

# Nanosecond UV resonance Raman examination of initial steps in $\alpha$ -helix secondary structure evolution

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## Introduction

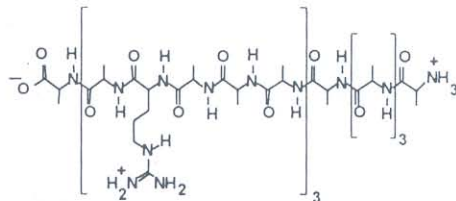
For most proteins the primary sequence encodes the native structure as well as the dynamics of the folding process. Recent theoretical models propose complex energy landscapes, which efficiently funnel the folding towards the native form(s) [1-4]. It is essential to develop methods to predict the structure of functioning and mutated proteins from their primary sequences in order to devise strategies for alleviating human disease.

We report here the first nsec transient UV resonance Raman spectroscopy (UVRS) to investigate the earliest events in protein structural evolution. We examined the thermal unfolding of the  $\alpha$ -helical peptide  $A_5[AAARA]_3A$  (AP).

## Results and Discussion

We Raman shifted the  $\sim 3$  nsec,  $1.06 \mu\text{m}$  fundamental of a Coherent, Infinity YAG to  $1.9 \mu\text{m}$  to heat the water. The T-jump was independently measured using shifts in the  $\sim 3400 \text{ cm}^{-1}$  water band. We probed peptide structural evolution by exciting the UVRS with delayed 204 nm pulses generated by the 5-th  $H_2$  antiStokes shifted 3<sup>rd</sup> harmonic of the YAG. UVRS were measured from the surface of a 0.6-mm diameter thermostatically controlled sample solution stream.

Fig. 1 shows the static 204-nm UVRS of AP at  $-5.5$ ,  $30$  and  $70^\circ\text{C}$ . The spectra are dominated by the Am I ( $1655 \text{ cm}^{-1}$ ), Am II ( $1547 \text{ cm}^{-1}$ ),  $C_{\alpha}$ -H bending ( $1382 \text{ cm}^{-1}$ ) and Am III ( $1244 \text{ cm}^{-1}$ ) bands. The high temperature AP spectrum is very close to that of random coil peptides [5]. The UVRS indicate an  $\alpha$ -helical content increase as the temperature decreases. Fig 1 (curves 2 and 3) shows the calculated pure random coil and  $\alpha$ -helix spectra of AP, which were used to calculate the secondary structure temperature dependence. Fig. 1 also compares the static AP UVRS at  $4^\circ\text{C}$ , and 95 nsec after a T-jump to  $69^\circ\text{C}$ , while Fig. 2 shows delay time dependence of the spectral changes. The top curve displaying the static difference between AP samples at  $37$  and  $4^\circ\text{C}$ , shows peaks at  $1236$ ,  $1374$ ,  $1534$  and  $1678 \text{ cm}^{-1}$  which are clearly due to an increased random coil conformation. Fig. 2 also shows transient difference spectra for a  $33^\circ\text{C}$  T-jump at different probe delay times. At the shortest delay times, we see small features due to the temperature downshifts of the amide bands. We only observe random



coil formation at longer delay times. However, even at 95 nsec the difference features have not evolved to the magnitude of those in the static temperature difference.

By assuming monoexponential kinetics, we calculate a relaxation time of  $180 \pm 60$  ns for a  $33^\circ$  T-jump and  $120 \pm 50$  and  $70 \pm 30$  ns relaxation times for  $43$  and  $65^\circ$  T-jumps. Using the equilibrium constant, we calculate reciprocal of the  $\alpha$ -helix melting rate constants of  $210 \pm 60$ ,  $130 \pm 50$  and  $70 \pm 30$  ns at  $37$ ,  $48$  and  $69^\circ$  C, respectively. In contrast, the reciprocal of the  $\alpha$ -helix formation rate constant was estimated as  $1.1^{+1}_{-0.3} \mu\text{s}$  at  $37^\circ$  C.

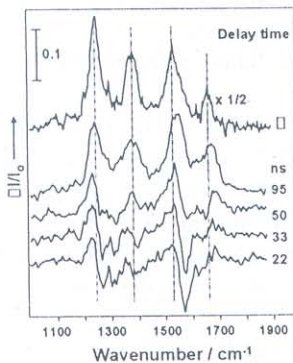
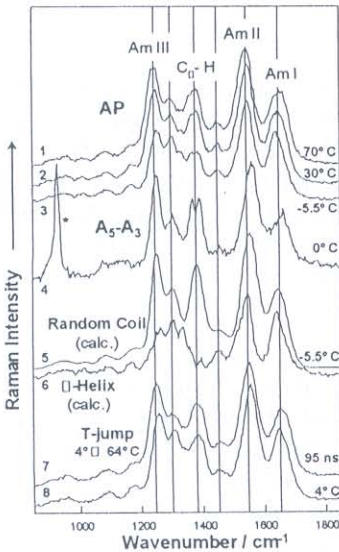
The  $\alpha$ -helix melting rate constant temperature dependence shows Arrhenius-type behavior with a  $7 \pm 2$  Kcal/mol activation energy. Assuming a two-state model, we can calculate the temperature dependence of  $\alpha$ -helix formation:  $k_h = K k_c$ . We find that  $k_h$

decreases as the temperature increases with an apparent negative activation energy of  $-7 \pm 2$  Kcal/mol, indicating a failure of the two state model for AP thermal unfolding.

We believe that these are the first measurements of activation barriers for the earliest stages in peptide or protein folding and unfolding, clearly indicate the complexity of the protein folding mechanism. Although monoexponential relaxation is expected for simple systems, macromolecules utilize much more complex multidimensional reaction coordinates. The slower folding kinetics and their unusual temperature dependence signals a folding bottleneck which may involve  $\alpha$ -helix nucleation.

Fig. 1. Steady state and time-resolved 204 nm UVRS of AP. (1-3) UVRS of AP measured at different temperatures. (4) UVRS which models the "internal" AP Ala residues obtained from the difference UVRS between penta-Ala and tri-Ala. Pure (5) random coil and (6)  $\alpha$ -helix UVRS of AP calculated from the UVRS at different temperatures. Transient UVRS of AP measured at (7) 95 ns after a T-jump of  $\sim 65^\circ$  and (8) a static UVRS of AP at  $4^\circ$  C without a T-jump.

Fig. 2. Transient difference UVRS of AP initially at  $4^\circ$  C measured 22 ns, 33 ns, 50 ns, and 95 ns after a T-jump of  $\sim 33^\circ$  C. The static UVRS of AP at  $4^\circ$  C is subtracted from each of the transient spectra. Static difference UVRS between spectra measured at  $37$  and  $4^\circ$  C represents a transient difference spectrum at an infinite delay time.



## References

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