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THE VASOINHIBIN SOLUTION STRUCTURE APPEARS UNFOLDED, DYNAMIC, AND FEATURES AGGREGATION

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BACKGROUND

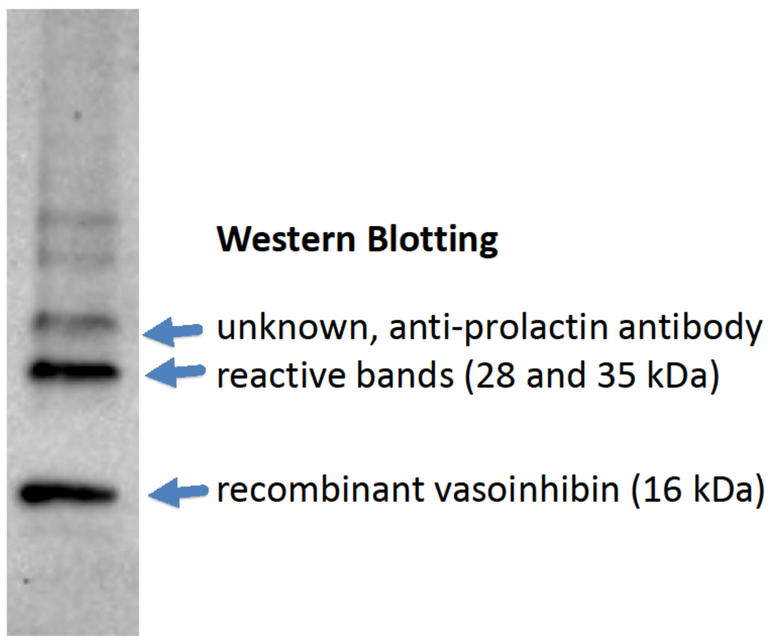
The pituitary hormone prolactin, the precursor of vasoinhibins, is structurally classified as a class-I helical cytokine with a four-helix bundle core and only a minimal degree of dark regions. Experimental data on the solution structure of vasoinhibins are not available.

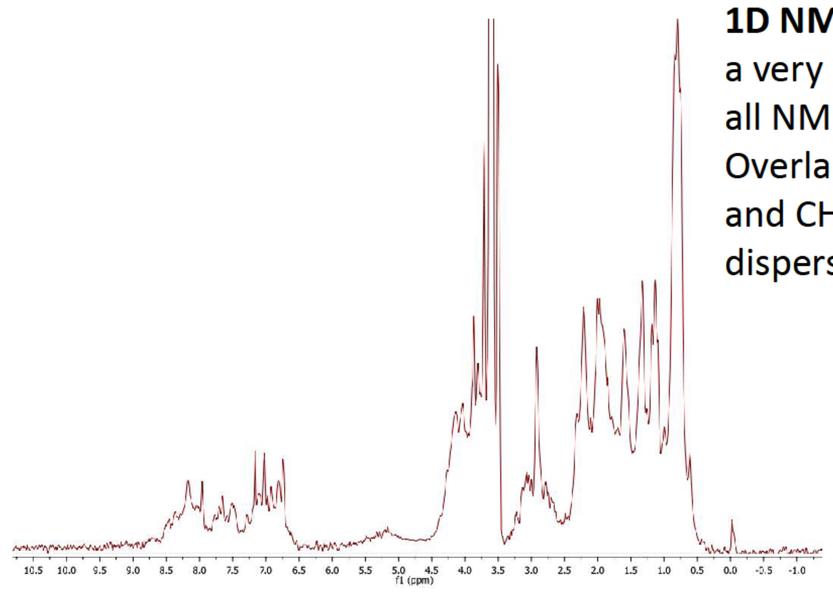
METHODS

A recombinant, human vasoinhibin with a molecular mass of 16.7 kDa, comprising amino acids 29-176 of prolactin, was expressed in E. coli and purified. Nuclear magnetic resonance (NMR) spectra were obtained (1H 1D, 2D TOCSY, 500/700 Mhz, and 2D NOESY, 950 Mhz). The purified vasoinhibin sample was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and Western Blotting was performed using polyclonal and monoclonal, epitope-mapped anti-prolactin and anti-vasoinhibin antibodies.

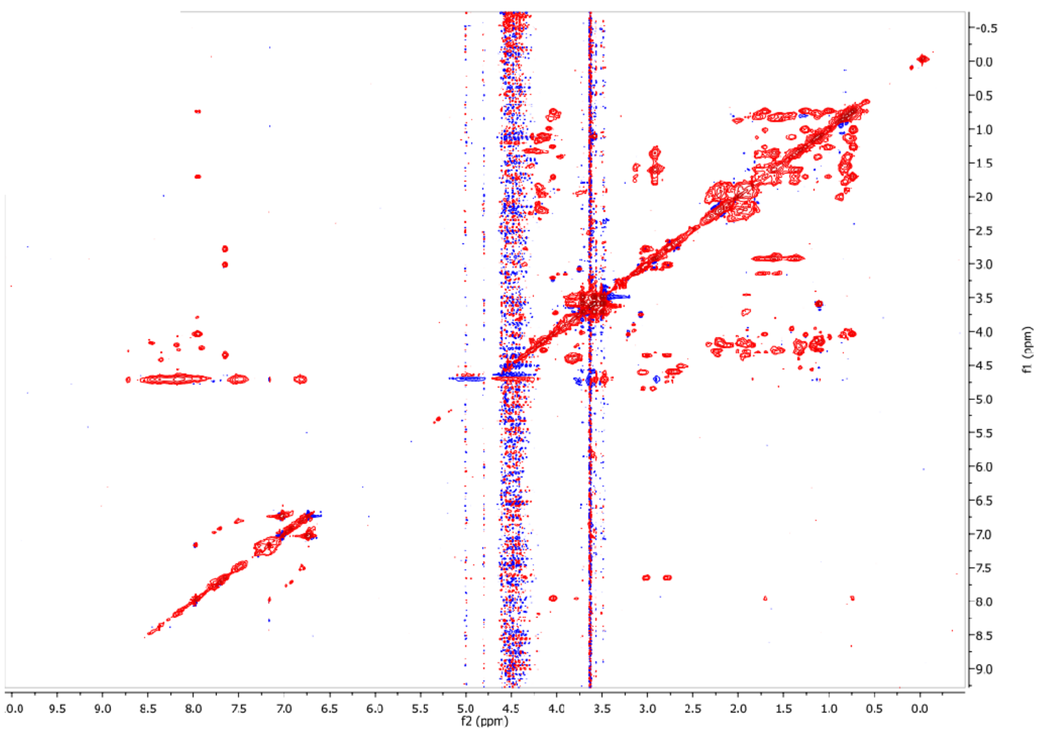
RESULTS

1D NMR spectra at several conditions of pH, buffer and temperature demonstrated broad vasoinhibin signals in a very narrow region with small differences between all spectra. Titration with trifluoroethanol and lithium chloride had minimal effect. TOCSY and NOESY experiments corroborated the results of all 1D experiments. Sodium dodecyl sulfate polyacrylamide gel electrophoresis and Western Blotting analyses demonstrated an anti-vasoinhibin antibody immunoreactive band with an apparent molecular mass of 16 kDa, consistent with the presence of the recombinant vasoinhibin-protein. Further immunoreactive bands of unknown identity with molecular masses of 28 and 35 kDa were detected.

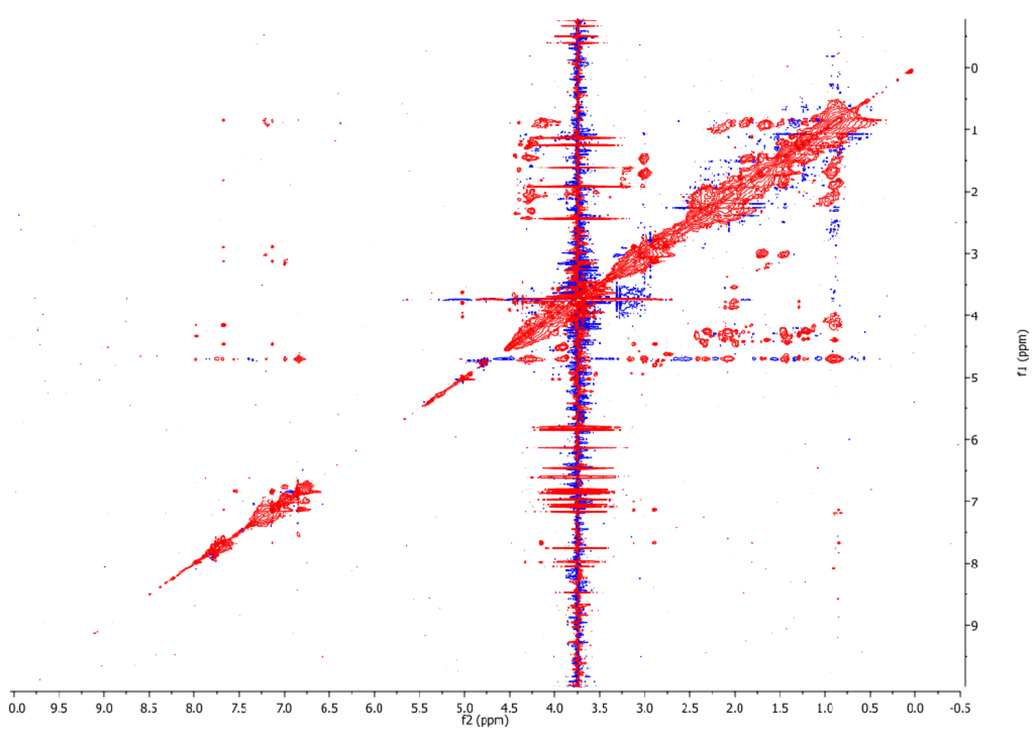




1D NMR Experiment shows a very narrow region where all NMR signals appear. Overlap is really crowded and CH3 groups are not disperse.



NMR TOCSY experiment shows reasonable cross signals in the aliphatic region. NH region lacks of enough correlations.



NMR NOESY experiment shows the appearance of very few cross signals in NH region and aliphatic protons. Tris is at 3.7 ppm.

CONCLUSION

Broad and little dispersion of few NH signals could indicate an unfolded vasoinhibin-protein in fast motion. Further NMR-studies are also complicated by possible aggregates of higher molecular masses.

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