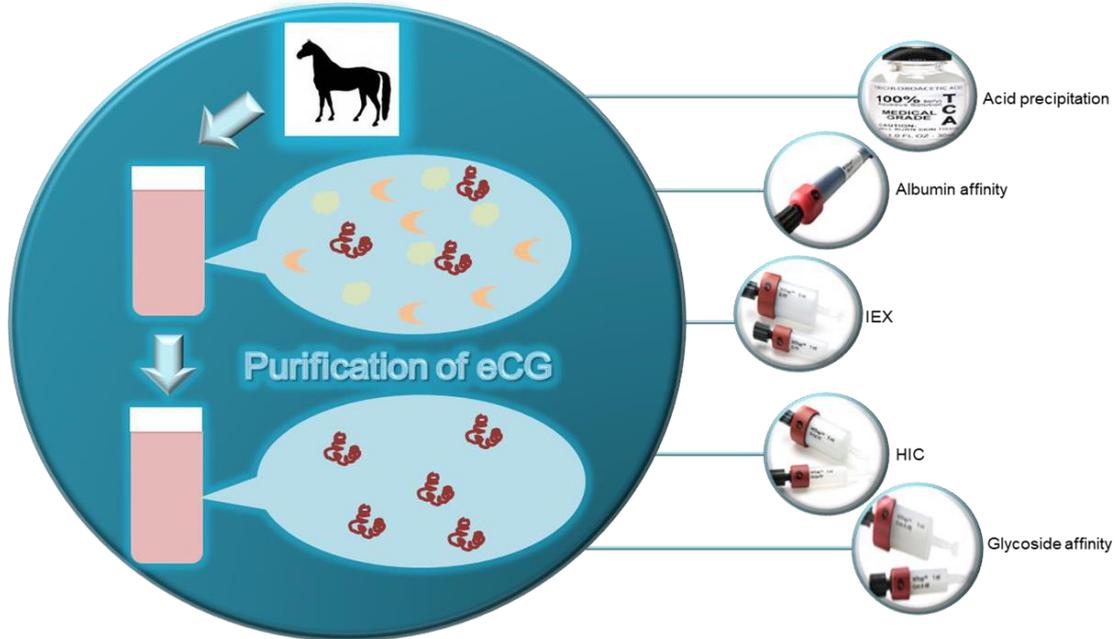


The purification of equine chorionic gonadotropin (eCG) from serum

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Graphical Abstract The purification of eCG from the serum of pregnant mares using various precipitation and chromatographic techniques.

Introduction: eCG is a glycoprotein hormone found in the serum of pregnant mares. It exhibits both follicle stimulating hormone and luteinizing hormone activity in non-equid species and is used in livestock reproduction. Recombinant production of eCG is hindered by the inability to produce the correct glycosylation profile needed for *in vivo* biological activity of the protein. eCG purification from the serum of pregnant mares is currently the only production method but remains problematic due to the presence of the abundant and characteristically similar equine serum albumin (ESA).

Methods: eCG was purified from horse serum spiked with commercial eCG in a preliminary attempt to optimize the classical purification method. Precipitation with 0,5 M Metaphosphoric acid (MPA) was compared to precipitation with Trichloroacetic acid (TCA) of various concentrations. Subsequently albumin depletion was attempted using a HiScreen Blue Sepharose FF column which has high affinity for albumin. Anion exchange chromatography (IEX), hydrophobic interaction chromatography (HIC) as well as glycoside affinity was performed in an attempt to separate ESA and eCG.

Results and Discussion: Precipitation with MPA removed approximately 55% of unwanted proteins but yielded only 45% eCG. TCA fared similarly in precipitating unwanted protein but gave a better yield of 85% eCG. The HiScreen Blue Sepharose column showed an equal affinity for eCG and ESA, therefore ruling out its future use for eCG purification. IEX showed that the column binds ESA but not eCG, while the glycoside affinity column binds eCG and not ESA. No separation of eCG and ESA was observed with HIC.

Conclusion: We found that precipitation with a 15% v/v TCA solution offered the best results when compared to precipitation with MPA. ESA depletion remains problematic due to eCG's similar characteristics to ESA. Preliminary IEX and glycoside affinity chromatography results, however, appears promising.

Keywords

Equine Chorionic Gonadotropin, eCG, protein purification, equine serum albumin, ESA

References

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