

I. Here's an octapeptide to sequence. Neither the N-terminal end nor the C-terminal end is blocked. Its composition is:

(ala₃,asp,glu,lys,met,phe)

[The convention is that amino acid composition is designated with commas separating the amino acids, which are listed in alphabetical order. Amino acid sequence is designated from amino end to carboxyl end, with hyphens between the amino acids.]

a. Digestion of the original peptide with staph V8 protease yields two peptides which can be separated by ion exchange chromatography at pH values between 5 and 10. If the digest is reacted with dansyl chloride without any attempt to separate the peptides, the only dansylated amino acid which can be detected is DNS-ala.

This result suggests that the N-terminus is ala, but there is a second ala which is C- to an asp or a glu (which one is indeterminate at this point).

b. Digestion of the original peptide with trypsin yields two peptides which can be separated by ion exchange chromatography at pH values between 5 and 10. If the digest is reacted with dansyl chloride without any attempt to separate the peptides, the only dansylated amino acid which can be detected is DNS-ala.

This result has now accounted for the third ala: it is C- to a lys. The separation of the two tryptic peptides indicates that the charged side chains are not evenly distributed between the two tryptic peptides, but it is premature to draw further conclusions at this point.

c. Digestion of the original peptide with chymotrypsin yields two peptides which can be separated by ion exchange chromatography at pH values between 5 and 10. If the digest is reacted with dansyl chloride without any attempt to separate the peptides, both DNS-ala and DNS-lys can be detected.

Now we're getting some useful information. There is a sequence stretch -phe-lys-ala- in the middle somewhere, and again the charge distribution in the two chymotryptic peptides is not uniform.

d. Exhaustive digestion of the octapeptide with carboxypeptidase A releases only two amino acids, even after long incubations. The two amino acids are ala and glu.

We're almost finished now. The limitation on carboxypeptidase A digestion must be due to the lys which prevents further cleavage. We already know that the ala is C- to the lys, so the glu must be C- to the ala, and must also be the C-terminus of the entire peptide. That also tells us that the ala which was C- to an acidic amino acid in a. above must in fact be C- to the asp (the glu is the C-terminal residue).

e. After the octapeptide is treated with cyanogen bromide, two peptides are generated. One of the peptides is a dipeptide which is shown to contain homoserine lactone on amino acid analysis.

Now we're really finished. The dipeptide which contains homoserine lactone must have been derived from the N- side of the cleavage, and must have the met. Since it has only two amino acids, the other residue must be the N-terminal ala.

Write the sequence of the peptide below:

 H₂N-ala - met - asp - ala - phe - lys - ala - glu-COOH

Inspection shows that one of the two V8, tryptic, and chymotryptic peptides has a charge of 0 and the other has a charge of -1 at pH 7 (it's obviously not the same two peptides generated by each of the cleavages).

II. You have accidentally mixed together three samples which were given to you. Sample A is the tripeptide **lys-val-asp**. Sample B is the dipeptide **glu-gly**. Sample C is the free amino acid **phe**. You would like to separate them. Next to each separation strategy, indicate whether the properties of material are most consistent with A and/or B and/or C. For each answer you may use one or more than one letter, or you may indicate NONE if none of the samples fits the description of the material.

1. A Sephadex G-10 is a bead-formed gel filtration medium which can separate even rather small molecules. This material comes off first from the Sephadex G-10 column.

In gel filtration, the largest material comes off first.

2. ABC The mixture is put into an electrophoresis apparatus in a buffer at pH 11.5. This material moves towards the anode.

At pH 11.5, all the α - and the ϵ -amino groups are at least partially titrated and deprotonated, while the deprotonated α - and β -carboxyl groups are fully negatively charged.

3. NONE The mixture is put into an electrophoresis apparatus in a buffer at pH 9.8. This material doesn't move towards the anode or the cathode.

At pH 9.8, only the α -amino groups are at least partially titrated and deprotonated, while the α -, β -, and γ -carboxyl groups are still fully negatively charged, so the components all still move to the anode.

4. C The mixture is put onto a cellulose column and is eluted with a solvent of pyridine and benzene. This material comes off the column first.

Cellulose tends to bind hydrophilic materials due to bound water. The aromatic solvent will elute the phe first.

5. C The mixture is successfully separated into its three components, each of which is subjected to hydrolysis in 6 N HCl overnight. The pH of the acid hydrolysate of this component is then adjusted to pH 1.5 and then titrated with NaOH until all titratable groups are in fact titrated. This requires the addition of two equivalents of OH^- per equivalent of component.

After acid hydrolysis, the only component with two titratable groups is the single amino acid, phe. The other components generate multiple amino acids, each with its own α -amino and α -carboxyl group.

6. NONE The acid hydrolysate of this component is titrated from pH 1.5 until all the titratable groups are in fact titrated. This requires the addition of four equivalents of OH^- per equivalent of component.

Since the dipeptide has a titratable side chain (glu), there are five groups to be titrated after acid hydrolysis, the 2 α -amino groups, the 2 α -carboxyl groups, and the side chain.

7. A The acid hydrolysate of this component is titrated from pH 1.5 until all the titratable groups are in fact titrated. This requires the addition of eight equivalents of OH^- per equivalent of component.

The tripeptide has 3 α -amino and 3 α -carboxyl, and 2 side chain groups which can be titrated.

8. AC This component is comprised solely of L-(s)-amino acids.

Glycine is optically inactive (has no center of chirality).

9. A The entire mixture is subjected to two cycles of Edman degradation, followed by reaction of the products with dansyl chloride. This material yields a single α -dansylated product.

Every time a cycle of the Edman degradation is run, the N-terminal amino acid is converted to its thiohydantoin.

Dansyl chloride reacts with the newly liberated N-terminal amino group. If two cycles of Edman degradation are run before the reaction with dansyl chloride, the only free N-terminal amino group will be that of asp. All the other amino acids will be in the form of thiohydantoins and cannot react with dansyl chloride.

10. AB This material could conceivably have been released efficiently from digestion of a larger peptide by chymotrypsin.

Since chymotrypsin is an endopeptidase, it wouldn't liberate a free amino acid. The other two peptides would have to come from the C- side of the chymotryptic cleavage.

III. You are trying to determine whether the dogma about primary and tertiary structure of proteins is true, so you take a water-soluble extracellular enzyme and treat it with β -mercaptoethanol and urea. Which observations are likely to be TRUE (write out the word) and which are likely to be FALSE?

1. ___T___ If the native enzyme has no disulfide bonds, it might not be necessary to bother with β -mercaptoethanol addition to completely denature the enzyme with urea.

β -mercaptoethanol breaks disulfide bonds, but urea doesn't. Complete denaturation involves rupture of all S-S bridges.

2. ___F___ If the native enzyme is like ribonuclease, the β -mercaptoethanol/urea treatment will destroy all enzyme activity, but very careful removal of the β -mercaptoethanol first, followed by removal of the urea, should result in almost complete restoration of enzymatic activity.

By removing the mercaptoethanol first, you would allow for incorrect pairing of the sulfhydryls in a denatured solution of ribonuclease, and you wouldn't be able to recover activity.

3. ___F___ The urea is likely to destroy enzyme activity because it disrupts the hydrogen bonds which stabilize the enzyme in its native conformation.

Urea is a chaotropic agent, favoring disruption of clatherates and eliminating the driving force for hydrophobic interactions.

4. ___T___ When the enzyme is in β -mercaptoethanol/urea, its hydrophobic side chains may point in any number of directions, but when β -mercaptoethanol and urea are removed in a way to recover enzyme activity, the hydrophobic side chains are likely to be pointing in fixed directions.

In urea/mercaptoethanol, the enzyme is in the random coil, whereas in the native conformation, the side chains are packed in the interior in such a way as to maximize van der Waals interactions.

5. ___T___ If the native enzyme were in fact found buried within the interior of a biological membrane, it might be removed from the membrane in the presence of a solution of 8 M urea in water, but once the urea was removed, the enzyme would be especially likely to aggregate and come out of solution.

Integral membrane proteins are typically highly insoluble in water in the absence of chaotropic agents or detergents because of their abundant surface hydrophobic residues.

6. ___F___ When urea is dissolved in water, it lowers the temperature of the water, but the effect of urea on a water-soluble enzyme is best understood in terms of the capacity of the urea to lower otherwise unfavorably high entropy.

The disruption of clatherates by urea is an example of raising an otherwise unfavorably low level of entropy (increasing disorder).

7. ___F___ The successful recovery of ribonuclease activity after treatment with β -mercaptoethanol and urea followed by their removal is a reflection of the essential role of a disulfide bond in the catalytic mechanism of this enzyme.

The disulfide bonds help stabilize the native conformation, but don't directly participate in catalysis.

8. ___F___ If the treatment with urea and β -mercaptoethanol was tried on lysozyme, the enzyme should recover its ability to selectively hydrolyze polypeptide chains.

Lysozyme cleaves polysaccharides, not polypeptides.

9. ___T___ The successful restoration of catalytic activity of trypsin or chymotrypsin after treatment with urea and β -mercaptoethanol would require a fairly precise restoration of the relative orientations of the side chains of a serine, histidine, and aspartic acid.

The ser, his, and asp form the catalytic triad which all serine proteases have in common.

10. ___F___ The properties of the normal form of PrP, the prion protein, would suggest that after urea and β -mercaptoethanol treatment and subsequent removal, the only conformation this protein can adopt is the conformation it adopts in normal tissues, analogous to the result observed for ribonuclease, thus confirming the dogma about primary and tertiary structure. The spontaneously aggregating conformation which PrP has in prion plaques requires a mutation in the protein.

The normal and the aggregatable conformations of the prion protein are both attainable from the same amino acid sequence. They normally don't interconvert except in the presence of a "template" provided by some aggregated conformation and a chaperone.

11. ___F___ From what is known about the protein found in the plaques in the brains of Alzheimer's patients, if after treatment with urea and β -mercaptoethanol and subsequent removal, the protein aggregated, you would suspect that it had acquired a high percentage of α -helical structure.

Like so many aggregating proteins, the Alzheimer protein A β forms stacked β sheets.

IV. Mutations have been found in what appear at first sight to be almost every position on the hemoglobin molecule. For each description of a mutation, circle the best description of the likely functional consequences. Remember, these are mutant hemoglobins and may be different from normal hemoglobin A.

1. This tetrameric hemoglobin has the F8 histidine which is normally present in each of the four polypeptide chains of hemoglobin A replaced with a glycine.

- The Bohr effect will be reduced by approximately 50%
- The hemoglobin will bind four molecules of oxygen, but the oxygen-binding curve will resemble that of myoglobin.
- The hemoglobin will likely not bind oxygen, because the hemes will slip out of the protein.
- The hemoglobin will bind only two molecules of oxygen, because the F8 histidine has a unique function in the β chains but not in the α chains.
- The hemoglobin will not be digested by carboxypeptidase B at low pH.

The F8 histidine in all the polypeptide chains of hemoglobin is the "heme-linked" histidine. If it were removed (gly has no side chain), the hemes might indeed slip out of the protein.

2. This next tetrameric hemoglobin has a C-terminal aspartic acid on each of its β -chains, the side chain of which points towards the side chain of a histidine residue within the same β -chain when the protein is deoxygenated but the aspartic acid and the histidine side chains are no longer pointing towards each other when protein is allowed to bind oxygen again.

- It is quite possible that the contribution of the β chains to the Bohr effect in this protein will strongly resemble their contribution to the Bohr effect in normal hemoglobin A.
- It is likely that the contribution of the β -chains to the Bohr effect in this protein will be in the opposite direction (with respect to the effect on oxygen affinity) from that which is seen in hemoglobin A.
- It is unlikely that a C-terminal aspartic acid side chain could interact with a histidine side chain within the same β -chains, regardless of whether the protein was oxygenated or deoxygenated.
- This arrangement will give a Bohr effect which is only detectable around pH 4 because the C-terminal residue is now aspartic acid.
- The Bohr effect of this hemoglobin will now be unaffected if the protein is digested with carboxypeptidase A.

These are the same amino acid side chains which participate in the intra- β chain salt bridges in hemoglobin, only their positions are reversed. However, the pK change would still occur on the histidine, and the Bohr effect might be virtually unaffected.

3. This next hemoglobin has a distinctive region of the β chains which forms part of a negative charge-lined cavity only in the oxygenated protein.

- If this is the only charge-lined cavity in the protein, the effect of 2,3-DPG on the properties of this hemoglobin is likely to be very similar to that on normal human hemoglobin A.
- It is possible that addition of polylysine to this hemoglobin would cause an increase in the oxygen affinity of the protein.
- Calgon, a polyphosphate polymer which is used to soften water, has the same effect as 2,3-DPG on human hemoglobin A. Addition of Calgon should cause an increase in the oxygen affinity of this hemoglobin.

d. It is possible that addition of polylysine to this hemoglobin would cause an effect which is very similar to that of 2,3-DPG on human hemoglobin A.

e. The idea of a charged-lined cavity is incompatible with formation of a hemoglobin tetramer - the molecule is likely to dissociate into subunits.

Polylysine is a polycation which could bind to the anionic cavity, but it is functioning in this example as a positive allosteric effector, not a negative effector, and it would cause an apparent increase in oxygen affinity.

4. This next hemoglobin has a charged amino acid replaced by a hydrophobic side chain at a position which contacts a hydrophobic patch on an adjoining hemoglobin molecule only in crystals of the oxygenated protein. The resulting interaction is quite strong, but is dependent on oxygenation as indicated.

a. It is possible that a patient who was heterozygous for this hemoglobin (50% mutant/50% normal hemoglobin A) would develop symptoms of vaso-occlusion (plugging of blood vessels) under conditions of high altitude or restricted blood flow.

B. It is possible that an individual who was homozygous for this hemoglobin (100% mutant) would die due to vaso-occlusion in the lungs, where formation of deformed red cells would be greatest.

c. It is possible that in individual who was homozygous for this hemoglobin would have symptoms which are triggered by the same conditions which trigger a sickle cell crisis.

d. It is possible that the pathologic effects of this hemoglobin could be relieved if the ionic strength of the environment could be raised by increasing the salt concentration.

e. It is possible that this hemoglobin would cause red cells to deform once they were exposed to 100% nitrogen.

This mutation is reminiscent of that in sickle cell disease, but since the interactions only occur in the oxygenated protein, the protein would aggregate most severely at the site where hemoglobin is most oxygenated - the lungs.

5. The same mutant hemoglobin in question 4 is being studied further to identify conditions which would diminish the pathologic effects. Which factor is most relevant to reducing the pathologic effects?

a. If the kinetics of aggregation were like the kinetics of aggregation in sickle cell hemoglobin, the rate of aggregation would be halved if the concentration of the mutant hemoglobin were halved.

B. If the kinetics of aggregation were like the kinetics of aggregation in sickle cell hemoglobin, reducing the concentration of the mutant hemoglobin by just a few percent could extend the time before the protein aggregated from seconds to several minutes.

c. If the kinetics of aggregation were like the kinetics of aggregation in sickle cell hemoglobin, raising the pH to increase the extent of oxygenation could extend the time before the protein aggregated, assuming that the effect of pH was like that on hemoglobin A.

d. Inhibiting the reaction which forms 2,3-DPG in the red cell could alter the affinity of the protein for oxygen in such a way as to extend the time before the protein aggregated, assuming that the effect of DPG was like that on hemoglobin A.

e. Removing the C-terminal residues of the mutant hemoglobin might alter the affinity of the protein for oxygen in such a way as to extend the time before the protein aggregated, assuming that these residues functioned as they do in hemoglobin A.

Aggregation in hbS shows typical polymerization kinetics, with a nucleation phase and an elongation phase. The lag time before polymerization takes off is inversely proportional to the concentration of the protein raised to a power equal to the number of molecules in a "nucleus" - between 15 and 30! Thus diluting the protein profoundly lengthens the lag time.

6. This next mutant hemoglobin is very similar to hemoglobin A, but it has undergone two post-translational modifications: the N-terminal amino groups of the α and β chains are acetylated, and the side chains of the β chain C-terminal residues have been methylated.

- This protein may bind oxygen with an affinity close to that of myoglobin.
- This protein may have a negligible Bohr effect
- This protein may not bind 2,3-DPG as well as hemoglobin A
- This protein may not release oxygen to tissues until the tissues are almost completely depleted of oxygen.
- All of the above characteristics are likely to be applicable to this post-translationally modified hemoglobin.

The modifications would break four out of the eight salt bridges, raising oxygen affinity, reducing the Bohr effect, and diminishing the stability of the T state so that DPG wouldn't bind as well.

V. You have been studying a number of proteins to see if their behavior can be explained better by the model of Monod, Wyman, and Changeux, or by the model of Koshland, Nemethy, and Filmer. Indicate next to each observation whether it is most consistent with the MWC or KNF model, whether both models are equally likely to explain the observation, or whether neither model can explain the observation (because the observation itself seems to be incorrect).

1. KNF This tetrameric enzyme (Enzyme 1) is dissolved in a solution of distilled water. It binds its substrate with an S-shaped binding curve (v vs S). As each molecule of substrate is bound, the enzyme expels a proton, lowering the pH of the solution. The proton concentration in the solution increases linearly with substrate binding.

This is like the Bohr effect in hemoglobin, reflecting a conformational change. A linear relationship between conformational change and ligand (or substrate) saturation is a feature of the KNF model.

2. KNF It appears that it is difficult to fully saturate this tetrameric enzyme (Enzyme 2) with substrate, so that the concentration of substrate required to achieve 90% maximum velocity is over 100 times greater than the concentration required to achieve 10% of maximum velocity.

This is a description of negative cooperativity, which can be explained only by the KNF model.

3. BOTH This dimeric enzyme (Enzyme 3) has been crystallized in two conformations, one of which comes out of the crystallization solution when substrate is present and the other of which comes out of the solution when substrate is absent.

The observation that an enzyme can assume two conformations which are associated with presence and absence of substrate is accommodated by both models. This observation does not exclude the possible existence of other conformations; it only notes that two conformations are preferentially crystallized under the two conditions.

4. MWC You have made antibodies which react rapidly with a conformation which this dimeric enzyme (Enzyme 4) assumes when substrate is bound. The antibodies react with the enzyme when substrate is absent, but very slowly, as if the concentration of enzyme were very low. Another set of antibodies reacts rapidly with the enzyme when substrate is absent, but these antibodies now react very slowly with the enzyme when substrate is present.

This is the result of an actual experiment on hemoglobin, which is interpreted as a proof of the MWC model. The antibodies which are described here are likely to be conformation specific, and since both antibodies react with fully liganded and fully unliganded protein (albeit at different rates reflecting the shifting distribution of the two conformations) the result indicates that both conformations are present at all times, and are therefore presumably in true equilibrium.

5. NEITHER This dimeric enzyme (Enzyme 5) binds its substrate with a very high degree of positive cooperative interactions. When you try to quantitate the cooperativity by making a Hill plot, the value of the Hill parameter, $n_H = 4.3$.

The Hill parameter can never exceed the number of subunits, even in the most cooperative protein ($4.3 > 2$).

6. MWC The conformation of this tetrameric enzyme (Enzyme 6) has been studied using circular dichroism. When substrate is bound, the amount of α -helix in the enzyme is decreased. The percentage of change in the circular dichroism spectrum has been graphed as a function of the amount of substrate bound, and the relationship is seen to be markedly nonlinear.

The nonlinear relationship between extent of conformational change (reflected in α -helical content) and extent of ligand (or substrate) binding is a fundamental feature of the MWC model, as opposed to the linear relationship which is due to induced fit in the KNF model.

VI. Time for some enzyme kinetics now. Circle the correct answer in each statement. Here's some general information which you may need to answer the questions below: The breakfast enzyme catalyzes the conversion of chicken eggs into omelets with Michaelis-Menten kinetics. It can also convert duck eggs into omelets, but at a rate so slow as to be negligible. Needless to say, there is some significant resemblance between duck eggs and chicken eggs, but the enzyme clearly distinguishes between them with respect to its ability to convert them into omelets. The transition state for the conversion of the substrate to product looks like a very distorted egg; the nearest analog in shape to the transition state isn't a duck egg, but rather a rattlesnake egg, which looks a little bit like a chicken egg, but doesn't get turned into an omelet. Addition of one cup of vinegar to the enzyme reduces the rate of omelet formation by 50% regardless of how many chicken eggs are added.

1. Given the features of duck eggs and rattlesnake eggs, you would conclude that:

a. duck eggs are competitive inhibitors and rattlesnake eggs are activators.

b. duck eggs are noncompetitive inhibitors and rattlesnake eggs are competitive inhibitors.

C. duck eggs and rattlesnake eggs are both competitive inhibitors, but they are not likely to be bound with comparable affinities.

d. duck eggs are competitive inhibitors and rattlesnake eggs are noncompetitive inhibitors.

Both duck eggs and rattlesnake eggs have critical features of competitive inhibitors - one looks like the substrate (it's a substrate itself but a very poor one - still an inhibitor of binding of the good substrate) and the other looks like the transition state into which the substrate is transformed as it gets converted to product. The transition state analog is likely to be bound with much greater affinity than a true substrate analog, which may fit the active site as well as the substrate, but not likely several orders of magnitude better.

2. In the presence a fixed amount of breakfast enzyme, addition of 1000 or 2000 chicken eggs per bowl yields omelets at a rate of 10 per minute. Addition of 120 eggs per bowl yields omelets at a rate of 5 per minute. The K_m of the breakfast enzyme for chicken eggs is

a. 5 omelets per minute.

b. 500 eggs per bowl.

C. 120 eggs per bowl.

d. without knowing how much enzyme was added, the K_m cannot be determined.

The simple definition of K_m is that it's the substrate concentration at which the velocity is half the maximal velocity. The invariance of the velocity in the presence of 1000 to 2000 eggs suggests that it must be V_{max} . Therefore, the substrate concentration at which the velocity is half as great as it is in the presence of 1000 eggs must be K_m .

3. Since duck eggs look more like chicken eggs than rattlesnake eggs look like chicken eggs:
- a. the enzyme probably binds duck eggs with greater affinity than rattlesnake eggs.
 - B.** the enzyme probably binds rattlesnake eggs with much greater affinity than chicken eggs, but it could actually bind duck eggs with lower affinity than chicken eggs.
 - c. the enzyme probably binds duck eggs with much greater affinity than chicken eggs, but the affinity of the enzyme for rattlesnake eggs is likely to be lower than that for chicken eggs.
 - d. the enzyme probably binds duck eggs with much greater affinity than chicken eggs, but the affinity of the enzyme for rattlesnake eggs might be greater than OR less than that for chicken eggs.

This question returns to the distinction between an ordinary substrate analog (duck eggs) and a transition state analog (rattlesnake eggs). See question 1.

4. Using the breakfast enzyme, you have decided to generate a series of Lineweaver-Burk plots for chicken eggs as substrate in the presence of a set of increasing fixed concentrations of duck eggs.
- a. The numerical value of the Y-intercept (sign not considered) on each plot becomes larger as the fixed concentration of duck eggs is increased.
 - b. The numerical value of the Y-intercept (sign not considered) on each plot becomes smaller as the fixed concentration of duck eggs is increased.
 - C.** the numerical value of the X-intercept (sign not considered) on each plot becomes smaller as the fixed concentration of duck eggs is increased.
 - d. the numerical values of both the X- and Y-intercepts (sign not considered) on each plot become larger as the fixed concentration of duck eggs is increased.

We're looking at Lineweaver-Burk plots of competitive inhibition. V_{\max} (the Y-intercept) isn't affected and the absolute value of the X-intercept ($-1/K_m$) gets smaller.

5. The described effect of vinegar on the breakfast enzyme would lead you to suspect that:
- a. vinegar increases the apparent value of K_m for chicken eggs.
 - b. vinegar decreases the apparent value of K_m for chicken eggs.
 - c. a series of Lineweaver-Burk plots for chicken eggs generated with increasing fixed concentrations of vinegar will have progressively decreasing slopes.
 - D.** vinegar decreases the apparent value of V_{\max} for chicken eggs.

Because the percentage of inhibition by a fixed concentration of vinegar is independent of substrate concentration, it's likely to be a noncompetitive inhibitor, which decreases V_{\max} only.

6. You are wondering whether you can turn omelets into chicken eggs with the breakfast enzyme. At the end of several hours, you notice that a bowl which was originally filled with 200 chicken eggs in the presence of some breakfast enzyme now has 190 omelets and 10 chicken eggs left. You decide to employ the rules of mass action and add 200 omelets to another bowl, along with some breakfast enzyme. After several hours you discover:

- a. that the enzyme cannot catalyze the conversion of omelets into chicken eggs.
- B.** that there are still 190 omelets left in the bowl, but there are also 10 chicken eggs.
- c. that there are 190 chicken eggs and 10 omelets left in the bowl.
- d. that half the omelets in the bowl have been turned into chicken eggs.

Enzymes are catalysts - they speed the equilibrium, but they don't shift it.

7. You are trying to quantitate the potency of duck eggs as an inhibitor of conversion of chicken eggs into omelets by the breakfast enzyme, so you carefully establish that 10 duck eggs will reduce the rate of conversion of 120 chicken eggs per bowl into omelets by 50% in the presence of the enzyme. You have decided to confirm this by measuring the effect of 10 duck eggs per bowl on the rate of conversion of 12 chicken eggs per bowl into omelets, and then measuring the effect of 10 duck eggs per bowl on the rate of conversion of 600 chicken eggs per bowl into omelets. You discover:

a. that no matter how many chicken eggs you add to the bowl, the presence of 10 duck eggs reduces the rate of omelet formation by 50%.

b. that the more chicken eggs you add to the bowl, the more effective duck eggs seem to be as inhibitors.

C. that the more chicken eggs you add to the bowl, the less effective duck eggs seem to be as inhibitors.

d. that the rate of conversion of chicken eggs to omelets increases with increasing concentration of chicken eggs in the bowl, but the effect of the duck eggs is always to reduce the rate of omelet formation by the same percentage.

Because duck eggs are competitive inhibitors, their efficacy as inhibitors diminishes with increasing substrate concentration, until it disappears at very high substrate concentrations where $v = V_{\max}$.

8. After a very careful search, you have found that turkey eggs are also a substrate for the breakfast enzyme. The K_m for turkey eggs using this enzyme is numerically twice that for chicken eggs. On the basis of the information you have been provided about the breakfast enzyme, you would expect that:

a. the K_i for rattlesnake eggs is still larger than the K_m for turkey eggs.

b. the K_i for rattlesnake eggs lies somewhere between the K_m values for chicken eggs and turkey eggs.

C. the K_i for rattlesnake eggs is much smaller than the K_m for turkey eggs.

d. the information is insufficient to draw any conclusions about the K_i for rattlesnake eggs.

The K_i for a transition state analog is always much smaller than that for just about any substrate (certainly one that's not as good a substrate as chicken eggs).

9. You have found a more sophisticated enzyme to catalyze the conversion of chicken eggs into omelets. One of the interesting aspects of this enzyme is that the concentration of chicken eggs which results in a rate of omelet formation equal to 90% of the value of V_{\max} is ten times the concentration of chicken eggs which results in a rate of omelet formation equal to 10% of the value of V_{\max} . Your most reasonable conclusion is that:

a. the new enzyme is displaying expected Michaelis-Menten kinetics.

B. the new enzyme has multiple subunits and displays positive cooperativity.

c. the new enzyme has multiple subunits and displays negative cooperativity.

d. the new enzyme is a tetramer, but its cooperativity cannot be ascertained from such information.

When the ratio of the substrate concentrations $S_{90\%}/S_{10\%} < 81$, the enzyme displays positive cooperativity.

The gourmet breakfast enzyme handles two substrates, chicken eggs and cheese, which it binds sequentially in catalyzing the synthesis of a cheese omelet. You are trying to find out the order in which eggs and cheese bind to the enzyme. Assume all the information concerning the properties of rattlesnake eggs and duck eggs you have already been given is applicable to the gourmet breakfast enzyme as well.

10. If cheese binds to the gourmet breakfast enzyme before eggs, Lineweaver Burk plots of the data for rate of cheese omelet formation as a function of chicken egg concentration determined at a fixed cheese concentration and a set of increasing fixed concentrations of duck eggs:

- A. will all intersect on the Y-axis
- b. will form a series of lines with parallel slopes
- c. will indicate that the apparent K_m for chicken eggs is decreasing.
- d. will show that duck eggs are now uncompetitive inhibitors for chicken eggs.

Even this is now a bisubstrate enzyme, we're still dealing with duck eggs and chicken eggs only, and we'll see Lineweaver-Burk plots for simple competitive inhibition.

11. If cheese binds to the gourmet breakfast enzyme before eggs, Lineweaver-Burk plots of the data for rate of cheese omelet formation as a function of cheese concentration determined at a fixed chicken egg concentration and a set of increasing fixed concentrations of duck eggs:

- a. will all intersect on the X-axis.
- b. will indicate that the apparent K_m for cheese is increasing.
- C. will indicate that the apparent V_{max} is decreasing, but the apparent affinity for cheese is increasing.
- d. will indicate that the apparent V_{max} and the apparent affinity for cheese are both increasing.

This is the case of uncompetitive inhibition - the duck eggs are inhibitors, and decrease V_{max} as a function of cheese concentration, but since they combine with the enzyme-cheese complex only, they appear to increase the affinity of the enzyme for cheese.

12. You would like to see a series of Lineweaver-Burk plots which are consistent with uncompetitive inhibition. Which of the following sets of plots will show the pattern you are seeking?

- a. if eggs bind before cheese, plots of data for rate of omelet formation as a function of cheese concentration determined at a fixed chicken egg concentration and a set of increasing fixed concentrations of duck eggs.
- b. if eggs bind before cheese, plots of data for rate of omelet formation as a function of chicken egg concentration determined at a fixed cheese concentration and a set of increasing fixed concentrations of duck eggs.
- c. if cheese binds before eggs, plots of data for rate of omelet formation as a function of chicken egg concentration determined at a fixed cheese concentration and a set of increasing fixed concentrations of duck eggs.
- D. if cheese binds before eggs, plots of data for rate of omelet formation as a function of cheese concentration determined at a fixed chicken egg concentration and a set of increasing fixed concentrations of duck eggs.

Again, a competitive inhibitor of the second substrate is an uncompetitive inhibitor of the first substrate.

VII. Some miscellaneous useful bits of knowledge:

1. There are several examples of the kind of conformational change which is undergone by a protein on the surface of the Human Immunodeficiency Virus in the course of infecting a target cell. Which statement best characterizes the conformational change?

a. Before attachment to the target cell, a hydrophobic domain is buried within the interior of the protein to avoid an otherwise unfavorably high entropy which would be generated if that domain were interacting with the aqueous environment.

B. A domain on the protein can exist in two environments: one hydrophobic environment is within the interior of the protein itself, while the other hydrophobic environment is within the membrane of the target cell.

c. A domain on the protein can exist in two environments: one environment results in extensive hydrogen bonding within the interior of the protein, while the other environment results in extensive hydrogen bonding to the surrounding water.

d. A domain on the protein can exist in two environments: one environment is associated with a high degree of entropy associated with hydrophobic bond formation, while the other environment is associated with a high degree of entropy associated with clathrate formation.

Many viruses infect cells with so-called fusion proteins, or fusion peptides as domains of attachment proteins. These domains are typically hydrophobic and are buried in the interior of the protein until the virus attaches to its target cell. Then a conformational change results in insertion of the fusion peptide into the hydrophobic interior of the membrane of the target cell, facilitating entry of the virus.

2. Which statement best describes the strategy employed by the neutrophil to penetrate the extracellular matrix of connective tissue?

a. neutrophil elastase activates the TIMPs, which in turn activate neutrophil collagenase, leading to synergistic proteolytic degradation of connective tissue.

b. neutrophil collagenase and neutrophil elastase activate one another from their inactive precursor forms, while alpha-1-proteinase inhibitor and the TIMPs inactivate one another.

C. neutrophil collagenase inactivates alpha-1-proteinase inhibitor while neutrophil elastase inactivates the TIMPs, leading to unopposed proteolysis by both enzymes.

d. alpha-1-proteinase inhibitor stimulates the neutrophil to secrete elastase, while the TIMPs stimulate the neutrophil to secrete neutrophil collagenase.

The reciprocal inactivation of the two endogenous antiproteinases by the two classes of proteinases in the neutrophil skews the proteinase-antiproteinase balance.

3. In the course of the formation of collagen, several unique events occur. Which one of these events is correctly described below:

a. hydroxyproline is synthesized within the fibroblast and then incorporated uniquely into collagen.

b. the repeating sequence of three amino acids within fibrillar collagens causes them to fold up into alpha-helices.

C. in the presence of high levels of selenium, the extracellular crosslinking of tropocollagen molecules is inhibited due to diminution of lysyl oxidase activity.

d. tropocollagen fibrils cannot form until two intracellular proteolytic cleavages remove the N - and C-propeptide domains from procollagen.

Processing of procollagen as well as crosslinking of tropocollagen are all extracellular events, the latter resulting from interaction of lysine and al-lysine residues, which are in turn generated by lysyl oxidase.

4. Dowex-1 can be used to manage hypercholesterolemia because:

A. as an anion exchange resin with a polystyrene backbone, it can bind bile acids strongly through multiple forms of interaction.

b. as a cation exchange resin with a polystyrene backbone, it can bind bile acids strongly through multiple forms of interaction.

c. as an anion exchange resin with a cellulose backbone, it can bind bile acids strongly through multiple forms of interaction.

d. as a cation exchange resin with a cellulose backbone, it can bind bile acids strongly through multiple forms of interaction.

Dowex 1 is an anion exchange resin, which can bind negatively charged bile acids (they are acids, after all) through a combination of electrostatic and hydrophobic interactions (the rings of the bile acids interact with the polystyrene backbone of the Dowex resin).