The Primary Structure of the β-Lactoglobulin of the Waterbuffalo (*Bubalus arnee*)

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The complete amino acid sequence of the β-lactoglobuline of the waterbuffalo (*Bubalus arnee*) was established. The sequence of peptides obtained by cleavage with BNPS-Skatole, CNBr and trypsin were determined automatically by the help of the sequenator. Only two differences were found in the β-lactoglobulin of the waterbuffalo compared with the bovine β-lactoglobulin B.

We present the complete primary structure of the β-lactoglobulin (β-lg) of the Italian waterbuffalo (*Bubalus arnee*). The sequence of bovine β-lg AB [1, 2] containing 162 amino acids was the first which has been established entirely with the sequenator [3]. The primary structure of the bovine variants C [4] and D [5], goat [6] and sheep [7] β-lg have also been determined. The function of this milk protein is unknown.

Experiments

β-lactoglobulin was prepared and crystallized from buffalo milk by the method of Aschaffenburg and Drewry [8] modified as previously described [9]. The preparation exhibited a single band in polyacrylamide gel electrophoresis and isoelectric focusing which had same mobility as the bovine β-lg B. No polymorphism of the buffalo protein was detected with these methods and sequence determination. This is in agreement with previous results [10, 11]. The primary structure was determined from the first 45 residues of the S-carboxymethylated [12] polypeptide chain and from seven appropriate peptides obtained by cleavage with CNBr [13], BNPS-skatole [14] and trypsin with a sequenator [3]. The peptides were isolated by gelfiltration, preparative paper electrophoresis and ion exchange chromatography.

Large peptides and the polypeptide chain were degradated in the "Quadrol"-programme [3], smaller peptides in the "Propyne"-programme [15, 16]. Thin layer chromatography [16] and high pressure liquid [17] chromatography were used for identification of PTH-amino acids.

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Results and Discussion

The amino acid sequence of the buffalo β-lactoglobulin (Figure 1) differs in two positions from the bovine β-lg B.

	buffalo	bovine B
Pos. 1	Ile	Leu
Pos. 162	Val	Ile

These differences were known from the determination of the N- [18] and C-terminal [10] amino acids and from the sequence of the first 17 residues [19] which differs in some points from the primary structure we have established.

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Ile-Ile-Val-Thr-Gln-Thr-Met-Lys-Gly-Leu-Asp-Ile-Gln-Lys-Val-Ala-Gly-Thr-Trp-Tyr-Ser-Leu-Ala-Met-Ala-Ala-Ser-Asp-Ile-Ser-
                                10                                  20                                  30
-Leu-Leu-Asp-Ala-Gln-Ser-Ala-Pro-Leu-Arg-Val-Tyr-Val-Glu-Glu-Leu-Lys-Pro-Thr-Pro-Glu-Gly-Asp-Leu-Glu-Ile-Leu-Leu-Gln-Lys-
                                40                                  50                                  60
-Trp-Glu-Asn-Gly-Glu-Cys-Ala-Gln-Lys-Lys-Ile-Ile-Ala-Glu-Lys-Thr-Lys-Ile-Pro-Ala-Val-Phe-Lys-Ile-Asp-Ala-Leu-Asn-Glu-Asn-
                                70                                  80                                  90
-Lys-Val-Leu-Val-Leu-Asp-Thr-Asp-Tyr-Lys-Lys-Tyr-Leu-Leu-Phe-Cys-Met-Glu-Asn-Ser-Ala-Glu-Pro-Glu-Gln-Ser-Leu-Ala-Cys-Gln-
                               100                                 110                                 120
-Cys-Leu-Val-Arg-Thr-Pro-Glu-Val-Asp-Asp-Glu-Ala-Leu-Glu-Lys-Phe-Asp-Lys-Ala-Leu-Lys-Ala-Leu-Pro-Met-His-Ile-Arg-Leu-Ser-
                               130                                 140                                 150
-Phe-Asn-Pro-Thr-Gln-Leu-Glu-Glu-Gln-Cys-His-Val
                               160
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Fig. 1. Amino acid sequence of buffalo β-lactoglobulin.

Some investigators [20–22] previously found that buffalo and bovine β-lg B are nearly identical which is confirmed by this sequence.

The low number of differences in the primary structure of β-lactoglobulins are probably due to a special biological function of this protein.

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