BE.342/442 Tuesday, September 27, 2005
Topic: Beta Sheets

Administrative announcements: Take-home exam at the end of next week.

Introduction to Beta-sheets:

Beta sheets can appear in a variety of arrangements. Last time, we saw that alpha-helices can organize into a higher-order structure: coiled coils. Beta sheets, too, pack together into macrostructures that can be seen with the naked eye! A common one is the silk fiber: a silk scarf is composed of 80% beta sheets! A single thread of silk is an extended arrangement of beta-sheets, so stretched that it has little elasticity. Spider's silk, in constast, is only 50% beta-sheets, which provide strength. The remaining 50% of loops and alpha-helical coils provide elasticity. Thus, the content drastically changes the properties of this material.

Demonstration: insect cocoon. Its fibers have a high content of beta-sheets.

The major configurations of beta sheets are:
parallel: adjacent strands are aligned with the C-termini and the N-termini on the same side
anti-parallel: adjacent strands are aligned with C-termini aligned to adjacent N-termini

Higher-order structures of these arrangements include Greek keys and jelly rolls. Another motif discovered in 1993 is the Bet-helix.

The sort of packing observed in beta sheets is seen in other structures, including:
- Architecture, such as tiles on the Cathedrial of Siena, Italy.
- Block copolymers, which can pack similarly to proteins

Spider's silk is composed of stacks of antiparallel beta sheets. In its soluble, gelatinous form (inside the spider), the silk proteins group into four 20-nm antiparallel strands. When expelled, they compress into insoluble higher-order structures.

A silk fibroin arranges orients into anti-parallel beta-sheets with alanine staggered on one side, and glysine on the other. Each fibroin contains 3,000 amino acids. Since each residue on a beta-sheet spans 3.4 angstroms, meaning each fibroin spans only 1 micron. As the silk is spun into a cocoon, it forms a single fiber that, stretched out, would span a mile! In contrast, cotton fibers are composed of relatively short segments that are overlapped and joined together. Hair, too, is made of short proteins joined in an overlapping arrangement.

What gives silk proteins this remarkable property?
Compare the compositions of silk, wool, and collagen. W. Austburg at the University of Leeds, England conducted diffraction experiments to compare these materials. All three contain large fractions of amino acids with small side groups:

- Glysine is predominant in silk, followed by alanine and serine. (Serine differs from alanine only by an –OH group.) Silk contains no cysteine.

Wool also contains substantial fractions of glysine, alanine, and serine, but it contains large quantities of glutamine and cysteine. Cysteine forms covalent crosslinks, which can account for the curliness of wool! It contains an even distribution of a variety of other amino acids.

Collagen contains 33% glysine. Every third amino acid in collagen must be glysine to allow it to pack in a specific way that will be discussed later.

Primary sequences that preferentially form beta-strands and beta-sheets alternate hydrophobic and hydrophilic amino acids.

Examples:

Residues 41-48, 53-60, and 71-78 of human plasma retinol-binding protein.

Engineered proteins that alternate lysine and alanine, to self-assemble into beta-sheets. Incorporation of a helical dipole can allow the proteins to change conformation from an alpha-helix to a beta-sheet.

The tiny molecule EFK8 (with the sequence FEFKFEFK), only 2.5 nm long, 1.4 nm high, and 0.9 nm thick, forms an extremely tight beta-sheet arrangement, because even this short sequence can establish a pattern of alternating hydrophobic and hydrophilic groups. The molecule self-assembles so as to maximize hydrogen-bonding through an S4 antiparallel configuration of adjacent beta-sheets. Left-handed helical strands form and pair into a left-handed double helix, dynamically forming a hydrogel network.(Source: Marini, et al., *NanoLetters*, April, 2002.)

Bonding between beta-strands:

Antiparallel strands are separated by 4.7 to 4.8 angstroms and held together by hydrogen bonds between N-H and C=O groups on adjacent strands, with the side groups “facing together” in a 2-D line drawing.. Parallel beta-sheets bond in a different arrangement, with the side groups “facing apart” in a 2-D line drawing. Mixtures of parallel and anti-parallel packing are possible.

The sequence and arrangement of parallel and antiparallel beta sheets determines the lengths of the linking loop region between the strands. (Demonstration: topology diagrams fir adjacent hairpin motifs, some of which were not observed in nature at the time that they were discovered.)

<u>Motifs in packing and folding:</u>

A common motif observed in nature is the beta-alpha-beta motif, in which two beta sheets are parallel with each other and antiparallel to an alpha helix. Since the sequence of the structures is antiparallel, the loops joining the 3 structures are quite short.

Another important motif is the Greek key, a 4-stranded structure in which beta strands 2 and 3 fold over such that strands 2 and 3 align antiparallel to 1.

The Greek key motifs can form a partial beta-barrel, an arrangement that is important in the green fluorescent protein originally discovered in jellyfish, GFP. Later, a red protein RFP was discovered in choral. BFP and CFP were later synthesized. Amazingly, these fluorescent molecules are made entirely out of protein, without any other compound.

Another example of Greek key beta-strands can be seen in Staphylococcus nuclease. (The structure of this protein was discovered in the 1960's at MIT, with 1.5-angstrom resolution.)

Greek keys can pack together into a crystalline structure, such as in the *gamma*-crystallin molecule found in the lens of the human eye. Cateracts occur when this protein aggregates uncontrollably and crystallizes into an opaque phase.

The trans-packing motifs occur when two different domains of beta-sheets cross over, with a strand of one domain interacting with another domain. Many diseases involve disruption of this intramolecular interaction. Later, we will see how a real disease utilizes this "domain swapping."

An up-and-down beta barrel motif contains 8 beta strands connected by hairpin loops.

This arrangement is sometimes found embedded into membranes, with the hydrophilic groups aggregated at the core and hydrophobic groups along the outside. If removed from the membrane, the protein drastically changes conformation, turning inside-out.

The up-and-down motif contains 4 antiparallel beta strands connected by hairpin loops.

Neuraminidase from influenza virus: the headpiece subunit contains six up-and-down motifs.

Up-and-down motifs and Greek keys motifs are found together in the the cancer suppressor protein p53.

The jelly roll motif appears similar to a safety pin. A chain 8 of beta-sheets connected end-to-end folds once at the center, and then again perpendicularly to the first fold, to form this structure. Pairs of strands 1 and 8, 2 and 3, 6 and 7, and 4 and 5 end up bonding in opposite arrangements.

Beta-sheets in medicine

Beta-sheets have a high tendency to self-assemble into fibrous structures, which act as nucleation seeds for protein conformational diseases, such as Alzheimer's. However, the assembled filaments can also be used as nanofiber scaffolds for medical applications.

For example, the protein transthyretin can be changed through mutation and other aggregation inducers to form an insoluble twisted beta-tape. A "molecular straw" arrangement is composed of a twisted, hollow higher-order structure of beta-sheets. These structures could have applications in materials science.

Beta-helices

Several beta-strands can form a parallel beta-configuration with variable size and flexible loops.

The primary sequence often contains repeated sequences rich in Ser, Asn, Asp, or Pro, and separated by Gly. Typical loop sequences would be:

-Gly-Gly-X-Gly-X-Asp-X-(Val/Leu/Ile)-X-

The helix has repeating turns that can bind ions such as calcium.

Two-stranded and three-stranded beta helices have the (two/three) beta sheets in every repeating turn, connected by loops of variable length. Phages utilize the three-stranded beta helical structure.

The Perutz helix is similar in structure of the beta-helix, but is tightly coiled into closed circles, or "Q's." Hydrogen bonds between the rings stabilize the structure.
Source: M.F. Perutz et al., *Proc. Natl. Acad. Sci.,* **99**, 5591, 2001.

Beta-hairpins

These short loops connect antiparallel beta-strands. Longer loops can be essential for the catalytic activity of the protein: they're the fingers on the beta-sheet arms! Shorter loop regions are more common than longer ones. The most common lengths for loop regions are 2 or 6 residues.