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Interaction analysis of buffalo pregnancy associated glycoprotein-1 in silico

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ABSTRACT

Pregnancy associated glycoprotein-1, a member of the aspartic proteinase family possesses placentogenic and embryo protective functions in numerous domestic species. The present study was conducted to deduce binding and interaction properties of buffalo pregnancy associated glycoprotein-1 (PAG-1) *in silico*. Buffalo PAG-1 DNA, RNA binding sites deduced using BindN server revealed buffalo PAG-1 protein sequence possess 93 residues with 80% and 56.96% DNA binding specificity and sensitivity, respectively. RNA binding sites are also restricted to identical clusters of amino acid residues as DNA binding sites with 78 residues with RNA binding potential with 80% specificity and 53.95% sensitivity. The potential domains with a high degree of DNA and RNA binding property are present at conserved microsequences residues of buffalo PAG-1 protein sequence. Ligand binding properties from PDBSUM database reveal buffalo PAG-1 possesses ten clefts having potential ligand binding sites consisting of aliphatic and positive amino acid residues. Analysis with the STRING database showed buffalo PAG-1 interaction with cytokines viz. phosphoprotein associated with glycosphingolipid microdomains 1, placenta growth factor precursor, alpha-fetoprotein precursor and SP1 transcription factor. These factors are found to be active during the embryonic stage exerting their functions through angiogenesis, endothelial cell growth, proliferation, migration and differentiation. In conclusion this study reports the various binding properties and putative functional interactions of buffalo pregnancy associated glycoprotein-1 with other cytokines for exerting its biological action.

Key words: Buffalo, Pregnancy, Pregnancy associated glycoprotein-1, Protein interaction

Pregnancy involves cascade of cytokines and their putative interactions for varied biological functions. Interaction of protein with macromolecule plays critical role in controlling gene expression pattern (Takahashi *et al.* 2013) and induce or inhibit other genes/proteins essential for uterine receptivity signaling and eventually facilitating pregnancy (Spencer and Bazer 2004). This is accomplished by the interaction of several growth factors (Schäfer-Somi 2003, Sousa *et al.* 2006). Pregnancy associated glycoproteins (PAGs) have a predominant role in the placentogenesis, placental modeling and embryogenesis during pregnancy in domestic species (Xie *et al.* 1991, Barbato *et al*. 2013). Many PAG genes were cloned and identified in cattle, sheep, goat, pig and wild ruminant species (Xie *et al*. 1994, Garbayo *et al*. 2000, Szafranska *et al.* 1995). PAGs share their identity with other members of aspartic proteinase family viz. rennin (Xie *et al.* 1991, Guruprasad *et al*. 1996).

Predicted structure of buffalo pregnancy associated glycopreotein-1 revealed the presence of aspartic acid residues near the active site (Jerome *et al.* 2011a). Buffalo PAG-1 protein homology modeling and its structural stability study revealed its stable nature (Jerome *et al*. 2011b) . Study on ligand and macromolecule binding ability and protein-protein interaction of buffalo pregnancy associated glycoprotein-1 is still lacking. Considering the paucity of knowledge the present work was carried out to decipher the binding properties and interaction buffalo pregnancy associated glycoprotein-1 *in silico.*

MATERIALS AND METHODS

Various databases and server were used to deduce properties of buffalo PAG-1 i.e. ligand, DNA, RNA binding properties.

Buffalo PAG-1 protein binding sites and pockets: The functional properties of any protein can be deduced by its ability to bind macromolecules viz. DNA and RNA. DNA and RNA binding sites was deduced using BindN server (Wang and Brown 2006, Kelly and Stemberg 2009).

Buffalo PAG-1 protein ligand binding site: Ligand binding site consisting of gaps and clefts present in buffalo PAG-1 were predicted by PDBSUM server (Laskowski *et al.* 1997).

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Using the comparative modeling program MODELER 9.9 (Sali 2011) along with PyMOL molecular graphics system (Version 1.2r3pre), the location of clefts buffalo PAG-1 was visualized.

Buffalo PAG-1 network modeling and interaction analysis: Interaction of buffalo PAG-1 with other potential related molecules involved in cellular stage was determined by the STRING database (Szklarczyk *et al.* 2011). Analysis with the STRING database revealed the nature and strength of interaction with various cytokines. Prediction of these interactions with other molecules will provide a clear insight to understand the putative role of pregnancy associated glycoprotein in cellular processes.

RESULTS AND DISCUSSION

In the present study buffalo PAG-1 ligand and macromolecule binding ability and protein-protein interaction was deduced through *in silico* studies.

Buffalo PAG-1 binding properties: Buffalo PAG-1 protein sequence was analyzed for binding properties for macromolecules viz. DNA, RNA and proteins. Analysis with BindN server revealed potential DNA and RNA binding sites in buffalo PAG-1 amino acid sequence (Fig. 1). It was deduced 93 residues have the potential for binding DNA with 80% specificity and 56.96% sensitivity. RNA binding sites are also restricted to same clusters of amino acid residues as DNA binding sites with 78 residues having the potential to bind RNA with 80% specificity and 53.95% sensitivity (Fig. 2). DNA and RNA binding sites were localized near specific amino acid residue clusters beyond the signal peptide sequence (MKWLVLLGLVAFSEC). Analysis reveals potential domains having high degrees of DNA and RNA binding located at residues 18–34, 47–67, 103–136, 209–213, 226–229 and 301–341 of buffalo PAG-1 amino acid residues (Xie *et al.* 1997, Barbato *et al*. 2013). Similar RNA binding sites were restricted to the residues 18–35, 54–61, 102–136, 210–215, 256–288, and 323–344 residues. The amino terminal of buffalo PAG-1 (residues 91 to 98, VVFDTGSS) does not possess sites for DNA binding when compared to carboxyl terminus (residues 278–284, LVDTGTS) which possess sites for binding for macromolecules. It was also deduced microsequences of buffalo PAG-1 amino acid sequence *viz*. YS (position 46, 47), LSQISF (position 48–58), RGSNLTTH (position 59–66), PLRN (position 67–70) and IKDLVYMGNITIGTP (position 71–81) which are conserved across ruminant PAG proteins have the binding properties of DNA or RNA or both. This in conjunction with earlier studies of protein–RNA interface in relation to residue conservation (Chen and Lim 2008, Barbato *et al*. 2013).

In comparison with earlier studies DNA and RNA binding regions lie on the exposed residue which are hypervariable and correspond to surface loops in protein structure. These hypervariable regions represent surface domains where amino acid substitutions will occur without disrupting the structural integrity of buffalo PAG-1 protein. The conserved regions deduced in earlier studies are structurally and functionally important for retaining the overall three dimensional fold of buffalo PAG-1. Furthermore, these regions possess low binding properties of macromolecules such as DNA or RNA as these residues are buried in the protein structure. This difference in binding properties can be attributed to the functional interacting role of the native protein during various critical processes including gene transcription, translation, and signal transduction (Szilagyi *et al.* 2005, Takahashi *et al.* 2013).

Buffalo PAG-1 clefts and ligand binding sites: Buffalo PAG-1 protein possesses nest, clefts and binding sites as deduced by *in silico* analysis. Nests are structural motifs that are functionally important in protein structure. A total of 6 nests were deduced in buffalo PAG-1 protein (Table 1). Clefts in protein structure possess the highest degree of probability of ligand binding property (Fig. 3). Analysis of buffalo PAG-1 revealed 10 clefts in the buffalo PAG-1 structure having the potential ligand binding sites (Table 2). It is evident these clefts possess the ligand binding sites consists of conserved residues as deduced in earlier studies (Jerome *et al.* 2011a). Moreover the clefts of buffalo PAG-1 contain aliphatic and positive residues in comparison to other residues. The cleft and cavities have a greater volume owing to the presence of aliphatic, aromatic and positive residues exhibiting their putative role in ligand binding and functional property. It is also evident the number of residues with accessibility vertices are comparatively more than buried vertices predicting the

Table 1. Various nests present in buffalo PAG-1 with their properties

Nest	Score	Residue range	Residue	Ramachandran region	Residue conservation
1	1.96	Ile290-Ala292	Ile290	Right	1.00
			Gly291	Left	0.89
			Ala292	-	1.00
2	1.85	Cys261-Asp263	Cys261	Left	1.00
			Ser262	Right	1.00
			Asp263	Right	0.55
3	1.83	Gln198-Ile201	Gln198	Right	0.77
			Arg199	Left	0.70
			Ala200	Right	1.00
			Ile201	-	0.86
4	1.76	Ser297-Tyr299	Ser297	Right	1.00
			Ala298	Left	0.49
			Tyr299	-	0.79
5	0.89	Lys371-Arg373	Lys371	Right	0.66
			Asp372	Left	1.00
			Arg373	-	1.00
6	0.80	Arg334-Arg336	Arg334	Right	0.80
			Gly335	Left	0.89
			Arg336	-	

Fig. 1. DNA binding sites of buffalo PAG-1. (+) denotes binding residues and (-) denotes non - binding residues with confidence level from 0 to 9.

Fig. 2. RNA binding sites of buffalo PAG-1. (+) denotes binding residues and (-) denotes non - binding residues with confidence level from 0 to 9.

Table 2. Gap regions (clefts and cavities) in the buffalo PAG-1 protein surface. The gaps are ordered by decreasing volume (in $Å^3$) with other parameters is included as shown in Fig. 3

Gap region	Volume	Accessiblevertices		Buriedvertices		Averagedepth	
1	3785.48	65.11%	5	10.50%	5	14.17	1
2	2064.66	64.90%	6	11.98%	3	13.87	3
3	1803.52	80.26%	1	15.02%	1	14.02	2
4	1512.84	76.93%	3	13.89%	2	11.49	4
5	1307.81	59.44%	9	9.25%	7	10.76	5
6	748.41	52.47%	10	7.84%	8	8.71	7
7	701.58	76.94%	2	10.32%	6	9.84	6
8	649.27	59.67%	8	6.65%	10	7.83	9
9	418.08	62.22%	7	7.60%	9	7.30	10
10	849.23	70.12%	4	11.00%	4	8.12	8

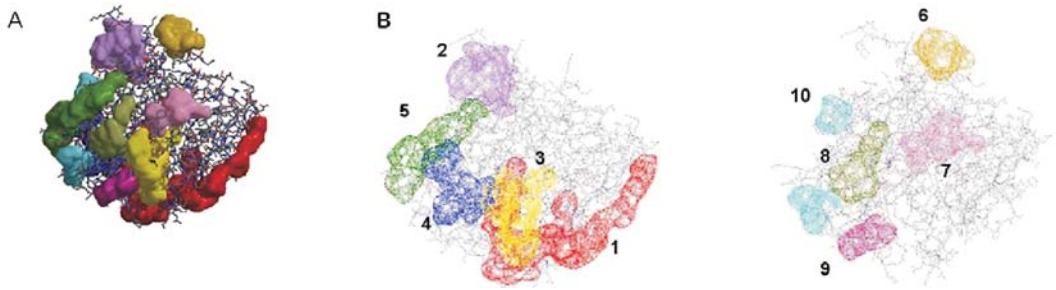

Fig. 3. A, Ligand binding sites (gaps and clefts) of buffalo PAG-1 protein structure; B, Location of various ligand binding sites (gaps and clefts) as in Table 2.

presence of functional active residues.

Buffalo PAG-1 protein interactions prediction: The protein-protein interaction of buffalo PAG-1 was deduced with STRING database. Using this database direct (physical) and indirect (functional) associations of known and predicted protein interactions was deciphered (Fig. 4). Buffalo PAG-1 retrieved bovine PAG-1 along with other cytokine sequences and the major predicted interacting partners of buffalo PAG-1 are phosphoprotein associated with glycosphingolipid microdomains 1, placenta growth factor precursor (PGF), alpha-fetoprotein precursor (AFP) and SP1 transcription factor (SP1). Interestingly, these factors are found to be active

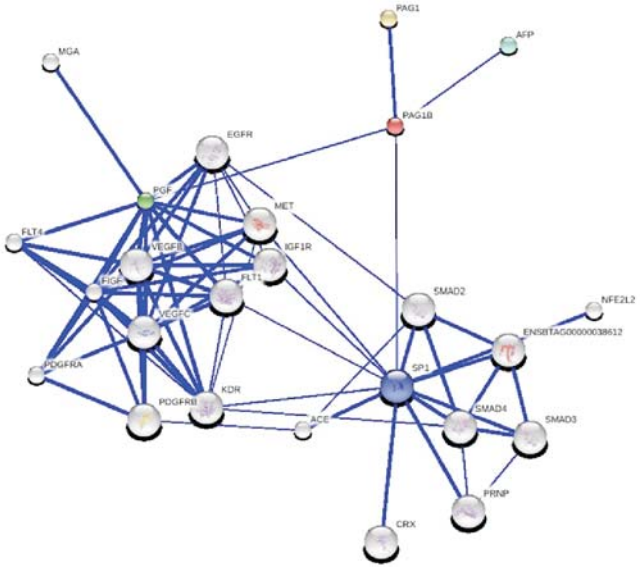

Fig. 4. Protein interacting partners of buffalo PAG-1. Thickness of the connecting line predicts the strength of interaction.

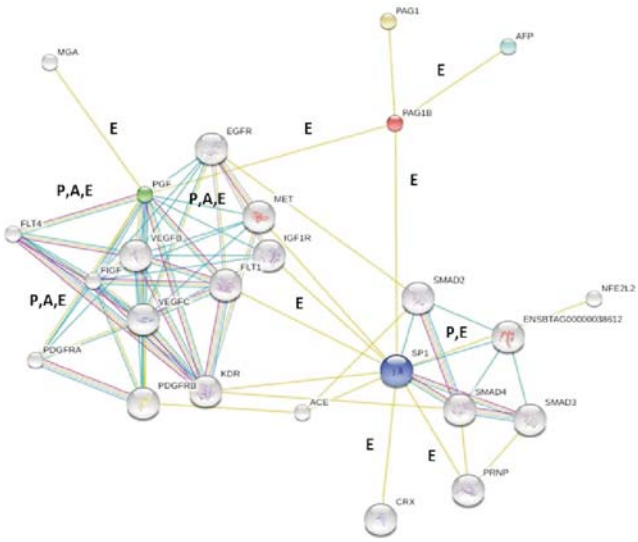

Fig. 5. Nature of protein interaction with buffalo PAG-1 (A, Activation; E, Expression; P, Post-Translational).

during the embryonic stage exerting the functions of angiogenesis, and endothelial cell growth, stimulating their proliferation and migration and differentiation. Moreover detection of alpha-1-fetoprotein as one of the major interacting partners justifies it was named protein A when compared to PAG as protein B in earlier reports. In addition alpha-1-fetoprotein binds copper, nickel, and fatty acid facilitating embryonic growth and survival.

Placenta growth factor precursor (PGF) interacts with a series of growth factors as shown in Fig. 4. Its predicted functional partners are vascular endothelial growth factor-receptor, kinase insert domain receptor (KDR), tyrosine kinase receptor, vascular endothelial growth factor B (VEGFB), vascular endothelial growth factor C (VEGFC), platelet-derived growth factor receptor A and B (PDGFRA/B), insulin-like growth factor 1 receptor Precursor (IGF1R), and epidermal growth factor receptor fragment (EGFR). These cytokines are involved in cellular proliferation, migration, differentiation, angiogenesis etc. SP1 interacts with cytokines *viz*. major prion protein precursor (PrP), mediator of signal transduction (SMAD4), transcriptional modulator activated by TGF-beta, nuclear factor erythroid 2-related factor 2 (NFE2L2) and globin transcription factor 1 (GATA1). These cytokines are mostly involved in mediators of transcription and signal transduction during cellular process.

The pregnancy associated glycoprotein 1 interacts with both PGF and SP1 but the interaction of PGF with other cytokines viz. VEGFB/C, EGFR, PDGFRA/B and IGF1R show a stronger interaction when compared to SP1 interaction with SMAD 2/3/4AC and ACE (Angiotensin converting enzyme). Stronger interaction between the cytokines and growth factors can be predicted due to their intrinsic nature of the molecule in cellular and biochemical functions.

Further analysis revealed buffalo PAG-1 triggered the expression of both PGF and SP1 mediated cytokines. In turn these cytokines exert various roles of activation, binding, catalysis, expression and post-translation of other cytokines as shown in Fig. 5. The importance and interaction of these cytokines are essential for various cellular function *viz*. cellular proliferation, division, migration, angiogenesis, inhibition or activation of other cytokines (Szilagyi *et al*. 2005, Hashizume 2007). The importance of the interaction study is to unravel the molecular mechanisms of biological systems as proteins perform their function in association with other molecules in biological processes. In conclusion, prediction of physical networks of interactions and interacting partners of buffalo PAG-1 was deduced which will pave way for modeling various cellular functional processes. The nature of these interacting partners reveals their putative role early during pregnancy. This is the first report of buffalo PAG-1 interaction with other cytokines to execute its biological and cellular functions (Hashizume 2007).

In the present study, binding and interaction properties of buffalo pregnancy associated glycoprotein-1 (PAG-1) was deduced using various bioinformatics tools. Analysis reveals buffalo PAG-1 possesses DNA, RNA and ligand binding sites located at conserved microsequences residues of buffalo PAG-1. *In silico* analysis with the STRING database revealed interacting partners buffalo PAG-1 *viz*. cytokines factors which exert their functions of angiogenesis, endothelial cell growth, proliferation, migration and differentiation during embryonic development.

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Abbreviations

ACE, Angiotensin converting enzyme; AFP, alpha-fetoprotein Precursor; DNA, deoxyribonucleic acid; EGFR, epidermal growth factor receptor; GATA1, globin transcription factor 1; IGF1R, insulin-like growth factor 1 receptor precursor; KDR, kinase insert domain receptor; NFE2L2, nuclear factor erythroid 2-related factor 2; PAG,1, pregnancy associated glycoprotein-1; PDGFR A/B, platelet derived growth factor receptor A / B; PGF, placenta growth factor precursor; PrP, prion protein precursor; RNA, ribonucleic acid; SMAD, mediator of signal transduction; SP1, SP1 transcription factor; TKR, tyrosine kinase receptor; VEGF B/C, vascular endothelial growth factor B/C; VEGFR, vascular endothelial growth factor-receptor.

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