

Chem 431A-L15-F'07

admin: Quiz #5 at 10:20 am

Last lecture:

- (1) we talk about peptide bond (Chapter 4 material)
- (2) 2° structure – α -helix and β -sheet.
- (3) Ramachandran plot (Ψ and ϕ)

Today: (we won't finish all today due to quiz)

- 1) turns
- 2) fibrous proteins : α - and β -keratins
- 3) general principles for protein structure
- 4) Stability of protein
- 5) Predicting 2° and 3° structure...

1) Some proteins have both α helix and β sheet structures within the same chain. They are connected often by the following:

beta turn: (tight turns) -usually forced by Pro.

beta bulges occur between two normal beta structure with 2 residues in 1 strand and only 1 residue in the other strand.

Some general principles describing protein structure: folding of protein in 3 dimensions is called 3° structure. all the info need to fold the protein into its native tertiary structure is contained within the 1° structure of the peptide chain itself.

(denaturat'n of native proteins led to renaturation)

Some principles have become more apparent:

a) 2° struc (α and β struc) form whenever possible (result of extensive H-bond)

b) α -helices and β sheets tend to associate and pack close together in a protein.

c) segments between secondary structures tend to be short and uncomplicated

d) proteins fold to form most stable strucs possible. Stability of proteins arise from: *formation of large no's of intramolec H-bonds, and *reduction in this surface area accesible to solvent.

Fibrous proteins: actually α keratin can be quite rigid due to lots of cross linking via Cys-Cys bonds. consists of a helices arranged in coiled coils which are arranged in pairs to form protofilaments which form the filaments (made up of 4 protofilaments).

collagen= triple helix.=rigid inextensible in connective tissue including tendos cartilage, bones, teeth, skin, blood vessels. strength of the collagen allows us to do high impact activities like jogging, running, jumping. Basic structure is tropocollagen, 3-ple helix.

<p>2) general rules of protein folding:</p> <p>a) all globular proteins have a defined inside and outside. The inside contains hydrophobic residues while the outside surface contains hydrophilic residues. (inside: Leu, Ile, Phe; outside: Lys, Glu, Asp, Arg, His; inside: polar form Hbonds, or may be in cavity)</p> <p>b) β-sheet usually twisted and wrapped into <i>barrel</i> structures. (folding patterns are of 2 kinds: α helix packing; β sheet structure framework.)</p>	<p>c) turning corners by β-turns (so called because they often connect antiparallel successive β strands). Other: γ-turns, Ω loops (6-16 aa; often on surface; most proteins with > 60 aa). Pro often in turns. Acting as breaker of α helices.</p> <p>d) not all parts of protein can be classified as β, α, β turns, Ω loops, or bends. That's the random coil region.</p>
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<p>2) <u>Native</u> struc. physiological and active structures. denaturation factors:</p> <p>1) heating – thermal denaturation, over narrow T range. (cooperativity) most proteins melt below 100°C (except thermophilic bacteria).</p> <p>2) pH variations changes charge distribn, and Hbonding reqments.</p> <p>3) detergents assoc with nonpolar residues</p> <p>4) chaotropic agents: guanidinium, $\text{H}_2\text{N}-(\text{C}=\text{NH}_2^+)-\text{NH}_2$ and urea $\text{H}_2\text{N}-(\text{C}=\text{O})-\text{NH}_2$ (5-10M; increase solubility of nonpolar substances in water; disrupt hϕ intxn in protein)</p>	<p>Denaturation experiments. Anfinsen, Christian (1957):</p> <p>used ribonuclease (RNase), a single subunit. 124aa. added 8M urea with 2-mercaptoethanol, ($\text{HSCH}_2\text{-CH}_2\text{-OH}$) which reduces the Cys-Cys disulfide bonds.</p> <p>RNase has 8 cys. chances of forming combo: $(7)(5)(3)(1) = 105$ ways.</p> <p>dialyze out the urea+ 2 mercapto: get reversible native. intro)₂ at pH8 (oxidizes SH groups to form disulfides), renaturation is $\approx 100\%$ active.</p>
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<p>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 conformation under physiological conditions. => proteins 1° struc dictates its 3° struc.</p>	<p>Reversible thermal denaturation expt: shows that native state is most stable. Suggests thermodynamics of folding:</p> <p>Thermo factors:</p> <p>a) conformational entropy: works against folding into the native state.</p> <p>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</p>
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