A STUDY OF κ-CASEIN COMPONENTS.
I - Preparation. Evidence for a common C-terminal sequence.

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Various authors (SCHMIDT, 1964 ; MACKINLAY and WAKE, 1964 ; NEELIN, 1964 ; WOYCHIK, 1964, 1965) have found that the A and B genetic types of κ-casein can be resolved into several fractions. Genetic studies (GROSCLAUDE et al., 1965) have shown a common segregation of a major and two minor fractions within each variant. This observation suggests either the existence of a single locus specifying the structure of these fractions, i.e. identity between their polypeptide chains, or the existence of several closely linked loci (at least bi-allelic), specifying different polypeptide chains, which together would constitute the socalled "phenotype A" and "phenotype B" of κ-casein.

MACKINLAY and WAKE (1965) MACKINLAY et al. (1966), and SCHMIDT et al. (1966) pointed out that the different mobilities of κ-casein fractions could be explained by the existence of different amounts of carbohydrates, particularly sialic acid, linked to a basic polypeptide unit. The comparison of para-derivatives released from κ-casein fractions by rennin treatment (MACKINLAY et al., 1966), supports the assumption of a common polypeptide chain.

In this paper a comparison of C-terminal sequences of highly chromatographically purified fractions is reported. The molecular weights, obtained by C-terminal analysis of the seven κ-A fractions, are presented. The results add evidence for identity or very close relationship of the polypeptide chains in the seven fractions which account for more than 90 % of κ-casein preparations.

MATERIALS and METHODS

κ-caseins A and B were prepared by the method of ZITTLE and CUSTER (1963) from whole caseins of homozygous cows for the κ-Cn^A and κ-Cn^B alleles, respectively, and also for the β-Cn^A allele, which facilitates the distinction of fast moving κ-casein fractions from β-casein. Three κ-Cn^A cows supplied identical whole κ-caseins. Whole κ-casein B was prepared from the milk of a single cow.

Chromatographic analyses of whole κ-casein (sample loads of 1.2 g) were carried out on DEAE-cellulose columns (3.2 x 20 cm) as described by RIBADEAU-DUMAS et al. (1964) for whole casein, in imidazole-HCl buffer (0.02 M, pH 7.0) containing urea (3.3 M) and 2-mercaptoethanol (3 g/l). A linear NaCl gradient (0.02 M to 0.20 M in 1,600 ml) was used for the elution. Each fraction was purified by further chromatographies (2 or 3) with appropriate gradients chosen according to the NaCl concentrations observed at its elution in whole κ-casein chromatography. The fractions were collected and dialyzed against water until precipitation. The precipitates were dissolved at pH 7 by addition of 0.1 N NaOH, filtered on Whatman n° 3 paper and freeze dried. Before analysis they were dried over vacuum and P_2O_5 for 48 hours.

Whole κ-casein or fractions were tested for purity by starch gel electrophoresis (SGE) in urea and 2-mercaptoethanol at pH 8.6 (WAKE and BALDWIN, 1961 ; SCHMIDT, 1964) and pH 3 (PETERSON, 1966). Concentration of samples : 2 %.

Each fraction was incubated at 40°C in Tris 0.013 M-HCl buffer pH 8.5 with Carboxypeptidase A(CPA), (Worthington, DFP treated, 3 x cryst.), E/S = 1/40 (w/w). Aliquots of the solution were removed after 5, 15, 30 min. and 1, 2, 24 hrs. After evaporation to dryness, part of each aliquot was analyzed by paper chromatography in butanol-acetic acid-water. Another part was eventually fingerprinted on paper to examine for the presence of Asn and Gln. This was carried out by chromatography as described above, then by electrophoresis (pH 1.9, acetic-formic buffer) in the rectangular direction. The remainder of each aliquot was kept for amino-acid analysis.

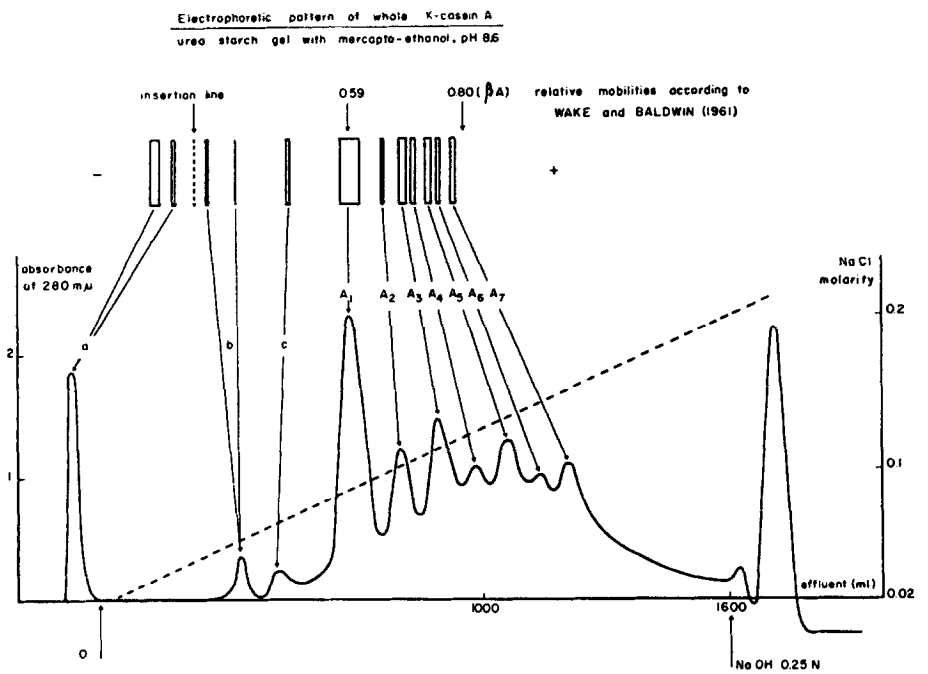
The release of Asn and Gln was followed in a separate experiment by the method described by KASPER et al. (1965) (Dialysis of the incubation mixture at 40°C, in 0.04 M ammonium bicarbonate buffer pH 7.7 ; E/S = 1/125). Every 30 min. for 5 hrs, the outer solution was removed, freeze dried, and the resulting residue was hydrolyzed at 110°C with 5.7 redistilled HCl for 15 hours under vacuum, then evaporated to dryness and analyzed.

Amino-acid analyses were carried out on a Phenix auto-analyzer. Only acidic and neutral amino-acids were determined since no basic amino-acid could be detected on paper even after treatment with carboxypeptidase B.

RESULTS AND DISCUSSION

A typical elution pattern of whole κ-casein is shown on Fig.1. Ten fractions could be isolated from either κ-A or κ-B casein.

Figure I



The fraction (a) is not retained on the column and its yield varies with preparations of κ-A (0.5 to 4 %).

This fraction shows two main bands identical to the two components of para-κ-caseins after SGE at pH 8.6 and 3.0. No further study was carried out on this fraction, but it may be assumed that it arises from limited proteolysis of κ-casein during its preparation, at the same points attacked by rennin.

Very low yields of fractions (b) and (c) were obtained. These fractions had the same mobilities on SGE whether they were obtained from κ-casein A or κ-casein B ; therefore they were not regarded as κ-casein and were no longer studied.

The average yields of the seven (crude) fractions A_1 to A_7, in g/100 g of whole κ-casein A are 24, 9, 18, 9, 13, 7 and 12 respectively. These fractions account for more than 90 % of the preparation ; their relative amounts were quite reproducible on different chromatographic patterns from the same κ-casein or from different preparations of κ-casein. The seven fractions were purified until single bands were obtained by SGE at pH 8.6. Together these bands give the complete electrophoretic pattern of whole κ-casein.

Seven components, B_1 to B_7, and only seven, were also found in κ-casein B. A_1 and B_1 correspond to the so-called "major" genetically variable components ; it must be emphasized that each of them represents only 25 % of the corresponding seven fractions. The dispersion of the mobilities of B fractions by SGE at pH 8.6 is greater than that of A fractions, in such a manner that B_7 migrates at about the same level as A_7. However, at pH 3.0, the fractions A_1 to A_7 are identical to the fractions B_1 to B_7, respectively.

This is in agreement with SCHMIDT's data (1966) on the amino-acid differences between A_1 and B_1 : A_1 would have one more aspartic acid, protonated at pH 3. Furthermore it suggests a similar difference within each pair of A_i-B_i fractions, and therefore supports the assumption of a common polypeptide unit in each genetic series.

Carboxypeptidase A releases eleven amino-acid residues from each of the fractions A_1 to A_7. These amino-acids are identical in nature, rate of release and number of residues in the seven fractions. (Tables 1 and 2). Assuming that κ-casein has a single, linear peptide chain, the following tentative suggestion may be made for the C-terminal sequence :

-Thr-Ile-Asn-Val-Thr-Gln-Ser-Val-Thr-Ala-Val-COOH

It is only in partial agreement with the results of JOLLES et al. (1961) and KONING et al. (1966) obtained from whole κ-casein and from caseino-macropeptides A and B respectively.

Assuming a unique linear peptide chain for each of the seven fractions, one can calculate their minimum molecular weights from the amount of Ala, Ser, Ile released after 24 hrs of CPA action. From A_1 to A_7, one finds 17,100, 18,600 , 23,600 , 21,700 ; 22,900 , 21,000 and 23,500 ($\pm$ 1,000) respectively.

The observed increase of these numbers is consistent with the existence of a basic polypeptide unit on which increasing amounts of carbohydrates are fixed. A_1 might represent this basic polypeptide chain ; its molecular weight (17,100) is similar to that calculated by KALAN and WOYCHIK (1965) for the polypeptide part of κ-B (17,900) from its amino-acid composition.

The N-terminal sequence of the caseino-macropeptide (derived from the C-terminal end of κ-casein) was recently given by DELFOUR et al. (1966). Its amino-acid composition has been determined by several authors, JOLLES et al. (1961), KALAN and WOYCHIK (1965), KONING et al. (1966). From the results of these authors and the C-terminal analysis presented here, one can state that neither phosphoric acid nor carbohydrates are attached to either its N-terminal or C-terminal sequence. In addition carbohydrates are not linked to aspartic acid since the N and C-terminal sequences account for all the Asp residues.

Table I

	A_1							A_2						
aa	5'	15'	30'	1h	2h	24h	Entier	5'	15'	30'	1h	2h	24h	Entier
Thre		0.33		1.50		2.86	3		0.286	0.79	1.64		2.86	3
Ser		0.20		0.73		1.06	1		0.07	0.36	0.715		1.00	1
AspN+GluN				0.87		1.66	2			0.286	0.715		1.72	2
Ala	0.53	0.80		0.80		1.00	1	0.43	0.86	0.86	1.00		1.00	1
Val	0.80	1.00		2.06		2.60	3	0.86	0.93	1.43	2.14		2.93	3
Ileu						0.94	1				0.05		1.00	1

	A_3							A_5						
aa	5'	15'	30'	1h	2h	24h	Entier	5'	15'	30'	1h	2h	24h	Entier
Thre			0.975	1.95	2.65	2.92	3				1.74	2.75	2.84	3
Ser			0.044	0.89	1.06	0.975	1				1.01	1.28	1.01	1
AspN+GluN			0.354	0.89	1.59	1.33	2				1.29	1.75	1.56	2
Ala	0.62		1.06	1.06	1.06	1.06	1	0.55			1.10	1.19	0.92	1
Val	1.06		1.59	2.48	2.92	2.84	3	1.01			2.66	3.12	3.2	3
Ileu				0.141	0.71	0.975	1				traces	0.73	1.01	1

	A_4							A_6						
aa	5'	15'	30'	1h	2h	24h	Entier	5'	15'	30'	1h	2h	24h	Entier
Thre		0.25	0.75	0.67	2.50	3.00	3		0.256	0.85	1.54	2.40	2.74	3
Ser	0.083	0.33	0.67	1.00	1.33	1.50	1-2			0.43	0.77	1.02	1.11	1
AspN+GluN				1.67	1.25	1.75	2		0.17	0.256	0.77	1.54	1.63	2
Ala	0.415	1.08	1.08	1.17	1.08	1.25	1	0.43	0.77	0.77	0.77	0.77	1.11	1
Val	1.00	1.17	1.58	2.41	2.92	3.08	3	0.85	1.02	1.45	2.05	2.57	3.07	3
Ileu					0.58	1.00	1				0.17	0.685	0.94	1
Leu*		0.167	0.25	0.33	0.415	0.415	<1							

	A_7						
aa	5'	15'	30'	1h	2h	24h	Entier
Thre	0.13	0.57	1.45	2.42	2.73	2.78	3
Ser	0.026	0.22	0.66	0.97	1.01	1.01	1
AspN+GluN		0.13	0.57	1.23	1.45	1.54	2
Ala	0.705	0.89	1.01	1.06	0.97	1.01	1
Val	0.80	1.36	2.30	2.86	2.86	2.91	3
Ileu			0.044	0.485	0.925	0.97	1

Moles of aminoacids released from one mole of each κ-casein A fraction by carboxypeptidase A.

The integers in the column "entier" are the proposed numbers of C-terminal amino acid residues for the seven fractions.

* Leucine and an abnormally high amount of serine could be detected only in A_4. In this fraction a fairly high amount of paracasein could be seen by SGE. Degradation involving up to 40-50% of chains may have occurred in this fraction at the particularly fragile point of rennin attack.

Table 2

aa	15'	30'	1 h	1.30 h	2 h	2.30 h	3.30 h	4.30 h	5.30 h
Asp	0.01	0.03	0.06	0.08	0.16	0.26	0.46	0.60	0.66
Thr	0.02	0.07	0.39	0.77	0.99	1.27	1.56	1.67	1.73
Ser	0.06	0.09	0.30	0.51	0.63	0.73	0.80	0.82	0.82
Glu	0.02	0.06	0.19	0.39	0.55	0.68	0.77	0.80	0.81
Ala	0.10	0.30	0.57	0.65	0.67	0.70	0.71	0.72	0.72
Val	0.14	0.32	0.58	0.98	1.30	1.52	1.66	1.69	1.69
Ile					0.04	0.13	0.33	0.47	0.59

Release of amino acids (μ moles) by CPA from ca. 15 mg of A_1 (40° C, in 0.04 M ammonium bicarbonate buffer pH 7.7, E/S = 1/125 w/w). The amino acid analyses were carried out after hydrolysis of the material released by CPA.

From these data it appears that κ-casein, previously defined either as a casein fraction able to protect α_s and β-caseins against precipitation by calcium, or as the rennin sensitive casein, can be characterized as a polypeptide chain produced by the locus κ-Cn.

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