

7.88 Lecture Notes - 7
7.24/7.88J/5.48J
The Protein Folding and Human Disease

Tropomyosin and S-peptide

- **Sequence determinants of Coiled Coil Structure**
- **Tropomyosin**
- **Circular Dichroism**
- **Tropomyosin thermal denaturation/renaturation**
- **Chain Recognition, Association, Registration**

A. Revisit Sequence determination of Coiled Coils

H - X - X - H - P - X - P - H - X - X - H - P - X - P - H - X - X - H - P - X - P

Lets draw as helical wheel:

Note side chain interactions above and below plain:

Now lets flip the sequence 18:

Sequence:

- 1,4 residues: hydrophobic core
- 5,7: selectivity, polarity
- 2,3, 6: Quaternary interactions; cellular functions

Perhaps: But for TM and other coiled coils, easy to see that they encode:

Lets review muscle structure:

Theta in degrees;

Light waves are transverse vibrations; can be vibrating in various planes perpendicular to direction of propagation.

B. Tropomyosin

IN vitro unfolding/refolding of a coiled-coil: Tropomyosin:
Properties of Tropomyosin
Denaturation/Renaturation of TM

Reversibility

Coupling of secondary and tertiary structure

From thermal denaturation curve: Conformation = f (sequence + environment)

Possible pathways

Question: Is this conformation/subunit organization encoded by amino acid sequence; perhaps protein folds in association with other components of muscle structure?

However, for these structures, only fiber diffraction; no 3-D crystals, no high res structure; not in PDB. In fact in first edition of Branden and Tooze no mention of coiled coils, because not present among globular soluble proteins!!

Why tropomyosin: Given that alpha helices are critical conformation of keratin polypeptide chains:

What about examining keratin folding into keratin helices using the in vitro methodology of Anfinsen???

Not possible: severe limitations in these systems;

Mature fibrils highly covalently crosslinked

Solubilization requires severe treatment and does not yield homogenous population of protein chains.

Only just recently has it been possible to obtain the precursor forms of keratin chains prior to folding and assembly into hair - from tissue culture and from products of cloned genes:

Tropomyosin function and organization:

Remind you of structure of striated muscle:

- Thin filaments from M band
- Thick filaments which are bipolar:

Tropomyosin Structure:

- rodlike, 2nm x 41 nm when observed in the electron microscope as soluble molecules.
 - molecular weight 65.5 kd.
 - two chains of 284 residues, a and b, with slight differences in sequence (39 residues)
 - Almost 100% alpha helical and consists of two alpha helical chains wrapping around each other.
 - Linear: No turns
 - Relatively high concentration of charged amino acids, but unusually stable to changes in Ph on both acid and alkaline sides, indicating that electrostatic interactions not important for maintenance of alpha helical coiled coil structure
 - Interact with actin filaments to form long filamentous structures that lie in each of the two long period grooves on opposite sides of thin filaments in muscle.
-
- pack head to tail with an 8-9 residue overlap
 - Bind Troponin Subunit - Troponin the other regulatory protein in the thin filaments is bound to each tropomyosin molecule.
 - Actin : tropomyosin : troponin present in 7:1:1 stoichiometry.

Its function is control rod restricting axis of myosin head groups to actin filament; moving back and forth during muscle contraction cycle; regulated by calcium

Purified Tropomyosin has never been observed to form three D crystals; So no crystal structure; (not even in Index of Branden and Tooze) However forms 2-D open crystal nets;

Original structural data came from X-ray scattering and electron microscopy of unusual two dimensional crystalline form; kite-like

C. Circular dichroism

Light represents transverse vibration; can be polarized, restricted to certain planes; A plane polarized beam is one in which electric vector vibrates in just a single direction in plane perpendicular to propagation

Plane of polarization can rotate, so circularly polarized, either clockwise or not, left or right; polaroids have crystallites which absorb one component not opposite; formed under stress so that crystallites are aligned;

Chiral L amino acids differentially absorb light with beams of left and right circularly polarized vectors; two components therefore travel at different speeds through polypeptides and proteins, which results in rotation of the plane of polarization

Differential absorption of left and right circularly polarized light;

- Far UV signal (200-230)- Monitors backbone conformation peptide bond itself,
- near UV - ring absorption at longer wavelength –

Function of wavelength; so scan ellipticity versus wavelength

Analogous to absorption spectra; scan versus wavelength

Expressed as theta, Molar ellipticity, measure differences between left and right polarized light compared to input light.

For helical proteins, net negative, so more negative, more helical:

Best for alpha helix because of a double dip ; Distinguishes alpha helices, beta sheets and random coils reasonable well.

D. Denaturation and Renaturation of Tropomyosin

First: Consider thermal-denaturation for proteins you are familiar with:

- Egg white: lysozyme plus ovalbumen?
- Milk: Casein + alphas lactalbumen?
- Collagen (gelatin) ?
- Gluten?
- Actin

Outcome of thermal denaturation processes depend on protein itself, not generally feature of polypeptide chains;

Now lets consider the folding and association of purified tropomyosin chains:

Does tropomyosin refold from the denatured state?? That is, does tropomyosin amino acid sequence by itself determine coiled-coil conformation??

Equilibrium Denaturation: All measurements after process has come to equilibrium or steady state; often confused in the literature, but I will be presenting true equilibrium data.

These are to be distinguished from kinetic analysis; reaction as a function of time.

This was RNase data.

Tropomyosin exhibits distinct melting transition when heated:

Lori L. Isom, Marilyn Emerson Holtzer and Alfred Holtzer (1984) alpha-helix to Random-Coil Transition of Two-Chain, Coiled Coils. Experiments on the Thermal Denaturation of alpha-Tropomyosin and beta-Tropomyosin Macromolecules, 17 2445-2447.

Purified reduced tropomyosin:
Rabbit cardiac muscle:

- Two chains alpha and beta;
- differ by 39 residues,
- 11 of which are at hydrophobic positions:

Each point sits 10 minutes, fully reversible; true equilibrium;
Draw test-tubes: change water bath:

Or multiple samples put in different water baths:

T_m is around 50°C.

What is state of polypeptide chain??

Certainly not alpha helix: but amino acid sequence remains the same; but we said sequence determines fold!!??

Fold = f (Sequence ; environment)

However, when cool (draw dashed line); identical curve:

It is possible to determine the molecular weight of the molecules in these samples by a number of techniques:

- Light scattering
- Sedimentation velocity
- Sedimentation equilibrium

Determine molecular weight above T_m = equals 1/2 of starting MW.

This material is half the molecular weight of starting material!!

So it is monomeric.

What is its conformation: not alpha helical, "disordered, random"

Thus alpha helicity is coupled to dimerization, and vice versa, loss of alpha helicity is coupled to dissociation.

What is Pathway for Coiled Coil Formation??

What would you predict as the physical state under these conditions?

So What are steps in reaction?

How could you test model?

Is there evidence for gain of alpha helicity prior to increase in molecular weight??
No.

Concentration dependence: Might depend on square of concentration? Diluted by two; No change??

In fact dilute by 1,000 - not easy detect: Questionable:

More direct route: Use light scattering to follow size of molecule:

Data: L Number of chains versus temperature for reduced or block chains, to prevent air oxidation cross linking.

Light scattering curve follows CD curve exactly:

Denaturation and dissociation tightly coupled:

NB: These alpha helical amino acid sequences are not alpha helices when free in solution:

secondary structure is function of tertiary structure (!?):

Important to avoid intellectual error of thinking that conformation is simple f(sequence);

always f(sequence/environment)

So:

- Registration information built into amino acid sequence:
- What would one predict about helicity;
- Pathway: Form helices then associate:
- What prediction: helicity versus temperature??
- Pathway: No helix until associate: Stability should be f(concentration)
- Yes: But very hard to detect;
- We will come back to this

In fact tropomyosin molecules pack parallel, in register.

Could these helices associate if in register??

So not just **Folding, Association**, but also **Registration!**

Could there be out of register intermediates??

1.....14.....284
1.....14.....284

Tropomyosins differ in different muscle, for example between Striated skeletal muscle, and smooth cardiac muscle; variation is generated by differential splicing at messenger RNA level: 13 exons

Note that lots of sequence available to do other stuff:
What is Role of Remaining Sites in Sequence??

Residues "b,c and f" lie on the outermost surface of the coiled coil molecule and probably have a special role in the aggregation of the coiled coil molecule into a larger assembly and in the function of the molecules.

In muscle this is clear: these sites are involved in interaction with actin monomers in actin filament!

Conclusion: **Folding information dispersed through Amino Acid Sequence!!**

In order to understand rules, need to know which amino acids carry the information for conformation.

If heptad repeat determines coiled coil structure, and any heptad repeat can form coiled coil, how do they find their correct partners?

Recognition/ specificity in chain association; But since association not uncoupled from folding, can't uncouple folding from recognition.

In the 1980s there were significant advances in the design and construction of spectrometers that could measure circular dichroism, and also a more sophisticated appreciation of the coiled coil structure:

To give you a sense of this aspect of problem, let me describe more recent studies on tropomyosin, and how alpha recognizes beta chain: A very penetrating study was carried out by Sherwood Lehrer and colleagues at the Boston Biomedical Research Institute:

They were interested in the question initially of whether in muscle there were different populations of tropomyosins: playing different regulatory roles; skeletal muscle versus cardiac muscle

- Alpha/alpha
- Beta/beta
- Or alpha beta:

It became clear that in the muscle major species was alpha/beta; That raised the question of how alpha and beta chains select heteropartner over homopartner:

Sherwin S. Lehrer, Yude Qian and Soren Hvidt (1989)

“Assembly of the Native Heterodimer of *Rana esculenta* Tropomyosin by Chain Exchange” *Science*, **246**, 926-928.

Lehrer's experiments were done with TM from **Frog Rana**

Equilibrium unfolding: Incubate sample at temperature for set amount of time; record spectra; express as % helix:

Curve for alpha/alpha: 50°C

Curve for beta/beta = 36°C

Curve for alpha beta; looked like two populations: but physical characterization indicated one.

How think about midpoints: could be two stable populations; but very rare:

Aha: Not a single transition; two transitions; one at lower temperature 36° second sharper one at higher temperature, midpoint 50°C.

Possibilities:

- Heterogeneity in sample: TM is a mixture of two species:
- Homogenous sample: Two different domains, one high tm, other low tm

On cooling, observed higher temperature transition, but lost lower temperature transition, and didn't regain same level of helicity as wild type:

Perhaps one domain refolds, other doesn't; or one species refolds, or other doesn't

Proceeded to purify by ion exchange chromatography (different salts) alpha chains and beta chains.

Under physiological conditions alpha/alpha dimers, β/β dimers:

Carry out melting:

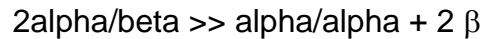
- Alpha/alpha shows single transition with midpoint about 50°C
- β/β shows transition at much lower temperature: around 30°C

Both indicate that dissociation of dimer is coupled to loss of alpha helicity:

The unfolding of the refolded material; curve fit perfectly by sum of alpha/alpha and beta/beta

Under these conditions; two chains fold back to homo dimers; melting curve is of mixture:

The first transition of native TM alp[ha/beta must be an exchange reaction of the form



since losing helicity; but still have a species present that shows cooperative transition identical to alpha/alpha

Under conditions where native structure being destabilized; fast equilibrium between
 $[Native]_2, > ?[I]_n < > 2[U]_2$

No species long lived enough to directly characterize:

Lets examine the rate of this process: takes about 50-100 seconds to cool from 60°C to 34°C;

Helicity regained rapidly:

Now continue to incubate for another 5,000 seconds about 8 hours:

Helicity and thermostability returns

E. Chain Recognition, Association, Registration

Recognition process; initiation of helicity > registration> propogation>

Careful investigation of Chain Recognition:

Erin O'shea, Rheba Rutkowski, Walter F. Stafford III, Peter S. Kim (1989)
Preferential Heterodimer Formation by Isolated Leucine Zippers from Fos and Jun
Science, 245, 646-648.

DNA binding proteins Fos and Jun each contain leucine zipper regions with five leucines at every seventh residues. Partial homology to GCN\$.

Synthesized peptides with cysteines and two glycines as spacers at N-terminus.
Run on HPLC;

Reduced, two single peaks of each peptide;
Oxidized, single peak;

So no fos/fos or jun/jun at equilibrium under these conditions;
Control: denature in GuHCL, oxidize, now recover all three species

Prepare all three dimers, examine by circular dichroisms:

All three very helical:

What happens if mix together homodimers;

In absence of oxidizing agent:

In presence of oxidizing agent - Only heterodimer!

So exchanging below melting transition;

Examine stability:

- Fos/jun T_m around 54
- Jun/Jun around 53
- Fos/fos 37C.

Fos is +4 net charge:

Jun is -4 net charge;

So electrostatic stabilization from heterodimer

Electrostatic repulsion in homodimers;

Classes of helices:

- Fibrous proteins – Insoluble, helicity deduced
- In globular proteins, observed by X-ray diffraction –ridges into grooves packing
- In coiled/coils, X-ray scattering and diffraction – is this packing ridges into grooves??
- isolated??

Coiled Coils stabilized by:

- Hydrophobic strip
- Charge charge interactions/salt bridges
- H- bonds
- buried hydrophobic core

Today we will focus on the formation of an alpha helix within a non helical polypeptide chain and the sequence specific interactions which stabilize and destabilize such species.

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