

MULTIPLE FORMS OF PIG, SHEEP AND GOAT α -LACTALBUMIN*

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SUMMARY

α -Lactalbumins isolated from pig, goat, and sheep milk have two electrophoretically distinct forms as determined by disc gel electrophoresis. Both forms of each species are active in the lactose synthetase reaction. The protein forms are charge isomers. Results from peptide mapping and amino acid analyses suggest a slight variation in amino acid composition.

INTRODUCTION

Lactose synthetase (UDP galactose:D-glucose 1-galactosyltransferase, EC 2.4.1.22) catalyzes the formation of lactose and this reaction requires a galactosyltransferase and α -lactalbumin for significant rates^{1,2}. α -Lactalbumin acts as a protein modifier of the galactosyltransferase and lowers the K_m for glucose so that it becomes an efficient substrate³⁻⁶.

α -Lactalbumin has been isolated from various sources^{7,8} and all those isolated to date are active in the lactose synthetase reaction⁹. The purpose of this paper is to report on the isolation and characterization of multiple forms of α -lactalbumin isolated from pig, goat, and sheep milk.

EXPERIMENTAL PROCEDURES

Materials

α -Lactalbumin was isolated from skim milk of the goat, pig, sheep and other species as previously described⁷. The galactosyltransferase was isolated from bovine skim milk and represented the HA₁₁ step of purification³. Other reagents were the same as previously described⁷.

Methods

The assay for α -lactalbumin¹⁰ and the procedure for peptide mapping⁷ were described previously. Disc gel electrophoresis was done according to the instructions

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with the Canalco Model 6 instrument. Separating gels were 7% in acrylamide in Tris-glycine, pH 8.9. All gels were pre-electrophoresed for 30 min at 5 mA per gel and protein separations were at 5 mA per gel at a running pH of 9.5.

Preparative disc gels (1 cm × 10 cm) containing 7% gel, pH 9.5 with 8 ml of separating gel were used for separating larger amounts of the proteins. The gels were run at room temperature and pre-electrophoresed for 1 h at 16 mA per gel. The proteins (10 mg/ml) were dissolved in the electrophoresis buffer containing 10% sucrose. About 0.6–0.7 ml of protein was placed on the gels. Bromophenol blue (20 μ l of 0.005% in 10% sucrose) was used as a tracking dye. After electrophoresis for 1 h they were placed in a solution of 0.025% Coomassie Blue in 7% acetic acid until the protein bands were barely detectable (5–10 min). The lightly stained bands were sliced from the gels and the mashed pieces were washed 3 times with 0.1 M NH_4OH at 37 °C with shaking. The NH_4OH was removed by lyophilization and the samples were dissolved in 10 ml of water, dialyzed 2 days against water and lyophilized.

Amino acid analysis was done on a Beckman Model 120C amino acid analyzer. About 1 mg of each protein band was hydrolyzed for 24 and 48 h as previously described⁷. The samples were made 4% in thioglycolic acid prior to the addition of the HCl to prevent tryptophan destruction in α -lactalbumin^{11,12}. Norleucine was used as an internal standard. After acid hydrolysis, the HCl and thioglycolic acid were removed in a rotary shaker and the sample was stored in an evacuated desiccator for 24 h to remove the remaining thioglycolic acid prior to dissolving in 1.3 ml of the pH 3.453 sample buffer.

RESULTS

The various α -lactalbumins isolated⁷ gave a single sharp band when subjected to starch gel electrophoresis at pH 3.3 and 8.6. However, two bands were observed when pig, goat and sheep α -lactalbumin were subjected to disc gel electrophoresis in Tris-glycine, pH 9.5 in 7% acrylamide gels (Fig. 1). The α -lactalbumin from the human (American Indian, Negro and Caucasian), guinea pig, bovine and buffalo appeared as a single protein band. Disc gel electrophoresis at pH 4.3 (Canalco) and 7.3 in 30 mM sodium phosphate gave a single band for all the α -lactalbumins tested. Two bands were observed also for the pig, goat and sheep α -lactalbumin when the Tris-glycine buffer, pH 9.5 was replaced by 50 mM borate, pH 9.5. The presence of 6 M urea in the gels did not alter any of the patterns observed at pH 4.3 and 9.5. Disc gel electrophoresis in the presence of 0.1% sodium dodecylsulfate gave rise to one major band for the pig, sheep and goat as well as the other α -lactalbumins. This result suggested that the multiple forms observed were not weight isomers. The method of Hendrick and Smith¹³ was used to determine if the multiple forms observed with the pig, goat and sheep α -lactalbumin were charge or weight isomers. The data presented in Fig. 2 for pig α -lactalbumin gave two parallel lines indicating that the two proteins are charge isomers. Similar parallel line patterns were observed for the two protein isomers of goat and sheep α -lactalbumin but only a single line was observed for the bovine and human α -lactalbumins. At the higher but not lower percent gels several very faint protein bands were seen and when plotted as in Fig. 2 the lines extrapolated to a common point near zero percent gel which indicates that these slight impurities may be weight isomers.

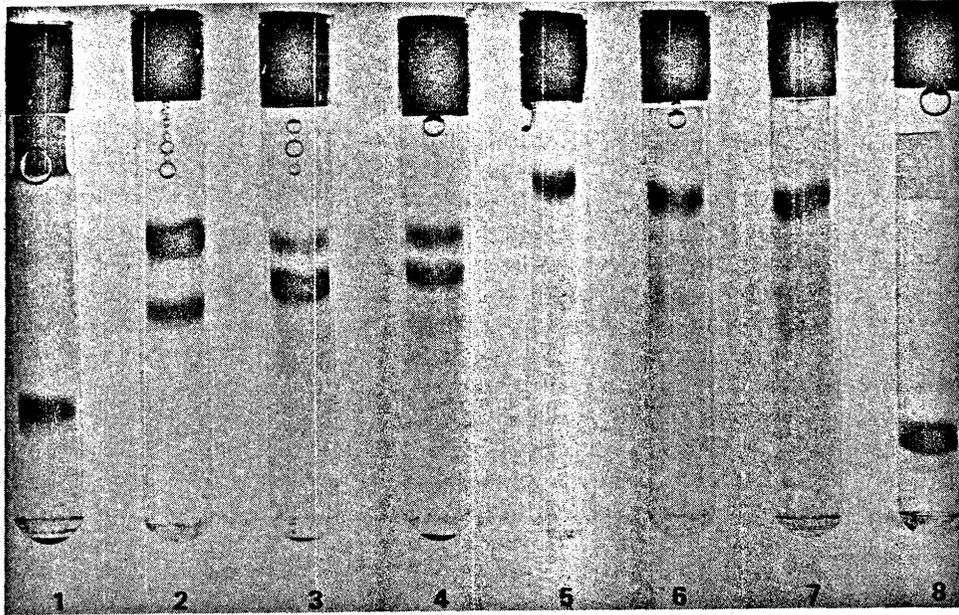


Fig. 1. Characteristic patterns of various α -lactalbumins on disc gel electrophoresis in Tris-glycine, pH 9.5. The α -lactalbumins were: (1) guinea pig, 40 μ g; (2) pig, 60 μ g; (3) goat, 40 μ g; (4) sheep, 45 μ g; (5) Caucasian, 30 μ g; (6) Indian, 30 μ g; (7) Negro, 30 μ g; (8) buffalo, 35 μ g.

The protein bands from the pig, goat and sheep α -lactalbumin were extracted from the disc gels (*Methods*) and assayed for α -lactalbumin activity in the lactose synthetase reaction. The data presented in Fig. 3 for the pig α -lactalbumin shows that both protein bands had activity in the lactose synthetase reaction and that the activity was approximately proportional to the protein concentration. The major amount of activity was in the slower moving band whereas in the goat the major amount of activity and protein was in the faster moving band. In the sheep (Fig. 4) there is an indication of the possibility of a small amount of a third faster moving band as discerned by activity measurements. None of the α -lactalbumins isolated

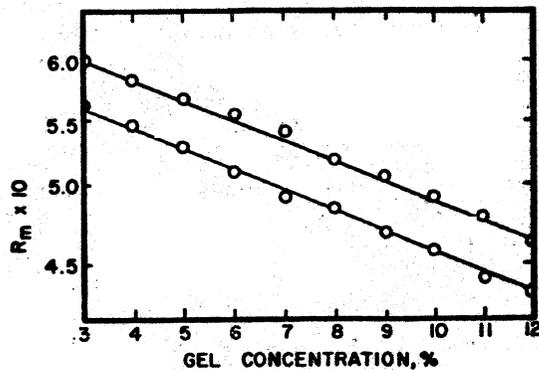


Fig. 2. Relative migration of pig α -lactalbumin on disc gel electrophoresis as a function of the percent gel.

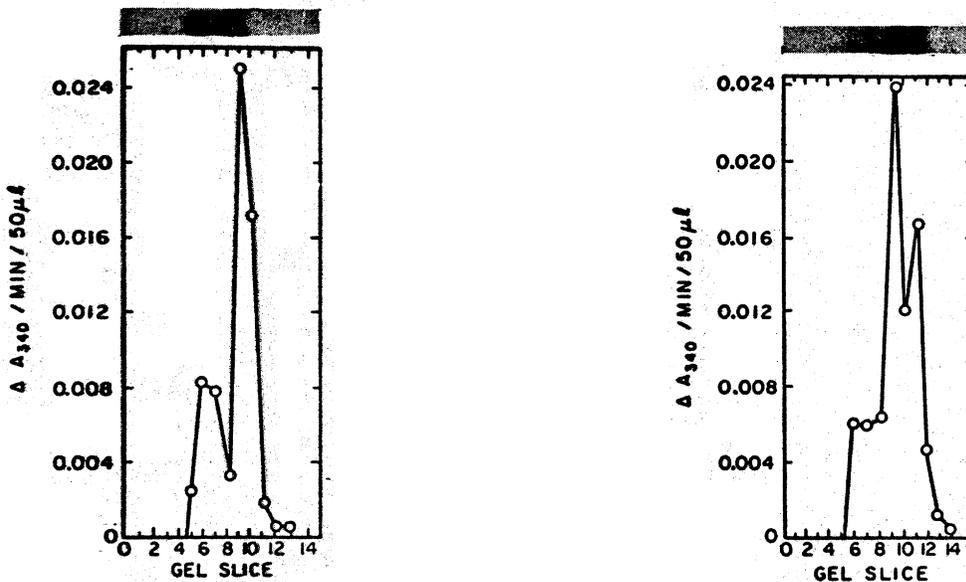


Fig. 3. Lactose synthetase activity of pig α -lactalbumin extracted from disc gels. Pig α -lactalbumin (75 μg) was separated on 7% disc gels at pH 9.5 and the gel was sliced in 0.3-cm sections. Each section was mascerated with 0.2 ml of 20 mM glycine, 0.1 M KCl (pH 8.5) and an aliquot was assayed for lactose synthetase activity which is expressed as the change in $A_{340 \text{ nm}}$ /min per 50 μl of sample. At the top of the figure is a duplicate gel stained with amino blue black.

Fig. 4. Lactose synthetase activity of sheep α -lactalbumin (60 μg) extracted from disc gels. The procedure is described in Fig. 3.

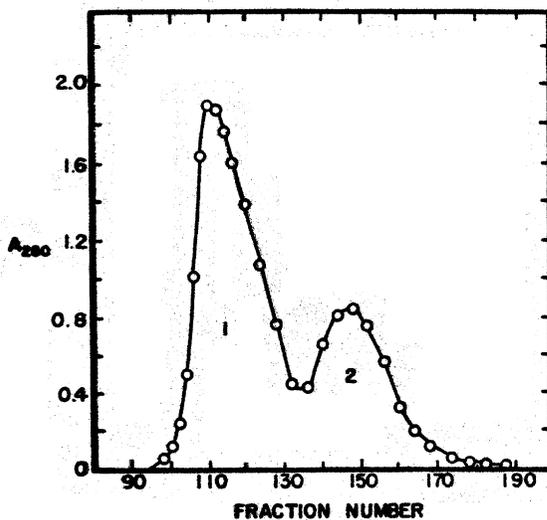


Fig. 5. Separation of pig α -lactalbumin on a DE-32 cellulose column. The DE-32 column (2 ml of DE-32 in a 5-ml disposable pipette) was equilibrated with 5 mM glycine, pH 9.5 and washed with 40 mM of the same buffer. Pig α -lactalbumin (25 mg) was dissolved in the same buffer and eluted with a linear gradient, 5 mM (80 ml) to 0.8 M (80 ml) glycine, pH 9.5. 0.7-ml fractions were collected and the $A_{290 \text{ nm}}$ was measured.

from the sheep, goat, pig, human or bovine stained as a glycoprotein on the disc gels¹⁴.

An effort was made to separate the two protein bands of pig α -lactalbumin by chromatography on DE-32 cellulose and two protein peaks were obtained as shown in Fig. 5. The results from disc gel electrophoresis experiments showed that the peak tube in the first peak eluted off the column contained mainly the slower moving protein (Fig. 1) whereas the peak tube from the second peak contained mainly the faster moving protein and only a trace of the slower component. Re-chromatography of the first peak resulted in an essentially single protein (slower moving band) band. The two protein bands of sheep and goat α -lactalbumin were not separated on DE-32 under the conditions described in Fig. 5 and no satisfactory conditions were found for their separation by column chromatography.

The separate protein bands of pig α -lactalbumin gave single and identical precipitin bands in a double diffusion experiment using antisera prepared against pig α -lactalbumin (both bands). The pig antisera did not react with human or ruminant α -lactalbumins.

The two protein bands from the goat and sheep α -lactalbumin were extracted from the polyacrylamide gels as described in *Methods*.

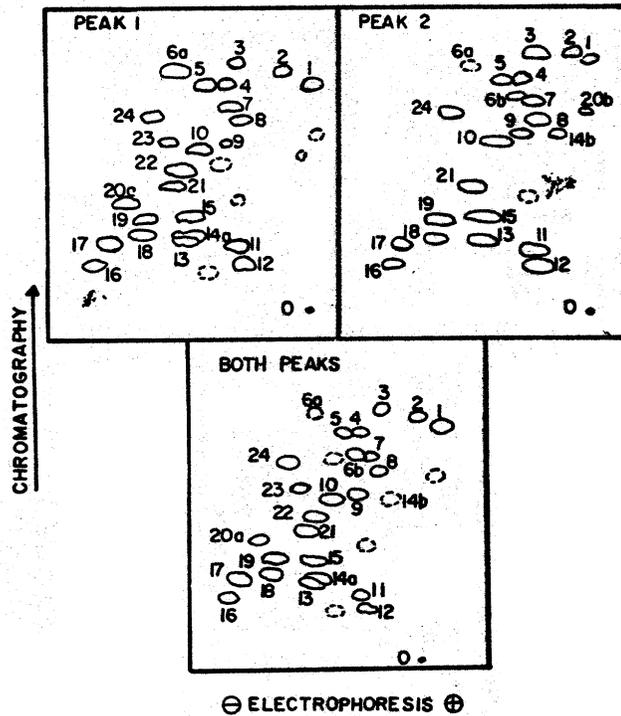


Fig. 6. Peptide maps of tryptic digests of pig α -lactalbumin. The peptides were separated in the first direction by thin-layer electrophoresis (pH 5.5, 2 h 300 V, 4 °C) and in the second direction by ascending chromatography in *n*-butanol-pyridine-glacial acetic acid-water (150:100:30:120, by vol.) for 10.5 h at 25 °C. Peak 1 and Peak 2 refer to the two forms separated by chromatography (Fig. 5) and O is the origin.

Peptide maps of pig α -lactalbumin (both peaks) and Peak 1 (slower moving, Fig. 3) and Peak 2 (faster moving, Fig. 3) from the DE-32 cellulose were prepared (*Methods*) and the results are presented in Fig. 6. Peptides 14a, 20a, 22 and 23 are present in Peak 1 but are not present in Peak 2 and Peptides 6b and 14b are only present in Peak 2. These results suggest that there are differences in the peptide maps which are apparently due to amino acid differences present in the two forms of pig α -lactalbumin.

Peptide maps were made of the slow and fast moving forms of goat α -lactalbumin isolated from the disc gels and only a few minor differences were noted. Peptide maps obtained from the two forms of sheep α -lactalbumin were unsatisfactory in that they streaked in the direction of electrophoresis.

The two protein bands from the sheep, pig and goat α -lactalbumin were separated by preparative disc gel electrophoresis as described in *Methods* and approxi-

TABLE I

COMPARISON OF THE AMINO ACID COMPOSITION OF THE TWO FORMS OF GOAT, SHEEP AND PIG α -LACTALBUMIN

Average of 24 and 48 h hydrolysis for both the slow and fast forms (Fig. 1). Calculations for the comparisons were made by arbitrarily setting leucine at 11 residues.

Amino acid	Goat α -lactalbumin			Sheep α -lactalbumin			Pig α -lactalbumin		
	Both*	Slow	Fast	Both*	Slow	Fast	Both*	Slow	Fast
Lys	11	11.7	12.0	10	10.0	10.0	9	10.8	10.8
His	2	2.5	3.0	2	2.3	2.2	2	2.7	2.6
Arg	1	1.5	1.8	1	1.0	1.1	1	1.5	2.0
Asp	19	22.2	21.9	18	23.5	23.8	18	18.4	16.5
Thr	5	5.2	5.3	5	4.7	5.3	6	6.1	6.5
Ser	4	6.5	6.5	4	4.8	3.8	6	4.4	11.2
Glu	12	12.3	12.9	11	13.3	11.8	12.5	18.5	15.4
Pro	3	6.1	6.0	2	4.0	5.2	3	5.6	7.3
Gly	4	6.4	9.9	4	6.0	6.0	6	7.6	8.3
Ala	5	4.9	5.3	5	5.6	5.4	3	3.0	3.0
Val	5	4.6	4.6	4	5.2	4.1	2	1.9	2.0
Met	Trace	Trace	Trace	Trace	Trace	Trace	3	2.4	2.3
Ile	7	5.9	4.0	6	6.0	7.2	9	9.4	9.4
Leu	12	11.0	11.0	11	11.0	11.0	10	11.0	11.0
Tyr	3	3.2	3.8	3	4.4	3.4	3	3.7	3.6
Phe	3	3.3	3.5	3	3.3	4.3	3	3.7	3.6
Trp	5	1.9	Trace	4	2.7	Trace	6	3.1	3.0

* From previous results.

mately 2 mg of each band were isolated, hydrolyzed and analyzed for amino acids and these results are presented in Table I. The differences in the amino acid composition of the slow and the fast bands of goat α -lactalbumin were for the most part minor except for glycine, isoleucine and tryptophan but it would appear that tryptophan recovery was poor. In the sheep α -lactalbumin, only minor differences were noted. In the pig, there were apparent differences in aspartate, serine and glutamic acid.

Two forms of pig, sheep and goat α -lactalbumin were observed upon disc gel electrophoresis at pH 9.5 in Tris-glycine or borate buffers but only a single band was observed at pH 4.3 and 7.3. The results of disc gel electrophoresis experiments in sodium dodecylsulfate and varying the percentage gels showed that the forms are charge isomers. Each of the two forms of pig, sheep and goat α -lactalbumin was active in the lactose synthetase reaction when assayed with bovine galactosyltransferase. The results of peptide mapping experiments and amino acid analyses are consistent with the view that they differ in amino acid composition though the exact nature has not been elucidated especially in the goat and sheep where it was difficult to obtain sufficient material for more detailed studies.

Genetic variants of bovine α -lactalbumin have been observed^{15,16} by electrophoretic separations and the evidence indicates that they vary in a few amino acid residues. It is possible that the two forms observed in the pig, goat and sheep α -lactalbumin represent genetic variants but further studies are necessary since the milks were obtained from single animals.

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