LOCATION OF AMINO ACID DIFFERENCES IN THE GENETIC VARIANTS OF κ-CASEIN A AND B

P.J. de Koning, P.J. van Rooijen and A. Kok

Netherlands Institute for Dairy Research (NIZO), Ede, Holland

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After reduction of the S-S bonds, the whole κ-casein from bulked milk can be resolved by starch-gel electrophoresis in concentrated urea into two major components and several minor ones (MacKinlay and Wake, 1964). These major components appear to be genetically controlled, and in order of decreasing mobility in starch-gel electrophoresis they are designated as κ-casein A (0.60) and B (0.52) respectively (Neelin, 1964; Schmidt, 1964; and Woychik, 1964, 1965).

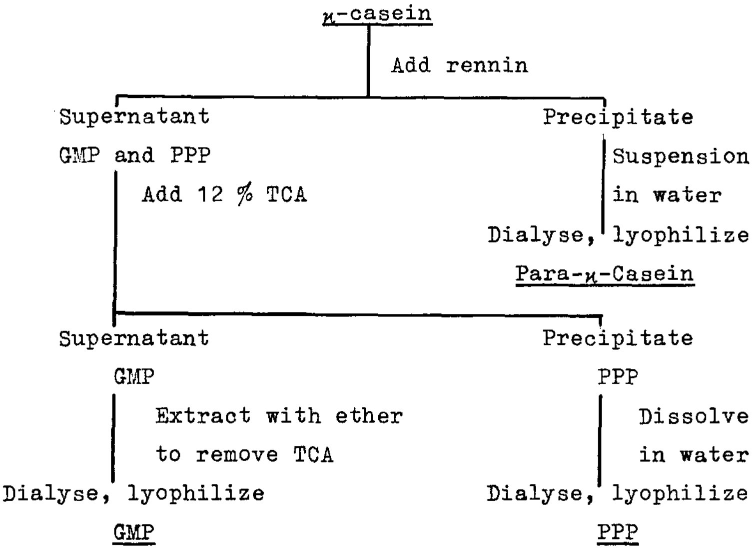
During the transformation of whole κ-casein into para-κ-casein by the action of rennin, a polypeptide portion is split off, consisting mainly of a glycomacropeptide (GMP) soluble in 12 % trichloroacetic acid and a small amount of a polypeptide (PPP) soluble only at low concentrations (2 %) of trichloroacetic acid (Alais, 1956; Wake, 1949 and Tsugo et al. 1960). The PPP, which is devoid of carbohydrate, is derived from the major components (κ-casein A (0.60) or κ-B (0.52) respectively). It was suggested by MacKinlay and Wake, 1965 and also by Grindrod and Nickerson, 1965, that the genetic polymorphism of κ-casein is located in the polypeptide part split off by rennin.

In the present paper it will be shown that the amino acid replacements which give rise to the difference of the genetic variants of κ-casein do indeed occur in these polypeptide moieties (GMP and PPP). Furthermore it has been demonstrated that the amino acid composition of GMP and PPP isolated from the same genetic variant is identical, and they probably have the same primary structure. The amino acid composition of the para-κ-caseins is in close agreement with these results.

Materials and methods

Whole κ-caseins A and B were isolated by the procedure of McKenzie and Wake, 1961, from the milk of individual cows homozygous for either variant. The major components, κ-casein A (0.60) and B (0.52) were isolated from whole κ-casein A and B respectively by chromatography on DEAE-cellulose columns in the presence of 2-mercapto-ethanol as described by Schmidt et al., 1966.

The above preparations were used to isolate para-κ-casein, GMP and PPP by treatment with rennin (Alais, 1956) as illustrated in the following diagram:



Whole para-κ-casein A and B and GMP A and B were prepared from whole κ-casein A and B. From the isolated major components κ-A (0.60) and κ-B (0.52) the corresponding para-κ-caseins A (0.60) and B (0.52) and PPP A and B were obtained.

The amino acid composition of the various components was determined by the method of Piez and Morris, 1960 using a Technicon Amino Acid Analyser as described previously (de Koning and van Rooijen, 1965).

The number of amino acid residues was calculated on the basis of a molecular weight of 5800 for the peptidic part of GMP and PPP as was found by Jollès et al., 1961. A value of 20,000 was taken for the molecular weight of the para-κ-caseins, being the difference between the molecular weight of κ-casein (28,000, Swaisgood et al., 1964) and that of GMP (8000, Jollès et al., 1961).

Results and Discussion

The results of the determination of the amino acid composition of the GMP's, PPP's and para-κ-caseins isolated from the genetic variants A and B are presented in Table I. The amino acid composition of the GMP's and PPP's A and B clearly confirms the suggestion of MacKinlay and Wake, 1964, and of Grindrod and Nickerson, 1965, that the amino acid substitutions in κ-casein A and B occur in the polypeptide part split off by rennin. The data indicate that the GMP's as well as the PPP's have identical replacements i.e. aspartic acid and threonine in variant A are replaced by alanine and isoleucine in variant B. According to the genetic code (Pelc, 1965) it seems feasible that the amino acid mutations involving ASP/THR and ALA/ILEU may be rationalized as a change in the triplets of the m-RNA sequence GAU/ACU by GCU/AUU.

The N-terminal amino acids of the PPP's were detected as the 2,4 dinitrophenyl (DNP) derevatives by using chromatography on thin-layers of silica gel (Stahl, 1962). Only one ether--soluble DNP-amino acid derivative was shown to be present, and this could be identified as DNP-methionine in the proportion of one mole per mole PPP.

The action of carboxypeptidase A (Sigma, Chemical Company, DFP treated) on PPP resulted in the liberation of valine and alanine. Amino acid analysis after increasing time of incubation showed valine as the C-terminal amino acid and alanine as the penultimate amino acid. Traces of serine and glycine also appeared during the incubation

From these results the partial structure formulae shown in Table II are proposed for the polypeptide part split off by rennin.

TABLE II

Partial Structure Formulae of the PPP of κ-Casein A and B

Polypeptide Part (PPP)		N-terminal Group	Amino acid Replacement	C-terminal Sequence
A	:	MET--- ---	(ASP)---(THR)---	(GLY SER)ALA.VAL
B	:	MET--- ---	(ALA)---(ILEU)--	(GLY SER)ALA.VAL

TABLE I

Amino Acid Composition of the Glycomacropeptide (GMP) Polypeptide (PPP) and Para-κ-Casein Portion of the Genetic Variants of κ-Casein A and B.

	Polypeptide Part Number of Residues[a] per 5800				Para-κ-Casein Part Number of residues[a] per 20,000			
Amino acid	GMP A	GMP B	PPP A	PPP B	$A^{+)}$ (0.60)	$B^{+)}$ (0.52)	$A^{++)}$	$B^{++)}$
ASP	4.3	3.5	4.3	3.6	11.2	10.9	11.6	11.4
THR[b]	9.8	8.8	10.0	9.0	6.1	6.2	5.4	5.5
SER[b]	4.7	4.8	4.8	4.7	11.0	11.0	10.1	10.4
GLU	8.8	8.7	9.1	9.1	26.8	27.2	27.2	27.4
PRO	6.6	6.4	7.0	7.0	18.1	18.6	17.5	17.9
GLY	1.1	1.1	1.2	1.2	2.8	2.9	3.6	3.3
ALA	4.2	4.9	4.2	5.1	13.7	13.6	13.1	12.7
VAL	4.9	4.9	4.9	5.0	8.6	8.9	9.0	9.2
CYS[c]	--	--	--	--	2.4	2.2	2.3	2.2
MET	0.7	0.7	0.8	0.8	1.5	1.6	1.2	1.9
ILEU	4.9	5.7	4.9	5.8	9.5	9.5	9.2	9.2
LEU	1.0	1.0	1.0	1.1	11.3	11.5	12.2	12.3
TYR	--	--	--	--	13.4	13.2	13.1	12.4
PHE	--	--	--	--	6.0	6.1	6.2	6.4
NH_3[b]	11.2	15.4	5.7	4.9	24.9	24.2	25.6	24.1
LYS	2.8	2.9	2.6	2.7	9.3	9.3	9.4	9.7
HIS	--	--	--	--	4.3	4.3	4.0	4.5
ARG	--	--	--	--	7.5	7.2	7.2	7.2
TRY	--	--	--	--	2.3	2.3	2.6	2.4

$^{+)}$para-κ-casein isolated from κ-casein A (0.60) or B (0.52)

$^{++)}$para-κ-casein isolated from whole κ-casein A and B respectively

a) average of duplicate analysis of hydrolysates at 24, 48 and 72 hrs

b) Linearly extrapolated to zero time

c) The cystine content was estimated from the cystine peak obtained during the amino acid analysis

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These formulae are in close agreement with those proposed by Jollès et al., 1962, and by Delfour et al., 1965, 1966, for the glycomacropeptide isolated from whole κ-casein, and provide evidence that both substances (PPP and GMP) are identical in their primary structure.

Comparing the amino acid composition of the para-κ-casein fractions isolated from whole κ-casein A and B, as well as from the chromatographically isolated major components κ-A (0.60) and κ-B (0.52), no replacements of amino acids are observed (see Table I). The amino acid compositions of both para-κ-casein variants are nearly identical when isolated from the same starting product. However, comparing the amino acid composition of the two para-κ-casein variants isolated from whole κ-casein with those isolated from the major components κ-A (0.60) or κ-B(0.52) respectively, small but real differences are found for THR, SER, PRO, GLY, ALA and LEU. These results could be explained by the presence of minor components in the κ-casein complex with slight differences in amino acid composition of the insoluble para-derivative (MacKinlay and Wake, 1966). Curiously, it is also found that the content of PRO is higher in the case of the para-κ-casein's B. The same observation was also made by Schmidt et al., 1966, when studying the amino acid composition of κ-casein B (0.52), in which one more residue of PRO was observed as compared with κ-casein A (0.60). It appears therefore that the peptide chain of κ-casein B is one residue longer than κ-casein A.

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