P–A4–45

ROLE OF CYSTEINE RESIDUES IN THE STRUCTURE OF THE CAPSID PROTEIN OF EIAV (P26)

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Purpose: The aim of this work is the structural characterization of the major capsid protein of the equine infectious anaemia virus (EIAV), p26, focused on the role of the three cysteine residues in maintaining the protein structure.
Methods: Recombinant wild-type and mutant C48S were obtained. Circular dichroism (CD) and fluorescence spectroscopy were used.
Results: CD and iodide quenching studies indicated differences on the structure of the native and oxidized proteins. Also, the denaturation curves, obtained measuring the ellipticty at 208 nm versus temperature, showed differences among the diferent forms of p26.
Conclusions: The structure and thermal stability of p26 are dependent on the oxidation state of the cysteine residues, two of which are conserved in other lentiviral core proteins. Moreover, it has been described that they form a disulfide bond in HIV p24.

P–A4–46

TEMPERATURE EFFECTS ON PROTEIN STRUCTURE IN H_2O AND D_2O STUDIED BY FTIR AND GLOBAL ANALYSIS

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Purpose: Monitoring of temperature-induced changes in protein secondary and tertiary structure.

Methods: Fourier transform infrared spectra of 15 proteins in H_2O and D_2O buffer containing phosphate-buffered saline were measured from 20°C to 80°C at 2°C intervals. The data were globally analysed using models for the spectra and thermodynamics of protein unfolding.

Results: Seven or eight bands were sufficient to fit the full data set of spectra ranging from 1400 to 1760 cm^{-1} with a root mean square error of 1-2% of the maximum. Subsequently, the estimated band amplitude curves which showed a sigmoidal progression with increasing temperature could be fitted with a two-state thermodynamic model. Surprisingly, even α-casein showed a transition of its amide-I secondary structure band (1646 cm^{-1}) with a midpoint temperature of 75°C and a Van't Hoff enthalpy of ~250 kJ/mol.

Conclusions: Transition enthalpies and midpoint temperatures obtained compare well with published literature values. For several proteins HD exchange points to a change in tertiary structure prior to unfolding of the secondary structure.

P–A4–47

THE ROLE OF CONTEXT ON α-HELIX STABILIZATION.

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Purpose: To investigate if the α-helix propensities of amino acids are the same regardless of the model peptide used. Propensities of all natural amino acids have been determined from the following model peptide:
succYSEEEEKAKKAXAEEAEKKKKamide
Calculated propensities are compared with those derived from other models.

Methods: The peptides have been synthesized using standard Fmoc chemistry. The helicity has been determined with CD. The influence of salt, temperature and concentration on the helicity have been investigated. Propensities have been calculated using previously published methods.

Results: The propensities of the various amino acids correlate with values measured in both charged and neutral model peptides. However charged residues show a lesser correlation.

Conclusions: We conclude that the α-helix propensities for this model system correlate with other model systems and calculations. This shows that propensities in small peptides are the same no matter what the nature of the context is.

P–A4–48

G PROTEIN INSTABILITY AND A GTP PRE-EQUILIBRIUM STEP ON THE G PROTEIN ACTIVATION PATH

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PURPOSE: G protein activity deteriorates irreversibly in the absence of GDP or GTP. We seek to understand the detailed mechanism of this instability.

METHODS: Tryptophan fluorescence in response to various nucleotides was recorded.

RESULTS: Hyperbolic saturation in both speed and amplitude of fluorescence increase, required 100-fold stoichiometric GTPγS excess without causing significant change in apparent forward rate constant. Without added nucleotide, intrinsic fluorescence deteriorated irreversibly with visible protein aggregation. This could not be reversed by subsequent addition of nucleotides.

CONCLUSIONS: The parameters we measure are not rate limited by GDP release from transducin. Kinetic modeling suggests a low fluorescence preliminary G•GTPγS binding state from which GTPγS can dissociate to allow continued denaturation in subsaturating GTPγS.
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