Chem 431A-L15-F'07
admin:

Last lecture:

1) turns
2) fibrous proteins
3) general principles for protein structure
4) stability: Anfinsen Expt

Today:

1) look at α- and β-keratins
2) Stability of protein: Anfinsen Expt
3) Predicting 2° and 3° structure…

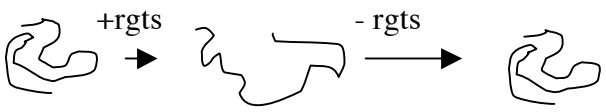
* Look at a) α-keratin, b) β-keratins c) collagen a) **α-keratin**- strong materials; give examples know: α-helix(right handed), 2-chain coiled coil (5.2Årepeat, left handed, parallel, rich in hydrophobic residues, 4° structure), protofilament, protofibril, intermediate filament(IF), stabilized by disulfide bonds. b) **silk fibroin**: predominantly β sheet. Not extensible but flexible. Predominantly Ala, Gly	b) **Collagen** – has repetitive 2° structure but <u>neither</u> α or β structure. provides strength, know examples, left-handed helix unique to collage, 3 aa's Gly-X-Pro, or Gly-X-4-Hyp (4-Hyp = 4hydroxylproline), right handed triple helix with glycines in the area of contact.; 3000Ålong,15Å thick. Staggered. Crosslinked. Tight wrapping of 3-ple helix more tensile strength than steel wire. Vitamin C allows 4-Hyp to be formed. Absence of 4-Hyp due to vit C deficiency => scurvy, degeneration of connective tissue.

2) <u>Native</u> struc. physiological and active structures. denaturation factors: 1) heating – thermal denaturation, over narrow T range. (cooperativity) most proteins melt below 100°C (except thermophillic bacteria). 2) pH variations changes charge distribn, and Hbonding rqments. 3) detergents assoc with nonpolar residues 4) chaotropic agents: guanidinium, H_2N-(C=NH_2^+)-NH_2 and urea H_2N-(C=O)-NH_2 (5-10M; increase solubility of nonpolar substances in water; disrupt hϕ intxn in protein)	Denaturation experiments.Anfinsen,Christian(1957): used ribonuclease (RNAse), a single subunit. 124aa. added 8M urea with 2-mercaptoethanol, ($HSCH_2$-CH_2-OH) which reduces the Cys-Cys disulfide bonds. RNase has 8 cys. chances of forming combo: (7)(5)(3)(1) = 105 ways. dialyze out the urea+ b mercapto: get reversible native. intro $)_2$ at pH8 (oxidizes SH groups to form disulfides), renaturation is ≈ 100% active.

But if RNase is reoxidized in 8M urea, then 1% activity after removal of 8M urea. What if we introduce a trace of mercaptoethanol? 100% activity reversibly obtained. indicates that proteins can fold spontaneously into its native confomation under physiological conditions. => proteins 1° struc dictates its 3° struc.

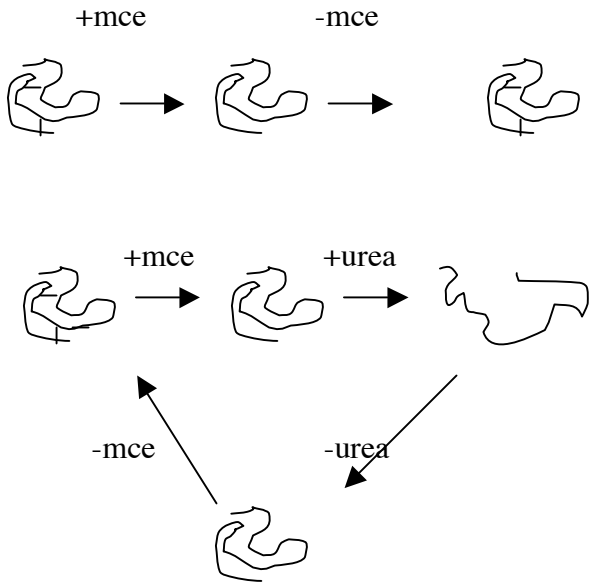
For RNAse, 105 possibilities. So only 1/105 of total is active. So if RNase is reoxidized in 8M urea, then 1% activity after removal of 8M urea.

What if we introduce a trace of mercaptoethanol? 100% activity reversibly obtained. indicates that proteins can fold spontaneously into its native confomation under physiological conditions. => proteins 1° struc dictates its 3° struc.



Native denatured native

Include the role of cystine cross linkages



what happens if you remove mce first? What % of the renatured enzymes will be active?

Reversible thermal denaturation expt: shows that native state is most stable. Suggests thermodynamics of folding:

Thermo factors:

a) conformational entropy: works against folding into the native state.

Recall: $\Delta G=\Delta H-T\Delta S$. since $\Delta S<0$ for the single folded state vs the more random random coil state, it makes a + contribution which goes against spontaneity

b) ΔH: works for folding bec. It is usually – due to more favorable:
   i) charge charge intxn. Between + and – charge side chain groups.
      Eg, Lys –Glu salt bridge.
   ii) Internal H bonds. eg Ser, Thr:
   iii) VdWaals intxn bet uncharged groups.

c) favorable entropy change due to burying Hphobic groups within molec.

Further stabilization by use of disulfide bonds.

Note: it is found that thermodynamically, proteins are still just marginally stable under physiological conditions.(only.4kJ/mol aa needed to denature).

*protein folding: not random conformational search but directed folding pathway, stability increases sharply as folding proceeds **molten globule*=result of *hydrophobic collapse* (likely scenario since proteins have hydrophobic cores) * prions (*pr*oteinaceous *i*nfectious *on*ly) – discuss creutsfeldt-jakob disease (mad cow) * molecular chaperones - shelters the hydrophobic sidechains of heat-denatured chaperonins. Hsp = *h*eat *s*hock *p*roteins. *Chaperonins* are more elaborate.	* look at ribbon representation of proteins(Fig 4-18) * *domains*, (Fig 4-19); stable globular units of proteins >200 aa ≈1000 unique domains **Motifs* (Fig 4-20) = common folding pattern of 2° structures also called supersecondary structure * go over the heating curves. Fig 4-26: shows cooperativity. Differentiate the curves. **Quaternary* structure = proteins >100 kD MW:composed of more than 1 polypeptide chain. *Multimers*. *Oligomers*. Spatial arrangement of these subunits = 4° struc