# GENETIC VARIANTS OF $\alpha_{s1}$-CASEIN; AMINO ACID COMPOSITION OF THE VARIANTS B, C AND BC

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The genetic polymorphism of the various casein components is well established (Thompson et al. 1965). At the moment three $\alpha_{s1}$-casein variants are known, which have been denominated as $\alpha_{s1}$-casein A, B and C, in order of decreasing electrophoretic mobility in urea-starch-gel electrophoresis. It was found by Kiddy et al. (1964) that the different forms of $\alpha_{s1}$-casein could occur singly (A, B and C) or in pairs (AB, AC or BC).

In the present study the amino acid composition of variants B, C and BC has been determined. As we did not have the disposal of cows producing the other three phenotypes A, AB and AC, no data could be given of the amino acid composition of these variants.

## Materials and Methods

The $\alpha_{s1}$-caseins B and BC were isolated from the milk of individual Dutch cows, and $\alpha_{s1}$-casein C was isolated from a sample of whole casein kindly provided by Dr. J. Garnier*). Homogeneous fractions were isolated by column electrophoresis over cellulose powder, as described by Schmidt and Payens (1963). No contaminating proteins could be observed, when analysing

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the purified samples by starch-gel electrophoresis according to Wake and Baldwin (1961).

Before analysis the lyophilized protein samples were dried intensively over $P_2O_5$. From the dried proteins, aliquots of 10 mg were taken to be hydrolysed in 6 N HCl in sealed evacuated tubes at 110 °C for 24, 48 and 72 hrs. Norleucine, used as an internal standard, was added to the protein samples before hydrolysis. The hydrolysates were evaporated to dryness in a rotating evaporator at 50 °C.

The amino acid composition was determined by the method of Piez and Morris (1960), using a Technicon auto-analyzer. Tryptophan was determined separately by the method of Spies and Chambers (1949).

The number of amino acid residues was calculated on the basis of a molecular weight of 30,000 (see Table 1).

Peptide patterns of tryptic digests of the genetic variants were obtained according to the method of Ritschard (1964) by performing chromatography on thin-layers of silicagel (n-BuOH:3, AcOH:1, $H_2O$:1) in one direction, and high-voltage electrophoresis (pyridine acetate, pH 3.6) in the other.

## Results and Discussion.

The amino acid composition of the studied variants is given in Table 1. Considering the total recovery of amino acid residues per 100 g of protein, a deviation from 100 per cent is observed. This may be caused partially by contamination with cellulose based material isolated during the purification of the $\alpha_{s1}$-casein samples by electrophoresis over cellulose columns. The recoveries in terms of protein nitrogen were in good agreement; 98.74 and 98.01 and 97.90 per cent for

Table 1. Amino Acid Composition of $\alpha_{s1}$-caseins B, C and BC.

| Amino Acid | Amino Acid residues[a] per 100 g of protein | | | Calculated number of residues per 30,000 | | | Proposed number of residues per mole | | |
|---|---|---|---|---|---|---|---|---|---|
| | B | C | BC | B | C | BC | B | C | BC |
| ASP | 6.46 | 6.29 | 6.62 | 18.8 | 18.5 | 19.0 | 19 | 19 | 19 |
| THR[b] | 1.83 | 1.83 | 1.95 | 6.0 | 6.1 | 6.4 | 6 | 6 | 6 |
| SER[b] | 4.58 | 4.46 | 4.22 | 17.6 | 17.4 | 16.4 | 17 | 17 | 17 |
| GLU | 19.03 | 18.33 | 19.40 | 49.3 | 48.1 | 49.6 | 49 | 48 | 48-49 |
| PRO | 5.95 | 6.01 | 6.18 | 20.5 | 21.0 | 21.0 | 21 | 21 | 21 |
| GLY | 1.91 | 2.08 | 1.99 | 11.2 | 12.4 | 11.5 | 11 | 12 | 11-12 |
| ALA | 2.43 | 2.41 | 2.37 | 11.5 | 11.5 | 11.0 | 11 | 11 | 11 |
| VAL[c] | 4.10 | 4.11 | 4.21 | 13.8 | 14.1 | 14.0 | 14 | 14 | 14 |
| MET[c] | 2.35 | 2.29 | 2.40 | 6.0 | 5.9 | 6.0 | 6 | 6 | 6 |
| ISO[c] | 4.58 | 4.56 | 4.66 | 13.5 | 13.7 | 13.6 | 14 | 14 | 14 |
| LEU | 7.14 | 7.05 | 7.16 | 21.1 | 21.1 | 20.9 | 21 | 21 | 21 |
| TYR | 5.86 | 5.80 | 5.93 | 12.0 | 12.0 | 12.0 | 12 | 12 | 12 |
| PHE | 4.39 | 4.31 | 4.37 | 9.9 | 9.9 | 9.7 | 10 | 10 | 10 |
| TRY[d] | 2.03 | 2.05 | 2.07 | 3.6 | 3.7 | 3.7 | 4 | 4 | 4 |
| LYS | 6.55 | 6.43 | 6.60 | 17.1 | 17.0 | 17.0 | 17 | 17 | 17 |
| HIS | 2.57 | 2.54 | 2.52 | 6.3 | 6.3 | 6.1 | 6 | 6 | 6 |
| ARG | 3.43 | 3.37 | 3.52 | 7.3 | 7.3 | 7.4 | 7 | 7 | 7 |
| $NH_3$[b] | 1.62 | 1.64 | 1.81 | 33.9 | 34.7 | 37.3 | 34±3 | 34±3 | 34±3 |
| $PO_3H$ | 2.89 | 2.89 | 2.89 | 12.1 | 12.3 | 12.0 | 12 | 12 | 12 |
| Total | 89.69 | 88.44 | 90.95 | | | | | | |
| % total N recovered | 98.74 | 98.01 | 97.90 | calculated minimum molecular weight[e] | | | 30.020 | 30,023 | 29,926 |
| N-content | 14.37 | 14.3 | 15.01 | | | | | | |
| P-content[f] | 1.12 | 1.12 | 1.12 | | | | | | |

a. Average of duplicate analysis of hydrolysates at 24, 48 and 72 hrs
b. Linearly extrapolated to zero time
c. Values found at 72 hrs of hydrolysis
d. Determined by the method of Spies and Chambers (1949)
e. The average from the content of Asp, Thr, Ser, Gly, Ala, Met, Iso, Tyr, Phe, Lys, His and Arg
f. These values were taken from Schmidt and Payens (1963)

$\alpha_{s1}$-casein B, C and BC respectively. For the calculation of the number of residues per mole, the experimental values of the amino acid residues given in Table 1 were corrected to a basis of hundred per cent recovery.

Considering the number of residues per mole it is obvious that the genetic variants bear a close resemblance in their amino acid composition. Compared with $\alpha_{s1}$-casein C, $\alpha_{s1}$-casein B contains one more residue of glutamic acid and one less residue of glycine. These results agree well with the observed electrophoretic mobilities in urea-starch-gel at pH 8.6. Furthermore a single amino acid mutation of glu/gly involving the triplets AAG/GAG seems feasible in the genetic codes described by Eck (1963) and Leder et al. (1964). A comparison of the amino acid composition of $\alpha_{s1}$-casein BC with that of the $\alpha_{s1}$-caseins B and C shows a good agreement. The slight differences observed with serine and glutamic acid has to be ascribed to the high rate of destruction during hydrolysis of serine, while in the case of glutamic acid the accuracy of the analytical method (approx. 1-2 %) becomes the limiting factor. Neither cystine nor cysteine was observed in the hydrolysates of the studied variants.

The differences in one more residue of valine, tyrosine and phenylalanine for $\alpha_{s1}$-casein B as compared with $\alpha_{s1}$-casein C mentioned by Gordon and Basch (1963) were not confirmed in our analyses.

Fingerprinting of tryptic digests of the studied variants showed identical patterns of about 30 peptides. As trypsin only hydrolyses arginine and lysine containing peptide bonds, a number of 25 peptides may be expected when the molecular weight of $\alpha_s$-casein is 30,000. The rather good agreement

between the observed and expected number of peptides confirms this supposition. Furthermore, the difference in amino acid composition of not more than one residue in both variants is additional support that the molecular weight of $\alpha_{s1}$-casein must be of this order.

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