Pregnancy-Associated Glycoproteins (PAGs) in Bos taurus taurus and Bos taurus indicus

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Introduction

In 1991, Zoli et al. purified a pregnancy-associated glycoprotein from bovine fetal cotyledons (PAG: later designated bPAG1). In the same year, molecular biology studies showed that PAGs belong to the aspartic proteinase family like pepsinogen, renin, chymosin, cathepsin D and E (Xie et al., 1991). The bPAG1 showed a molecular weight of 67 kDa and four isoforms with different pIs (5.4, 5.2, 4.8 and 4.4). These glycoproteins can be detected in the maternal circulation by week 3 after breeding and have been currently used as a gestation marker in cows (Sasser et al., 1986; Zoli et al., 1992).

The bPAG2, initially isolated by Beckers et al. (1988) as a bovine chorionic gonadotropin, was later characterized by Xie et al. (1994) as an other bovine pregnancy-associated glycoprotein presenting a binding site to the LH receptors. This glycoprotein is structurally related to bPAG1 (57.9% amino acid sequence similarity) and seems to be expressed uniformly throughout gestation (Green et al., 1999).

PAG molecules were also isolated in other ruminant species like sheep (Zoli et al., 1995) and goat (Garbayo et al., 1998). The characterization of the caprine PAGs revealed 3 different forms having molecular weights of 55, 59 and 62 kDa, and different amino acid sequences.

Moreover, new members of the PAG family were identified by molecular cloning of cDNA in horses (Green et al., 1999), pigs (Szafranska et al., 1995), zebras (Gan et al., 1997) and cats (Gan et al., 1997).

Although the PAGs clearly belong to the aspartic proteinase family, most of them are considered to be enzymatically inactive because of mutations around their active site (Xie et al., 1991). With regard to PAG expression during the pregnancy period, molecular biology studies showed that it can vary spatially and temporally throughout gestation (Garbayo et al., 1999; Green et al., submitted).

Aim

The aim of this study was to compare crude placental extracts of B. taurus taurus and B. taurus indicus and purified bPAG1 against two different antisera by means of **Double Radial Immunodiffusion** (DID).

Materials and Methods

1. Antigens and antisera:

1.1. Antigens:

A) Placental extracts and purified bPAG1:

Approximately 500 g of 6th month aged cotyledons from B. taurus taurus and B. taurus indicus were used. The tissue was minced and homogenized with a hand mixer in 50 mM phosphate buffer containing PMSF (0.2 mM) and EDTA (0.2% w:v), with ratio of buffer to tissue of 5:1 (v:w). The homogenate was stirred overnight. It was then centrifuged at 27 000 X g for 1 h, and the pellet was discarded. The supernatant was dialyzed against 5 mM ammonium bicarbonate (pH 7.8), lyophilized and the final powder stored at 4°C. The antigen preparation steps are summarized in *Figure 1*. The dry powder from placental extracts was reconstituted at a concentration of 20 mg/ml in phosphate buffer before use in DID.

Only a pure preparation of bPAG1 was used as standard (the bPAG2 was not available as pure preparation; Beckers et al., 1988b; Xie et al., 1994). This antigen was diluted in phosphate buffer to obtain a concentration of 1 mg/ml.

Figure 1: Protocol of antigen preparation

B) Albumins:

The bovine and porcine albumins (BSA and PSA) were used to control the specificity of the antisera. This proteins were dissolved in phosphate buffer to obtain a concentration of 1 mg/ml and then tested against all the antisera.

1.2. Antisera:

A) Antisera against bPAGs:

Two groups of antisera stored in the Department of Physiology of Reproduction of University of Liège were used in this study *(Table 1)*.

Table 1: Groups of antisera tested and their origin.

Groups	Numbers tested	Source and reference
Anti-bPAG1	497	Zoli et al., 1991
	726	
	727	
Anti-bPAG2	436	Beckers et al., 1988a,b
	437	
	438	

Table 1: Groups of antisera tested and their origin

The first group of antisera (anti-bPAG1) was raised in rabbits previously immunised with purified bPAG1. The second group (anti-bPAG2) was produced against semi-purified bPAG2 preparation. All the antisera were obtained in rabbits according to the method of Vaitukaitis (1971). The rabbits received injections of 500 mg of protein homogenized at 2-wk intervals and were bled 3 months after the first immunization.

B) Antisera against albumines:

Antisera against BSA and PSA were used to verify if the reactions between anti-bPAG and crude placental extracts were not due to the albumine contamination in the extract with. These antisera were obtained by the same protocol described above.

2. Double Radial Immunodiffusion (Ouchterlony, 1949):

An agarose solution of 2% in veronal buffer was poured onto clean microscope slides, and after solidification, 0.5 mm diameter wells were cut with a 7-well cutter. After filling the samples in the wells, the slides were left to stand for 24 h in the wet chamber. The soluble proteins were then washed out by 2 changes in 0.9% NaCl solution and by 2 changes in distilled water. The slides were stained with Coomassie blue (R250) solution for 3 min and the excess stain was washed out by repeated changes in ethanol:acetic acid:distilled water (4:1:5 v/v/v).

2.1.Double radial immunodiffusion - Semi-quantitatif Test:

This test was used to verify the specificity of the antiserum against the albumin. The antisera (anti-bPAG1, anti-bPAG2, anti-BSA and anti-PSA) with their following dilutions (1/1, 1/2, 1/4, 1/8, 1/16, 1/32) were placed in the peripheral wells. The central well was filled by the bovine albumin (BSA) or by the porcine albumin (PSA) (Figure 2).

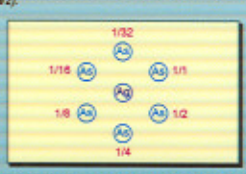

Figure 2: BSA or PSA were placed separately in the central well. Antisera (anti-bPAG1, anti-bPAG2, anti-BSA and anti-PSA) with different dilutions (1/1; 1/2; 1/4; 1/8; 1/16; 1/32) were filled in the peripheral wells.

2.2.Double radial immunodiffusion - Qualitatif Test:

This test was used to compare purified bPAG1 and crude placental extracts of B. taurus taurus and B. taurus indicus against two groups of antisera (Figure 3).

Figure 3: Comparison of bPAG1, crude placental extracts of B. taurus taurus and B. taurus indicus. The undiluted anti-bPAG1 or diluted anti-bPAG2 at a ratio of 1:4 were filled in central well. The peripheral wells were filled with crude placental extracts of B. taurus indicus (wells a-a) B. taurus taurus (wells b-b) and pure bPAG1 (wells c-c).

Results

1. Control of antiserum specificity:

BSA and PSA (data not shown) did not cross-react with any of the antisera (anti-PAG1 and 2) *(Figure 4A)* but they cross-reacted clearly with anti-BSA and anti-PSA, respectively *(Figure 4B)*.

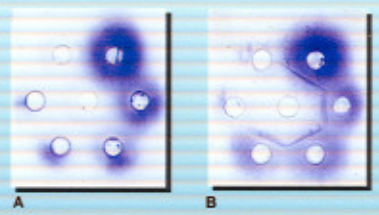

Figure 4. In both slides (A and B) the BSA was filled in the central well. Peripheral wells were filled with different dilutions (1/1; 1/2; 1/4; 1/8; 1/16; 1/32) of the antisera anti-bPAG (# 497 e.g.) *(Figure 4A)* and anti-BSA (# 292 e.g.) *(Figure 4B)*.

2. Comparison of placental extracts and bPAG1:

In the bPAG1 system *(Figure 5A)*, there was one clear precipitation line between the purified PAG1 protein and anti-bPAG1. This line was also observed with placental extracts of B. taurus taurus and B. taurus indicus. One more concentric line -closer to the central well- was also observed for both extracts.

In the bPAG2 system *(Figure 5B)* no precipitation line appeared with the pure bPAG1 and anti-bPAG2 confirming the immunological difference between the two PAGs. When placental extracts were compared B. taurus indicus gave one precipitation line and B. taurus taurus gave two lines.

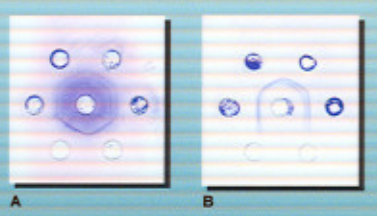

Figure 5: The central wells were filled with non-diluted anti-bPAG1 antiserum (A) and with diluted (1:4) anti-bPAG2 antiserum (B). Peripheral wells in A (bPAG1 system) and B (bPAG2 system) were filled with crude placental extracts from B. taurus indicus (a), B. taurus taurus (b) and bPAG1 purified (c).

Conclusions

In conclusion, B. taurus indicus seemed to be similar to B. taurus taurus in the bPAG1 system, while appearing different in the bPAG2 system.

The clear difference concerning the bPAG2 is worthy for further investigations using the SDS-PAGE and Western blotting methods.

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