Lecture 1 Course overview and intro to enzymes

Web page http://courses.ucsd.edu/rhampton/bibc102
Ideas in metabolism
  non-iterated structures but common structural themes
  metabolic pathway
Why study metabolism
  relevant to all life
  numerous diseases and conditions
  catabolism and anabolism
  we think on many levels
    from atomic to ecological
Proteins
  amino acids
  peptide bond
  4 levels of structure
    alpha helix, beta sheet
Cofactors
  heme, ions, vitamins, etc.
Enzymes: the key to metabolism
  features and action
Rate enhancement
  typical numbers
  description on energy diagram ( lowering DG‡)
Stabilizing transition state
Four modes of catalysis
  entropy reduction, acid-base, metal ion, covalent intermediate

Lecture 2 Enzyme kinetics and regulation

Activation energy and the reaction coordinate
  ΔG‡: the free energy of activation
  effect of enzyme on ΔG‡
Computing the effect of changing ΔG‡ on a reaction rate
  $e^{-\frac{\Delta G}{RT}}$ as a multiplicative factor
Ligand binding: features and the Kd
Relation of binding isotherms to Kd and Bmax
Viewing enzyme action as a process started by binding
Michaelis-Menton equation and isotherm
Relationship between Kcat and Vmax
Lineweaver-Burk yo! Flippin' M & M…
  use in visualizing data for mechanism and inhibition
Enzyme inhibitors
  competitive
uncompetitive
suicide
Chymotrypsin mechanism
Induced fit
xylose vs. glucose and ATP hydrolysis
Covalent modification of enzymes
phosphorylation and dephosphorylation
kinases and phosphatases
example: glycogen phosphorlase
other modifications: adenylylation, uridylylation, methylation
ADP ribosylation

Lecture 3  More enzymes and bioenergetics

Cooperative vs. simple binding: hemoglobin vs. myoglobin
Cooperative enzyme an analogous curve
structural ideas
features of a cooperative enzyme
heterotypic vs. homotypic regulator
Positive and negative allosteric regulators
Example: aspartate transcarbamoylase
Example: chorismate mutase: an allosteric dimer
Metabolism overview: anabolism and catabolism
convergence of all anabolism and catabolism to a few molecules
ATP, NADH, NADPH, FADH2
converging catabolism diverging anabolism
recurring patterns
Def of DG: equation with H and S
structural ideas
Cooperative vs. simple binding: hemoglobin vs. myoglobin
Cooperative enzyme an analogous curve
structural ideas

Lecture 4 Bioenergetics

ΔG: a combination of enthalpy and entropy
some aspects of entropy
ΔG° : relation to K'eq
equation relating actual ΔG to ΔG°
concentration ration determines true ΔG
ATP : the 20 dollar bill of the cell
hydrolysis of ATP
a variety of phosphorylated compounds
free energy is additive
general meaning
Example of spontaneity due to sequence of reactions
  polynucleotide synthesis and pyrophosphate hydrolysis
General idea of coupling reactions
  To lift a big weight, you need to drop a larger weight
    "Big Bear/Little Bear
Example of coupling two reactions by forming a covalent intermediate
  formation of glutamine by addition of P to glutamate
Other types of ATP hydrolysis
Examples of coupling two reactions by forming a covalent intermediate
  formation of palmitoyl-CoA by addition of AMP to palmitate
  firefly reaction
substrate-limited reactions vs. enzyme-limited reactions in metabolism
Redox chemistry
  half-cell reactions
  calculating half-cell potentials
E'\text{\textregistered}\) for redox reactions
  nernst equation
  finding half-reactions in sugar oxidation
  predicting reactions from half-cell potentials
NAD/NADH and NADP/NADPH
FAD/FADH2 and FMN/FMNH2)

**Lecture 5 Glycose and glycolysis**

Glucose metabolism overview
Glycolysis overview
  preliminary phase
  payoff phase
Individual reactions and enzymes
  Hexokinase
  phosphohexose isomerase
    anomers to understand reaction
  phosphofructokinase (PFK-1)
  aldolase
    two 3-carbon compounds
  triose phosphate isomerase
glyceraldehydes 3-phosphate dehydrogenase
  active site and mechanism
  phosphoglycerate kinase
    substrate level phosphorylation
  phosphoglycerate mutase
    a bis-phosphorylated intermediate
  enolase
    keto-enol chemistry
  pyruvate kinase
Uses of glycolysis
  single cells and large animals

**Lecture 6  More glycolysis and pentose phosphate**

Uses of glycolysis
  single cells and large animals

Entry of other sugars into glycolysis
  Sucrose: fructose and glucose
  Lactose: glucose and galactose
  Fructose
    6 or 1 phosphorylation
    aldolase of 1-P in liver
  Galactose: addition to UDP, epimerization

Glucose from glycogen
  structure and phosphorolysis

Fermentations: continuous energy in the absence of O2
  lactate production
  ethanol production

Regulation of glycolysis
  energy landscape reveals regulated enzymes
  isozymes of hexokinase
  PFK and multiple regulators
  allosteric control of glycogen breakdown

Pentose phosphate pathway
  source of NADPH, ribose and many sugars
  oxidative steps
  non-oxidative steps

**Lecture 7 PDH and Krebs Cycle (delivered by Steve Mason)**

Overview of Krebs cycle and respiration
  acetate is central
  first Krebs, then respiration

Coenzyme A, or CoA-SH: the acetate carrier

Pyruvate Dehydrogenase Complex (PDH)
  the reaction
  CoA-SH
  lipoic acid
  the catalytic cycle
the layout of PDH TPP, lipoic acid, FAD, NAD
Krebs cycle
reactions and logic
source of reducing equivalents
individual reactions
Citrate synthase
Aconitase
Isocitrate dehydrogenase
alpha ketoglutarate dehydrogenase complexes
analogy to PDH
Succinyl CoA synthetase
produces GTP
Succinate dehydrogenase
FAD/FADH2
inhibitors
Fumarase
specificity
Malate dehydrogenase
summary of input and output

**Lecture 8 More Krebs Cycle**

Prochirality in aconitase reaction
“three point landing” model
Sum of Krebs cycle reactions: energy revenue
Amphibolic nature of Krebs cycle
anapleurotic reactions
pyruvate carboxylase
PEP carboxykinase
PEP carboxylase
malic enzyme
biotin and carboxylation
Enzyme complexes
Regulation of Krebs cycle
citrate synthase
isocitrate dehydrogenase
alpha keto glutarate dehydrogenase
Glyoxylate cycle
making glucose from acetate groups
isocitrate lysase
malate synthase
glyoxysome: cell biological aspects of metabolism
Coordinate regulation of Krebs and glyoxylate
isocitrate lysase: allosteric
isocitrate dehydrogenase: phosphorylation control by
Lecture 9 Respiratory chain and oxidative phosphorylation

Functions and structures of the mitochondrion
Descriptions of electron carriers
  ubiquinone
  heme
  iron-sulfur centers
Respiratory chain ideas and studies
  E values and an ordered cascade
  use of inhibitors
  biochemical studies of isolated complexes
    (Efraim Racker)
Electron donors
  ubiquinone; CoQ
  cytochromes
  iron-sulpher centers
Predicting/hypothesizing the electron transport chain
  order of electron carriers
  use of blockers
  biochemical studies
Complex composition of respiratory chain components
Complex I: NADH dehydrogenase
Complex II: succinate dehydrogenase
Complex III ubiquinone:cyt c oxidoreductase
  the Q cycle
Complex IV: cytochrome c oxidase
Making a proton gradient
  two components: chemical (concentration gradient) and
  electrochemical (voltage)

Lecture 10 Chemiosmotic Hypothesis and the F1 ATPase

Electron transport chain
  proton-motive force
    osmotic component
    electrochemical component
Chemiosmotic hypothesis
  Statement of hypothesis
  Experimental observation of coupling
O2 consumption and ATP production
  Observation of coupling
  Uncouplers as membrane perturbants
  Weak hydrophobic acids
  Direct reconstitution
F1 ATPase
  how energy is used: product extraction
Structure of the ATPase
  F1 active sites
  Fo membrane channel
  rotary concept
    direct rotary experiment Noji et al.
Other rotary engines
  bacterial ATP synthase
  direct mechanical energy from H+ grad
Use of uncoupling
  hybernation
Coordinate regulation
  ADP limitation: “acceptor control”
  coordinated regulation of entire glucose oxidation
Bookeeping
Mitochondrial diseases

**Lecture 11 Remaining mitochondrial stuff; Gluconeogenesis, and coordinated regulation of glucose**

Malate-Aspartate shuttle: getting electrons into and out of the mito
  redox and transaminations
  carbon skeletons are a good way to follow complex reaction
    schemes
Uncoupling as a useful function: thermogenin and heat
Coordinated regulation of respiration, Krebs and glycolysis
  Acceptor control: ADP availability regulates respiratory chain
  NADH, ATP/ADP and other intermediates regulate Krebs
  similar regulation of glycolysis
Mitochondrial diseases
Anabolism: gluconeogenesis
  overview of glucose synthesis in animals and plants
  bypass steps in glycolysis
    PC/PEPCK
      regulation of PC vs PDH by acetyl-CoA
    two routes of pyruvate to PEP depending on cytosolic
      NADH

  FBP-1
G-6 phosphatase
Regulation of gluconeogenesis
Fr2,6BP activator of glycolysis and inhibitor of gluconeogenesis
coordinate control of PFK1 and its FBP1
synthesis by PFK-2 and FPolase-2: one polypeptide!
Control of Fr2,6BP by phosphorylation
insulin stimulates dephosphorylation (elevates Fr2,6BP)
glucagons stimulates phosphorylation (lowers Fr2,6BP)

Glycogen
description of structure
glycogenin, branch structure, 1-4 and 1-6 linkages
granules
synthesis of glycogen
UDP-glucose, glycogenin, glycogen synthase
branching enzyme
breakdown of glycogen
glycogen phosphorylase, debranching enzyme

Control of glycogen metabolism
allosteric control of glycogen phosphorylase phosphorylation
hormonal control of GP phosphorylation
insulin decreases activity (less phosphorylated GP)
glucagons (in liver) increase activity (more phosphorylated GP)
hormonal control of GS phosphorylation
insulin, glucose increase activity (decrease phosphorylation)
glucagon and epinephrine inhibit GS (increase phosphorylation)

Liver as a glucostat
various ways glucose is processed in liver
normal blood glucose levels
Cori cycle: trans tissue regeneration of glucose
response of liver to blood glucose levels: a buffer
glucagons effect on glycogen met. and glucose met.
insulin effect on glycogen met and glucose met.

Muscle vs. liver: the selfish tissue
The pancreas as source of glucagon and insulin

finish lecture 11
regulation of glucose storage and utilization

Lecture 12 photosynthesis and carbon fixation

Carbon fixation
molecular aspects of carbon fixation
Chloroplast
structure and origin
Light: The Biggest Bear
Pigments that participate
structures
properties
action spectrum
Macromolecular organization
LHCII
phycobilosome
chloroplast photosystem
Bacterial photosystems
cycling electrons for H+ gradient
harvesting electrons for reducing equivalents
Chloroplast photosystem: the Z scheme
system II
water splitting and proton gradient
system I
making reducing equivalents
cytb6f: linking photosystems II and I
water splitting complex: where those e come from
Chloroplast compartments
lumen of the thylakoid membrane
Other light-harvesting complex
bacteriorhodopsin
Light independent processes
Calvin cycle
Rubisco
3 stages fixation, reduction, and regeneration
Regulation of photosynthesis
stromal conditions Mg+2 and pH
Calvin cycle enzymes reg. by redox
control of Fr2.6BP more in dark, less in light
more glycolysis when dark, more gluconeogenesis when light
Transport of products to cytosol
C4 plants
rubisco’s unanticipated substrate
cell biological solution to this problem

Lecture 13 catabolism of fats

Structure of phospholipids and triglycerides
Absorption of fats
uptake, conversion, packaging
chylomicrons
Use of leftover glycerol
one per phospholipid
CoASH as an acylcarrier
Carnitine and mitochondrial oxidation
rate-limiting step in b-ox
Beta oxidation: an interated pathway
Steps in beta oxidation
dehydrogenation
hydration
dehydrogenation
transfer to new CoASH

Effectiveness of beta-ox for energy and water
bear
camel

Special cases on B-oxidation
mono-unsaturated
polyunsaturated
odd-numbered
cobalamin

FAO in mitos and peroxisomes

Ketone bodies
excess AcCoA
acetoacetate and b-OH-butyrate
synthesis via HMG-CoA
HMG-CoA lysase
utilization
transfer to CoA
lyase

Presence of ketone bodies in diabetics and dieters

**Lecture 14 outline Fat Anabolism**

Fatty acid synthesis
carboxylation of AcCoA
FAS complex
ACP like CoA
FAS reactions
loading of malonyl coa
condensation
reduction
dehydration
reduction
transfer

NADPH
cyto
chloroplasts
source reactions
malic enzyme
pentose phosphate

Citrate as a carbon source
Regulation of FAS
insulin
glucagon
allosteric

Further steps in synthesis
elongation
desaturation
Cyclooxygenase
  protaglandins
  thromboxanes
Inhibitors of COX
  aspirin, etc “NSAIDS"
Phospholipid synthesis
  anatomy: headgroup and DAG
  chemistry of linkages
  CDP as carrier
  two strategies
Isoprenes and cholesterol
  four stages of cholesterol synthesis
    mevalonate
      similarities to KB synthesis
      rate limiting enzyme HMG-CoA reductase
      active isoprenes
      condensation to squalene
      production of cholesterol
  regulation of cholesterol synthesis
    both LDL receptor and HMG-CoA reductase regulated
  the statins: inhibitors of HMG-CoA reductase
  thousands of isoprene compounds in biology
  many sterols are important in physiology
Lipoproteins
  chylomicrons, VLDL, LDL, HDL

Lecture 15 Amino acid catabolism and urea cycle

Nitrogen from AA
Three routes to liver
  ammonia from ingested AAs
  aspartate from muscle
  glutamine from muscle and other tissue
Final fate of nitrogen
  ammonia, urea, uric acid
Transamination to aKG
  PLP an active aldehyde
  reactions after addition of AA
    CO2 removed to give amine
    H removed and replaced to give D form
    N removed to give alpha keto acid
Glutamine as N carrier
  glutamine synthetase and glutaminase
Aspartate as N carrier
The urea cycle, also called the ornithine cycle
  two compartments
mito, and cyto
structure of urea
reactions
carbamoyl P production
addition to ornithine to make citruline
addition of AMP
replacement with aspartate to make arginosuccinate
removal of fumarate to make arginine
cleavage of urea to regenerate ornithine
regulation of UC
acetyl glutamate
Medical enhancement of N removal
synthetic allosteric
giving bulk metabolites
Essential and non-essential amino acids
Aa catabolism and the Krebs cycle
One carbon metabolism
SAM, tetrahydrofolate, biotin
Diseases of AA catabolism
genetic blocks to the pathways
PKU

Lecture 16  Metabolism of Amino acids and Nucleotides

The nitrogen cycle
nitrogen fixation
ammonia, nitrates, nitrites and N2
nitrogen-fixing bacteria
nitrogenase
legumes
 glutamine as a N source
gln synthetase
reaction
regulation
adenylylation, guanylylation
glutamine amidotransferases
Synthesis of AA
familiar sources Krebs, glycolysis, pp pathway
regulation
classic feedback
crosstalk between pathways
Synthesis of things from AAs
glutathione, neurotransmitters, porphyrins, creatine
Nucleotide structure
names and structures
nucleotides and nucleosides
bases purines and pyrimidines
Synthesis of nucleotides
PRPP
purine synthesis
many molecules contribute
construction on PRPP ring
IMP and subsequent conversion
regulation of multiple steps
PRPP synthetase
glutamine-PRPP amidotransferase
pyrimidine biosynthesis
carbamoyl phosphate
first base, then addition to PRPP
UMP to UTP to CTP
regulation
CTP regulates ATP
balanced synthesis: purines override CTP

Synthesis and salvage of purines and pyrimidines
APRT
HPRT and Lesch-Nyhan disease
pyrimidine pathways as well
inhibitors of glutamine amidotransferases
azaserine, acivicin

Production of D's from R's
ribonucleotide reductase
glutathione or thioredoxin as a source
NADPH ultimately
regulation of RNR
primary regulation (ATP vs. dATP)
specificity site
balanced synthesis

Production of T from U
thymidylate synthase
THF as methyl donor, serine as replenisher
drugs that affect cycle of THF usage
methotrexate, FU (mammals)
trimethoprim (bacteria)

Purine catabolism
ADA deficiency
xanthine oxidase overactivity
gout and allopurinol

Lecture 17 Cancer, Ageing and Metabolism

Problems with being long-lived metazoan
cell quantity
cell quantity
Why cancer is so difficult
fighting self-derived cells
Cancer and oxygen
  HIF1-alpha
  glycolysis
  angiogeneic factors
Apoptosis
  definition
  cytochrome c
Differences between cancer cells and normal ones
  the citrate lysase connection
  source of AcCoA, and NADPH
  orphan drugs that block citrate lyase
Ageing: a universal feature of living things
  yeast, worms, flies, rodents, primates
Caloric restriction
  variety of organisms
  Primates and humans?
    Okinawa study
    Biosphere 2
    NIH study of CR in monkeys
      C58: the oldest rhesus monkey?
Genetics of ageing
  progeria indicates master ageing genes
  C. elegans
    daf2-the insulin connection
Yeast
  SIR2: the more the better
    NAD⁰-dependent protein deacetylase
CR may be connected to SIR2 by insulin
Molecules that stimulate SIR2 homologues: STACs
  resveratrol

Lecture 18 Exercise, Diet and Obesity

Liver as a metabolic integrator
  glucose pathways
  amino acid pathways
  lipid pathway
  glucostat
Adipocytes
  fat storage and synthesis
Metabolic stores of lipid, protein, and carbohydrate
  liver glycogen vs. muscle glycogen
Interplay between liver, muscle, and adipocyte
Muscle fuels
  no glucose-6-phosphate
  phosphocratine as phosphate buffer
  Cori cycle restores muscle by use of liver
Heart vs. muscle
Brain metabolism
  PET scanning for glucose ketone bodies
Glucose maintenance
  range
  insulin and glucagons
  other hormones
Insulin and diabetes
  molecule
  effects
  source
definitions of diabetes
    type I insulin production; 5-10% of cases
    type II insulin response: remaining cases
  rapidity of increase
  receptor removal from different tissues
  a novel strategy: glucokinase activation, by Dr. Joseph Grippo
    glucokinase: the liver-specific hexokinase
    structure and effect of synthetic allosteric activator

Obesity and diabetes
  Dramatic increase over time
  BMI
    correlates with disease, esp type II diabetes
  genetics of obesity
    ob/ob mice
    leptin is the altered gene
      an adipocyte "hormone of plenty"

CNS components of appetite and energy expenditure
  Leptin works centrally
  appetite-controlling peptides
    PYY decreases, ghrelin increases
  cannabinoid receptor and appetite control
    CB1 receptor for cannabinoids
    CB1 blockers suppress appetite
    rimonabant (Acomplia®)

The explosion in high-fat, high carb foods
  lifestyle vs. evolution
  the industrialization of the food industry
    Fast Food Nation by Eric Schlosser (info on website)
Exploring genetics of obesity and fat balance
  Pima people on western diet
    50% vs. 6-8% adult onset NIDDM
  Thrifty gene model (certainly multiple genes)
  model organisms
    Kaveh Ashrafi (UCSF)
      C. elegans (worms) and nile red
        fat stores can by visually scored
        fat-storage related genes can be discovered

Human behavior and obesity
calories and activity

interventions
- consumption, absorption, utilization
- gastric bypass surgeries
- low carb diets
  - inducing a lipolytic state
- 2003 studies on website for the interested