

Identification of Pregnancy-Associated Glycoprotein 1 (PAG-1) in zebu (*Bos Indicus*) placenta

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1 Introduction

- ▲ *Bos indicus* is used interchangeably with the term zebu which originates from the Tibetan word Zen or Zebu, which means "the hump of the camel". These Bovidae constitute 65% of the world's bovine population.
- ▲ Zebu cattle is an important source of animal protein in many countries, being more effective in extracting nutrients from low quality roughages and having a remarkable ability to tolerate high temperatures.
- ▲ Reproductive differences between domestic (*Bos taurus*) and zebu (*Bos indicus*) cattle have been described. Zebu cattle reach puberty later (16 to 40 months of age) than *Bos taurus* x *Bos indicus* crossbreeds or purebred taurine cattle. Different authors reported also longer pregnancy lengths in some breeds of zebu cattle as well as in crossbreed *Bos taurus* x *Bos indicus*.
- ▲ Although there is a relatively abundant literature on the characterization of ruminant placental proteins by the use of molecular biology techniques, till now, excepted Sousa et al. (2000) there was no report on the biochemical characteristics of the protein purified from zebu placenta.

2 Aim

The aim of this study was to investigate the characteristics of the pregnancy-associated glycoproteins extracted from zebu placenta

3 Materials & Methods

PLACENTA

- ▲ Three placentas from zebu females; gestational ages, as determined according to Rexroad et al. (1974), between 30 and 31 weeks
- ▲ Total weight of cotyledons: 1,920 g

FOLLOW-UP OF ISOLATION

- ▲ Follow-up of PAG-immunoreactive fractions
Homologous bovine PAG-1 radioimmunoassay (boPAG-1 RIA) (Zoll et al., 1992)
- ▲ Total protein (TP) determination
Lowry method (Lowry et al., 1951)

ISOLATION

- ▲ Acid precipitation: pH 4.5 with H_2PO_4 0.5 M
- ▲ Ammonium sulfate precipitation (A.S. ↓): 0-40% and 40-80% of saturation
- ▲ Anion exchange chromatography
DEAE-Sephadex A25 (Pharmacia*) in 0.01 M Tris-HCl, pH 7.6
Eluted with 0.02, 0.04, 0.08, 0.16, 0.32 and 1.00 M NaCl concentrations

ISOLATION (following)

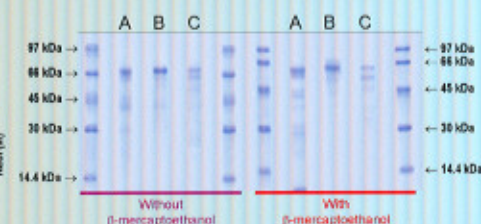
- ▲ Cation exchange FPLC
CM: Ceramic column (Pharmacia*) (1 cm x 3 cm)
0.01 M ammonium acetate, pH 5.2

CHARACTERIZATION

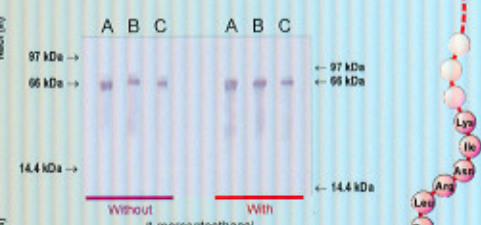
- ▲ SDS-PAGE 12% with and without reducing agent (β -mercaptoethanol)
- ▲ Western blot analysis
- ▲ 2-D SDS-PAGE
- ▲ NH_2 -terminal microsequence analysis
After transfer onto PVDF membrane
By automated Edman degradation
(Beckman LF3400D microsequencer)



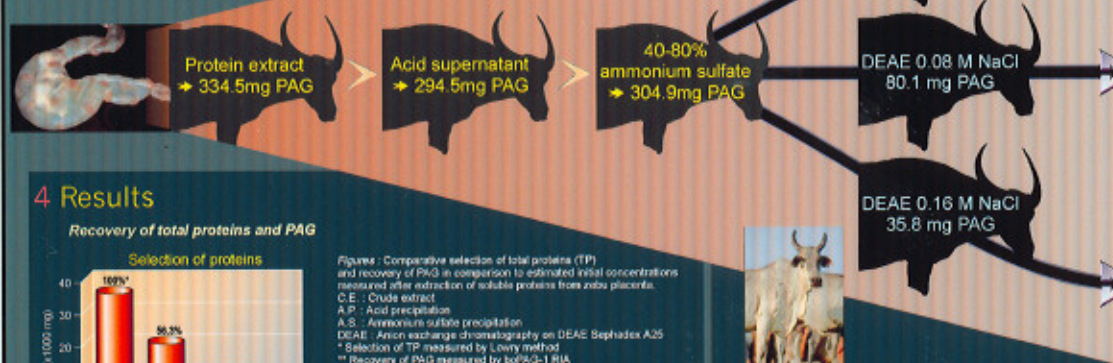
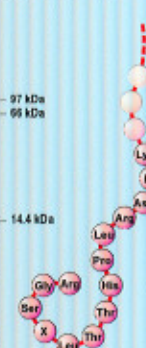
Characterisation of immunoreactive fractions by electrophoresis



Coomassie Blue-stained SDS-PAGE (12%). Gel run without and with reducing agent (5% β -mercaptoethanol). Lanes A, B, and C = most immunoreactive fractions from CM Ceramic column (steps 0.04, 0.08, and 0.16 M from DEAE-Sephadex A25, respectively).



Western-Blot analysis with antiserum anti-boPAG1 (AS # 497). SDS-PAGE was run without and with reducing agent (5% β -mercaptoethanol). Lanes A, B, and C = most immunoreactive fractions from CM Ceramic column (steps 0.04, 0.08, and 0.16 M from DEAE-Sephadex A25, respectively).



4 Results

Recovery of total proteins and PAG

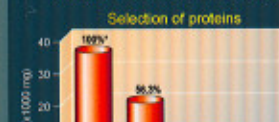


Figure 1: Comparative selection of total proteins (TP) and recovery of PAG in comparison to estimated initial concentrations measured after extraction of soluble proteins from zebu placenta.
C.E.: Crude extract
A.P.: Acid precipitation
A.S.: Ammonium sulfate precipitation
DEAE: Anion exchange chromatography on DEAE-Sephadex A25
* Selection of TP measured by Lowry method
** Recovery of PAG measured by boPAG-1 RIA

Localization of the main immunoreactive fraction

Table 1: Quality of immunoreactive PAG and total protein (TP) of the fraction eluted from the DEAE-Sephadex A25

DEAE fraction (NaCl concentration)	PAG* (mg)	TP* (mg)	Ratio PAG:TP
0.00 M	8.33	282.7	2.95 %
0.02 M	5.63	114.6	4.91 %
0.04 M	30.74	176.3	17.44 %
0.08 M	80.07	292.9	27.34 %
0.16 M	35.78	666.0	5.37 %
0.32 M	7.49	292.4	2.56 %
1.00 M	5.98	70.9	8.53 %
Total	174.02	1 895.8	-

* Determined by boPAG-1 RIA. ** Determined by Lowry method on BSA equivalent.

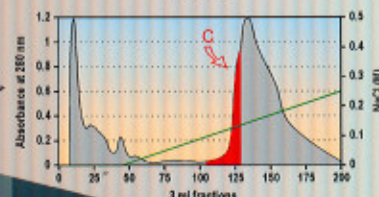
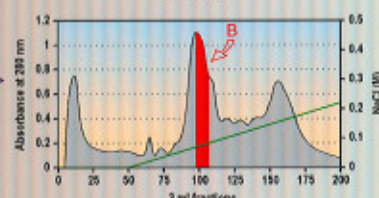
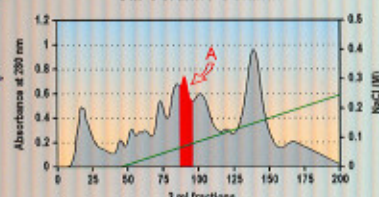


NH_2 -terminal microsequence analysis

- ▲ Microsequencing of the N-terminus from the most immunoreactive fraction from the 0.08 M DEAE-Sephadex A25 (eluted at 0.11 to 0.17 M NaCl on CM Ceramic column) revealed the R G S X L T T H P L R N I K sequence, characteristic of the boPAG-1 isolated by Zoll et al. (1991).

By using pepstatin affinity column for aspartic proteinase isolation and early pregnancy zebu placenta (3.5 months of age), we recently characterized a PAG with the same sequence and apparent MW of 67 000. So, the 69 000 MW form characterized in the present investigation can be the result of a higher carbohydrate content or post-translational differences in placenta collected later in pregnancy.

CM Ceramic Column



Bibliography

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Conclusion

This study confirms the existence of a pregnancy-associated glycoprotein in zebu placenta. Investigations are in progress in order to isolate other zebu pregnancy-associated glycoproteins expressed at different stages of pregnancy.