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Methods in Protein Sequence Analysis

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Amino Acid Sequence of Bovine β_2 -Microglobulin

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Crystalline bovine β_2 -microglobulin (β_2 -m) was prepared from colostrum and its complete primary structure elucidated. β_2 -m is a low molecular weight protein (11,600) widely found in various body fluids and bound to cell surface proteins. It is structurally related to the immunoglobulins and is non-covalently associated with histocompatibility antigens. Bovine β_2 -m was isolated from colostrum casein by DEAE- and CM-cellulose chromatography, gel filtration and finally by crystallization. The bovine protein is the only reported crystalline homologue.

Bovine β_2 -microglobulin was sequenced completely using only *S. aureus* V8 protease (Miles) under two sets of conditions to catalyze limited cleavage at glutamyl residues. Cleavage at pH 4 in 0.1 M ammonium acetate, 37°C, for one hour at an enzyme to protein ratio of 1/50 yielded only two fragments resulting from a single split at a glu-ile bond between positions 36-37. Catalysis mediated by the same enzyme at pH 8 in 0.5% ammonium bicarbonate, 37°C, for two hours at a ratio of 1/100 produced several peptides with the major additional cleavage of a glu-phe link at residues 68-69. Three sequences provided the primary structure data: intact carboxymethylated β_2 -m, sequenced 1-41; one pH 4 fragment, sequenced 37-72; and a pH 8 peptide, 69-98.

Bovine β_2 -m contains 98 amino acid residues as compared with 99 for the other species whose complete sequence has been reported. This difference represents a deletion at position 49 where the other β_2 -m molecules contain valine. The bovine protein is also unique in that it contains three di-prolyl sequences all located within the amino terminal third of the molecule. In a comparison of absolute differences among species, bovine β_2 -m ranges from a low of 24 substitutions as compared with the rabbit homologue and 26 for human to 32 for mouse and guinea pig. β_2 -microglobulin is certainly a highly conserved protein; of the 26 differences between bovine and human, 19 represent single base changes.

In single letter notation, the sequence of bovine β_2 -microglobulin is:

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                10                20
I Q R P P K I Q V Y S R H P P E N G K P

                30                40
N Y L N C Y V Y G F H P P Q I E I D L L

                50                60
K N G E K I K S E Q S D L S F S K D W S

                70                80
F Y L L S H A E F T P D S K D E Y S C R

                90                98
V K H V T L E Q P R I V K W D R D L
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