

Polymorphism of γ -Casein in Cow's Milk

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γ -Casein occurs in two forms, designated A and B, as measured by disc-gel electrophoresis. The results of the typing of milk samples from 165 cows for β - and γ -casein suggest a genetic basis for the polymorphism of γ -casein and a close relationship in the synthesis of β - and γ -casein.

Genetic polymorphism has been demonstrated by zone electrophoresis for all the major caseins in cow's milk. Variation in α_{s1} -, β -, and κ -casein is controlled by three separate loci. Two or more codominant alleles are known for each system. In the α_{s1} -casein system four alleles have been found so far and these determine the occurrence of α_{s1} -casein A, B, C, and D (1, 2). In β -casein three types—A, B, and C—can be distinguished by electrophoresis at alkaline pH in the presence of urea (3, 4). Further variation in the β -casein system is revealed by electrophoresis at acid pH and the A type is subdivided into A¹, A², and A³, (5, 6). Thus, there are now five known alleles at the β -casein locus. Mobility differences in κ -casein are observed by electrophoresis of the reduced protein (7, 8). Although interpretation of the results was originally complicated by the large number of minor bands associated with κ -casein, two major types, A and B, were found. These appear to be controlled by two codominant alleles (9, 10).

Several studies indicate that the α_{s1} - and β -casein loci are located on the same chromosome and are closely linked (11, 12). There is some evidence that the κ -Cn locus is located on the same chromosome as α_{s1} - and β -Cn (9, 10). In the present work two variants of γ -casein were found which are designated A

and B. There appears to be a genetic relationship between γ -Cn and β -Cn since the A types of both occur together as do the B types.

The possibility of genetic polymorphism was previously reported for γ -casein based on paper electrophoretic determinations in which two γ -casein zones were observed (3). The amount of protein in the two zones varied considerably among samples, and with some milks the bands were absent so that interpretation of the results was difficult. Several minor proteins in the caesin fraction of milk can now be clearly resolved using gel electrophoresis with urea at alkaline pH. A number of these minor proteins, including γ -casein, are found with mobilities less than the β -casein variants.

MATERIALS AND METHODS

There are several papers on the isolation of γ -casein (13-15). In the most recent method, casein is extracted at pH 4 and 2° followed by chromatography of the extract on DEAE-cellulose at 0.005 M phosphate buffer, pH 8.3 (15). The protein fraction eluted with the front is designated "temperature sensitive" since it is soluble at 2° but precipitates at 25°. The γ -casein fraction is eluted at 0.02 M phosphate concentration. Similar fractions are also obtained when a solution of casein is chromatographed on a DEAE-cellulose column without prior fractionation of the casein.

Individual cow's milk samples for typing were obtained from the U.S. Department of Agriculture herd at Beltsville, Maryland, and from herds of

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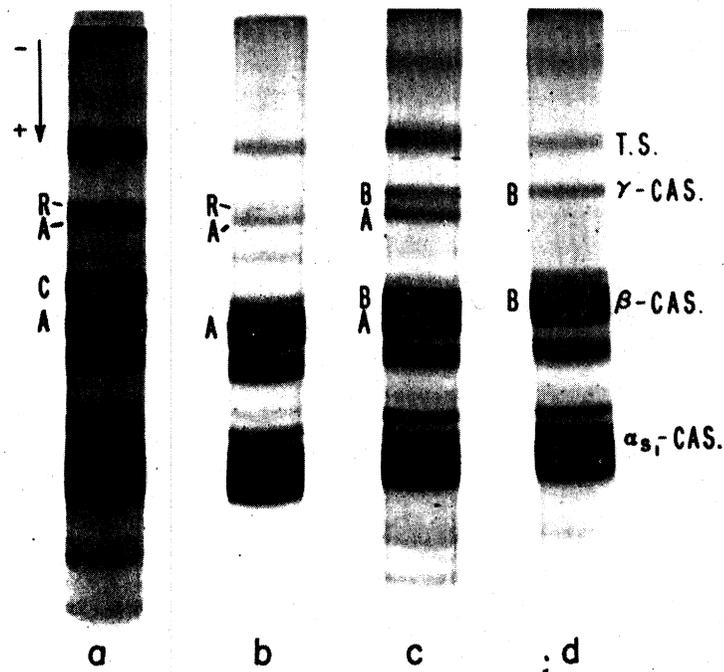


FIG. 1. Disc electrophoresis of casein samples from individual cows, run under standard conditions except that the gels contained 4 M urea: a = γ -casein A, β -casein AC, α_{s1} -casein B; b = γ -casein A, β -casein A, α_{s1} -casein B; c = γ -casein AB, β -casein AB, α_{s1} -casein B; d = γ -casein B, β -casein B, α_{s1} -casein B. The T.S.- and R-protein bands are also identified.

cooperating dairymen. Some of the samples were skim milks that were frozen and stored, while many were fresh milk samples. The casein fraction used for γ -casein typing was precipitated from skim milk at pH 4.6 and separated from the whey by centrifugation. The sedimented casein was washed, then recovered by lyophilization.

The standard disc-gel electrophoretic technique was used for typing γ -casein. Electrophoresis was carried out at alkaline pH, with a Canaleco² model 12 apparatus, using the polyacrylamide gel system of Ornstein (16) and Davis (17) except that the gels contained 4 M urea. All stock solutions and the ammonium persulfate solution were made to 4 M urea. In stock solution F the sucrose was replaced by urea. Tris buffer at the concentration required for solution A was insoluble in the presence of 4 M urea. Consequently the concentration of reagents in solution A was halved, keeping the final urea concentration at 4 molar. The formulation for the small-pore gel solution was then 4 parts A, 4 parts

² Mention of companies' or products is for the readers' convenience and does not constitute an endorsement by the U.S. Department of Agriculture.

C, and 8 parts ammonium persulfate solution. No urea was added to the electrode buffer in the reservoirs which contained 6 g Tris and 28.8 g of glycine dissolved in 2 liters of water. About 0.2 mg casein dissolved in 0.1 ml large-pore solution was used for the standard run in which electrophoresis was stopped when the marker dye reached the bottom of the columns. Individual samples showed large variation in the amount of minor proteins of the casein fraction and for some caseins larger samples were required.

RESULTS AND DISCUSSION

Figure 1 shows disc electrophoretic patterns of typical caseins from individual cows. The three γ -casein phenotypes A, AB, and B are shown in b, c, and d, respectively. The designation for the γ -casein variants follows that used for the α_{s1} - and β -caseins in which the band with the greater mobility is called A. The corresponding types of β -casein are also indicated in Fig. 1 together with the temperature-sensitive (T.S.) protein, R-protein, and α_{s1} -casein zones. In samples where

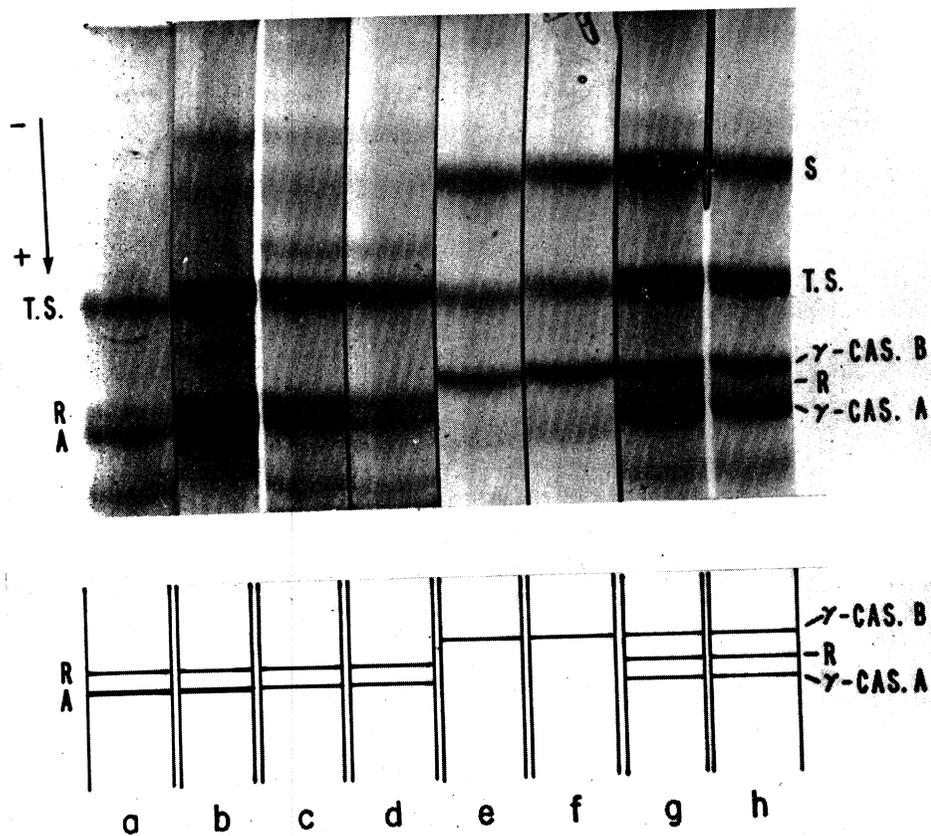


FIG. 2. Disc electrophoresis of caseins from individual cows as in Fig. 1 except the electrophoresis was extended to one-and-one-half times the standard run. a, b, c, d = γ -casein A; e, f = γ -casein B; g, h = γ -casein AB; e, f, g, h also show S-protein bands. The β -caseins (not shown) are the same types as γ -casein except with gels c and d where the β -casein is AC.

γ -casein is the A type, another band, designated R, is found with a mobility between that of the A and B bands of γ -casein (Fig. 1a and b). It does not appear to be a γ -casein since it is eluted from DEAE-cellulose with 0.005 M phosphate while the γ -caseins are eluted at 0.02 M phosphate buffer concentration. It also shows a different shade of blue relative to γ -casein when strained with amido black. Only a small amount of R-protein (Fig. 1b) is found in many of the γ -casein A samples, although with some cows it is found in an amount equal to that of γ -casein A as judged by the relative intensities of the stain. Most samples containing β -casein AC show the prominent R band besides γ -casein A, as illustrated in Fig. 1a. The presence of R-protein cannot be clearly identified in the

γ -casein AB sample when run under the standard conditions of disc electrophoresis; conditions in which most of the typing was done. Disc electrophoresis run one-and-one-half times as long as the standard run will resolve the γ -caseins A and B and the R-protein as illustrated in Figs. 2g, h and 3e. Drawings of the zones are included for each figure since photographic reproduction of some bands is difficult. The R-protein appears to be absent in samples where the γ -casein is of the B type (Figs. 1d and 2e, f). However, only a limited number of these have been found (Table I).

Casein samples containing γ -casein A alone and in combination with B (AB) have been partially fractionated on DEAE-cellulose. Figure 3 compares the electrophoretic pat-

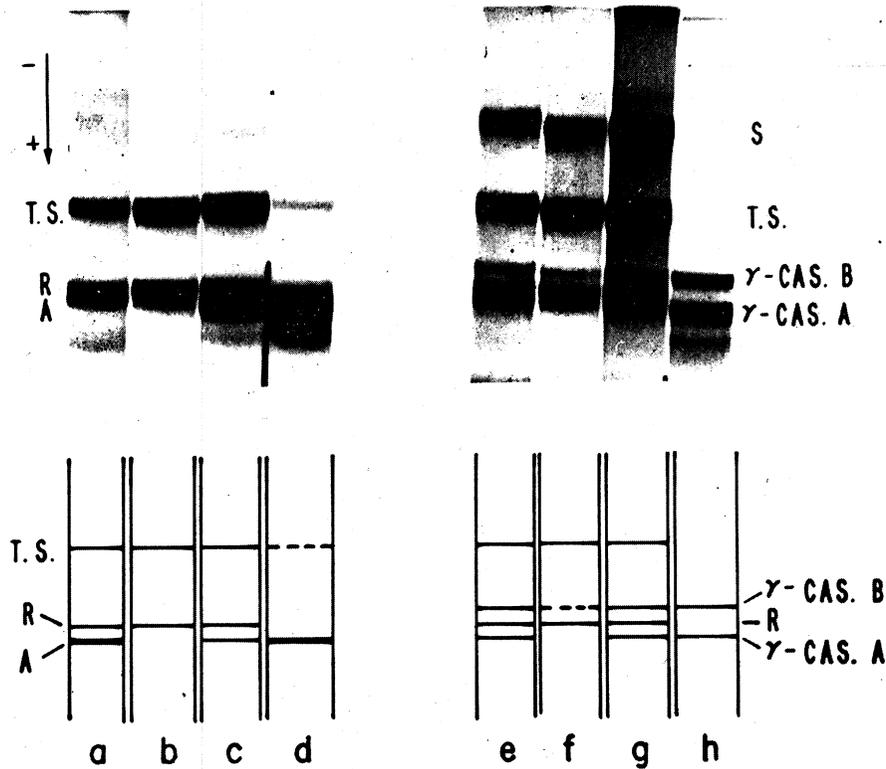


FIG. 3. Disc electrophoresis of caseins and some of their fractions after chromatography on DEAE cellulose. The faster moving β -casein and α_{s1} -casein bands are not shown. Disc electrophoretic samples were run one-and-one-half times as long as those in Fig. 1. a = casein-typed γ -casein A, β -casein A; b = temperature-sensitive fraction; c = mixture of temperature-sensitive fraction and γ -casein fraction; d = γ -casein fraction; e = casein-typed γ -casein AB, β -casein AB; f = temperature-sensitive fraction; g = mixture of temperature-sensitive and γ -casein fraction; h = γ -casein fraction. e, f, g, also show S-protein bands.

tern for the unfractionated caseins (a, e), the temperature-sensitive fractions eluted from the resin at 0.005 M phosphate buffer concentration (b, f) and the γ -casein fractions eluted at 0.02 M phosphate buffer concentration (d, h). The whole caseins containing γ -casein variants are shown in gels a (γ -casein A) and e (γ -casein AB) while b and f are the corresponding temperature-sensitive fractions, and d and h are the γ -casein fractions. Also, mixtures of the 0.005 M (temperature-sensitive) and 0.02 M (γ -casein) fractions are shown in c and g. The faster moving proteins for the casein samples, namely β -casein and α_{s1} -casein, are not shown, the latter having moved off the gels under these conditions. The γ -casein AB

samples show a prominent slow-moving band designated S, in the unfractionated casein (e) and in the temperature-sensitive fraction (f). In the γ -casein A samples (a, b) this prominent band is absent although two faint bands are observed in this area. Apparently the B and AB samples contain the protein corresponding to the S band (Fig. 2e, f, g, h) but it is either absent in the A samples (Fig. 2a, b, c, d) or found in trace amounts. The R-protein band, as already mentioned, is important because its mobility is near that of γ -casein. It is eluted in the temperature-sensitive fraction as shown in Fig. 3b and f. A small amount of γ -casein B (Fig. 3f) was also found in this fraction when the γ -casein AB sample was chromatographed.

TABLE I
PATTERN OF OCCURRENCE OF β -CASEIN AND
 γ -CASEIN TYPES IN MILK OF
INDIVIDUAL COWS

| β -casein type | γ -casein type | | |
|-------------------------------|-----------------------|----|----|
| | A | AB | B |
| A ¹ | 9 | 0 | 0 |
| A ² | 30 | 0 | 0 |
| A ¹ A ² | 26 | 0 | 0 |
| A ¹ A ³ | 1 | 0 | 0 |
| A ² A ³ | 2 | 0 | 0 |
| B | 0 | 0 | 11 |
| A ¹ B | 0 | 20 | 0 |
| A ² B | 0 | 45 | 0 |
| BC | 0 | 0 | 1 |
| A ² C | 16 | 0 | 0 |
| C ^a | 0 | 0 | 0 |

^a 2 samples.

Subsequent work indicates that the column was overloaded in this experiment. The light band which runs ahead of γ -casein A (Fig. 3a) is unidentified. The wideness of the band in pattern d is probably not due to the presence of this fast component, but rather to an overloading effect. Studies on the isolation of γ -casein A and B from milk of the respective types are continuing and will be reported later.

Milk samples from 165 cows were typed by the procedure described. Of these, 87 were γ -casein A, 65 were AB, and 13 were B. When it became apparent that γ -casein B occurred only in milks which contained the infrequent β -casein B, samples of milk from cows with the β -casein B gene were sought. Table I shows the pattern of occurrence of the two caseins in milk from individual cows. The data indicate that γ -casein B does not occur unless β -casein B is present. Although the data collected so far are not sufficient to allow analysis of segregation patterns, the association between γ -casein and β -casein indicates a genetic basis for the γ -casein variation. The locus symbol γ -Cn is tentatively proposed for use in designating the polymorphism in γ -casein. If control is by codominant autosomal alleles, as suspected, they would be designated γ -Cn^A and γ -Cn^B.

Samples from two cows homozygous for β -Cn^c were available and they showed a band corresponding in mobility to the R-protein

but no other band in the area of γ -casein. Further evidence for the absence of γ -casein was obtained when the casein from a β -Cn^c cow was chromatographed on DEAE-cellulose and very little protein was eluted at 0.02 M phosphate buffer where γ -casein A and B are eluted. The finding of only γ -casein A in 16 β -casein A²C samples and only γ -casein B in one β -casein BC sample also suggests the absence of a γ -casein C. Unfortunately very few β -Cn^c samples are available.

Considering the A and B variants of both the β - and γ -casein systems it would seem that either pleiotropy or close linkage is involved. Whether one or two loci are involved, the limited data on β -casein C suggests the possibility of an allele for the absence of γ -casein. This would be analogous to the situation in some blood group systems where alleles exist which confer no antigenic activity.

Polymorphism in γ -casein has recently been reported by El-Negoumy (18). Using a γ -casein fraction prepared by a modification of Hipps method (13), he finds by zone electrophoresis five bands, three of which occur in two forms.

Good resolution of the α_{s1} - and β -caseins was also obtained with the disc electrophoresis technique used in this study. Results on these proteins are not reported here since the types found agreed with those obtained with vertical slab gels.

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