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A1 And A2 Beta-Casein in Bovine Milk: Molecular Differences, Detection Methods and Implications for Human Health

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Introduction

Milk is composed of approximately 87% water and 13% solids, including lactose (4.9%), protein (3.4%), fat (4.0%), vitamins, and minerals. Among milk proteins, casein is the major fraction, and β-casein accounts for nearly 30% of total casein. β-casein consists of 209 amino acids with a molecular weight of approximately 24 kDa. The two most common variants are A1 and A2 β-casein.

History and Genetic Basis

Around 5,000 years ago, a mutation occurred in the beta-casein gene that resulted in the substitution of the amino acid proline with histidine at the 67th position of the beta-casein chain. Cows carrying this mutated beta-casein protein are referred to as A1 cows. Different mutations in bovine beta-casein have led to the formation of 13 genetic variants, among which A1 and A2 are the most common.

Cow Breeds and Milk Type

Crossbred cattle such as Holstein Friesian and crossbred Jersey commonly produce A1 milk or a mixture of A1 and A2 milk. Indigenous Indian dairy breeds such as Red Sindhi, Sahiwal, Gir, Tharparkar, and Rathi predominantly produce A2 milk.

Research studies involving blood sample collection from true-to-type animals in their respective breeding tracts were conducted to determine allelic status. DNA was isolated and a PCR-RFLP genotyping protocol was followed. The procedure included amplification of a 251 base pair region of beta-casein exon 7 containing the mutation site, followed by restriction

digestion using the TaqI enzyme and visualization on a 3 percent ethidium bromide-stained agarose gel.

Three restriction fragment patterns corresponding to different genotypes were observed:

A1A2: 251 bp and 213 bp fragments

A2A2: 251 bp fragment

A1A1: 213 bp fragment

Data collected from Indian native cattle breeds across various agro-climatic regions revealed that approximately 97.4 percent of animals possessed the A2A2 genotype, while 2.6 percent were heterozygous (A1A2). No animals showed the homozygous A1A1 genotype, except for a few individuals of Malnad Gidda and Kherigarh breeds that exhibited heterozygosity. All major Indian dairy breeds, including Gir, Tharparkar, Rathi, Red Sindhi, and Sahiwal, showed fixation of the A2 allele. Several other breeds such as Belahi, Konkan Kapila, Kangayam, Nimari, Red Kandhari, Malvi, Amritmahal, Kankrej, Hariana, and Mewati also showed complete absence of the A1 allele.

Metabolism and BCM-7 Formation

In A1 beta-casein, enzymatic digestion in the intestine produces beta-casomorphin-7 (BCM-7), which is an opioid peptide similar to morphine. Bovine beta-casein contains 209 amino acid residues, of which approximately 16.7 percent are proline residues distributed along the polypeptide chain. This distribution limits the formation of an alpha-helix structure. The molecular weight of beta-casein is approximately 24 kDa. Different mutations have resulted in 13 genetic variants of beta-casein: A1, A2, A3, A4, B, C, D, E, F, H1, H2, I, and G. Each variant differs from the others based on amino acid substitution at specific positions.

There are two major alleles of the beta-casein gene: A1 and A2. Each cow carries two copies of the beta-casein gene and may have one of three genotypes: A1A1, A2A2 (homozygous), or A1A2 (heterozygous). The A1 and A2 variants differ at amino acid position 67, where histidine is present in A1 and proline is present in A2 milk. This difference arises due to a single nucleotide polymorphism at codon 67 of the beta-casein gene. This substitution may alter the secondary conformation of the protein and influence its physical properties as well as its susceptibility to enzymatic digestion.

In A1 beta-casein, the peptide bond between isoleucine (position 66) and histidine (position 67) is susceptible to cleavage by digestive enzymes such as pepsin, elastase, leucine aminopeptidase (LAP), and pancreatin. This enzymatic cleavage releases a seven-amino-acid

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bioactive peptide known as beta-casomorphin-7 (BCM-7), consisting of the sequence Tyr60–Pro61–Phe62–Pro63–Gly64–Pro65–Ile66.

In contrast, in A2 beta-casein, the bond between isoleucine and proline is resistant to enzymatic hydrolysis due to the rigid structure of proline. As a result, BCM-7 is not released during digestion of A2 milk. Instead, longer peptides such as BCM-9 may be formed. BCM-7 can be further degraded into smaller peptides such as BCM-5 and BCM-3 by the enzyme dipeptidyl peptidase-IV (DPP-IV), which is present on the surface of enterocytes and in the bloodstream.

Biotechnological Detection Methods

- **Single Nucleotide Polymorphism (SNP) Genotyping and High-Resolution Melting (HRM):** These techniques detect allelic variations in the CSN2 gene responsible for A1 and A2 beta-casein. The rhAmp SNP genotyping method has been reported to be more sensitive than HRM in detecting small proportions of A1 milk in predominantly A2 samples.
- **Real-Time PCR:** Highly sensitive real-time PCR methods allow direct detection and quantification of A1 and A2 alleles from raw milk samples. These techniques can detect A1 alleles at levels as low as 2 percent in A2 milk.
- **Cycleave PCR:** It is a real-time PCR method that uses a chimeric DNA-RNA-DNA probe and RNase H for highly specific SNP detection. The probe contains a single RNA base at the mutation site. When the probe perfectly matches the target DNA, RNase H cleaves the RNA base, separating the fluorophore from the quencher and producing fluorescence. If there is a mismatch, cleavage does not occur and no signal is generated. This method is highly suitable for distinguishing A1 and A2 alleles.
- **Liquid Chromatography–Mass Spectrometry (LC-MS):** LC-MS-based methods use characteristic peptides as biomarkers to directly quantify A1 and A2 beta-casein variants. Unlike PCR, which detects the gene, LC-MS measures the actual protein or peptide products present in milk or digested samples.
- **Ultra-Performance Liquid Chromatography coupled with High-Resolution Mass Spectrometry (UPLC-HRMS):** This technique enables detailed protein fingerprinting and quantitative profiling of different beta-casein variants.

Effect of Heat Treatment and Fermentation on BCM-7

Pasteurization at 72°C for 15 seconds has minimal effect on BCM-7 activity. Ultra-high temperature treatment (135–150°C) causes greater protein denaturation and structural changes but does not completely destroy BCM-7 because it is relatively heat stable.

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Fermentation is considered the most effective method for reducing BCM-7 activity. During fermentation, lactic acid bacteria produce proteolytic enzymes that further degrade BCM-7 into smaller inactive peptides. In the human intestine, enzymes such as peptidases and DPP-4 further break down BCM-7 into smaller amino acids.

Impact of BCM-7 on Human Health

A1 beta-casein digestion produces BCM-7, an opioid peptide that interacts with mu-opioid receptors. It has been suggested that BCM-7 may be associated with health conditions such as type 1 diabetes, coronary heart disease, atherosclerosis, autism, schizophrenia, sudden infant death syndrome, and digestive inflammation. However, scientific evidence remains inconclusive and requires further well-controlled clinical studies.

In A2 milk, the presence of proline at position 67 prevents significant release of BCM-7, which is why A2 milk is often considered easier to digest.

Conclusion

The difference between A1 and A2 milk arises from a single amino acid substitution in β-casein. This small molecular variation influences digestive behavior and peptide release. While A2 milk is considered the ancestral form, the health implications of A1 milk remain under investigation. Continued scientific research is essential for clear conclusions and informed consumer decisions.

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