

Lecture #2

Lecture 2

2/6/04

Notes: DS viewer Pro is PC only. This is the program that will be used in Techniques and Discussion, but Swiss PDB viewer and Rasmol are both available free online, and work on both PC and Mac.

Also, remember that you are expected to know all the amino acids, their one letter codes and their structures for this course. This is some of the basic vocabulary of biochemistry, so memorize them!

Review from previous lecture:

	Prokaryotes	vs	Eukaryotes
1. Size (important when thinking about metabolism)	1 to 2 microns		10 to 100 microns
2. Compartmentalization	no discrete organelles		organelles with membranes
3. Cytoskeleton	recently discovered in bacteria		very important for cell shape, organelle movement
4) DNA (remember different structures when thinking about replication and repair mechanisms)	<i>E.coli</i> -circular DNA		histone/DNA complexes

5) Larger surface to volume ratio for bacteria- important in nutrient fluxes such as diffusion of oxygen and other molecules. In eucaryotes the surface to volume ratio is much smaller and therefore some some molecules require carriers, myoglobin for example delivers oxygen?

Similarities in cellular MACHINERY in prokaryotes and eukaryotes allow us to apply what we learn from the simpler prokaryotic system to the more complex eukaryotic systems. Fatty acid synthases (FAS), the ribosomes, the machines that fold proteins and the machines that degradation proteins are all conserved. Proteins related to redox active metal homeostasis (copper and iron) and oxidative stress are also conserved.

CELL INTERIOR

When you break open cells, the solution appears viscous, and sometimes “snotty”

The interior of cells is very CROWDED! In bacteria, the concentration of macromolecules is estimated to be 340 mg/mL ! The crowded interior of cells has thermodynamic and kinetic consequences for how things work inside the cell.

See module 1 handout for an artist's rendition of the "stuff" crowding the cellular interior- including cartoon images (to scale) of ribosomes, globular proteins, DNA, etc... Question: Is the interior of cells just a bag of enzymes, or is there a higher order of protein-protein, protein-nucleic acid interactions?

The density of the crowded cell affects both thermodynamics and kinetics. This crowdedness is a key difference when studying a system in living cells vs *in vitro*. In a typical enzyme assay (think 5.32 lab or 7.02) the concentration of enzyme is nM-microM. Compare these concentrations to a cellular environment in which the concentrations of enzymes are typically microM and in the case of enzymes in the glycolysis pathway can be as high as 100 microM or even mM. i.e. Rate limiting step *in vitro* may be the time it takes for proteins to find each other, and this may not be true *in situ*.

Think of the time it takes a passenger to check in at Logan Airport-(min to hours) depending on how crowded it is. Do you have an e-ticket or do you have to wait in line for a single ticket agent? Do you have to take off your shoes at security? This is an analogy for reactions within the cell. If a protein interacts with many other proteins, it may be "sidetracked" before interacting with correct protein. The concentrations and affinities of proteins for one another govern the reactions that occur.

Also in handout 1, notice the page with of SIZES and TIMESCALES. These are important considerations when choosing physical methods. Each method has a timescale associated with it.

1. What are the concentrations of small molecules inside the cell? How much of your small molecule is bound to a macromolecule? How much is free? A new field called METABOLOMICS is now trying to identify all of the metabolites within the cells, their concentrations and how these concentrations change depending on growth conditions. The concentration of "free" metabolite is difficult to determine as one molecule plays many roles within the cell. All methods to measure concentrations, in general disrupt the binding to macromolecules unless it is unusually tight.

Example: Serine

- 1) Serine is covalently attached to tRNA, and is required for protein synthesis
- 2) Serine is the major one carbon donor (at the methyl, aldehyde, acid oxidation states) and is essential for purines and pyrimidine biosynthesis
- 3) Serine is converted to pyruvate by a pyridoxal phosphate requiring enzyme that catalyzes beta eliminations. Pyruvate plays a metabolite between glycolysis and the Krebs cycle
- 4) Serine is also found in phospholipids: phosphatidyl serine
Point: almost all small molecules are involved in numerous metabolic pathways. Sorting out the free concentration vs the amount bound to macromolecules is an active area of research.

Recently, 100s of structures of proteins at high resolution have been solved. Some proteins unexpectedly are found to have small molecules (ADP, zinc, CoA) bound. They

are so tightly bound that they remain bound through extensive protein purification protocols. Some are so tightly associated researchers may not realize they are there until a crystal structure is done! This tight binding again interferes with establishing the concentrations free small molecules.

2. The large molecule *E. coli* beta-protein (structurally homologous to PCNA in humans) is part of the “real” DNA polymerase (DNA polymerase III). It forms a clamp on DNA and facilitates reactions of proteins that are involved with synthesis and metabolism of DNA. The beta protein is involved in replication, repair and recombination. A recent paper suggests that beta-protein interacts with as many as 10 other proteins in *E. coli*. Its size is not sufficient to allow it to bind to this many proteins at one time, so DYNAMIC PROTEIN/PROTEIN INTERACTIONS must be occurring. The other proteins must come on and off the beta-protein. The concentrations of the beta protein and its partners and the affinity of the beta protein and each of its partners controls metabolism.

3) Nature, 415, 141 (2002) (TAP- tandem affinity purification scheme)

Proteomics and mapping protein interactions is another very important area of research. Researchers find protein/protein interactions by purifying a protein and seeing what purifies with it. EX. Yeast has 6000 total genes, and 1739 of them have been tagged at the DNA level so that the expressed protein will bind a column through its tag. The protein is purified on a column that recognizes and binds the tag, and proteins with non-specific interactions are washed away. Proteins that specifically interact with the tagged protein will come off the column with it. Using this technique, 200 protein complexes have been proposed from the 1739 tagged proteins investigated.

Remember our question: Is the cell just a bag of enzymes?

Protein/protein interactions are important - there are both tight and weak protein interactions, and interactions are governed by the concentration of protein and may be governed by the concentration of small molecule metabolites.

PREVIEW of what is to come in Modules 2, 3 and 4

Fatty acid synthase (FAS) as a paradigm for biosynthesis of a large number of natural products that are pharmaceutically and agriculturally important: polyketide synthases (PKS) and non-ribosomal polypeptide synthase (NRPS). All polymerization reactions involve- initiation, elongation, termination, fidelity. FAS involve non-template dependent polymerizations. No templates- so how does the cellular machinery control chain length and fidelity?

FAS also require post-translational modification for activity. Post-translational modification is a major mechanism of expanding the number of proteins. The number of genes in the yeast genome is about 5500 (the number keep changing). However, the

modification by phosphorylation, acetylation, ubiquitination, glycosylation, hydroxylation, methylation etc greatly expands protein function.

Mammalian FAS is homodimer and its size based on cryoEM is 180x130x75 angstroms, How does this protein catalyze 50 reactions!

In modules 3 and 4 we will be examining similar size machines the ribosome, the foldosomes the proteosomes.

For next week, read the chapter on fatty acid biosynthesis in an introductory biochemistry textbook like Voet&Voet.