

## IDENTIFICATION OF THE BOVINE $\alpha$ 1-ACID GLYCOPROTEIN IN COLOSTRUM AND MILK

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### Introduction

The acute phase protein alpha1-acid glycoprotein (AGP) is an immunomodulatory protein expressed by hepatocytes in response to the systemic reaction that follows tissue damage caused by inflammation, infection or trauma. AGP features at least two different biological activities, apparently very different each from the other: AGP may immunomodulate the inflammatory response, and, meanwhile, act as a plasma transport protein. As the other acute phase proteins, AGP is produced mainly by hepatic cells, but local expression has been reported in human breast epithelial cells, stimulated alveolar macrophages and human endothelial cells. The detection of two of the major bovine APP, haptoglobin and serum amyloid A (SAA) in milk during mastitis may suggest a role for APP also in the immunomodulation of local inflammatory reaction in the udder. This communication presents the detection of the bovine AGP (boAGP) in mammary secretions (colostrum and milk) and mammary gland tissue.

### Material and Methods

Bovine AGP was purified from whey by means of ionic exchange and reverse-phase chromatographic procedure. The amount of boAGP was determined by means of RID test. The cDNA sequence was determined following conventional molecular biology procedure.

### Results

Bovine AGP was detected by Western blotting in all the samples analyzed, and could be quantified in colostrum at 162 ( $\pm$  63.7) mg/ml and 114.5 ( $\pm$ 67.8) mg/ml during the first 12 hrs and 24 hrs respectively. In mature milk the boAGP concentration clearly decreased and was no longer detectable using the Radial Immunodiffusion (RID) technique. Mature milk boAGP's concentration was therefore semi-quantified using an anion-exchange chromatographic procedure that allowed the concentration of the protein to be determined. The presence of AGP in bovine milk was confirmed by the internal sequence analysis performed following the purification to homogeneity of the protein from milk. The concentration of AGP in bovine milk with low SCC (< 250000) was very similar to that from bovine with high SCC (> 250000). In order to investigate the origin of AGP in bovine milk, a search for mRNA was carried out in somatic cells and mammary gland tissue and cells: mRNA expression of boAGP gene was detected in mammary gland tissue, but not in somatic cells. Finally, cDNA sequence of boAGP was determined, and hereby presented: the cDNA sequence (658 bp) codes for 219 aminoacid residues that accounts for a MW of 20410 kDa. Prosite analyses revealed five potential N-glycosylation sites and, interestingly seven potential phosphorylation sites.

### Discussion

What can be the biological significance of the presence of AGP in bovine milk? As an immunomodulatory molecule, boAGP could play several roles during inflammatory challenges in the mammary gland, including the modulation of the undesirable inflammatory reactions during mastitis due to excessive phagocytic activities that can cause severe mammary secretory cell damages. Bovine AGP can be found also at high concentrations in colostrum in non-pathological conditions. Therefore, it is conceivable that boAGP may also contribute to the complex framework of the immunoregulatory molecules expressed in bovine colostrum.