

A convenient method is presented for obtaining the genetic variants of β -casein of high purity utilizing DEAE-cellulose chromatography in the presence of 3.3 M urea as a dissociating agent. Furthermore, the separation of heterozygous mixtures of the β -casein variants may be accomplished with column chromatography by simple manipulation of salt elution gradients. β -Caseins A, B, and C contained 15.18, 15.33, and 15.45% nitrogen, respectively, and 0.59, 0.57, and 0.50% phosphorus. The genetic forms of β -caseins may be conveniently identified by their relative electrophoretic mobilities on starch-gel and polyacrylamide-gel.

During the past seven years considerable attention has been given to the isolation and properties of α_s - and κ -caseins. β -Casein, however, which comprises at least 30% of whole casein, has been the least studied of the major caseins, although significant contributions have been made by Sullivan et al. (7), von Hippel and Waugh (12), and Payens and van Markwijk (6). It is generally agreed that β -casein, with a molecular weight of about 25,000, undergoes temperature- and concentration-dependent association.

The scope of this paper is not to report association behavior of β -caseins, although such studies are the object of current research. It is intended to report the isolation and some properties of the three genetic forms (A, B, and C) of β -casein reported by Aschaffenburg (1), and confirmed by Thompson et al. (8).

MATERIALS AND METHODS

Sources of β -casein variants. β -Casein A was obtained from the milks of individual Holstein cows, β -casein B from Jersey milks, and β -casein C from a Guernsey milk. The latter casein was a gift from Dr. R. Aschaffenburg, Reading, England. Because of the low frequency (1,8) of homozygous β -casein C, it is often necessary to begin studies on β -C from a heterozygous mixture. β -AC is sufficiently common in Guernsey and Brown Swiss breeds, so that its procurement is not especially diffi-

cult. In this paper, a method for separating a β -AC mixture is reported. This should be useful because of the infrequent occurrence of homozygous β -casein C.

Preparation of β -caseins. β -Casein fractions were prepared from acid-precipitated caseins (9) by the urea fractionation method of Hipp et al. (4). Fifty to 100 g of whole acid casein was used as starting material. Yields were comparable to those reported for this method. Crude β -caseins were used for chromatography. Nitrogen, phosphorus, zonal electrophoresis, and absorptivity (A 1%) analyses were as previously described (9).

Column chromatography. β -Casein variants (500-800 mg of protein) were chromatographed on DEAE-cellulose in a buffer system of 0.01 M imidazole-HCl and 3.3 M urea as previously described (9). The elution schedule was as follows: A Technicon Autograd² mixing chamber with nine cylinders containing 150 ml of urea buffer and 0.05, 0.07, 0.09, 0.11, 0.13, 0.15, 0.17, 0.19, and 0.21 M NaCl, respectively, was used. The elution was monitored at 280 m μ , and 10-ml aliquots were collected. Selected fractions were dialyzed urea- and salt-free, pervaporated, and either lyophilized or solvent-dried for analysis.

The separation of β -casein A from C was accomplished by chromatographing 1 g of whole casein on a 2- by 20-cm DEAE-cellulose column with the imidazole-HCl, urea buffer used above. Elution of protein was achieved with

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¹ Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

² It is not implied the U. S. Department of Agriculture recommends the above company or its products to the possible exclusion of others in the same business.

a NaCl gradient achieved with the eight cylinders of the mixing device; each cylinder contained 300 ml urea buffer and 0.00, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, and 0.30 M NaCl, respectively. The elution of protein was monitored by a U.V. analyzer, and not quantitated, as our interest was only in the separation of β -A from β -C.

RESULTS AND DISCUSSION

Chromatographic behavior. Figures 1, 2, and 3 show the elution diagrams of β -caseins A, B, and C prepared by the method of Hipp et al. (4). The vertical broken lines indicate the area from which fractions were taken for rechromatography. The diagonal broken line indicates the linear salt gradient used in this study. The salt gradients are identical in

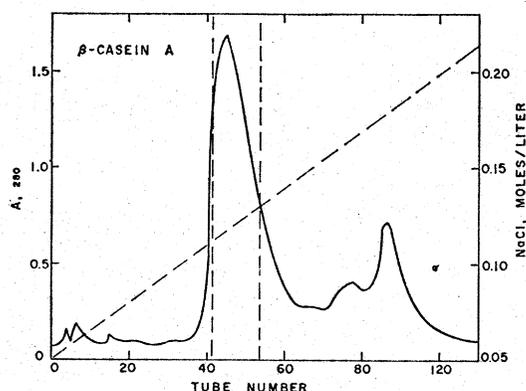


FIG. 1. Gradient elution diagram of β -casein A from DEAE-cellulose, 3.3 M urea, pH 7.0; 736-mg protein charge. Eluted protein monitored at 280 $m\mu$.

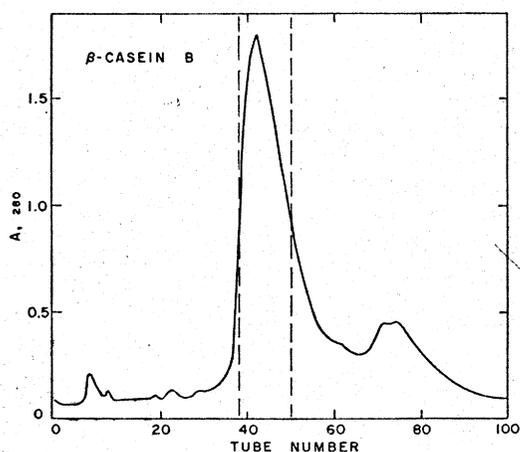


FIG. 2. Gradient elution diagram of β -casein B, from DEAE-cellulose, 3.3 M urea, pH 7.0; 736-mg protein charge. Eluted protein monitored at 280 $m\mu$.

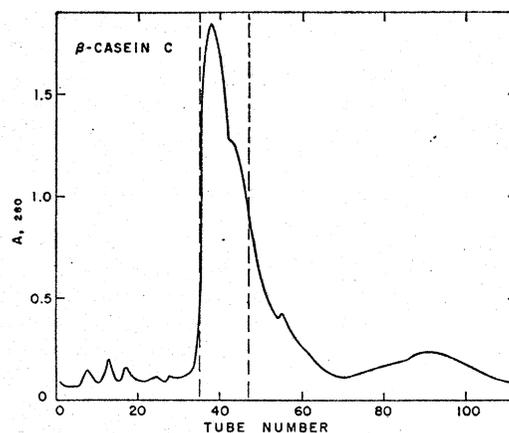


FIG. 3. Gradient elution diagram of β -casein C, from DEAE-cellulose, 3.3 M urea, pH 7.0; 736-mg protein charge. Eluted protein monitored at 280 $m\mu$.

Figures 2 and 3. With each variant some protein elutes at low ionic strength before the major peak. The casein eluted at the front (that is, at the retention volume of the column) is identical to the temperature-sensitive casein of Groves et al. (3). γ -Casein and unidentified casein components are also eluted. At higher ionic strengths beyond the β -casein, components are eluted in the α_s -casein region which do not correspond to the genetic variants of α_{s1} -casein. These components correspond to Wake and Baldwin's (13) zones 1.00 and 1.04, which are currently under investigation. Table 1 shows that β -caseins A, B, and C are eluted at ~ 0.14 , 0.12, and 0.10 M NaCl, re-

TABLE 1

Chromatographic properties of β -casein variants recovered from DEAE-cellulose columns (2 by 20 cm) at pH 7.0, imidazole-HCl-urea buffer, and relative mobilities by zonal electrophoresis

Variant	Position of elution moles NaCl/liter	% Protein in major peak	Relative mobility	
			SGE	PAE
β -A	0.14	79.8 ^{a, b}	0.80 ^c	0.65 ^f
β -B	0.12	65.2 ^{b, c}	0.76	0.61
β -C	0.10	73.0 ^{c, d}	0.70	0.54

^a Using A 1% of 4.6.

^b Excluding tailing portion of major peak.

^c Using A 1% of 4.7.

^d Using A 1% of 4.5.

^e Using zone 1.00 of Wake and Baldwin as reference (13).

^f Using corresponding SGE zone 1.00 applied to PAE.

ERRATA

Page 3, Fig. 4. Na^2EDTA should read Na_2EDTA .

Page 4, Table 2. Phosphorus. ± 0.10 value should be ± 0.0

spectively, whereas the genetic forms of α_{s1} -caseins elute at approximately 0.20 M NaCl. Recoveries of purified β -caseins in the main peak (Table 1) show that the urea fractionation method of Hipp et al. (4) gives principally β -casein, but with 25-35% impurities. This observation is consistent with the SGE patterns, published by Wake and Baldwin (13), on different β -casein preparations.

β -Casein C possesses anomalous chromatographic behavior (Figure 3). Invariably, a chromatographic shoulder, amounting to about 30% of the total peak, is observed. Rechromatography of either the main peak or the shoulder results in a bimodal elution curve suggestive of some association of β -C in 3.3 M urea; that is, monomer-polymer formation. β -Caseins A and B do not possess this characteristic.

Some preliminary observations on β -caseins prepared by the method of Aschaffenburg (2) suggest a higher degree of purity of the fraction than obtained by the above method. A sample of β -casein C supplied by Dr. Aschaffenburg, when chromatographed, revealed that about 90% of the eluted protein was in the main peak. Here, too, the anomalous chromatographic behavior was noted.

Upon rechromatography of the genetic variants only traces of contaminants were observed, and the position of elution of each variant was the same as in the initial chromatographic separation.

Figure 5 shows the results of efforts to separate β -casein A from C in a heterozygous mixture. Only the β -casein elution area of the chromatogram is presented. Obviously, a distinct separation of A from C has occurred, and the purity of each is excellent following only a single elution from DEAE-cellulose (Figure 6). Separation of β -B from β -C is accomplished with the same ease as the β -A from β -C separation. Preparative column chromatography of 2-g samples of the β -casein variants (3- by 15-cm columns and twice the elution volume used in eluting the proteins in Figures 1, 2, and 3) provided a convenient method for obtaining larger quantities of protein. These fractions were less pure than when smaller amounts of protein were applied to the column, but they were free of α_{s1} -like caseins.

Properties of β -caseins. Figure 4 shows polyacrylamide-gel electrophoresis (PAE) patterns of the purified β -casein variants after a single passage over the DEAE-cellulose column. β -Casein A is contaminated with a fraction immediately ahead of the principal zone. This contaminant is readily removed by rechroma-

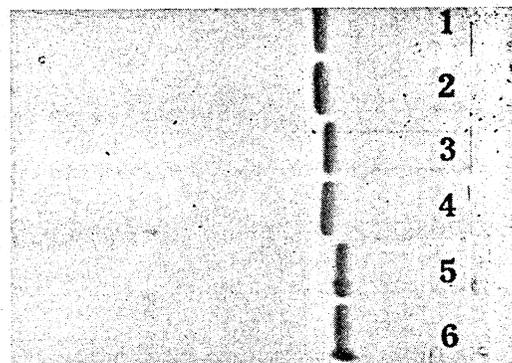


FIG. 4. Polyacrylamide-gel electrophoresis patterns (at pH 9.1-9.3, Tris-Na² EDTA-borate buffer, 4.5 M urea) of two different preparations each of column purified β -casein A (1 and 2), β -casein B (3 and 4), and β -casein C (5 and 6). 30 μ l of 0.7% protein solutions was applied to the gel.

tography. Gel electrophoresis studies at various protein concentrations have shown that (a) at low protein concentration only a single β -casein zone exists (i.e., no minor bands were masked) and (b) at high protein concentration twice-chromatographed β -caseins are free of contaminating proteins. On PAE the relative mobilities of β -caseins A, B, and C are 0.65, 0.61, and 0.54, respectively. Although these values are not the same as SGE (Table 1), the magnitude of the difference among the variants is the same and the results are reproducible.

Chemical analyses of the β -casein variants are shown in Table 2. The principal difference in the variants seems to be β -casein C where 4-g atoms of phosphorus per mole of 25,000

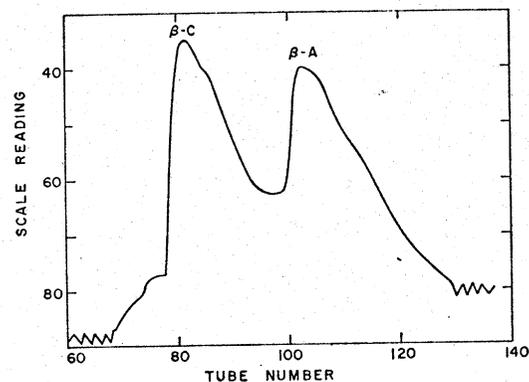


FIG. 5. Gradient elution diagram showing the separation of β -casein A from β -casein C on DEAE-cellulose, 3.3 M urea, pH 7.0. One gram of whole casein (typed β -AC, α_{s1} -BC) (see Figure 6) was applied to the column. The ordinate represents the scale reading of the U.V. analyzer chart. Only the β -casein elution area is shown.

Fraction	Reference to preparation	Nitrogen	Phosphorus	P/N ratio	A 1%
β -A	This study	15.18(\pm 0.10)	0.59(\pm 0.10)	0.0389	4.6
β -B	This study	15.33(\pm 0.10)	0.57(\pm 0.10)	0.0372	4.7
β -C	This study	15.45(\pm 0.10)	0.50(\pm 0.10) (0.52) ^a	0.0324	4.5
β -casein	(4)	15.47	0.64	0.0414
β -casein	(3)	15.35	0.48	0.0313

^a Sample supplied by R. Aschaffenburg.

molecular weight is calculated, whereas β -A and β -B contain about 5-g atoms of phosphorus using the same molecular weight. Hipp et al. (4) reported a phosphorus value of 0.64% for pooled β -casein (presumably the A variant) as compared to 0.48% by Groves et al. (3). Nitrogen analyses (Table 2) in all three studies are similar.

The absorptivities of all three β -caseins are low, as is expected from the low concentration of tyrosine and tryptophan in these proteins. Differences among the variants, however (Table 2), suggest a difference in either of these amino acids among the variants. This observation is consistent with unpublished amino acid analyses of β -caseins A, B, and C by Dr. W. G. Gordon of this laboratory. Furthermore, methionine was the only sulphur-containing amino acid present in the variants.

Nomenclature. In accord with the proposal of Aschaffenburg (1) the genetic variants of β -casein have been termed β -casein A, β -casein B, and β -casein C in order of decreasing electrophoretic mobility. For purposes of identi-

fication on SGE or PAE, the genetic form (for example, β -casein A) should be followed by the appropriate relative position on the gel. For β -casein A this would be 0.80 (SGE) and 0.65 (PAE). Thus, the complete identification of β -casein A on SGE would be: β -casein A (0.80).

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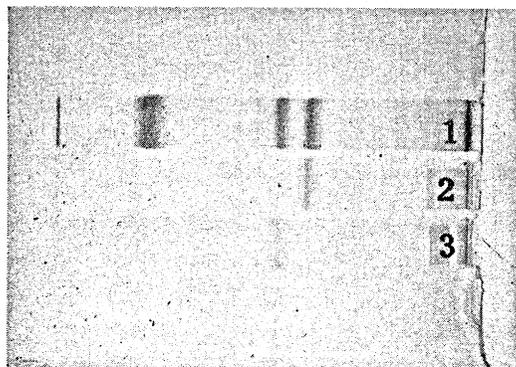


FIG. 6. Polyacrylamide-gel electrophoresis patterns, at pH 9.1-9.3, Tris Na_2EDTA -borate buffer, 4.5 M urea, of (1) whole casein typed β -AC, α_{s1} -BC, (2) β -casein C, and (3) β -casein A. Patterns 2 and 3 represent fractions eluted from DEAE-cellulose (Figure 5). Protein applied to gel as (1) 30 μ l of a 2% solution, (2 and 3) 30 μ l of a 0.4% solution.

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