Chem 431A-L13
admin: Quiz

Last lecture:

1) levels of protein structure

2) separation of proteins

Today:

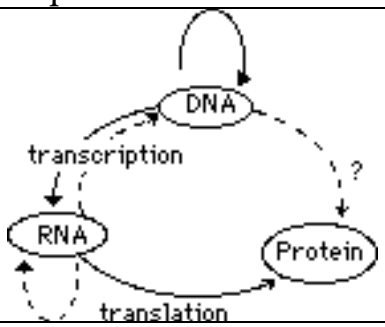
1) Review about proteins and 1° structure:

2) Covalent structure of proteins & sequencing.

Lecture

(1) Review: function of protein depends on 1° struc

Consider sequence of bovine insulin.

2) Proteins are long *unbranched* chains of amino acids formed together by peptide bonds. It turns out that the specific sequence of amino acids stringed together to form a protein is very critical to determining what protein's final structure will be and therefore what its function will be in a cell. The length of the *polypeptide* chain is typically over 100 to 1800 aa's (myosin). A muscle protein, called *titin*, has 17,000 aa's! average is about 270 aa's in eukaryotes (mw=31,700 amu, daltons).	(3) Just as an exercise, how many unique dipeptides are possible using the 20 aa's? well, there are 20 possibilities for the first and 20 possibilities for the second. so the total is 20x20=400 unique dipeptides. In general, for n-peptide, $(20)^n$! What would it be for the smallest proteins? $(20)^{100}$! Each protein has a unique sequence of aa's which give it its unique properties. this sequence is the primary structure (1°) of the protein. like DNA right? This sequence is encoded in the genetic
material's DNA molecule. By convention, the sequence is read from the N-terminal end of the polypeptide chain through to the C-terminal end of the chain. Each of these aa's are coded = matched to specific tRNAs ***Show the overhead on the aa's and the ***translation*** step.	

6) amino acid sequencing – now automated.

Understand and differentiate between

Sanger Method and Edman Degradation Methods (see figure)

First complete sequence reported 1953 by Frederick Sanger : bovine hormone insulin. (took 10 years and 100 g of insulin). Hundreds of thousands of polypeptide aa sequences are now known. (now: a few days, a few μg) Imptance of aa sequence info: 1. prerequisite for determining 3-d structure and molecular mechanisms of action 2. sequence comparison among proteins provide info on evolutionary relationships 3. many inherited diseases caused by mutations: assist in diagnostic tests and therapies.	N-terminal can be determined by Sanger's reagent. to determine the N terminal: use Sanger's reagent in alkaline solution. 1-fluoro-2,4-dinitrobenzene (FDNB) + H_2N-C-… -> DNP-N-… + HF After hydrolysis of the entire polypeptide, only the N-terminal will have dinitrophenol-aa, DNP-aa_1. To break –S-S- bonds, use either performic acid (oxidation) or dithiothreitol(DTT, reduction)

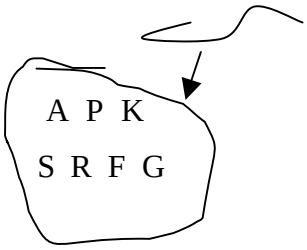
How to sequence a long protein chain? First cut it into several smaller segments. Each segment can be deduced by judicious use of *proteases*: Protease(peptidase) = enz. that cleave peptide bond or protein. 2 types: exo- & endopeptidases. A. *Exopeptidases* (cleave at terminal peptide bonds) have 2 types: *a) Carboxypeptidases*-cleave C-terminal *b) Aminopeptidases*-cleave N-terminal B. *Endopeptidases*(cleave internal peptide bonds). See below: R -N-(C-R_1)-(C=O)---NH-(C-R_2)-(C=O)-R ↑ (cleavage site) **enzyme** **R_1** **R_2** *trypsin* K, R ≠ P *Thrombin* R ≠ P *Bromelain* K, A, Y, G *Carboxypeptidase* C-terminal aa ≠P	Say we have: n-Phe-Ala-Lys-Arg-Pro-Ser-Gly-c Or, n-F-A-K-R-P-S-G-c (we start by determining all aas: A,K,F,R,S,G,P (7 aas; we don't know the sequence yet) _____ _____ _____ _____ _____ _____ _____ Use *FDNB* (i.e. Sanger's rgt) to det. n-terminal aa_1: Sanger's reagent labels Phe (F), therefore F _____ _____ _____ _____ _____ _____ Carboxypeptidase releases S & G only: So F _____ _____ _____ P (SG) (SG) Use trypsin: releases 2 peptides. One has F, K, A; the other contains S ,G, P, R So R is next to P F (AK) (AK) R P (SG) (SG) And also, K must be to the left of the cleavage so: F A K R P (SG) (SG) Use bromelain: releases 3 fragments containing: (F,A) (K) (R,P,G, S) so A goes with F, S is before G: Phe Ala Lys Arg Pro Ser Gly

6) Protein sequences and evolution

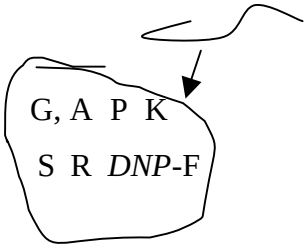
LET'S REPEAT THE PROCESS:

Given unknown polypeptide: 

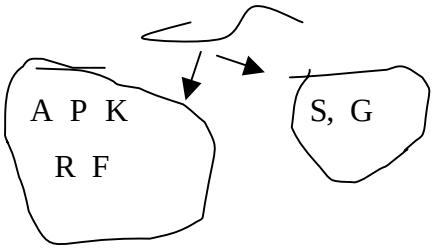
(1) Treat with acid hydrolysis: => you have 7 amino acids!



(2) Treat with FDNB, then hydrolyze => (1)F (2) (3) (4) (5) (6) (7)
the polypeptide:



(3) Treat with Carboxypeptidase. Obtain => (1)**F** (2) (3) (4) (5)P (6) (7)
a 5-peptide segment and release S, G

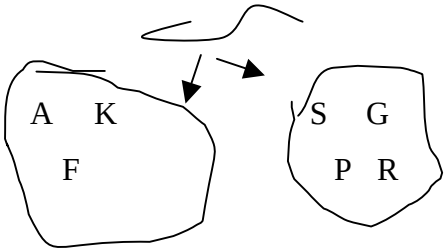


(4) (3) Treat with *trypsin*. Obtain => (1)**F** (2) (3) (4) (5)**P** (6) (7)

a 2 segments. Acid hydrolyze them => (1)**F** (2) (3)K (4) (5)**P** (6) (7)

to determine the aa's present in each: => (1)**F** (2) A (3) **K** (4) (5)**P** (6) (7)

=> (1)**F** (2) **A** (3) **K** (4) R (5)**P** (6) (7)



(5) Treat with *bromelain*, obtain 3 segments => (1)**F** (2) **A** (3) **K** (4) **R** (5)**P** (6) (7)G

Hydrolyze each to find their aa's. => (1)**F** (2) **A** (3) **K** (4) **R** (5)**P** (6) S (7) **G**

They contain:

(F,A) (K) (R,P,G, S). => VOILA!

=> (1)**F** (2) **A** (3) **K** (4) **R** (5)**P** (6) **S** (7) **G**