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Plasmin Cleaves Prolactin and Placental Lactogen and Generates Vasoinhibin-Like Peptides

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Background

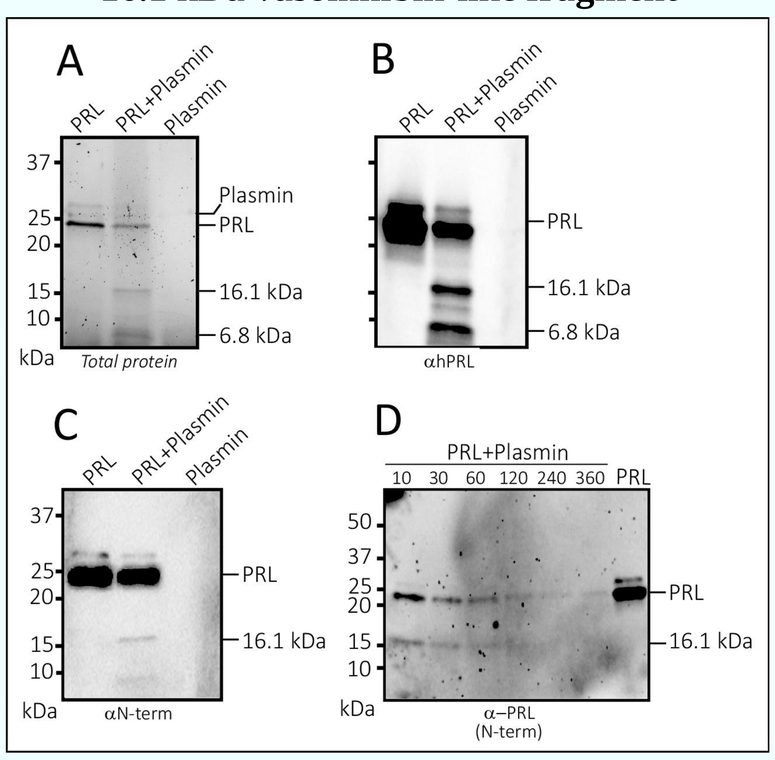
Vasoinhibin is an endogenous antiangiogenic and profibrinolytic peptide generated by the proteolytic cleavage of the pituitary hormone prolactin (PRL) by cathepsin D, matrix metalloproteinases and bone morphogenetic protein-1. Based on the phylogenetic relationship of growth hormone and placental lactogen (PL), these hormones may also function as precursors to generate vasoinhibin. Clinical significance of vasoinhibin is best demonstrated in diabetic retinopathy and peripartum cardiomyopathy, and a dysregulation in the generation of vasoinhibin was also reported in preeclampsia.

Material and Methods

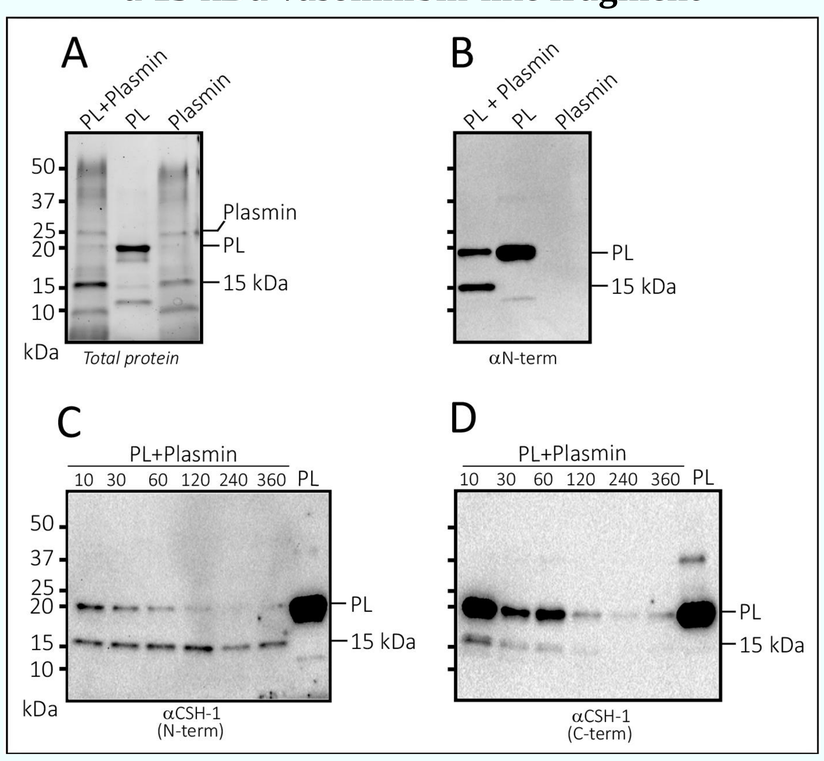
Proteolytic cleavage of recombinant PRL (23 kDa, expressed in HEK-cells) and recombinant PL (21 kDa, expressed in human placental tissue) by natural human plasmin were performed at 37 °C and pH 7.4. Resulting fragments were analyzed by SDS-PAGE and Western blotting using polyclonal and monoclonal, N- and C-terminal, epitope-mapped anti-PRL and anti-CSH-1 antibodies. Mass spectrometric analyses were carried out for additional sequence validation.

Results

Prolactin cleavage by plasmin generates a 16.1 kDa vasoinhibin-like fragment



Placental lactogen cleavage by plasmin generates a 15 kDa vasoinhibin-like fragment

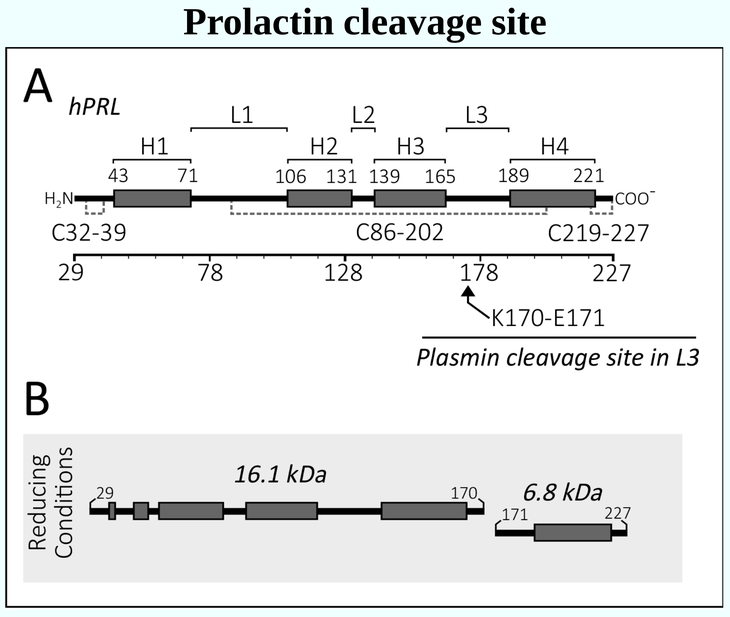


Molecular weight of vasoinhibin-like molecules generated by plasmin

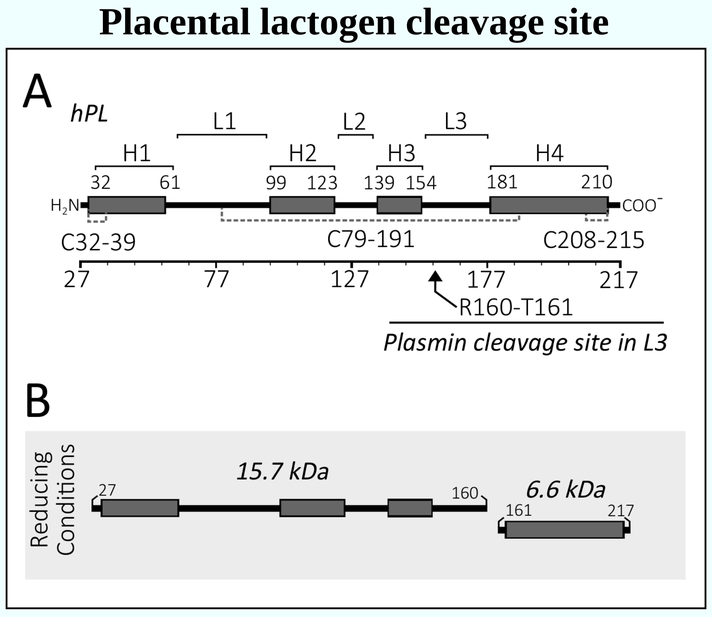
Precursor	Vasoinhibin-MW
Prolactin	16.1 kDa
Placental lactogen	15 kDa

- detection of a 6.8 kDa C-terminal PRL-fragment
- no detection of C-terminal PL-fragment
- all experiments were carried out under reducing conditions

Prolactin cleavage site



Placental lactogen cleavage site



Cleavage sites of vasoinhibin-like molecules generated by plasmin

Precursor	AA Position
Prolactin	170 (Lys) - 171 (Glu)
Placental lactogen	160 (Arg) - 161 (Thr)

- Plasmin prefers binding to loop 3 of the precursors PRL and PL
- assumed resulting fragmentation by plasmin under reducing conditions with indication of the molecular weights and cleavage sites

Conclusion

Plasmin generates vasoinhibin-like peptides by proteolytic cleavage of PRL and PL. These peptides may represent vasoinhibin isoforms with effects on vasculature, fibrinolysis, and behavior.