A Neutral, Water-Soluble, α-Helical Peptide: The Effect of Ionic Strength on Helix-Coil Equilibrium

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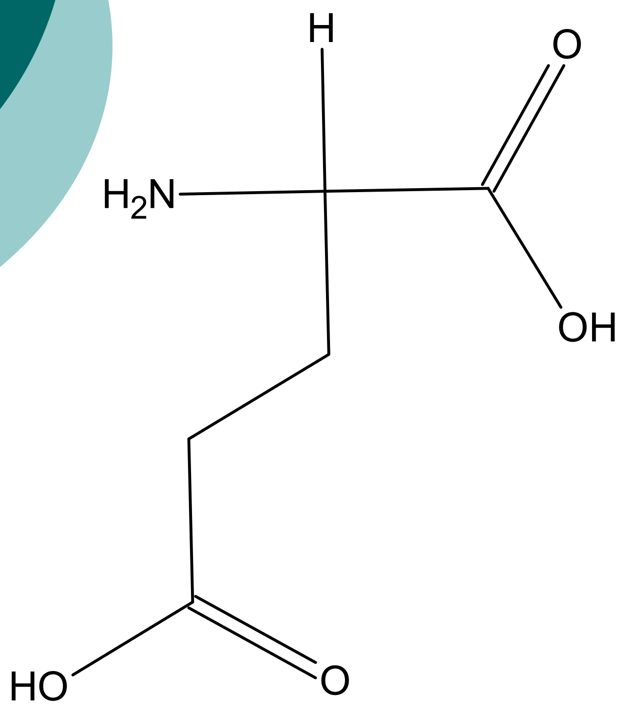
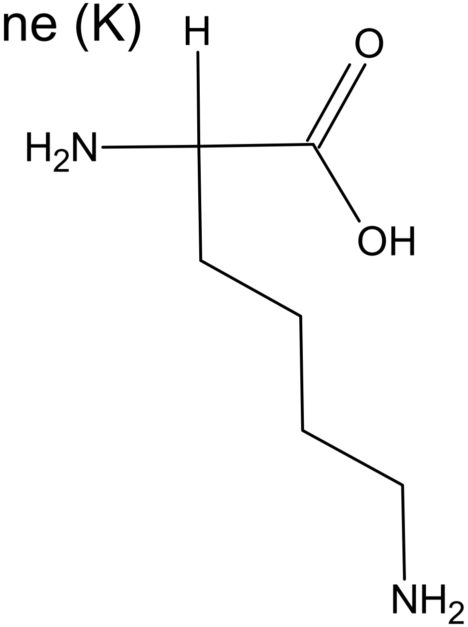
Background

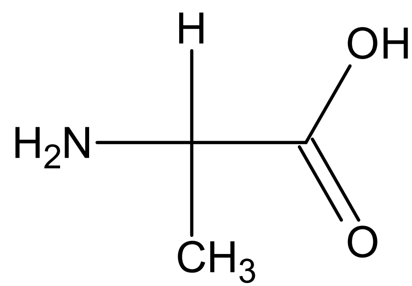
Glutamic Acid (E)



Lysine (K)

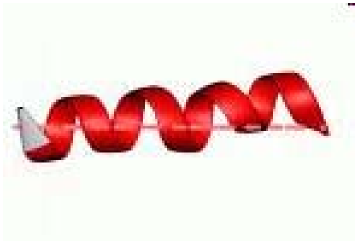


Alanine (A)



Alanine-based Peptides

- In a previous study it was suggested that the α-helical polypeptide backbone itself is responsible for stability of the helix in water.
  - Scholtz, J.M.; Marqusee, S.; Baldwin, R.L.; York, E.J.; Stewart, J. M.; Santoro, M; Bolen, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 2854.
- These peptides are being used to study helical propensities of amino acids.



Objective

- To characterize a synthesized uncharged alanine-based peptide.
- Test a prediction based on 1943 theory of Kirkwood for the thermodynamic interaction between a dipolar ion and an electrolyte.
  - Prediction: Increasing ion strength will stabilize the helix by shifting the equilibrium between the helix and random coil toward the helix.

Methods

- A 16 residue peptide used:

  $Ac\text{-}(AAQAA)_3Y(NH_2)$

- Circular Dichroism Spectroscopy
  - Uses circularly polarized light
  - Is used to determine secondary structure
    - Alpha helices give a characteristic CD spectrum

Thermal-unfolding Curve

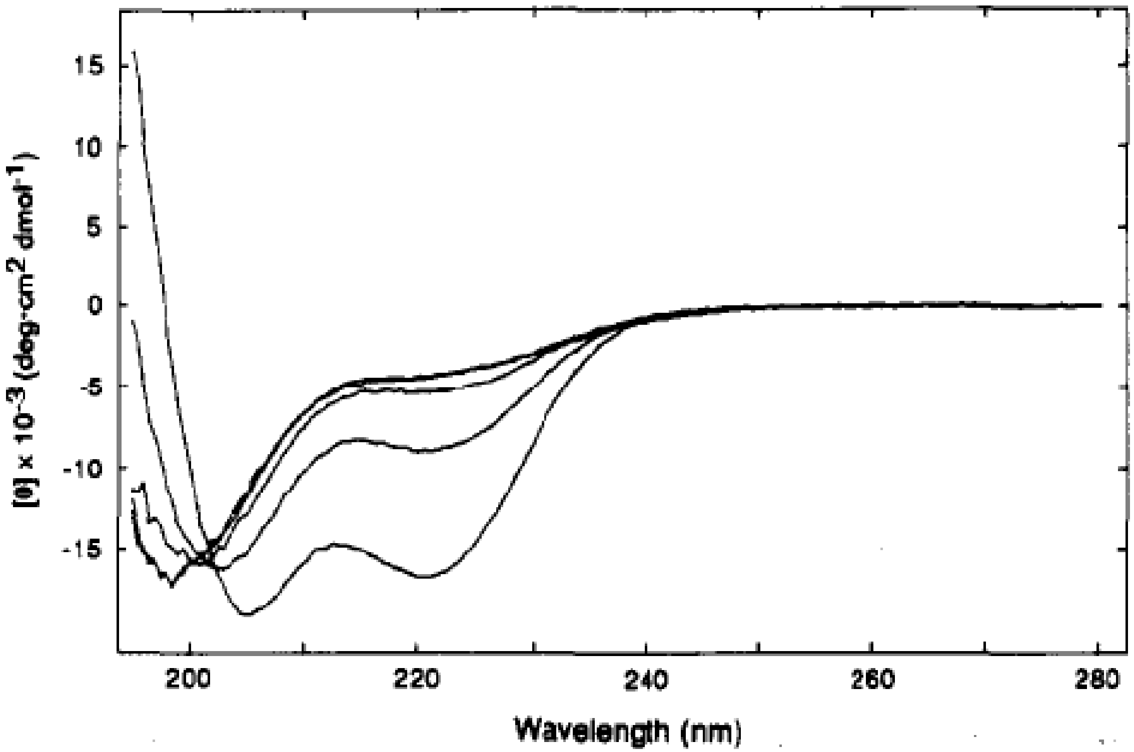
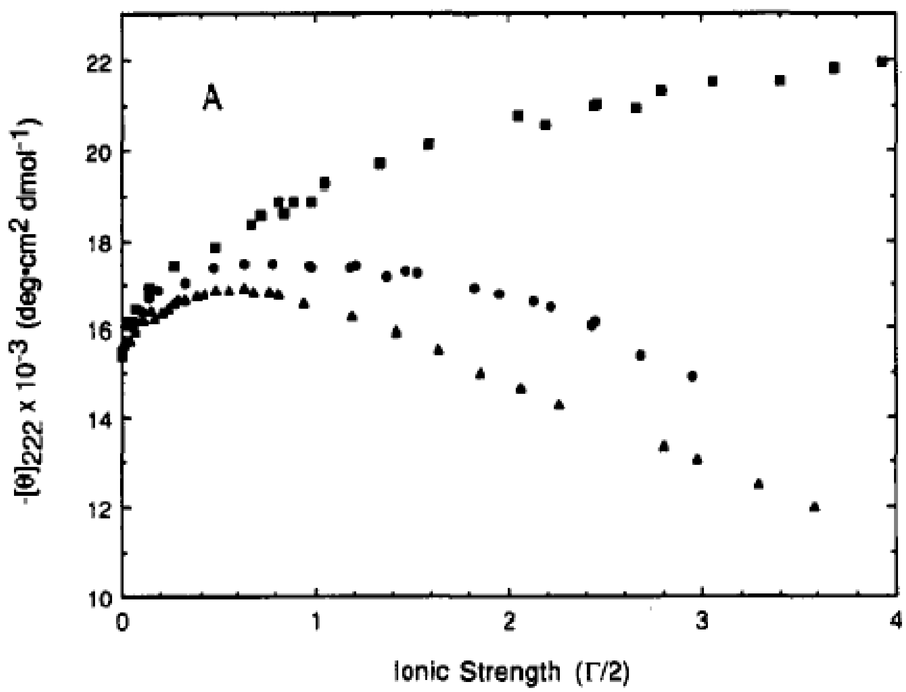


Figure 1. Circular dichroism (CD) spectra of $Ac(AAQAA)_3Y(NH_2)$ recorded at 0, 20, 40, 60, and 80 °C with an Aviv 60DS spectropolarimeter as described.[1–4] At 222 nm, the bottom curve represents the spectrum at 0 °C and the top curve at 80 °C. The peptide concentration was 24.2 μM in 1 mM KH_2PO_4 containing 0.1 M KF at pH 7.0.

Helical Content of Peptide as a Function of Ion Strength

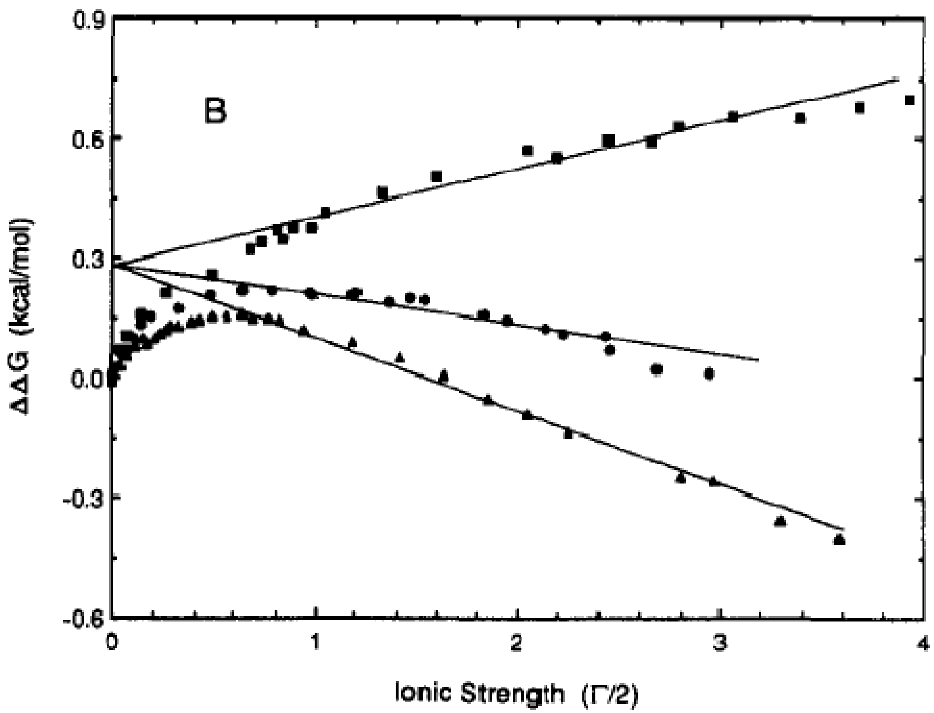


■-Na_2SO_4

●-NaCl

▲-$CaCl_2$

Changes in ΔG for Helix Stability as a Function of Ionic Strength



■-Na_2SO_4
●-NaCl
▲-$CaCl_2$

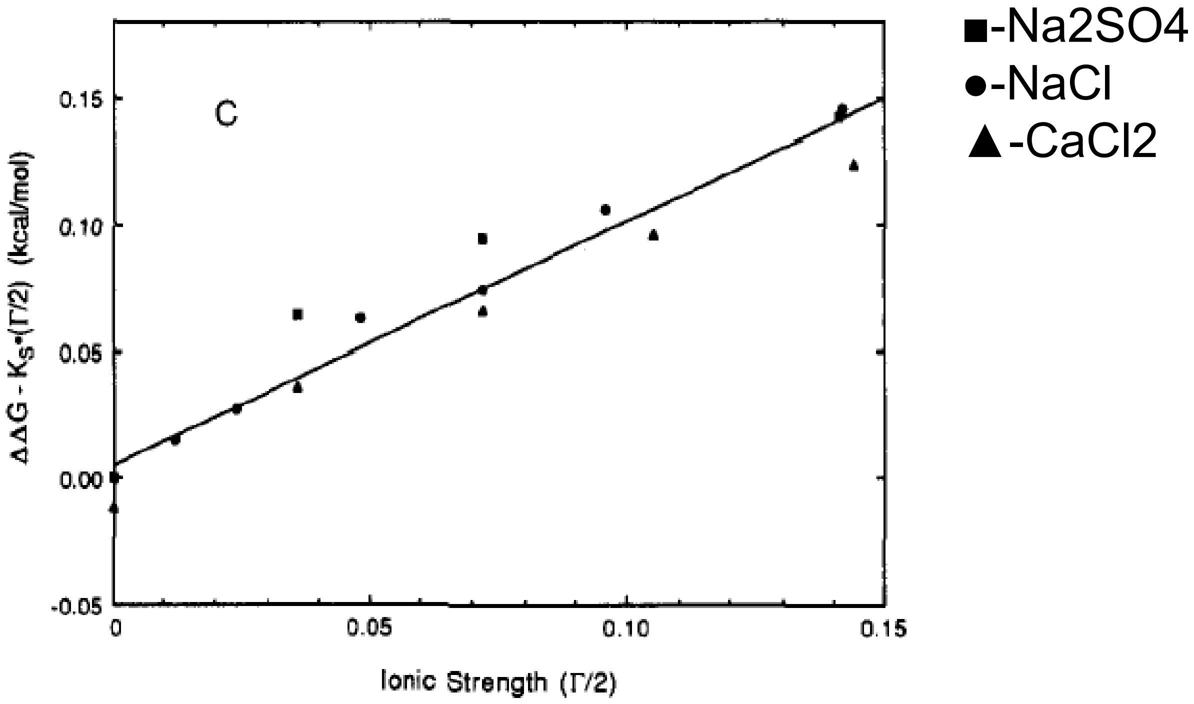
Equation used to calculate free energy:
$\Delta G = -RT \ln(\sigma s^{\pi-2})$

Hofmeister series

$$F^- \approx SO_4^{2-} > HPO_4^{2-} > \text{acetate} > Cl^- > NO_3^- > Br^- > ClO_3^- > I^- > ClO_4^- > SCN^-$$

$$NH_4^+ > K^+ > Na^+ > Li^+ > Mg^{2+} > Ca^{2+} > \text{guanidinium}$$

Changes in ΔG for Helix Stability after Subtraction of the Specific Hofmeister Effect



The magnitude of the dipole moment of the α-helix is estimated from the slope of the line and is 51D, yielding a 3.2D dipole moment per residue

Conclusion

- Agreement with previously determined values may be coincidental.
- A detailed investigation of Kirkwood's theory may reveal that electrolyte ions interact with partial charges NH and C=O near the ends of the helix that are not H bonded rather than with the macrodipole.