SOME PHYSICAL AND CHEMICAL PROPERTIES OF GOAT β-LACTOGLOBULIN [1]

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Goat β-lactoglobulin has been crystallized from ammonium sulfate by Askonas (1) and from isoelectric salt-free solution by Sen and Chaudhuri (2). Its molecular weight is 37,000 and isoelectric point pH 5.9 as compared to pH 5.2 for cow β-lactoglobulin under identical conditions. The present paper compares goat β-lactoglobulin with the three genetic polymorphs of cow β-lactoglobulin (A, B, and C) with respect to pH dependence of aggregation, amino acid composition and end groups.

One lot of goat β-lactoglobulin was prepared as hexagonal bipyramidal crystals from mixed herd milk by the method of Askonas (1). Another lot prepared by the method of Sen and Chaudhuri (2,3) yielded an oil which failed to crystallize even with the addition of seed crystals obtained from Dr. Sen. However, both preparations exhibited a single sharp band of considerably lower mobility than the cow β-lactoglobulins in polyacrylamide gel electrophoresis at pH 8.6. No genetic variants of goat β-lactoglobulin were detected by this procedure.

Sedimentation coefficients were determined at several pH's with a Spinco Model E Ultracentrifuge under identical conditions of speed, concentration, solvent, and pH as had been used for cow β-lactoglobulins (4,5,6). Runs at

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[1] Scientific Paper No. 5740, Minnesota Agricultural Experiment Station. Data presented herein are taken from a thesis presented by Nancy Phillips in partial fulfillment of the requirements for the degree of Master of Science, University of Minnesota, March, 1965.

pH 7.0 and 3.0 were made near 20° C., that at pH 4.6 at 5° C. The results summarized in Table I show that goat β-lactoglobulin, like cow β-lacto-

TABLE I

Sedimentation Constants of β-Lactoglobulins at Various pH's

	$S_{20,w}$ at 1% Protein Concentration			
	Cow β-lactoglobulin			
pH	A(5,6)	B(5,6)	C(4)	Goat β-lactoglobulin
7.0	2.8	2.8	2.8	2.7
4.6	4.9	2.9	2.9	2.7
3.0	2.3	2.3	2.4	2.1

globulins B and C, but unlike cow β-lactoglobulin A, does not aggregate at pH 4.6 (5° C.). At pH 3.0 the sedimentation coefficient of goat β-lactoglobulin is 2.1 as compared to 2.7 for the molecule at higher pH. This suggests either an expansion of the goat β-lactoglobulin molecule at pH 3.0 or, as is true for cow β-lactoglobulins A, B, and C, a reversible dissociation of the dimeric molecule into monomeric subunits of molecular weight about 18,000.

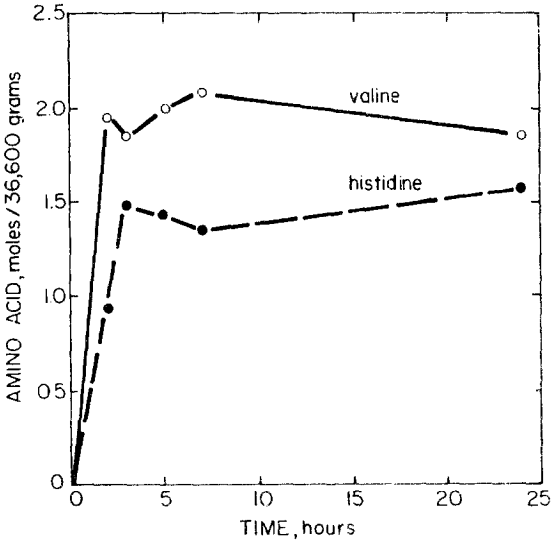

FIGURE 1. Action of Carboxypeptidase A on Goat Beta-lactoglobulin. Moles of Amino Acids Released per 36,000 Grams.

No carbohydrate was found in goat β-lactoglobulin by the Molisch test. Amino acid analysis was performed using a Beckman Spinco, Model 120, Amino Acid Analyzer. Separate aliquots were hydrolyzed at 110° C. for 12, 22, and 66 hours in nitrogen-filled, sealed tubes. The results for the three times of hydrolysis were averaged, except that the values for serine, threonine, and tyrosine were extrapolated to zero time, the 66 hour value was used for isoleucine, and the 12 hour values were used for glutamic and aspartic acids. Complete analyses were performed separately on both preparations and the results averaged. Nitrogen recoveries were 99.6 and 98.2% respectively for the two preparations. Tryptophan was determined on a separate sample by the method of Spies and Chambers (7), and by absorbance at 280 and 294.4 mμ according to Beaven and Holiday (8). Half-cystine was determined as cysteic acid (9), and as total sulfur minus methionine. One mole of cysteine per half molecular weight of goat β-lactoglobulin was found in this laboratory by titration with mercury and N-ethyl maleimide (10). Amide nitrogen was determined on a separate sample by a modification of the procedure of Hirs, Stein and Moore (11).

The results are shown in Table II. Goat β-lactoglobulin contains one more glycine, one more valine, two less leucine, and one less isoleucine residue per chain of MW 18,300 than cow β-lactoglobulin B. The amino acid differences between goat and cow β-lactoglobulins do not, in themselves, explain the higher isoelectric point and lower electrophoretic mobility of goat β-lactoglobulin at pH 8.6. Such differences may be due to conformational differences between the two proteins.

The C-terminal sequence was obtained by the action of carboxypeptidase A (Worthington Biochemicals) on goat β-lactoglobulin (15). Valine appears as the C-terminal amino acid and histidine the penultimate amino acid (Figure 1). Two moles of valine were released per 36,000 grams of goat β-lactoglobulin, suggesting that the molecule is composed of 2 chains, as are the cow β-lactoglobulin variants. Only trace amounts of other amino

TABLE II

	Amino Acid Residues Per 18,300 Grams			
	Cow β-Lactoglobulin			
Residue	A(12,13,14)	B(12,13,14)	C(14)	Goat β-lactoglobulin
Glycine	3	4	4	5
Alanine	14	15	15	15
Valine	10	9	9	10
Aspartic Acid	16	15	15	15
Glutamic Acid	25	25	24	25
Histidine	2	2	3	2
Isoleucine	10	10	10	9
Leucine	22	22	22	20
Tyrosine	4	4	4	4
Lysine	15	15	15	15
Serine	7	7	7	7
Proline	8	8	8	8
Methionine	4	4	4	4
Phenylalanine	4	4	4	4
Arginine	3	3	3	3
Threonine	8	8	8	8
Tryptophan	2	2	2	2
Half Cystine*	5	5	3***	5
Cysteine	1	1		1
NH_3**	14	14		14
Total Amino Acids	162	162		161

* Includes cysteine
** Values obtained by the authors
*** Uncorrected for destruction during hydrolysis

acids were present. The C-terminal and penultimate amino acids of cow β-lactoglobulins A and B are isoleucine and histidine respectively (15).

The N-terminal amino acid of goat β-lactoglobulin was obtained as its 2,4-dinitrophenyl (DNP) derivative (16). Chromatography according to Blackburn and Lowther (17) showed only one ether soluble DNP-amino acid derivative present, which was identified as either DNP-leucine or DNP-isoleucine. Upon regeneration of the amino acid from its DNP derivative (18), followed by descending chromatography of the amino acid (19), isoleucine was shown to be the N-terminal amino acid of goat β-lactoglobulin. Leucine has been reported as the N-terminal amino acid of all variants of cow β-lactoglobulin (14).

The C-terminal isoleucine/valine difference between cow and goat β-lactoglobulins may be rationalized as a ApUpU/GpUpU change in the m-RNA sequence which codes for these amino acids (20). Similarly, the N-terminal difference between the 2 proteins, leucine/isoleucine, if it does not represent a shortening of the protein chain, may be the result of a CpUpU/ApUpU change in the nucleotide sequence coding for the N-terminal amino acid.

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