

IDENTIFICATION AND LOCALIZATION OF ALKALINE PHOSPHATASE ISOFORM IN CANINE SEMINAL PLASMA AND GONADAL TISSUES

MA Kutzler¹, P Solter², W Hoffman², DH Volkmann³

¹*Department of Biomedical Sciences – Cornell University, Ithaca, New York 14853*

²*Department of Veterinary Pathobiology – University of Illinois, Urbana, Illinois 61802*

³*Department of Clinical Sciences – Cornell University, Ithaca, New York 14853*

INTRODUCTION

Alkaline phosphatase (AP) is a useful indicator of the presence of the sperm-rich (2nd) fraction in the canine ejaculate. Two AP isoenzymes originating from separate genes have been identified in the dog: tissue non-specific (TNS) and intestinal. Bone, liver, and kidney AP are all different isoforms of the TNS isoenzyme. The objectives of this study were to identify the AP isoform in canine seminal plasma and to localize its source. We hypothesized that the seminal AP (SAP) is a unique TNS isoform and is secreted by epithelial cells in the cauda epididymides.

MATERIALS AND METHODS

Semen from 5 male dogs (2 Beagles and 3 mongrels) was manually collected in fractions. The 1st/2nd fraction was centrifuged for 10 minutes and the seminal plasma was removed from the sperm pellet. Total SAP concentration was measured on seminal plasma using an automated assay (Roche, Indianapolis, IN). The seminal plasma was then diluted with an equal volume of 50 mM Tris buffer (pH 8.0) containing 0.1% sodium azide. Isoenzyme analysis was determined by levamisole inhibition assay. The glycosylation of SAP was compared to the liver, bone, and corticosteroid-induced (CIAP) isoenzymes by a combination of wheat germ lectin (WGL) agglutination and cellulose acetate electrophoresis. The dogs were subsequently castrated and their testes and epididymides fixed in 4% paraformaldehyde/0.1 M sodium cacodylate (pH 7.4), dehydrated through a graded ethanol series and embedded in JB-4 Plus (Polysciences, Warrington, PA). Native SAP activity was localized within the testes and epididymides using BCIP/NBT substrate (KPL, Gaithersburg, MD) applied directly onto the sections.

RESULTS

Total SAP concentration was >10,000 U/L in 4 of the 5 seminal plasma samples. Isoenzyme analysis revealed that the activity was due to the TNS isoenzyme. The electrophoretic patterns of SAP on cellulose acetate were relatively unique in that a very broad band resulted, indicating some variability in charge between individual molecules. In addition, SAP bound WGL, indicating the presence of N-acetylglucosamine residues, similar to bone AP and different from liver AP. The SAP activity was localized to the caput, corpora and cauda epididymal and seminiferous tubule epithelium, with the most intense staining in the cauda epididymis.

DISCUSSION

SAP is a unique isoform of canine TNS AP whose glycosylation is likely distinct from either of the TNS AP isoforms commonly found in canine serum. The ability to distinguish SAP from bone AP, liver AP and CIAP could be useful in determining the quality of the semen sample obtained. Localization of SAP activity was consistent with that reported by Frenette et al.

KEY WORDS Alkaline Phosphatase, Seminal Plasma, Epididymis, Testes, Dog

REFERENCE Frenette G, Dube J, Tremblay R. 1986. *Archives of Andrology* 16:235 – 241.