CHEMICAL PROPERTIES OF β-LACTOGLOBULINS A, B AND C

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Bell (1962) reported the discovery of a third genetically determined β-lactoglobulin, β-C, of cow's milk. This variant was shown to have a slightly lower mobility than β-lactoglobulin B in starch-gel electrophoresis at pH 8.6. Aschaffenburg and Drewry (1955, 1957a) had previously demonstrated the existence of β-lactoglobulins A and B. These latter proteins were shown by Gordon et al. (1960, 1961) and Piez et al. (1961) to differ in amino acid composition. β-Lactoglobulin A has two more aspartic acid and two more valine residues than β-B, whereas β-lactoglobulin B has two more glycine and two more alanine residues than β-A. We would like to report on some chemical differences present in β-lactoglobulin C.

β-Lactoglobulins A, B and C were prepared from the milk of single, typed cows by the method of Aschaffenburg and Drewry (1957b). We have found that in order to obtain maximum yields of β-C, the "pH 2.0 lactalbumin" precipitate must be carefully worked up, since most of the protein is precipitated at this step, in contrast to the A and B proteins. Each protein was recrystallized four times and lyophilized. The purity of each preparation was verified by polyacrylamide-gel electrophoresis at pH 8.6 where a single band was obtained for each protein and by DEAE-column

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chromatography (Piez et al. 1961) where a single peak was found for each protein. The material was also rechromatographed and found to have the same elution characteristics as the original.

The amino acid composition of the three proteins was determined by hydrolyzing samples in triplicate for 24, 72 and 96 hours in 6 N HCl in sealed evacuated tubes held at 110° C. The analyses were carried out using the automatic recording apparatus described by Piez and Morris (1960). Table 1 gives the results of these analyses. Only the grand averages in terms of residues per molecule are reported. The serine and threonine values were obtained by extrapolation to zero time using the method of least squares. The sulfur value was obtained gravimetrically and the tryptophan by ultraviolet spectrophotometry using molecular weights between 35,500 and 37,700 and specific extinction coefficients between 9.3 and 9.7. It is evident that β-lactoglobulin C is a "mutant" of β-lactoglobulin B, having two residues of histidine more and two residues of glutamic (or glutamine) less than the β-B variant. It appears to be similar in all other respects. The analyses of β-A and β-B confirm the previous work of Piez et al. (1961) and Gordon et al. (1961) on samples prepared from pooled milks.

Photooxidation by the method of Weil et al. (1951) of each of the proteins is shown in Fig. 1. The increased rate of O_2 uptake for the C protein and the greater extent of photooxidation is presumably due to its greater histidine content. The similarity of β-A and β-B is attributable to equal content of photooxidizable amino acids (met, his, tyr and try).

The three proteins have identical ultraviolet spectra. This confirms the equal content of tyrosine, cystine and tryptophan.

The proteins also have identical carboxyl-terminal sequences, -leu-his-ileu, as revealed by the action of carboxypeptidase on the native and S-sulfo proteins. The amino terminal sequence for all three proteins was shown to be leu-thr- by the Edman degradation as applied for paper strips by Fraenkel-Conrat et al. (1955) and Schroeder et al. (1961). That

leucine is N-terminal was verified by the Sanger procedure as described by Fraenkel-Conrat et al. (1955).

Table 1

Amino Acid Composition of β-Lactoglobulins

Residues of Amino Acid Per Mole (Mol Wt ~ 36,000)

	A	B	C
ASP	31.7	30.4	30.0
THR*	16.2	15.9	15.6
SER*	13.6	13.5	13.6
GLU+	49.1	50.6	48.2
PRO	17.5	17.2	17.4
GLY	6.2	8.2	8.2
ALA	27.6	29.8	29.8
1/2 CYS°	6.8	6.7	6.8
VAL+	20.0	18.2	18.2
MET	8.0	8.0	8.0
ILEU+	19.5	19.6	19.7
LEU	42.4	43.0	43.1
TYR	8.0	8.0	8.0
PHE	8.0	8.0	8.1
LYS	29.9	29.6	29.5
HIS	4.0	4.0	6.0
ARG	6.2	6.1	6.1
TRY	3.9-4.6	3.9-4.6	3.9-4.5
% S	1.50	1.46	1.40

*Extrapolation to zero time by method of least squares.

+Based on 72 and 96 hour hydrolysates only.

°Cys values uncorrected for destruction during hydrolysis.

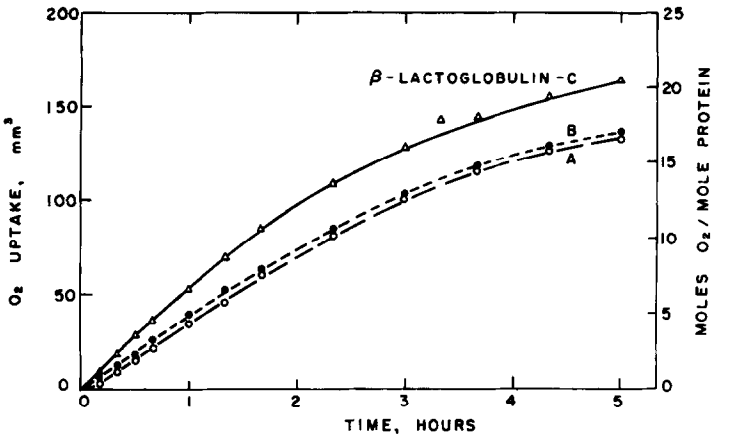

Fig. 1 Photooxidation of β-lactoglobulins A, B and C. Approximately 15 mg of each protein dissolved in 1.5 ml of phosphate buffer (0.067 M, pH 7.0); 0.5 ml of solution containing 0.1 mg of methylene blue added after equilibration at 10° C.

Peptide patterns for peptic, tryptic and chymotryptic digests of the native and the S-sulfo proteins were obtained using high voltage electrophoresis (pH 6.4) in the horizontal direction and ascending chromatography (n-BuOH:3, AcOH:1, H_2O:1). In most of the cases, the C protein revealed an additional his-containing peptide confirming the amino acid analysis.

Finally, the three proteins can be conveniently separated by column chromatography using DEAE-cellulose and an increasing NaCl gradient in a constant phosphate buffer (0.05 M, pH 5.8). This is shown in Fig. 2.

In conclusion, β-lactoglobulin C has been shown to be a genetic variant of β-lactoglobulin B, differing in glutamic (or glutamine) and histidine content. A single amino acid mutation involving his/gluN seems feasible in the genetic codes described by both Jukes (1963) and Eck (1963). This would involve the triplets ACC/AAC. However, a his/glu mutation does not seem as likely. Previous work had shown β-A and β-B to differ in glycine, aspartic, alanine and valine content.

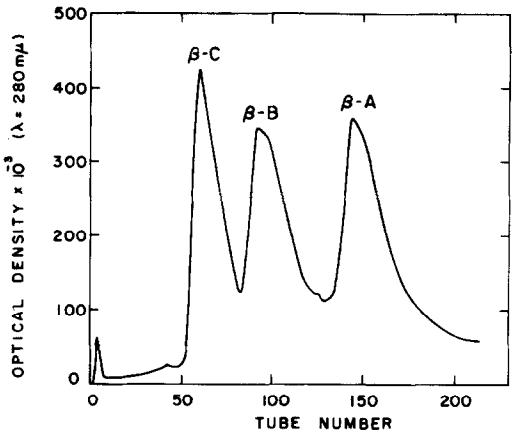

Fig. 2 Column chromatography of β-lactoglobulins A, B and C. A mixture of 200 mg of each protein previously dialyzed against phosphate buffer (0.05 M, pH 5.8) applied to a 1.8 X 35 cm DEAE-cellulose column. Elution accomplished at room temperature by an increasing NaCl gradient (7 chambers each of 500 ml of .01, .03, .05, .07, .09, .11, .14 M) in phosphate buffer (0.05 M, pH 5.8); 15-17 ml/tube.

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