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JOURNAL ARTICLE

Partial purification and localization of platelet-activating factor acetylhydrolase from bovine seminal plasma

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Platelet-activating factor (PAF) is a potent lipid mediator that is inactivated by platelet-activating factor acetylhydrolase (PAF-AH). Platelet-activating factor bioactivity has been detected in bovine sperm phospholipids and PAF-AH activity is extraordinarily high in bovine seminal plasma. The purpose of this study was to purify and characterize partially the PAF-AH in bovine seminal plasma. Platelet-activating factor acetylhydrolase was partially purified from bovine seminal plasma using gelatin-agarose and ion-exchange chromatography and nondenaturing polyacrylamide gel electrophoresis (PAGE). Enzyme activity was increased 11-fold over seminal plasma with a yield of 11%. Platelet-activating factor acetylhydrolase activity was eluted from a single band with a R(f) of 0.258 from a nondenaturing preparative PAGE gel along with several other proteins of varying molecular weights. Following separation by sodium dodecyl sulfate (SDS)-PAGE under reducing conditions, PAF-AH was identified as a approximately 60-kD band by western blotting using antiserum directed against human blood PAF-AH. N-terminal sequencing of the approximately 60 kD band, followed by amino acid-sequence similarity searching, demonstrated a single-sequence match with PAF-AH from bovine blood. Based on western blotting, a approximately 60-kD band corresponding to PAF-AH was detected in seminal vesicle fluid but not in samples of washed, sonicated sperm or sperm plasma membranes where activity was low (<5% and <0.3%, respectively, of that in seminal plasma), suggesting that seminal plasma PAF-AH does not bind tightly to sperm. Specific PAF-AH activity measured in seminal vesicle fluid was in the lower range of that in seminal plasma. These results demonstrate that PAF-AH activity in bovine seminal plasma is due to PAF-AH secreted by the seminal vesicles with sequence homology to the enzyme in human blood.

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