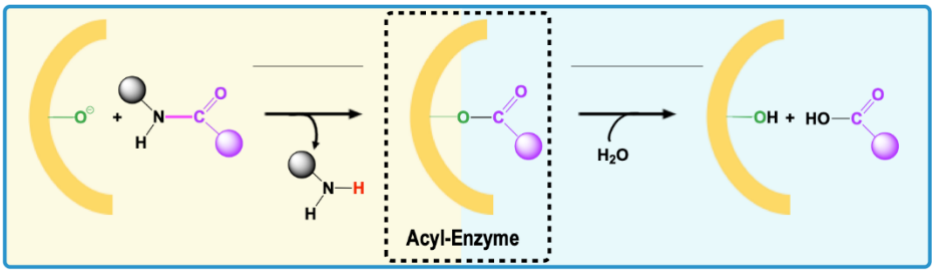
CONCEPT: CHYMOTRYPSIN'S CATALYTIC MECHANISM

●Chymotrypsin catalytic mechanism has _____ phases: 1) _____ *phase*. 2) _____ *phase*.



1) Acylation Phase

●Chymotrypsin's ____-step *acylation* phase: *covalent* catalysis forms an *ester* linkage & the *peptide bond* is _____.

Nucleophilic Acyl Substitution:

1 ***Substrate Binding***: peptide to be cleaved binds to chymotrypsin's _____ *site*.

2 ***Nucleophilic attack***: _____**-195** attacks carbonyl carbon of substrate to form unstable *tetrahedral intermediate*.

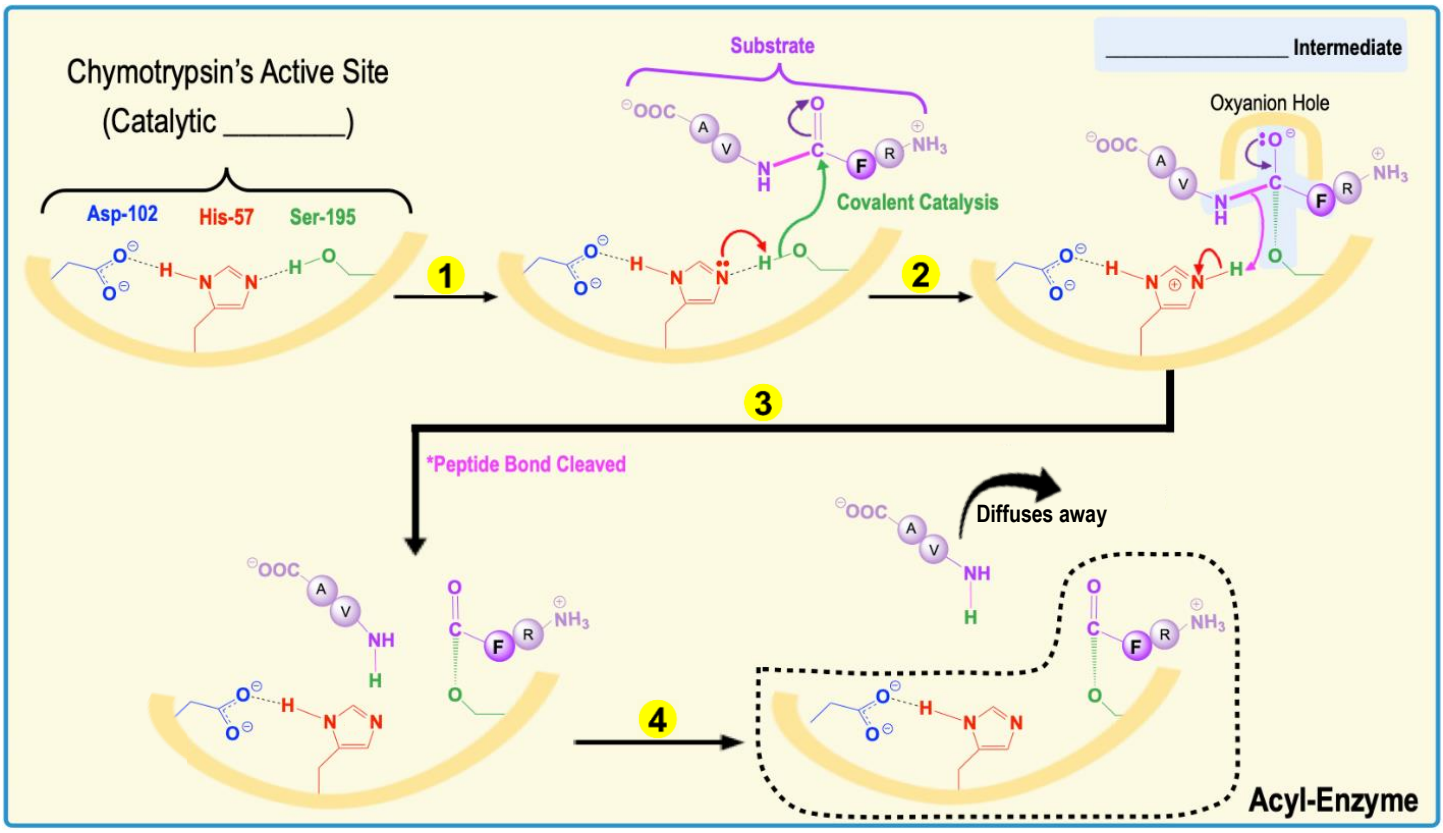
□ A region in chymotrypsin's active site (*oxyanion hole*) _____ the tetrahedral intermediate.

□ Newly formed _____ covalently links chymotrypsin's **Ser-195** to the carbonyl carbon of substrate.

3 ***Remove LG***: tetrahedral intermediate "*collapses*", **His-57** acts as an _____ & peptide bond is *broken*.

4 ***End of Phase***: enzyme is _____ & the newly formed *amine* portion of substrate *diffuses away*.

EXAMPLE: Acylation Phase of Chymotrypsin's Catalytic Mechanism.



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2) Deacylation Phase

●Chymotrypsin's ____-step *deacylation* phase: *ester linkage is hydrolyzed* to _____ the original chymotrypsin.

Ester Hydrolyzation:

5 ***Substrate Binding*:** _____ enters chymotrypsin's active site and a series of *similar* steps repeats.

6 ***Nucleophilic attack*: His-57** (*base*) *deprotonates* **H_2O**, creating ____ *nucleophile* that attacks carbonyl carbon.

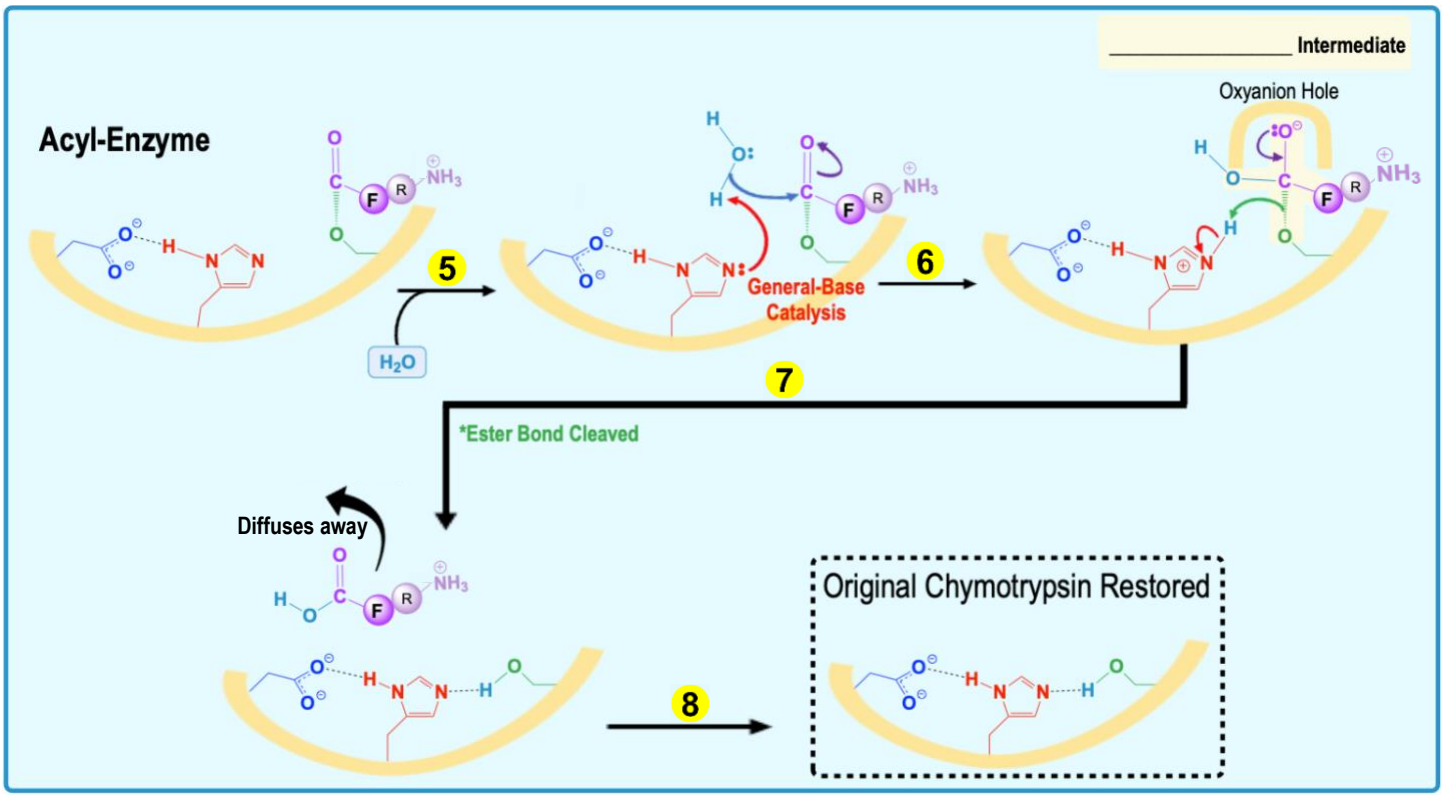
□ Once again, _____ *hole* in chymotrypsin's active site *stabilizes* the *tetrahedral intermediate*.

7 ***Remove LG*:** tetrahedral intermediate "*collapses*", **His-57** acts as an _____ & ester bond is _____.

8 ***End of Phase*:** enzyme is _____-acylated & newly formed *carboxylic acid* portion of substrate *diffuses away*.

□ Original chymotrypsin enzyme is _____ and ready for another round of catalysis.

EXAMPLE: Deacylation Phase of Chymotrypsin's Catalytic Mechanism.



PRACTICE: How is chymotrypsin's specificity for its substrate determined?

a) Conformational change upon binding of the substrate.

b) Binding of the N-terminus amino acid at the active site.

c) Covalent binding of the His residue to the substrate.

d) Binding of the proper amino acid into a deep pocket on the enzyme.

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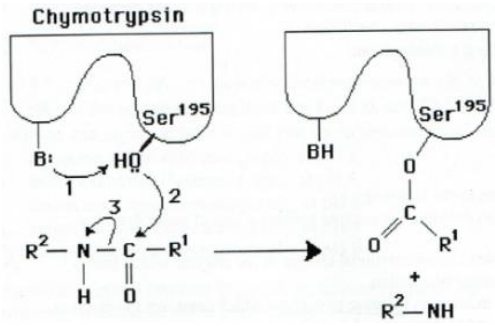
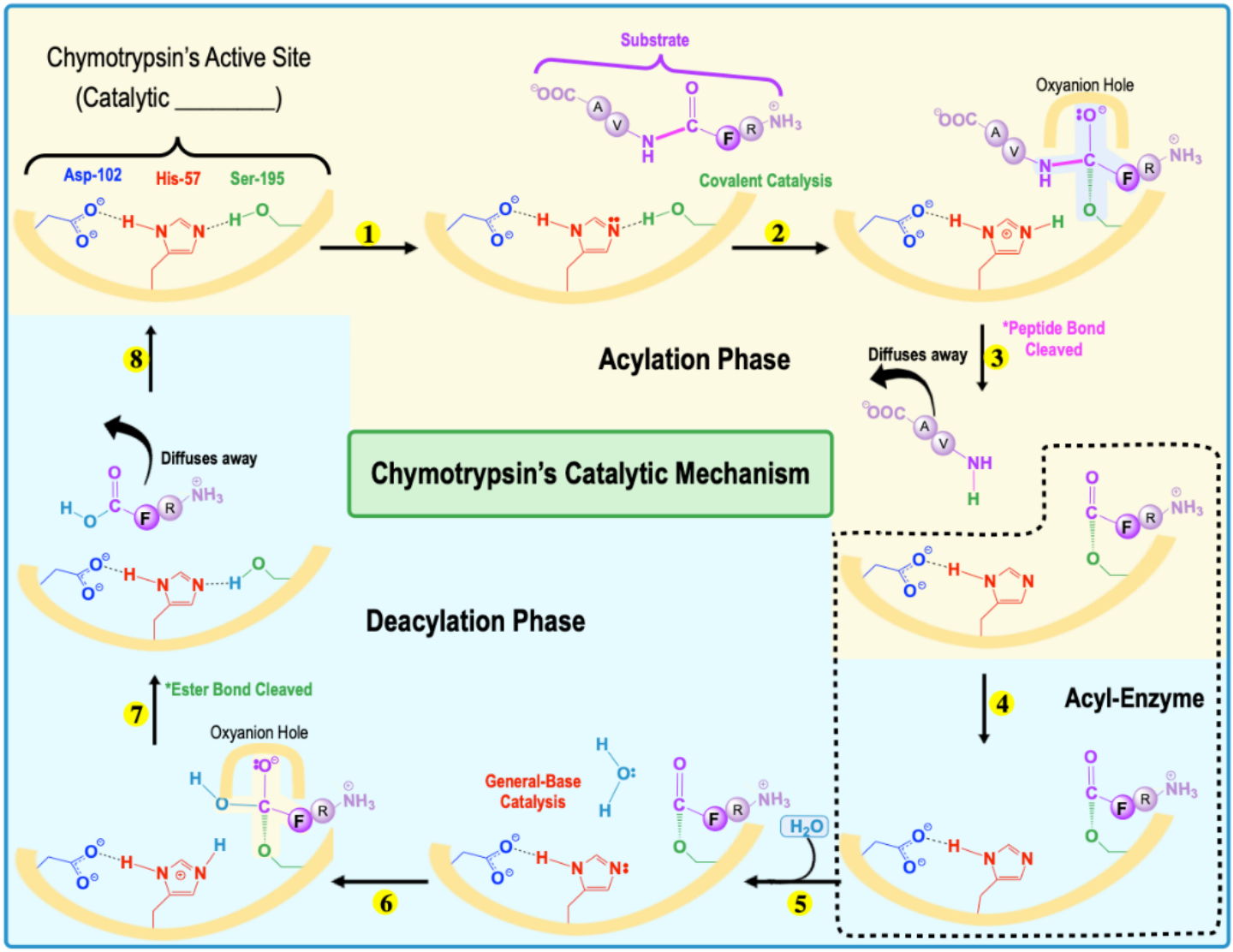
PRACTICE: Chymotrypsin's acylation phase is below. The catalytic process of _____ is illustrated by arrow # ____ and the catalytic process of _____ is illustrated by arrow # ____.

a) General-Base catalysis; 1 ; covalent catalysis; 2.
b) General-Acid catalysis; 1 ; covalent catalysis; 3.
c) Covalent catalysis; 2 ; covalent catalysis; 3.
d) Covalent catalysis; 1 ; General-Base catalysis; 3.



Recap of Chymotrypsin's Catalytic Mechanism

●Let's recap all the steps/phases of chymotrypsin's catalytic mechanism:



CONCEPT: CHYMOTRYPSIN'S CATALYTIC MECHANISM

PRACTICE: In the mechanism of chymotrypsin, serine-195:

a) Functions as a nucleophile.
b) Functions as an electrophile.
c) Forms a tetrahedral intermediate with part of the substrate.
d) Is reduced by Aspartate 102.
e) Is oxidized by Histidine 57.
f) A and C.
g) A, C and E.

PRACTICE: Sequentially number the following steps of Chymotrypsin's catalytic mechanism in the correct order from 1-8:

a) Released newly formed amine portion of the substrate diffuses away _____
b) His-57 deprotonates the Ser-195 hydroxyl group, generating a stronger nucleophile _____
c) Tetrahedral intermediate collapses & His-57 donates a H^+ to N of scissile bond (cleaved peptide bond) _____
d) Via general-base-catalysis, His-57 deprotonates a water molecule, generating OH^- _____
e) Tetrahedral intermediate collapses & His-57 donates a proton to Ser-195 (cleaving ester bond) _____
f) Released newly formed carboxylic acid portion of the substrate diffuses away & enzyme is restored _____
g) Hydroxide ion attacks the carbonyl group of the substrate, forming another tetrahedral intermediate _____
h) Nucleophilic Ser-195 attacks the carbonyl C of the substrate forming a tetrahedral intermediate_____