C483 Exam 1
Spring 2017

Name Key Seat Number _____

Student ID _____

The last page of this exam contains pKa values and other information you might find useful.
This exam contains 110 points. The highest score you may earn on this exam is 100 points.

1 _____/20pts

2. _____/10pts

3. _____/20pts

4. _____/10pts

5. _____/10pts

6. _____/10pts

7. _____/10pts

8. _____/10pts

9. _____/10pts

Total:

Regrading: All requests for regrades must be submitted in writing within 48 hours of the return of the exam. You must explicitly state what has been misgraded and why it is an error. The entire exam will be regraded, which could result in points being added or deducted overall.

Section 1: Short Answer (50 points)

1. 20 pts. Fill in the blanks (2 points each.)

A. In chymotrypsin, the amino acid Asp serves to stabilize a general base through a low-barrier hydrogen bond.

B. In DNA, the two polynucleotide strands are antiparallel; that is, their phosphodiester bonds run in opposite directions.

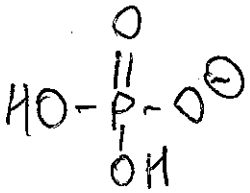
C. RNA is significantly more susceptible than DNA to hydrolysis due to its 2' -OH.

D. Restriction enzymes can be used to cut DNA as specific sequences, generally palindromes.

E. Tubulin is a structural protein made up of globular monomers that can act as "highways" for kinesin.

F. The sigmoidal shape of hemoglobin's oxygen binding curve can be attributed to hemoglobin's cooperative binding behavior.

G. Draw phosphate in its predominant ionization state at pH 5.6.



H. Irregular secondary structures, such as loops and turns, are more likely to be found on the exterior of a globular protein.

I. Two amino acids which might form a salt bridge in the core of a globular protein are D, E and K, R, H. accept any pair

J. Pancreatic proteases are released in Zymogen form so that they do not digest the cells in which they are made.

2. 10 pts. Write True or False (1 points each)

A. False The mRNA has the same sequence and 5'→3' directionality as the template strand of the DNA, except for the substitution of U for T.

B. True All α-helices are polar, but not all α-helices are amphipathic.

C. True Hyperventilation, which expels CO_2 from the body, will eventually lead to an increase in blood pH.

D. False Glycine is commonly found in α-helices, loops, and turns.

E. False The selectivity of complementary DNA strands for one another in a double helix does not depend significantly on hydrogen bonding.

F. False A mixture of proteins with identical pI usually cannot be separated using gel filtration chromatography.

G. False The pH of a solution of weak acid/conjugate base is always higher than the pKa of the weak acid if the [A-]/[HA] ratio is greater than zero.

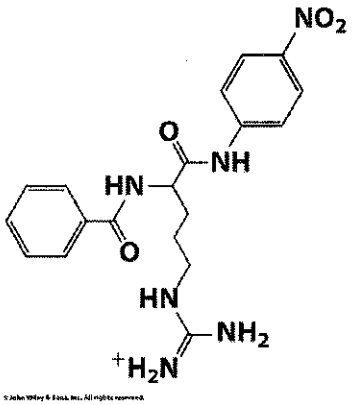
H. False The induced fit model of enzyme explains how an enzyme can bind to one substrate more selectively than another.

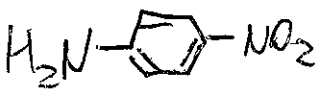
I. False At its isoelectric point, the α-amino group of lysine is protonated.

J. False The phosphodiester bond of a polynucleotide is thermodynamically and kinetically stable.

3. 20 pts. Short answer (5 points each)

A. The molecule below is a substrate for trypsin. Draw the products of the trypsin-mediated process and explain how it could be used in an assay.





This molecule absorbs UV/vis light, and Absorbance is used to measure enzyme activity.

3'AGTTCAG5'

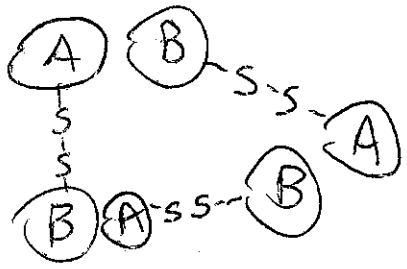
B. Part of the template strand of a gene is …TCAAGTC…

Write the sequence of the coding strand: 5'GACTTGA3' +2 sequence +1 direction

Write the sequence of the mRNA: 5'GACUUGA +2 (based on above

C. A protein was purified by gel filtration and determined to have a mass of 90 kDa. A sample of this purified protein was treated with urea. SDS PAGE analysis showed one band at 30 kDa. Treatment of a sample of the purified protein with urea and β-mercaptoethanol was analyzed by SDS PAGE to have two bands, at 14 kDa and 16 kDa. Draw a schematic of this protein that is consistent with all the data.

Trimer of dimers – A subunit 14 kDa
B subunit 16 kDa



D. Give two reasons that proline disrupts the regular structure of an α-helix.

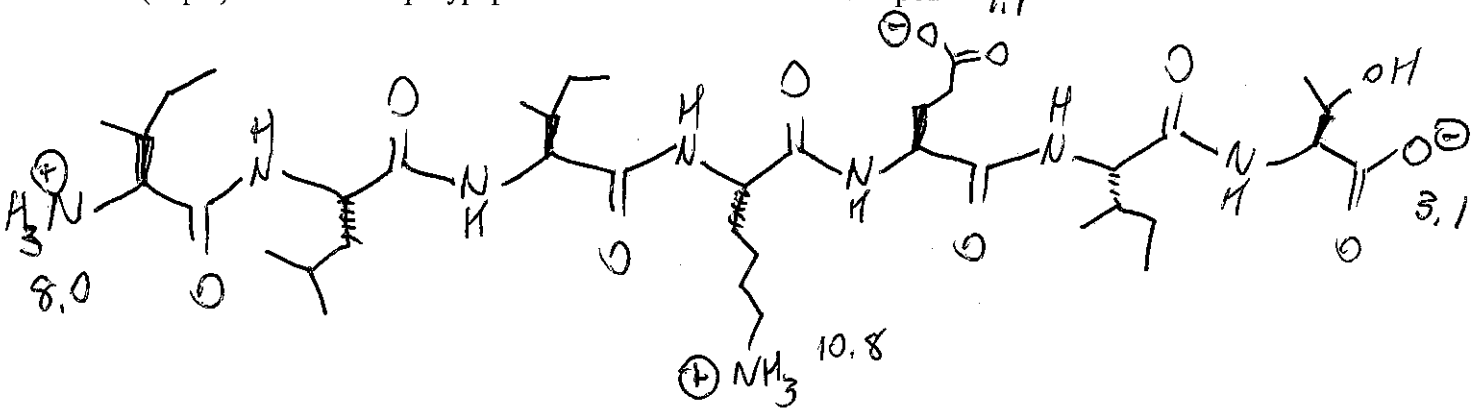
+3 constrained dihedral angle

+2 not capable of acting as H-bond donor

stereo +1
backbone +1
ionization +1
sidechain +5
+8

Section 2: Problems

4. (15pts) A. Draw the polypeptide ILIKEIT at its isoelectric point. 4.1



B. A sample of this peptide was placed in 1.00 L of 0.100 M ACES buffer at pH 6.9. How many moles of HCl or NaOH would have to be added to this solution so that the peptide is at its isoelectric point?

Isoelectric point is ~ 6.0 (+3)

Buffer: final

$$pH = pK_a + \log \frac{A^-}{HA}$$

$$6.0 = 6.9 + \log \frac{A^-}{HA}$$

$$\frac{.126}{1} = \frac{A^-}{HA}$$

% HA = 89%

Final HA = 0.089 Mol

initial

$$pH = pK_a$$

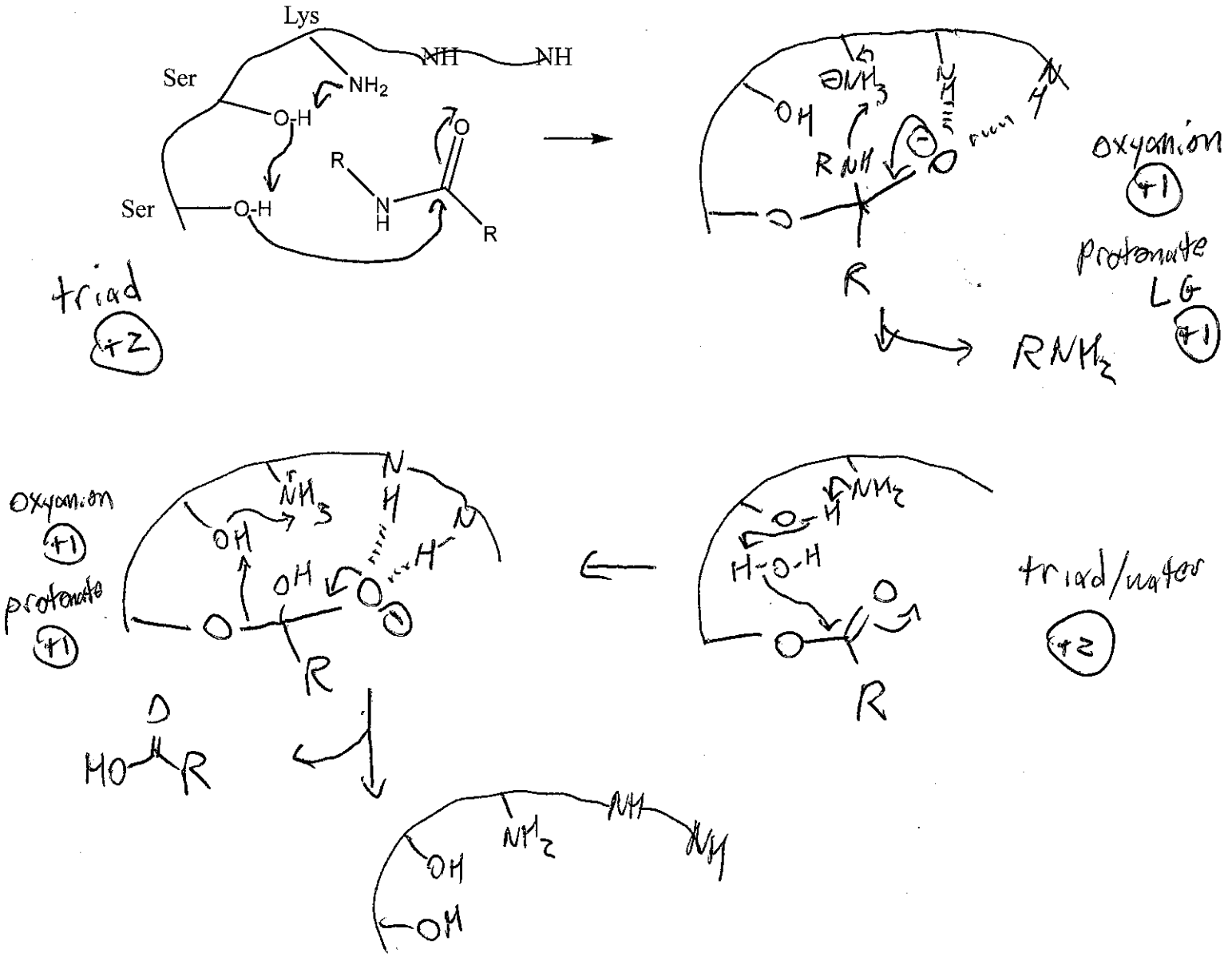
% HA = 50%

Initial HA = 0.050 mol

∴ A total of 0.039 mol of HCl need to be added.

(+4)

5. (10pts) A protease family has been discovered that is analogous to chymotrypsin (including an oxyanion hole), except that is has a Ser-Ser-Lys catalytic triad. Propose an arrow mechanism for the enzyme mediated hydrolysis of a peptide bond:



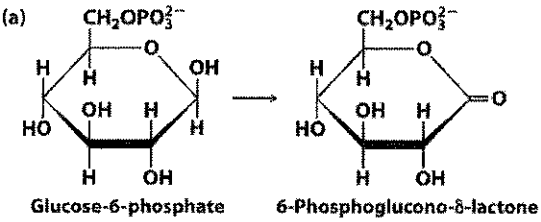
+1 Based on your mechanism, would you expect diisopropylphosphofluoridate (DIPF) to inactivate this enzyme family? yes

+1 Based on your mechanism, does this enzyme family demonstrate burst kinetics? yes

+2 each

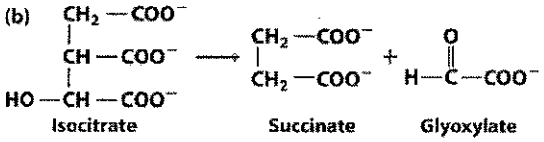
6. (10pts) Label each enzyme or enzyme-catalyzed reaction according to its enzyme class: oxidoreductase, transferase, hydrolase, lyase, isomerase, or ligase.

(a)



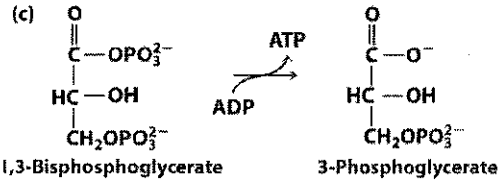
oxidoreductase (dehydrogenase)

(b)



lyase

(c)

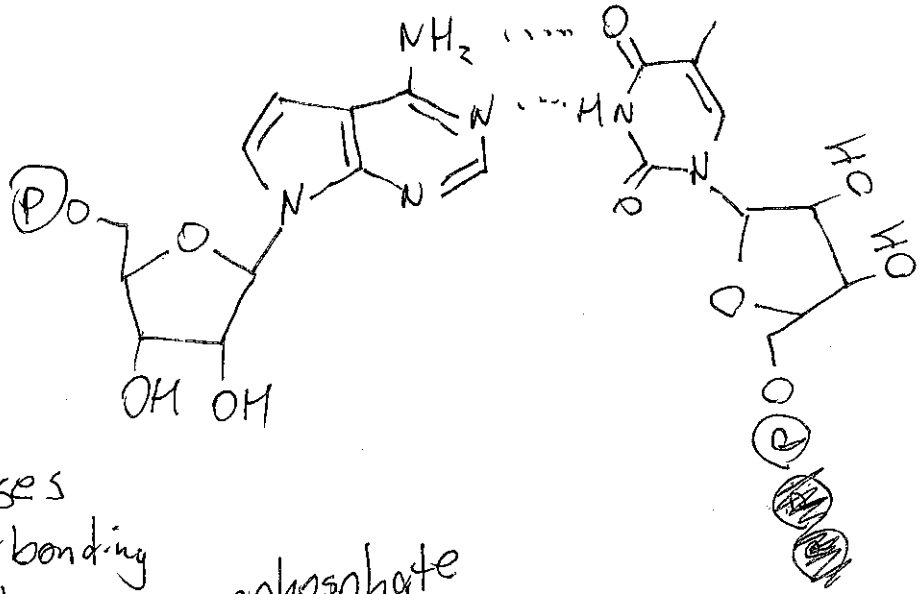


transferase (kinase)

d. pyruvate dehydrogenase — oxidoreductase

e. asparagine → aspartate — hydrolase

7. (5pts) Draw AMP and TMP base paired through hydrogen bonding. Use the drawing to explain the origin of the major and minor grooves in the DNA double helix.



+2 bases
+1 H-bonding
+1 ribose monophosphate
+1 ~~Monophosphate~~ major groove

8. (10pts) A. Fibrous proteins like keratin have seven-residue repeats like the ones shown below. What is the key feature that makes these sequences similar?

...IQEVERD...
...LDKMRNE...
...VNDLKDR...

Residues 1+4 are nonpolar
The others are charged/polar

+2

B. Describe the structure of keratin, and explain why the sequences lead to this structure.

+2 { Keratin is a coiled-coil protein. The nonpolar
1, 4 residues pack against each other, causing the
+2 { coiling. The other residues are on the surface,
and are hydrophilic.

C. Fibrous proteins like collagen have three residue repeats like Gly-Pro-Hyp. Describe the structure of collagen, and explain why Gly is invariant in this sequence.

+2 { It is a left handed triple helix.
Glycine packs in the middle of the triple
helix, where its H-sidechain provides no
+2 { steric hinderance. It is invariant because
any other side chains will disrupt the
structure significantly, causing denaturation
(low melting point...)

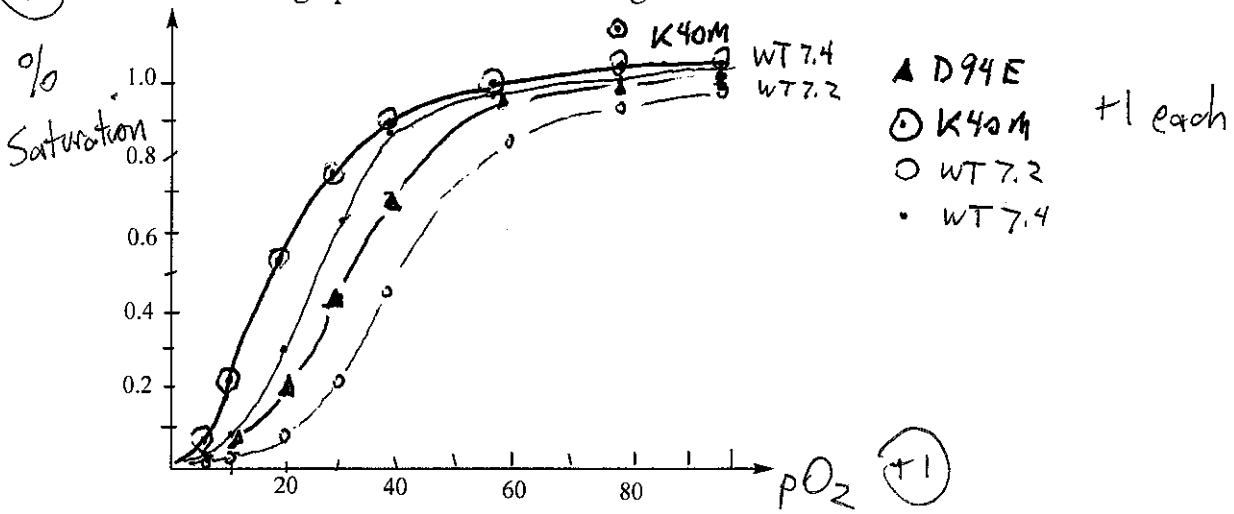
Section 3: Case study (10pts)

9. The Bohr Effect was investigate on three hemoglobin variants: wild type, the D94E mutant, and the K40M mutant. The fractional saturation (Y) of each was measured at pH 7.2 and compared to wild type fractional saturation at pH 7.4. The data is in the table below.

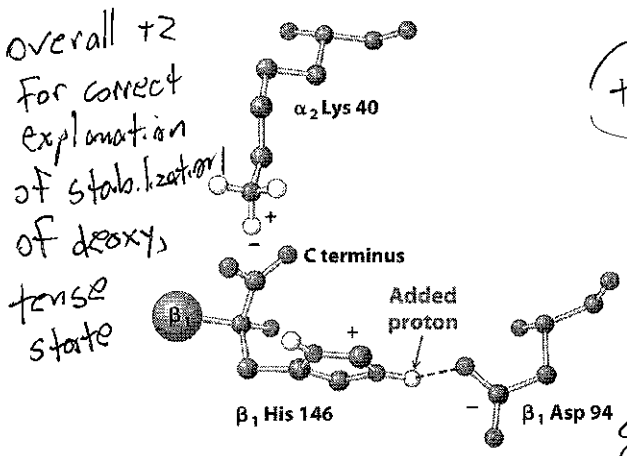
pO_2 (torr)	Wildtype pH 7.4	Wildtype pH 7.2	K40M pH 7.2	D94E pH 7.2
5	0.03	0.01	0.05	0.02
10	0.08	0.02	0.21	0.05
20	0.30	0.08	0.52	0.19
30	0.62	0.20	0.70	0.40
40	0.81	0.45	0.85	0.63
60	0.92	0.84	0.94	0.88
80	0.96	0.92	0.97	0.94
100	0.98	0.97	0.98	0.97

(+1)

A. Draw one graph with the four binding curves to scale. Label the axes units and each curve.



B. Using the structural data below for the wildtype hemoglobin at low pH and the data above, explain the roles of Lys40 and Asp 94 O_2 binding?



★ Replacing Lys with Met causes a significant shift of the binding curve left, meaning loss of the Lys 40/C-terminus salt bridge significantly destabilizes the deoxy Tense state.

★ Replacing Asp with Glu maintains the Bohr effect, but weakens it. (Curve still right-shifted, but less.) Distortion of His 146 conformation must partially disrupt the Lys 40 - C terminal salt bridge

Data Tables

Amino acid pKa values

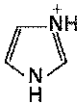
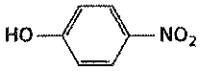
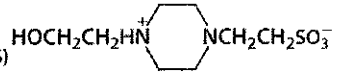
C-terminal	3.1
N-terminal	8.0
Aspartate, glutamate	4.1
Histidine	6.1
Cysteine	8.3
Tyrosine	10.9
Lysine	10.8
Arginine	12.5

TABLE 3-3 The Standard Genetic Code[a]

First Position (5′ end)	Second Position U	Second Position C	Second Position A	Second Position G	Third Position (3′ end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

[a]The 20 amino acids are abbreviated: Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartate; Cys, cysteine; Gly, glycine; Gln, glutamine; Glu, glutamate; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; and Val, valine.

TABLE 2.4 pK Values of Some Acids

Name	Formula [a]	pK
Trifluoroacetic acid	CF_3COOH	0.18
Phosphoric acid	H_3PO_4	2.15[b]
Formic acid	$HCOOH$	3.75
Succinic acid	$HOOCCH_2CH_2COOH$	4.21[b]
Acetic acid	CH_3COOH	4.76
Succinate	$HOOCCH_2CH_2COO^-$	5.64[c]
Thiophenol	C_6H_5SH	6.60
Phosphate	$H_2PO_4^-$	6.82[c]
N-(2-acetamido)-2-amino-ethanesulfonic acid (ACES)	$H_2NCOCH_2\overset{+}{N}H_2CH_2CH_2SO_3^-$	6.90
Imidazole		7.00
p-Nitrophenol		7.24
N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES)		7.55
Glycinamide	$^+H_3NCH_2CONH_2$	8.20
Tris(hydroxymethyl)-aminomethane (Tris)	$(HOCH_2)_3C\overset{+}{N}H_3$	8.30
Boric acid	H_3BO_3	9.24
Ammonium ion	NH_4^+ 9.25	
Phenol	C_6H_5OH	9.90
Methylammonium ion	$CH_3NH_3^+$	10.60
Phosphate	HPO_4^{2-}	12.38[d]

[a]The acidic hydrogen is highlighted in red; [b]pK_1; [c]pK_2; [d]pK_3.

TABLE 4-4

Specificities of Some Proteases

Protease	Residue Preceding Cleaved Peptide Bond[a]
Chymotrypsin	Phe, Trp, Tyr
Elastase	Ala, Gly, Ser, Val
Thermolysin	Ile, Met, Phe, Trp, Tyr, Val
Trypsin	Arg, Lys

[a]Cleavage does not occur if the following residue is Pro.