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Electrophoretic Pattern of Protein Molecules in Gut Associated Lymphoid Tissue of Prenatal Goat

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ABSTRACT

Gut associated lymphoid tissue (GALT) extracts of prenatal goat were subjected to 12.5% SDS-PAGE. The study revealed that there was less variation among different age groups of prenatal goat in same GALT extract. Ileal and thymic extract of 99 and 112 days old prenatal goat fractioned into 12 protein whereas in 50 days old foetal goat one protein in ileal and two protein in thymic extract were missing. In splenic extracts of 112 days old foetus three protein bands were missing when compared to 50 and 99 days old goat foeti. The mesenteric lymph node in 99 and 112 days old foetus was studied and its molecular weight of proteins ranged from 161.5 Kd to 16 Kd.

Introduction

Several pre-existing protein molecules play pivotal role in cell differentiation, maturation and proliferation.

Such protein moieties also provide the basis to cytoskeleton and also contribute to cellular secretion.

Thus a detailed electrophoretic pattern of protein molecules in thymic, splenic, mesenteric lymph node and ileum extracts will provide clues for involvement of specific protein fractions during GALT development and maturation.

Materials and Methods

The samples were collected in aseptic conditions from local slaughterhouse in Bhubaneswar and transported to the laboratory maintaining cold chain.

GALT like Spleen, thymus, ileum, and mesenteric lymph nodes were isolated from goat foeti aging 50, 99 and 112 days of gestation and stored at –25°C till further use.

Tissue extraction

2g of each sample was taken and mixed with 2 ml of PBS, pH 7.2 containing 2 µl of 1 mM PMSF (phenyl methyl sulfonyl fluoride) solution and then homogenized properly under chilled condition. Samples were collected in Eppendorf tube and centrifuged in 16000 rpm for 10 minutes at 4°C. The supernatants were collected in cryovials in different aliquots and stored at –25°C till further processing.

SDS-PAGE of gut associated lymphatic tissue extracts of prenatal goat was carried out as per the method of Laemmli (1970) with vertical mini slab gel system (Atto Ltd, Japan).

Reagents

Solution-A:	Acrylamide	29.2 gm
	Bisacrylamide	0.8 gm
	Made upto 100 ml with distilled water	
Solution-B:	1.5 M tris-HCL buffer, pH 8.8, 0.4% SDS	
Solution-C:	1.5 M tris-HCL buffer, pH 6.8, 0.4% SDS	
Solution-D:	10% ammonium persulphate prepared fresh.	
Solution-E:	0.05 M tris 0.192 M glycine, 0.1% SDS, pH 8.3.	
Solution-G:	Loading buffer	

Glycerol -2 ml
2– mercaptoethenol -1 ml
10%SDS -4.5 ml
Upper buffer -1.7 ml
0.1% Bromophenol blue -0.2 ml
Distilled water - 0.6 ml
Mixed and stored at -20°C

Preparation of gel

Gel concentration	10%	12.5%	15%	Stacker gel 5%
Solution-A: (ml)	12	15	18	1.8
Solution-B: (ml)	9	9	9	--
Solution-C: (ml)	--	--	--	3
Solution-D: (ml)	0.14	0.14	0.14	0.036
TEMED (ml)	0.02	0.02	0.02	0.012
Distilled water (ml)	15	12	9	7.2

Casting of gel

12.5% gel solution as per the Table 1 was prepared and poured carefully into gel casting space between the glass plates until about 75% of the space volume was filled. Water saturated n-butanol was layered over the gel and after polymerization of the separating gel; n-butanol was drained off by tilting the gel cast assembly. The gel upper surface was then washed with distilled water to remove n-butanol, if any. 5% stacking gel solution was layered over the separating gel after washing the upper surface by the same gel solution.

Slot forming comb was carefully inserted into the top of the gel casting area until both ends of the comb were stopped at top of the side-spacer. Water saturated n-butanol was over layered. After polymerization of stacker gel, the comb was removed slowly and carefully and the wells were washed thoroughly with solution E.

Preparation of sample

The GALT crude extracts prepared earlier were mixed with solution-G in 1:1 proportion and the samples were boiled for 5 minutes in hot water bath and cooled down to be used for loading.

Electrophoretic run

Samples were applied to each slot so that amount of protein was about 40 µg in each case. Electrophoresis was performed at a constant voltage mode of 80 volts/slab for 20 minutes and was increased to 120 volts/slab subsequently till the tracking dye reached the lower end of the gel. Electrophoresis was carried out at room temperature.

Staining and destaining of gels

The gels after electrophoresis were stained with the staining solution i.e. 0.25% coomassie brilliant blue R-250 in 10% glacial acetic acid and 50% methanol solution for 4 hours. The gels were then destained with several changes of 10% acetic acid and 40% methanol in distilled water. After through destaining the gels were stored in 7% acetic acid till photographed.

Determination of molecular weight by SDS-PAGE

Protein markers like Myosin-rabbit muscle, Phosphorylase–b Bovine serum albumin, Ovalbumin, Carbonic anhydrase, Soyabin trypsin inhibitors, Lysozyme, Aprotinin and Insulin having molecular weight 205 kd, 94.7 kd,66 kd, 43 kd, 29 kd, 20.1 kd, 14.3 kd, 6.5 kd, and 3 kd respectively were used. The mobilities of all the proteins and peptides were recorded as:

$$\text{Relative mobility} = \frac{\text{Length of gel before staining}}{\text{Distance of dye migration before staining}} \times \frac{\text{Distance of protein migration after destaining}}{\text{Length of gel after destaining}}$$

The standard curve was plotted by relative mobilities of the standard marker proteins against their corresponding log molecular weights from which the molecular weight of unknown proteins were calculated by plotting their corresponding relative mobilities in the graph.

Determination of molecular weight of GALT extracted proteins by SDS –PAGE

The molecular weights of different GALT extracted proteins of prenatal goats of various age groups were estimated by using molecular weight marker (Genei cat No.-PMW-H), by plotting the log molecular weights of the standard proteins against the corresponding Rm values (Fig. 2) and comparing the Rm value of unknown protein bands with the graph for the corresponding molecular weights. The molecular weights of unknown protein thus found out are illustrated in Table 1.

Results and Discussion

GALT extracts of prenatal goats were subjected to 12.5% SDS-PAGE to study the relative distribution of different proteins in various GALT of different age groups (Fig.

1). From the electrophoretic pattern it was clear that the ileum extracts of 99 and 112 days old goat foetus were resolved into 12 protein bands where as that of 50 days old ileum extract was fractioned into 11 bands. The molecular weight of ileum extracted protein of prenatal goats irrespective of age group ranged from 161.5 kd to 16 kd but in 50 days old goat foeti ileum there was missing of one protein having molecular weight of 64.5 kd. Wang and Hasnain (2017) reported that the Murine intestinal mucins are large heavily glycosylated proteins and typically have a molecular mass higher than 1000 kd. In case of spleen extracted protein the molecular weight also ranged from 161.5 kd to 16 kd, but three protein bands with molecular weights 131, 81 and 72.5 kd were not present in 112 days old prenatal goat spleen extract. Cunningham and Tang (1976) revealed that the molecular weight of cathepsin D in porcine spleen ranged from 34 to 35 kd**.** Donella-Deana *et al.,* (1996) isolated 57-kDa protein substrate of the tyrosine kinase Lyn from rat spleen.

Table.1 Molecular weight (Kd) of GALT extract in prenatal goat by SDS-PAGE (with age)

Ileum			**Spleen**			**Thymus**			**Mesenteric Lymph node**		**Protein Marker**
50 Days (11)	**99 Days (10)**	**112 Days (9)**	**50 Days (8)**	**99 Days (7)**	**112 Days (6)**	**50 Days (5)**	**99 Days (4)**	**112 Days (3)**	**99 Days (2)**	**112 Days (1)**	**Mol. Wt. (Kd)**
161.5	161.5	161.5	161.5	161.5	161.5	161.5	161.5	161.5	161.5	161.5	**205**
-	-	-	131	131	-	-	131	131	-	-	**97.4**
-	-	-	-	-	-	-	122	122	-	-	**66**
104	104	104	-	-	-	-	-	-			**43**
89	89	89	-	-	-	-	-	-	-	-	**29**
-	-	-	81	81	-	-	-	-	-	-	**20.1**
72.5	72.5	72.5	72.5	72.5	-	72.5	72.5	72.5	72.5	72.5	**14.3**
-	64.5	64.5	-	-	-	-	-	-	-	-	
-	-	-	-	-	-	61.5	61.5	61.5	61.5	61.5	
-	-	-	60	60	60	60	60	60	-	-	
55	55	55	-	-	-	-	-	-	**-**	**-**	
-	-	-	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.5	
47	47	47	-	-	-	47	47	47			
-	-	-	43.5	43.5	43.5	-	-	-	-	44	
-	-	-	-	-	-	-	-	-	40	-	
35	35	35	35	35	35	35	35	35	35	35	
31.5	31.5	31.5	-	-	-	-	-	-	-	-	
28	28	28	28	28	28	28	28	28	28	28	
24	24	24	24	24	24	24	24	24	24	24	
16	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	

* The number within parentheses indicates the sample type and corresponds to the number indicated in SDS PAGE (Fig. 1)

Fig.1 SDS - PAGE (12.5%) of GALT extract of prenatal goat

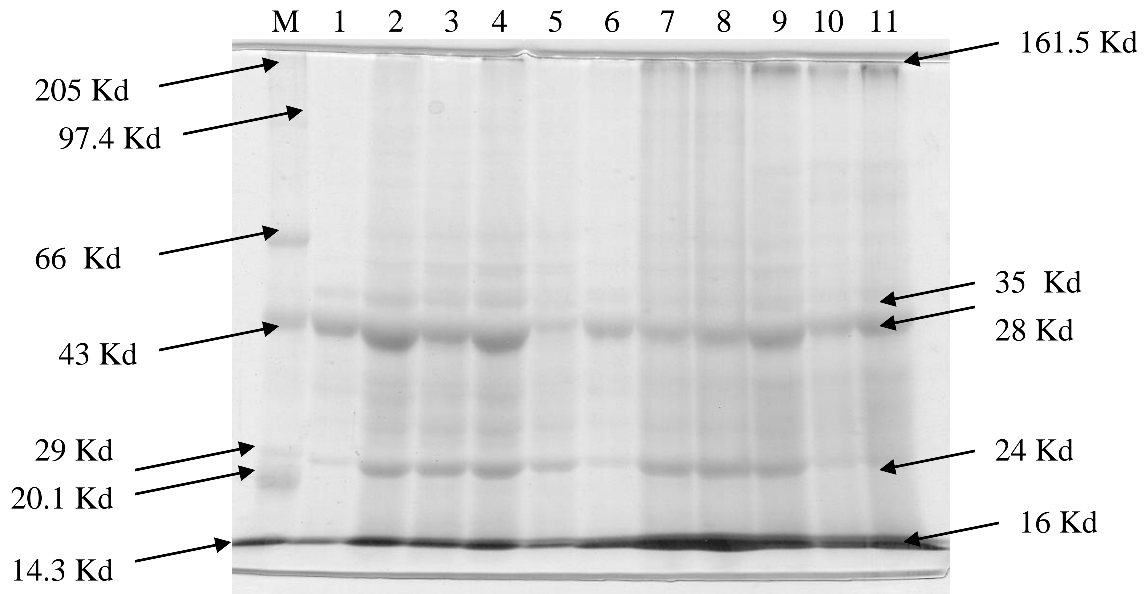
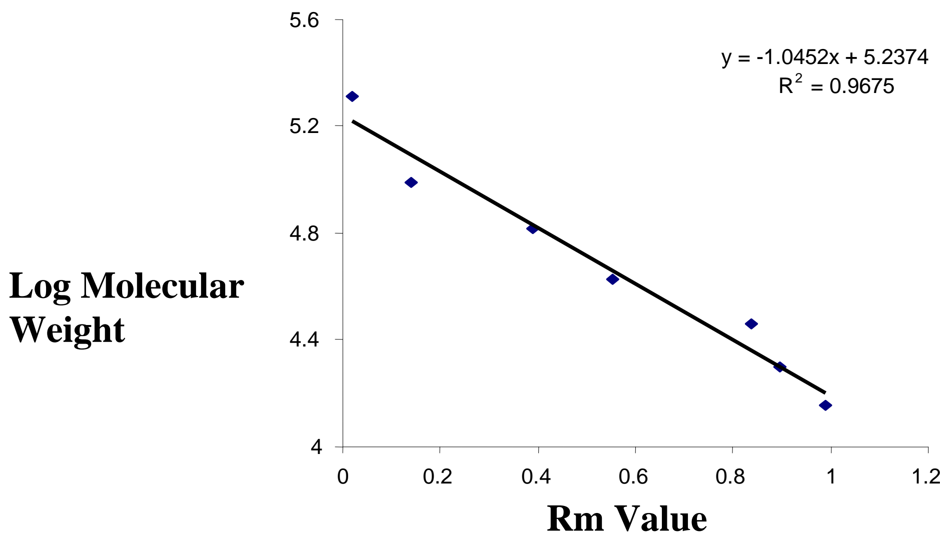


Fig.2 Calibration curve for Molecular weight estimation by SDS – PAGE



In thymus extract the molecular weight also ranged from 161.5 kd to 16 kd, but in 50 days old prenatal goat foetal thymus there was absence of two proteins having molecular weight of 131 and 122 kd. Rong and Carl (1990) observed that molecular weight and subunit composition of calf thymus ribonuclease H1 enzyme either was single polypeptide of 74 kd or consisted two to four subunit in the range of 21-34kd. In mesenteric lymph node extract the molecular weight ranged from 161.5 to 16 kd. In both the age groups studied but there is only variation in one protein. The presence of many protein bands in lower molecular weight range might be due to proteolysis of the proteins after extraction. The low molecular weight range proteins could be well resolved by using

gradient gel of higher concentration. In the present study the proteins having molecular weight 16 kd could only resolved. The proteins/peptides having lower molecular weight were also present which might be responsible for the innate immunity in the mucosal layers of these tissues.

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