

7.88 Lecture Notes - 8
7.24/7.88J/5.48J
The Protein Folding and Human Disease

- **S-peptide Helices Continued**
- **S-peptide**
- **Reprise Fos/Jun**
- **Helix Dipole (10 min)**
- **S peptide of ribonuclease S. (20 min)**
- **Helicity of S- peptide variants (30 min)**
- **Presta and Rose model for helix start and stop in proteins (reading)**

A. Reprise Fos/Jun

If prepare homodimers, analyze under conditions that suppress shuffling (acidic, no S-):

HPLC trace with both homodimers

Now incubate (room temp, 1 hour) with redox buffer – mixture of oxidized and reduced glutathion; these conditions promote S-S > Sh + SH > S-S-

Find a single peak of heterodimer: So chains exchanging, even though both species are below their melting temperatures:

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 groves??
- Isolated??

Coiled Coils stabilized by:

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

Continue with investigations of actual dynamics of chain motion and folding Helix:

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

B. Helix Dipoles

Originally proposed by Wada (1976), *Adv. in Biophysics*, 9, 1-63.

Alignment of peptide dipoles, parallel to the helix axis, gives rise to a macro-dipole of considerable strength.

Each peptide bond has a dipole: about 3.6 Debye units. In a helix, these point up axis:

For 10 residues 34D, as 97% of the peptide dipole points in direction of helix axis.

For points near the terminus of the helix the effect of the dipoles is the equivalent of

- half a positive charge at the N-terminus and
- half a negative charge at the C-terminus..

In a number of native proteins, the charge on the helical dipole is playing a functional role in the protein. For example in the sulfate binding protein.

The negatively charged SO_4^{--} ion is bound in a pocket defined by the amino terminus of three helices. Presumably the helix dipoles are contributing to the binding and charge neutralization.

Similar in a number of enzymes that bind a phosphate, the phosphate is associated with the N-termini of alpha helices in proteins.

However, what about the role of these macro-dipoles in the **folding** of the protein.

Hol, Wim G. J., Louis M. Halie, and Christian Sander. "Dipoles of the alpha helix and beta sheet: their role in protein folding." *Nature* 294 (1981): 532-536.

Hol et al examined effect of the dipole by examining potential effect on helix /helix interactions, which gives a qualitative feel for the effects. Quantitatively these values are suspect because of the difficulties of assessing local field strength in a complex system, but give a feel for the scale of the effects.

Here are two adjacent helices, their axes separated by a distance d . Rotating around their own axis has little effect, since peptides are radially symmetric. Critical variable is angle ω , rotation of their axes relative to each other.

Here is the calculated electrostatic interaction energy for 5-turn helices, 27A.

Most favorable interaction is among anti-parallel helices. In fact all-helix helical bundle proteins are characterized by close packing of the anti-parallel helices.

Most unfavorable with fully parallel.

The affect of course depends on the distance between the helices and is probably only a factor among adjacent helices. Separated

Also useful to examine the variation of this factor with helix length.

If we plot residues/helix on the bottom axis, and ΔE , energy of GNOw the strength of this interaction does not increase, but levels off. Thus the energy per residue is decreasing over this range, and one would expect other factors to begin to be stronger.

C. S-Peptide of ribonuclease S. (20 min)

Consider RNase or hemoglobin: Helices start and stop; not continuous as in coiled/coils.



Figure 8.0: RNASE

What about alpha helices before formed?

What factors in amino acid sequences control the chain>>alpha helix free in solution??

In absence of receptors or stabilizing surfaces

When the structure of hemoglobin was solved, numerous efforts were made to prepare peptide sequences corresponding to the individual helices, and examine their conformation in solution. The results were disappointing in that little evidence was found for isolated peptides taking helical conformations while free in solution.

Part of evidence that protein folding **highly cooperative**; all sequences present, but unable to form stably folded protein if connectivity is broken!

Though the helical regions of myoglobin and hemoglobin had revealed no evidence for helicity in solution, the existence of this activity suggested it might have conformation.

During the early studies of ribonuclease it had been noted that treatment to subtilisin>>scission between residues 20 and 21, yielding an S peptide + S-protein in complex, active.

The shortened S-protein was not biologically active. Reconstitution of these fragments, resulted in the reconstitution of the active ribonuclease, even though there was a chain discontinuity.

Paradox: How can randomly fluctuating unstructured peptide specifically bind to protein surface: Regardless of mechanism, provides simple model of protein folding system, either protein recognizing peptide, or vice versa or mutual, correctly docking in correct configuration

In the native protein:

- residues 3-13 are helical;
- residue 14 an asparagine breaks the helix, and is H-bonded to the C=O of a valine at position 47 which is in the Beta sheet of ribonuclease.

Note: No S-S bonds in S peptide: Major species is ribonuclease A. = 124 amino acids.

- 13,700
- 37% beta strand, 23% alpha helix
- 8 cysteines as four disulfides
- 26 - 84 between beta strand and helix
- 40 – 95 between loops connecting beta strands.
- 58-110 helix yo strand at other end of sheet
- 65-72. loop to strands

As I mentioned earlier, Ribonuclease was model protein for development of solid phase synthesis of polypeptide chains:

S peptide and S protein, smaller than entire chain, were early targets in these studies. Both fragments were highly soluble.

First S-peptide synthesis was carried out by -Irving Chaiken - involved in the synthesis of ribonuclease.

Now the sequence in wild type

**Lys- Glu - Thr - Ala - Ala - Ala- Lys - Phe - Glu - Arg - Gln - His - Met --Asn - Ser
- Ser - Thr - Ala - Ala**

A key step forward was carried out by:

Brown, J. E and Klee, W. A. *Biochemistry*10 (1971): 470-476.

Who observed that at low temperature – 5°C- peptide in solution gave circular dichroism spectr

When measure as a function of temperature, decreases, helicity was at limit of detectability at 25°C.

Robert Baldwin's lab then picked up system as way to examine alpha helicity in solution:

An important series of experiment were carried out by Peter Kim, Kevin Shoemaker and Robert Baldwin at Stanford on the role of charged groups in stabilizing alpha helices in solution.

The experiments I will review are at a later phase, and are done with a sequence modified from the original sequence.

The early work by Kim and Baldwin using 2D-NMR revealed that in a solution of the S-peptide, only residues **2-13 were helical**, and subsequent residues were not.

They therefore moved to study of shortened sequence – easier to synthesize; When they isolated a 13-mer corresponding to 1-13, this gave evidence of helicity.

Subsequent work then concentrated on this **C-peptide** species, since these were of reasonable length to produce synthetically.

In C-peptide, made by cyanogen bromide cleavage, have homoserine lactone. It was originally prepared by cyanogen bromide cleavage of the S-peptide, which leaves homoserine lactone.

Did CD as function of pH: Clear maximum at pH 5, thought that Glu9- interacting with His12+

Same side of helix; location of ion pairs???

D. Helicity of S- peptide variants (30 min)

In the early work on this Baldwin was interested in the possibility that an ion pair between Glu9 and His12 was stabilizing the helix. To test this they synthesized the peptide without the ion pair, and found in fact the contrary, getting rid of this Loss of glu9 stabilized the helix!?

Perhaps Lys1+ and Glu9-

Similarly when replaced lysine by alanine stabilized helix.

Each of these peptides with increased solution helicity was better experimental system to study control of helicity:

As this work evolved Replace this methionine - 13 by an alanine, to avoid problems in synthesis; similarly replaced this glutamine with alanine, because of easier synthesis.

Acetyl-Ala - Glu - Thr - Ala - Ala - Ala -Lys - Phe - Leu - Arg - Ala - His -Ala

This peptide shows the following properties. If you measure the far UV circular dichroism, at low temperature, 30C, see a well defined helical pattern, with the characteristic double dip at 222 and 205.

That this is due to the conformation of the peptide is supported by the loss of this pattern with increasing temperature. At 45°C essentially gone.

Now first question have to ask, is this structure a property of the peptide itself, or is it due to multimeric or aggregated form of the chain. How do we know that due to naked peptide; what was state of leucine zipper peptides?

In fact when examine the signal (use 222 as diagnostic) versus chain concentration, essentially independent of concentration.

In fact see fall with dilution, presumably due to absorption onto sites on quartz cell.

Could be something going on.

In fact by sedimentation equilibrium, no evidence for aggregation until one gets into the millimolar range >1mM.

[If mol weight is 1400, then one mole is 1400 grams/liter =1.4 grams/ml. = 1400mgs/ml. 1mM would equal 1.4mg/ml.]

Best estimate for

100% helicity (from CD of native ribonuclease + -S peptide) is 30,000 degcm²d²mole⁻¹ (0222).

Baseline value for 0% helix was $0.222 = 3,000 \text{ deg cm}^2 \text{ dmole}^{-1}$.

This indicates that **at 3°C, getting about 50% helix.**

At any moment 50% of molecules are in helical conformation, flipping out of them.

Now the titration behavior of circular dichroism with pH had indicated that glutamic acid 2 and histidine 12 were important in stabilizing helix in the region of peak helicity (pH5).

To test this, Shoemaker et al synthesized C peptides with

- glutamic acid 2 > alanine, and with
- histidine 12 > alanine.
- pK of histidine = 6.7
- glutamic acid = 3.8

They then measured circular dichroism as a function over pH over a wide range.

The results were as follows.

[Note: Results may be found in: Baldwin, Robert L. "Sediment Protein Folding." *TIBS* 11 (1986): 6-9.]

Now in this C-peptide the only groups titrating in the range pH2-5 is the glutamic acid, and the only group titrating in the range 8>5 is the histidine.

- Loss of glutamic acid results in loss of stability.
- Major increase in stability occurs in range of protonation of his-12. Consistent with his 12 + contributing to helicity.
- In acid region, removing glutamic acid removes change with acid pH.

Loss of histidine.

- In acid region helicity increases with ionization of glutamic acid.
- Helix stability does not reach level of control peptide
- no changes of helicity as titrate through high pH range.

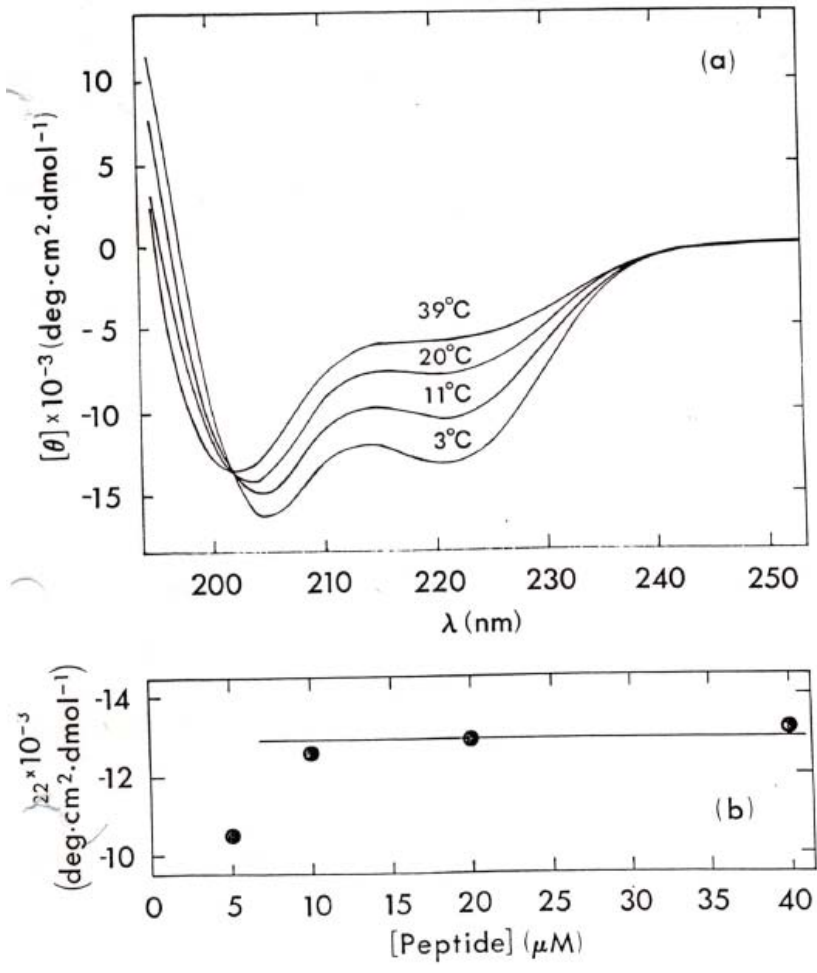
To a first approximation, looks like these two groups contributing independently to the overall stability.

Do not appear to be in salt bridge with each other.

Simplest hypothesis is that stabilization is due to the interaction of these charged groups with the helix dipoles.

To test this directly made a series of peptide derivatives, with differing charges at the N-terminii

(Reference peptide III)
 lysine, (+2)
 Alanine (+1)
 acetyl alanine (neutral)
 succinyl alanine (-1)



≈ ~50% helical

Courtesy of Robert Baldwin.

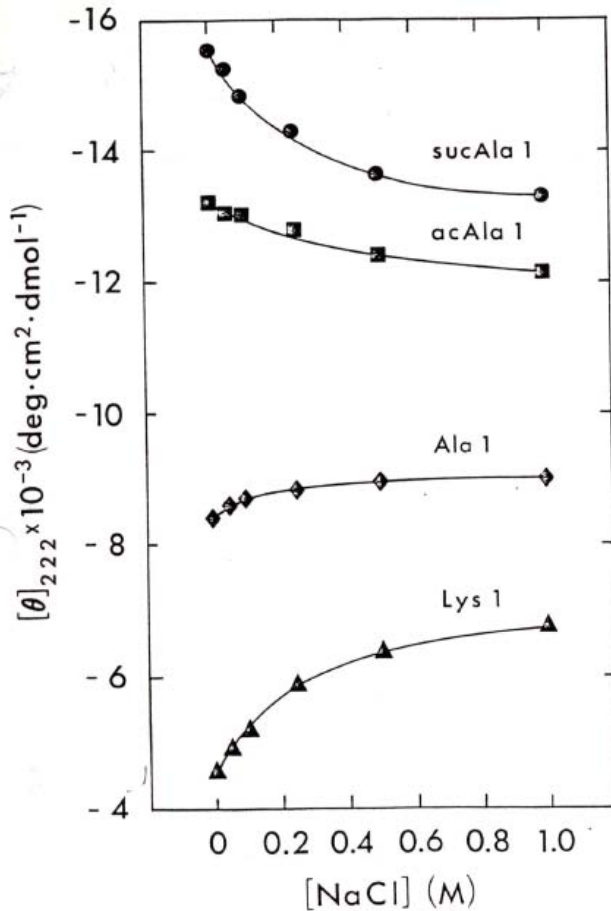
Figure 8.1: Peptide Graph (from Shoemaker, Kevin R., Peter S. Kim, David N. Brems, Susan Marqusee, Eunice J. York, Irwin M. Chaiken, John M. Stewart, Robert L. Baldwin. "Nature of the Charged-Group Effect on the Stability of the C-Peptide Helix." *PNAS* 82, no. 8. (Apr. 15, 1985): 2349-2353.)

Result is clear;
 Increased charge on N-terminus, increased helicity
 In all these cases helicity is temperature dependent, apparently well-behaved conformational parameter

Now, if it really is an ionic interaction, should be sensitive to ionic strength. In particular should be able to damp out at high ionic strength.

Indeed, differences highest at low ionic strength, damped out as increase NaCl concentration.

One should not overestimate this effect though...



screen stability, ionic interaction

mim ether

screen ⊕ fm + dipol

NaCl graph.: "Nature of the Charged-Group Effect on the Stability of the C-Peptide Helix." Kevin R. Shoemaker; Peter S. Kim; David N. Brems; Susan Marqusee; Eunice J. York; Irwin M. Chaiken; John M. Stewart; Robert L. Baldwin. PNAS, Vol. 82, No.

Figure 8.3: NaCl Graph (from Shoemaker, Kevin R., Peter S. Kim, David N. Brems, Susan Marqusee, Eunice J. York, Irwin M. Chaiken, John M. Stewart, Robert L. Baldwin. "Nature of the Charged-Group Effect on the Stability of the C-Peptide Helix." PNAS 82, no. 8. (Apr. 15, 1985): 2349-2353.

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