

## STRUCTURAL AND THERMODYNAMIC STUDIES OF CLONED FRAGMENTS OF SPECTRIN

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We are investigating the molecular mechanism of flexibility of the repeating unit domain of the cytoskeletal protein, spectrin. The linker regions between repeats are of particular interest as they may contribute to this unusual feature of spectrin, which facilitates the reversible deformation of red cells.

The five structures of four constructs of two connected repeating units of chicken brain  $\alpha$ -spectrin, solved by X-ray crystallography, suggest two models of spectrin flexibility [1]. The conformational rearrangement model features the transition of a loop region into a helix so that each repeat is raised towards the N-terminus, thus shortening the end-to-end distance of a chain of 20 units by 70%. The bending model features bending at the linker region to effect supercoiling of the chain so that its end-to-end distance can decrease by 50%.

All five crystal structures exhibit a helical linker region which is, therefore, compatible with at least two models of spectrin flexibility.

To further characterize the linker region, we have measured the thermodynamic stabilities of the two unit fragments and corresponding N-terminal one unit fragments by their urea and thermal denaturation [2]. The free energies of unfolding of two unit fragments ranged from 6.2 to 9.9 kcal/mol and were thus greater than those of the one unit fragments, which ranged from 3.7 to 4.4 kcal/mol, on both urea and thermal denaturation. These results also show that the two units in each two unit fragment unfold simultaneously, as confirmed by preliminary results of differential scanning calorimetry.

## REFERENCES

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2. MacDonald, R.I. and Pozharski, E.V. Free energies of urea and of thermal unfolding show that two tandem repeats of spectrin are thermodynamically more stable than a single repeat. **Biochemistry** 40 (2001) 3974-3984.