AMINO ACID COMPOSITION OF β-CASEIN GENETIC VARIANTS

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The presence of β-casein genetic variants has been demonstrated by ASCHAFFENBURG (1961). In order of decreasing mobility in urea starch-gel electrophoresis at pH 8.6, these variants have been termed β-casein A, B and C with the relative mobilities of 0.80, 0.76 and 0.70.

In the present paper the amino acid composition of these variants is reported. The analyses were performed as independent and cooperative studies at both Dairy Institutes.

MATERIALS and METHODS

Samples of β-caseins A and B were obtained from the milk of individual Dutch and French Friesian cows and β-casein C from one single individual Abondance cow* (SIMMENTAL type), all homozygous for these proteins. The chromatographic method described by GARNIER et al. (1964) for the preparation of homogeneous β-casein was followed. The purity of the β-casein preparations was checked by starch-gel electrophoresis according to WAKE and BALDWIN (1961) and by immunoelectrophoresis according to GRABAR and WILLIAMS (1955) under the conditions described by GARNIER et al. (1962). Before analysis the lyophilized protein fractions were dried over P_2O_5 for at least 24 h.

The amino acid composition was determined after hydrolysing the proteins samples in duplicate or triplicate for 24, 48 and 72 or 96 hours in 6 N HCl (HCl:protein ratio = 500:1 or 1000:1) in pyrex sealed evacuated tubes maintained at 110°C. The analyses were carried out on several preparations of each variant using two methods : MOORE et al. (1958) with a Phoenix amino acid analyzer (Jouy-en-Josas) and PIEZ and MORRIS (1960)

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with a Technicon amino acid analyzer (Ede).

Tryptophan was determined separately by the method of SPIES and CHAMBERS (1949) as modified by HARRISON and HOFMANN (1961) or by spectrophotometry according to BENCZE and SCHMID (1957).

Phosphorus determinations were made using the method of BAMANN et al. (1948).

Peptide patterns for tryptic digests of the three variants were obtained by high voltage electrophoresis (pH 1.9 Acetic-formic acids buffer) in the horizontal direction, and by descending chromatography (n-BuOH:200, AcOH:30, H_2O:75) in the other direction. Arginine containing peptides were detected by the SAKAGUCHI test. The numbers of amino acid residues were calculated on the basis of a molecular weight of 24,100.

RESULTS AND DISCUSSION

The amino acid compositions of the genetic variants obtained by the two laboratories are presented in Table I. They are quite close to that given by GORDON et al. (1949) who probably analysed a mixture of β-caseins A and B. The molecular weight was based on the average value calculated from the minimum molecular weight of Lys, His, Arg, Asp, Thr, Ser, Gly, Ala, Met, Ileu, Tyr, Try and Phe.

The results have been corrected for a non peptidic part usually varying between 4 and 10 % after deduction of PO_3H. This can be explained by a contamination with salts or/and cellulose material eluted from the ion exchanger and not removed by dialysis.

No cystine-cysteine was found in the hydrolysate from the three variants.

The results obtained by both Institutes are in good agreement within one amino acid residue for most of the amino acids (see Table I). Therefore we propose definite numbers of residues for each amino acid. The figures presented are based on a careful and critical examination of the cooperative results.

The observed spread of the results for Ser must be related to the high rate of destruction of this amino acid during hydrolysis (10 to 20 % per 24 h). This rate is reduced at a higher HCl to protein ratio (i.e. 1000:1). Although the extrapolated number of residues of Ser might have an error of ± 1, non-concomitant variation with the genetic type was found by both laboratories.

TABLE I

Number of amino acid residues (1) per mole of β-casein variants

Amino acid	β-casein A			β-casein B			β-casein C		
	Jouy	Ede	Proposed number of residues	Jouy	Ede	Proposed number of residues	Jouy	Ede	Proposed number of residues
Asp	9.1	9.7	9	9.0	9.2	9	9.0	9.4	9
Thr (2)	8.7	9.2	9	8.9	9.8	9	9.3	9.0	9
Ser (2)	14.9	15.3	15	14.0	14.9	15	16.2	13.5	15
Glu	39.5	40.2	40	39.0	38.7	39	38.5	40.1	39
Pro	33.4	33.3	33 - 34	33.7	32.4	33 - 34	33.1	34.1	33 - 34
Gly	5.4	5.2	5	5.1	5.3	5	5.4	5.2	5
Ala	5.2	5.2	5	5.2	6.1	5	5.1	5.3	5
Val (3)	20.0	19.1	20	19.8	18.7	20	19.5	19.4	20
Met	6.2	6.0	6	6.3	6.2	6	5.8	5.9	6
Ileu (3)	9.9	9.7	10	9.8	9.9	10	9.6	10.0	10
Leu	22.3	22.1	22	22.1	21.4	22	22.2	21.8	22
Tyr	3.9	3.9	4	4.3	4.5	4	3.9	3.9	4
Phe	9.0	9.1	9	9.1	8.9	9	8.8	9.0	9
Try	1.3	1.0	1	1.1	1.1	1	1.0	1.1	1
Lys	11.3	10.9	11	11.2	10.9	11	12.6	11.8	12
His	5.5	6.0	6	6.0	5.9	6	6.2	6.0	6
Arg	4.0	4.0	4	4.8	5.1	5	3.8	3.9	4
NH_3 (4)	(29.4)		(30)	(29)		(29)	(29.6)		(30)
PO_3H	4.5		5	4.4		5	4.3		5
M.W.			24,185-24,282			24,194-24,292			24,184-24,281
Total number of residues			209-210			209-210			209-210

(1) Average values of the times of hydrolysis for the different preparations of β-casein.
(2) Linearily extrapolated to zero time. (3) Values found at 72 or 96 hours of hydrolysis.
(4) Linearily extrapolated to zero time only when a linear increase with time was found.

The results differ between laboratories by one residue for Asp, Thr, Pro, Ala, Val, but as in the case of Ser, these differences cannot be related to a genetic variant (see Table I). Accordingly the number of residues nearest to the mean of the figures obtained has been proposed, except for Pro for which the concentration is too high to decide between 33 and 34.

It is obvious that genetic variation involves an extra Arg in β-casein B and an extra Lys in β-casein C as compared with β-casein A. The peptide mapping fully confirms this result and indicates, furthermore, that the replacement of more than one amino acid in the single polypeptide chain of β-casein is very unlikely, contrary to data already reported by THOMPSON and GORDON (1964) and DRESONER and WAUGH (1964).

Glu is the only amino acid of the β-caseins B and C which is present in a lower quantity than that in β-casein A (the results from a repeated analysis at Jouy of a hydrolysate of β-casein C made at Ede would also favour a similar low Glu content for B and C). With this in mind we propose the tentative amino acid replacement in the polypeptide chain shown in Table II. This replacement agrees with the content in amide groups of Table I, although this last figure is known at ± 3 residues and it explains the difference in electrophoretic mobility between the three variants. One Arg peptide of β-casein C has a different mobility from one Arg peptide of β-casein A and this indicates the presence of at least one Arg between the two amino acids replaced in the polypeptide chain.

TABLE II

Z*	β-casein	Amino acid	Genetic code
- 14	A	--Glu---Arg---Glu NH_2--	..GAA**...CGA...CAA..
- 13	B	--Glu---Arg-----Arg----	..GAA**...CGA...CGA..
- 12	C	--Lys---Arg---Glu NH_2--	..AAA**...CGA...CAA..

* Total approximative net charge per mole of β-casein at pH 8.6 according to the results given in Table I.

** The other pairs of triplets are GAG (Glu) → AAG (Lys).

No significant difference in the phosphorus content was found between the three variants (0.56 % ± 0.03) and the extinction coefficient (1 mg/ml, 1 cm) for the variants A, B and C at 276 mμ (max. of absorption) is 0.48 ± 0.02.

From the amino acid replacement proposed the β-caseins B and C are

probably two mutants of β-casein A. According to ECK (1963) and TRUPIN et al. (1965) these mutations would consist in a single base substitution of the coding triplets of the β-casein A messenger RNA, i.e. one adenine is replaced by one guanine for β-casein B and one guanine by one adenine for β-casein C (see Table II).

β-caseins, apart from the lack in cystine-cysteine, are remarkable for the high content in Glu, Pro and large non-polar amino acids ($\sum$ Val, Leu, Ileu, Pro, Phe, Try = 95 residues for a total of 209). These elements would deserve consideration in a study of the conformation of this unusual protein.

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