Amino Acid Composition of Chromatographically Separated β-Casein A^3

F. C. KOPFLER [1] and R. F. PETERSON
Eastern Utilization Research and Development Division, USDA
Philadelphia, Pennsylvania 19118
and
C. A. KIDDY
Dairy Cattle Research Branch, Animal Husbandry Research Division, USDA
Beltsville, Maryland 20705

Abstract

Polyacrylamide gel electrophoresis or starch gel electrophoresis at pH 3 demonstrates that the β-casein type A, as classified at pH 9, actually consists of three species, A^1, A^2, and A^3. Chromatography on cellulose phosphate has been used to separate an A^3/B β-casein into its constituents. Amino acid analyses and end group analyses of β-caseins A^3 were compared with those of the other genetic variants, A^1 and A^2 β-caseins. The three variants, A^1, A^2, and A^3, have 6, 5, and 4 histidine residues per molecule of ~24,000 Daltons. The analytical data indicate a substitution of proline for histidine.

Introduction

By typing caseins with polyacrylamide gel electrophoresis at pH 3, Peterson and Kopfler (5) demonstrated the existence of three distinct genetic variants of β-casein A, all previously typed as a single variant, A, by polyacrylamide gel electrophoresis at pH 9.2.

At that time they proposed renaming the β-caseins in order of decreasing mobility at pH 3 in acid polyacrylamide gels. According to this system, β-casein C was renamed A, β-casein B remained B, whereas the three types of β-casein A became C, D, and E in order of decreasing mobility.

It has since been decided to retain the original nomenclature and to designate the β-casein variants by superscripts, as A^1, A^2, and A^3 in order of decreasing mobility at pH 3. Peterson, Nauman, and Hamilton (6) reported the amino acid composition of β-caseins A^1 and A^2, and included a table clarifying the system of nomenclature of β-casein.

No cow homozygous for β-casein A^3 has been found by us; only 1.8% of the milk types have been shown to be β-casein A^3 (3). The milk of one cow has been found to contain β-caseins A^3 and B. This paper reports a method for the separation of β-casein A^3 (from B) from the casein of this cow, and its amino acid analysis after purification.

Methods and Materials

Fresh skimmilk containing β-casein typed A^3/B, from an individual cow, was adjusted to pH 4.6 with N HCl where the casein precipitates. β-Casein was prepared by the method of Aschaffenburg (1) and was solvent dried by successive washings with ethanol, acetone, and ether.

β-Casein prepared in this manner was found to contain a small amount of α_{s1}-casein and several slower-moving components in gel electrophoresis at pH 9.2.

Separation of β-caseins A^3 and B was accomplished by chromatography on phosphocellulose (Whatman P-70)[2] having a maximum capacity of 7.4 meq per gram. The phosphocellulose was suspended in water and decanted to remove fines and was recycled by suspending in 0.1 N NaOH, washed on a sintered glass filter with water, suspended in 0.1 N HCl, and washed free of acid. After several regeneration cycles, the phosphocellulose was suspended in 0.05 M sodium acetate–acetic acid buffer, pH 5.0; the pH of the slurry was adjusted to 5.0 with N NaOH, and the buffer was removed by suction on a glass Büchner funnel. The filter cake was washed with several volumes of buffer and finally suspended in the buffer.

A column 2 cm in diameter was poured to a depth of 25 cm under 0.35 kg/cm² air pressure. The column was equilibrated with several volumes of 0.05 M sodium-acetate (3.3 M urea) buffer (the starting buffer for chromatography).

Received for publication April 17, 1969.

[1] National Research Council–Agricultural Research Service Postdoctoral Research Associate. Present address: Gulf Coast Marine Health Sciences Laboratory, U.S. Public Health Service, Dauphin Island, Alabama 36528.

[2] Mention of commercial products does not constitute a recommendation by the U.S. Department of Agriculture over similar products not mentioned.

TABLE 1. Amino acid composition of β-casein residues of amino acid per mole (24,100).

	A^1	A^{2-1}	A^{2-2}	A^{2-3}	A^3
	His_6 Asp_9 Glu_{40}	His_5 Asp_{10} Glu_{39}	His_5 Asp_{10} Glu_{38}	His_5 Asp_9 Glu_{38}	His_4 Asp_9 Glu_{41}
Lysine	10.60 ± .05	10.77 ± .14	11.05 ± .20	10.71 ± .07	10.95 ± .33
Histidine	5.87 ± .08	5.10 ± .11	5.02 ± .06	4.84 ± .15	4.20 ± .16
Ammonia	25.3	24.6	24.5	24.1	24.0[a]
Arginine	3.88 ± .04	3.98 ± .07	4.16 ± .11	3.80 ± .09	3.85 ± .16
Aspartic Acid	9.16 ± .03	9.89 ± .16	9.76 ± .33	9.21 ± .07	9.31 ± .10
Threonine	9.07 ± .07	8.78 ± .12	9.19 ± .20	9.54 ± .57	9.32[b]
Serine	14.98 ± .13	13.56 ± .72	14.72 ± .47	15.33 ± .28	13.80[b]
Glutamic Acid	39.70 ± .27	39.40 ± .40	38.00 ± .53	38.43 ± .54	41.38 ± .74
Proline	33.70 ± .13	34.64 ± .04	33.34 ± .56	35.39 ± .49	35.49 ± .57
Glycine	5.00	5.00	5.00	5.00	5.00[c]
Alanine	5.01 ± .01	5.54 ± .05	5.41 ± .00	4.95 ± .03	5.00 ± .02
Valine	18.74 ± .15	19.05 ± .64	18.32 ± .76	18.10 ± .14	19.20[d]
Methionine	5.89 ± .00	5.82 ± .08	5.78 ± .08	5.79 ± .06	5.92 ± .16
Isoleucine	9.37 ± .08	9.98 ± .03	9.50 ± .43	9.28 ± .05	9.72[d]
Leucine	21.34 ± .17	21.43 ± .13	21.34 ± .28	21.29 ± .16	22.05[d]
Tyrosine	3.88 ± .02	3.84 ± .02	4.01 ± .09	3.71 ± .13	3.90 ± .16
Phenylalanine	8.85 ± .03	8.71 ± .04	8.76 ± .09	8.71 ± .08	8.99[d]
Tryptophan	1.0	1.0	1.0	1.0	1.0
Phosphorus	4.6	4.8	4.8	4.6	4.8
Mole wt	23,836	24,006	23,471	23,635	23,945

[a] Microdetermination following Stegemann (8).
[b] Extrapolated to zero time from 24- and 72-hr values.
[c] All values slightly readjusted to glycine = 5.00 residues.
[d] Seventy-two-hour values.

A sample of β-casein A^3/B, 2 g dry weight, was dissolved in 40 ml of urea buffer and the pH adjusted to 5.0 if necessary. A small amount of insoluble material, shown by gel electrophoresis to be α_{s1}-casein, was removed by centrifugation.

The sample was applied to the column at 0.14 kg/cm^2 and the inside walls of the column rinsed down with 5 ml of buffer. An elution rate of 80 ml per hour was maintained and 10-ml fractions were collected. β-Casein B remained visibly bound to the exchanger and was eluted only by changing the buffer to one which contained 0.4 M NaCl. β-A^3, however, was not retained by the absorbant and was collected in the void volume (Fig. 1). The eluant, containing the β-casein A^3, was thoroughly dialyzed against several changes of distilled water at 4 C. Removal of urea by dialysis at pH 5 caused the β-casein to precipitate in the dialysis sack. The precipitate was removed from the dialysis sack, centrifuged, washed with water, alcohol, acetone, and ether and finally air-dried.

Gel electrophoresis at both acid and alkaline pH showed that the β-casein A^3 was completely separated from β-casein B (Fig. 2). However, several minor bands moving more slowly than the β-casein in both the pH 3 and pH 9 dyed gels were part of the A^3 fraction. Chromatography on DEAE cellulose with 0.01 M imidazole-HCl buffer, pH 7.0, 3.3 M urea containing 2-mercaptoethanol (10), removed the contaminants from the β-casein A^3.

Amino acid analyses were performed by the automatic method of Spackman, Stein and Moore (7), using a Phoenix Precision Instrument Company K-5000 amino acid analyzer.

Amide nitrogen was determined by the method of Stegemann (8), which involves the diffusion of ammonia from an alkaline solution of the protein at room temperature into dilute sulfuric acid and nesslerization of the solution in the same flask. Phosphorus determinations were made by the method of Morrison (4).

The C-terminal amino acids of the β-casein A^3 were determined by the hydrolysis with carboxypeptidase-A followed by amino acid analyses of the trichloroacetic acid supernatants (2). The N-terminal amino acid was identified by labelling the β-casein with 2-dimethylamino-

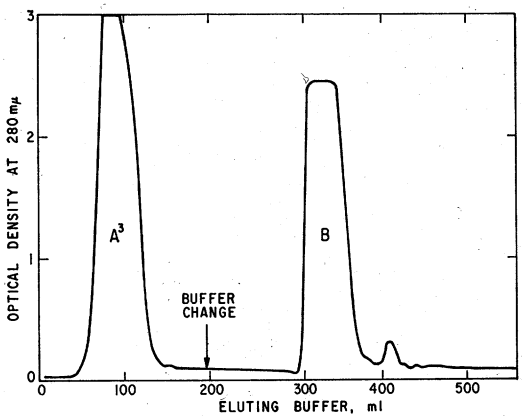

FIG. 1. Chromatography on cellulose phosphate in pH 5.0, 0.5 M sodium acetate–acetic acid buffer containing 3.3 M urea. At arrow buffer was changed to one of similar composition but 0.4 M in sodium chloride. Electrophoresis at pH 3.

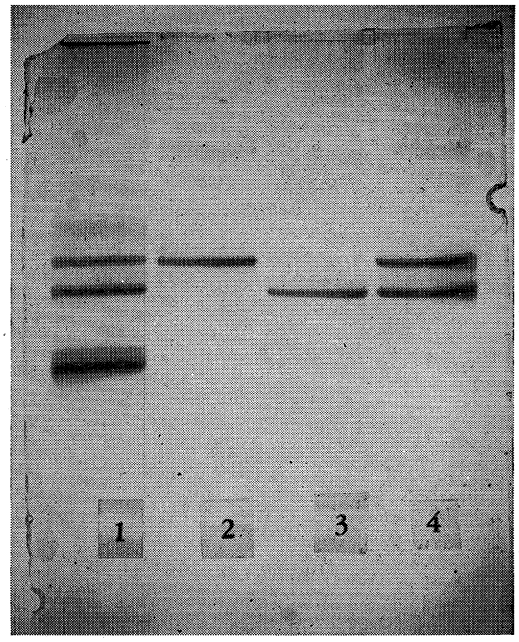

FIG. 2. Acrylamide gel electrophoresis of 1, whole casein; 4, β-casein A^3/B separated by urea fractionation; 2, β-casein A^3 from breakthrough peak; 3, β-casein B eluted with 0.4 M sodium chloride. Electrophoresis at pH 3.

naphthalene 5-sulfonyl chloride and identified by chromatography on polyamide sheets following the procedure of Woods and Wang (11). Tryptophan was determined by the procedure of Spies (9) using Method W.

Results

The amino acid analyses are summarized in Table 1. Here four other β-caseins A^1 and $A^{2\text{-}1,2,3}$ (6) are added to show the close resemblance among the β-caseins typed A by alkaline electrophoresis. The three β-caseins contain 6, 5, and 4 histidine residues per molecule of about 24,000 Daltons, the presence of which reflects their electrophoretic mobilities at pH 3. Variations in the other amino acids such as glutamic acid and aspartic acid are not sufficiently significant to produce discernible electrophoretic differences at alkaline pH. The proline variation, 34, 35, and 36 for A^1, A^2, and A^3 probably reflects a substitution of histidine for proline. Possible coding triplets for the histidine-proline substitution are: CA-U/C, CC-U/C. The site of the A^1-A^2 histidine-proline substitution was found to be located in a peptide [6,800 Daltons, (6)] obtained by trypsin hydrolysis of the protein. Tryptic peptide maps show that all three types of β-casein are very similar. Minor differences in the tryptic peptide maps of the A^2 types have been confirmed by amino acid analyses of these types in other laboratories.

The C-terminal sequences for all three β-caseins, A^1, A^2, and A^3 are identical, Ile, Ile, Val-COOH, which agrees with the studies of Kalan et al. (2) on alkaline typed β-casein A and β-caseins B and C. The N-terminal of β-casein A^3 is arginine, and the dansylation procedure gives the same results for β-caseins A^1 and A^2. N-terminal group analysis by difference on the amino acid analyzer for β-casein A^2 shows that one of the arginine residues per 24,000 (of the four present) does not appear in the chromatogram of the basic amino acids on the short column. This would appear to exclude the possibility that the production of isoleucine and valine was from separate chains (2). Both DNP-arginine and Dansyl-arginine are sufficiently resistant to acid hydrolysis that the recovery of the unmodified arginines on the short column is no greater than the expected three arginines.

References

(1) Aschaffenburg, R. 1963. Preparation of β-casein by a modified urea fractionation method. J. Dairy Res., 30: 259.

(2) Kalan, E. B., M. P. Thompson, R. Greenberg, and L. Pepper. 1965. Genetic polymorphism in caseins of cow's milk. V. End-group analyses of β-caseins A, B, and C. J. Dairy Sci., 48: 884.

(3) Kiddy, C. A., R. F. Peterson, and F. C. Kopfler. 1966. Genetic control of the variants of β-casein A. J. Dairy Sci., 49: 742.

(4) Morrison, W. R. 1964. A fast, simple and reliable method for the micro-determination of phosphorus in biological materials. Anal. Biochem., 7: 218.

(5) Peterson, R. F., and F. C. Kopfler. 1966. Detection of new types of β-casein by polyacrylamide gel electrophoresis at acid pH: A proposed nomenclature. Biochem. Biophys. Res. Commun., 22: 388.

(6) Peterson, R. F., L. W. Nauman, and D. F. Hamilton. 1966. Amino acid composition of six distinct types of β-casein. J. Dairy Sci., 49: 601.

(7) Spackman, D. H., W. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem., 30: 1190.

(8) Spies, J. R. 1967. Determination of tryptophan in proteins. Anal. Chem., 39: 1412.

(9) Stegemann, H. 1958. Eine Mikrobestimmung von amid-stickstoff speziell in Proteinen. Z. Physiol. Chem., 312: 255.

(10) Thompson, M. P. 1966. DEAE-cellulose-urea chromatography of casein in the presence of 2-mercaptoethanol. J. Dairy Sci., 49: 792.

(11) Woods, K. R., and K. T. Wang. 1967. Separation of dansyl-amino acids by polyamide layer chromatography. Biochim. et Biophys. Acta, 133: 369.