PREPARATION OF *ALPHA*$_{S1}$- AND *BETA*-CASEIN RELEASED DURING PREPARATION OF *KAPPA*-CASEIN FROM BUFFALO MILK

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(Received for publication August 2, 1971)

ABSTRACT

Methods were modified to improve preparation of α_{s1}- and β-casein from the precipitate obtained when *k*-casein was prepared by Hill's method. A concentration of 3.3 *M* urea was used instead of 4.6 *M* urea to precipitate all the α_{s1}-casein. β-Casein was precipitated from the second-cycle casein fraction P at pH 4.9 as described by others, the gummy precipitate was washed with alcohol, and then air dried. Starch-gel electrophoresis showed that α_{s1}- and β-casein prepared by this method followed by further purification through redissolving and reprecipitating, was essentially free from specific fractions.

Fractionation of casein had to be repeated with buffalo milk and new techniques developed to obtain individual casein components. Previously methods to prepare α_{s1}- and β-caseins were given by Warner *(12)*, Hipp et al. *(4)*, and von Hippel and Waugh *(10)*. These methods formed the basis for further modifications by Payens *(6)*. Schmidt and Payens *(8)* prepared α_{s1}-casein by using a combination of procedures described by Waugh and von Hippel *(13)* and Zittle and Custer *(14)*. They found by addition of Ca^{++} to the α-casein solution, two fractions resulted which appeared to be highly heterogeneous when tested by starch gel electrophoresis. Melnychyn and Wolcott *(5)* found that addition of polyphosphate to milk retarded or reversed the migration of *k*-casein and this reaction facilitated resolution of α_{s1}-casein in a high state of purity. Aschaffenburg *(1)* modified the method of Hipp et al. *(4)* for preparing β-casein.

In the present work a combination of methods has been used to prepare α_{s1}-casein and β-casein released when *k*-casein was prepared from buffalo milk by Hill's method.

MATERIALS AND METHODS

Preparation of α_{s1}- and β-casein

Whole casein was precipitated from 2 liters of skimmilk with 1 N HCl at pH 4.6, the precipitate was filtered through a Buchner funnel, washed with warm water, re-dispersed with the aid of 1 N NaOH, and precipitated again at pH 4.6. The precipitate was used for preparing *K*-casein by Hill's method *(3)*. The precipitate (second cycle Ca-caseinate) remaining after preparation of *k*-casein was used to prepare α_{s1}- and β-casein. It was suspended in water and Ca^{++} was removed from the suspension by addition of 200 ml of 1.5 *M* potassium oxalate and 0.2 *M* oxalic acid to maintain the pH at 7.0. The calcium oxalate was removed by centrifugation and filtration. The filtrate (second cycle casein fraction P) contained a_s- and β-casein which were precipitated by reducing the pH to 4.6, filtered off, dispersed in water, and 6.6 *M* urea was added to give a final concentration of 4.6 *M* urea. An oily precipitate was formed which consisted mainly of crude Ca-sensitive α_s-casein. The supernatant containing the β-casein was filtered through Whatman No. 1 filter paper, the pH of the filtrate adjusted to 4.9, and the β-casein precipitated by the method of Aschaffenburg *(1)*. The oily floccules of β-casein were then centrifuged and further purified by dispersion in water, addition of NaOH, and re-precipitation at pH 4.9. The crude Ca-sensitive (α_s-casein) was washed with 4.6 *M* urea, and dissolved again in 6.6 *M* urea solution with 2.12 g NaCl added per 100 ml. It was re-precipitated by diluting to 3.3 *M* urea to obtain α_{s1}-casein. Both α_{s1}- and β-casein precipitates were washed with alcohol and ether, dried in air, and finally crushed to a fine powder.

Starch-gel-electrophoresis

The procedure of Wake and Baldwin *(11)* and a modification of Schmidt's *(7)* method for using 2-mercaptoethanol were used to prepare the gel. A further modification was made in the present work, in which 13% starch hydrolysates were used instead of 12.2% since the 2-mercaptoethanol had a weakening action on the gel strength.

Application of the sample

The technique of Ganguli and Majumder *(2)* was used in which a solution of sample in urea was prepared according to the procedure of Aschaffenburg *(1)*. Strips of Whatman 3 mm wide filter paper 10 mm long were impregnated with pro-tem solution and then inserted into the gel bed.

Buffer systems

A discontinuous buffer system was used. The tris-citrate-urea buffer at pH 8.6, containing 0.02 *M* 2-mercaptoethanol was used to prepare the starch gel. The buffer system of 0.3 *M* boric acid titrated to pH 8.6 with NaOH was used for the electrode vessels.

Electrophoretic test

To allow solubilization of proteins into the gel bed, the electrophoresis was started 30 min after insertion of strips. Electrophoretic tests were carried out using a constant voltage of 200 for 16 hr at room temperature.

Staining and washing

The procedure described by Smithies *(9)*, was used.

RESULTS AND DISCUSSION

The method of preparing α_{s1}- and β-casein described in this paper depends on the nearly complete removal of *k*-casein and the use of the precipitate (second cycle Ca-caseinate). Since *k*-casein is insensitive to

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Ca ion concentration, a high concentration of $CaCl_2$ (4 *M*) can be used, as described by Hill *(3)*, for complete precipitation of the second cycle Ca-caseinate and to be sure that all *k*-casein remains in the supernatant. Moreover, the crude *k*-casein was centrifuged at high speed so that the recovery of α_s- and β-caseins is as complete as possible. The removal of Ca^{++} from the precipitate of second cycle Ca-caseinate gave a supernatant, second cycle casein fraction P, as described by Waugh and von Hippel *(13)*. For this reason, potassium oxalate in sufficient amount was used to precipitate all the Ca^{++}, and the calcium oxalate was removed by centrifugation. The supernatant was dialysed against distilled water to insure removal of excess potassium oxalate. As shown by Hipp et al. *(4)*, the property of differential solubility in urea of the casein components can be utilized for preparative purposes. Meanwhile, fractionation is achieved by step-wise dilution of a solution of all casein components. Therefore, by using this method for second cycle casein fraction P, with some modifications as reported in the present work, more effective separation of α_{s1}- and β-casein can be obtained. A concentration of 4.6 *M* urea has been used for precipitating all α_s-casein from the second cycle casein fraction P, and the β-casein remains in the supernatant of 4.6 *M* urea. The α_s-casein was redissolved in 6.6 *M* urea and diluted to 3.3 *M* urea instead of 4.6 *M* urea, with the addition of 2.12% sodium chloride solution, to precipitate all the α_{s1}-casein. Using 3.3 *M* urea improved precipitation of α_{s1}-casein over the original method of Hipp et al. *(4)*. The supernatant of 4.6 *M* urea with the β-casein, was filtered, and the pH of the filtrate adjusted to 4.9, to precipitate the β-casein as described by Aschaffenburg *(1)*. Figure 1 shows the electrophoretic densitometer tracing of α_{s1}- and β-casein samples. The bands have been numbered as to their relative position in the gel. Distance from the starting slot to front has been set at 1.00. Figure 1-a shows the peak in the position of 0.8 to 0.9 corresponding mainly to α_{s1}-casein, and very minor peaks in the position of 0.03 to 0.24 corresponding to *k*-casein. At the same time a very faint band appeared in the position of β-casein. Figure 1-b shows the peak in the position of 0.47 to 0.55 which corresponds to β-casein, and slower moving material which corresponds to *k*-casein was noted in the position of 0.13, 0.19, and 0.26.

Generally, starch-gel electrophoresis showed that α_{s1}- and β-casein prepared by this method, and after further purification by redissolving and reprecipitating, to be free from major impurities.

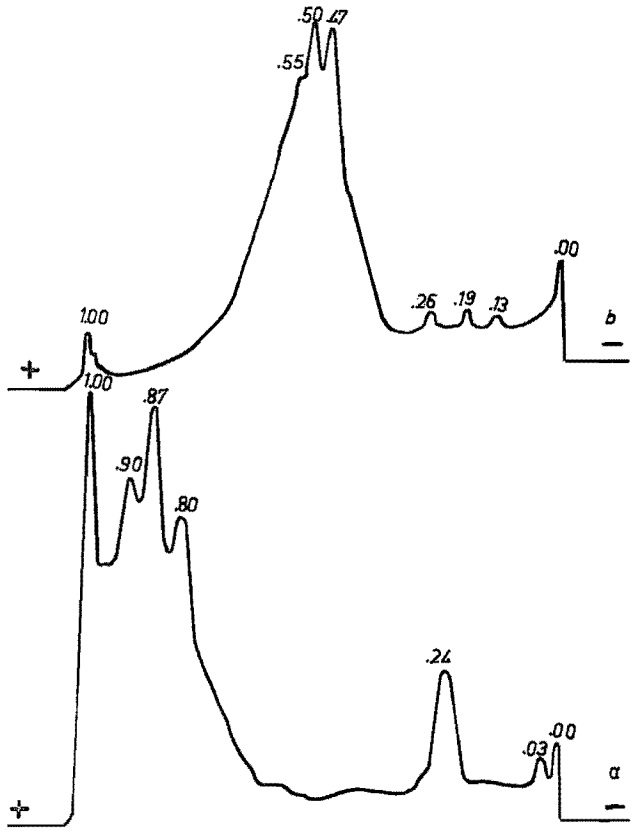

Figure 1. Densitometric tracing of electrophoretic patterns of α_{s1}-casein and β-casein. Starch gel electrophoresis conditions: 0.76 *M* Tris-citrate buffer pH 8.6; 7 *M* urea; 0.02 *M* 2-mercaptoethanol. a: α_{s1}-casein; b: β-casein.

ACKNOWLEDGMENT

The first author is grateful to the Council of Scientific and Industrial Research for awarding a Fellowship. Thanks also go to the National Dairy Research Institute, Bangalore, India, for providing facilities for this investigation.

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ASSOCIATION AFFAIRS

ANNOUNCEMENT CONCERNING THE SANITARIANS AWARD FOR 1972

Announcement is made that nominations will be accepted for the annual Sanitarians Award until June 1, 1972, and the members of the International Association of Milk, Food and Environmental Sanitarians, Inc. are requested to give consideration to the nomination of individuals whose professional work in the field of milk, food, or environmental sanitation has been outstanding.

The Award consists of a Certificate of Citation and $1,000 in cash, and is sponsored jointly by the Diversey Chemical Corporation, Klenzade Products, Inc., and Pennwalt Corporation. It is administered by the International Association of Milk, Food and Environmental Sanitarians, Inc., and is presented annually. The next presentation of the Sanitarians Award will be made at the 59th annual meeting of the Association which is to be held at Milwaukee, Wisconsin, in August 1972.

The Executive Board of the Association has established the following rules and procedures governing the Sanitarians Award.

Eligibility:

1. *General Criteria*

To be eligible for nomination the Sanitarians Award offered annually by the International Association of Milk, Food and Environmental Sanitarians, candidates must:

a. Have been a member of IAMFES in good standing for a period of five years prior to the date when the Award is to be presented;
b. Be a living citizen of the United States or Canada who, at the time of nomination, is employed as a professional sanitarian in the field of milk, food, and/or environmental sanitation by a county, municipality, state or federal government provided that in the odd years beginning with 1969 the Sanitarians Award will be limited to state and federal employees and the even years to county and municipal employees.

Members of the Executive Board, members of the Committee on Recognition and Awards of the International Association of Milk, Food, and Environmental Sanitarians, and industry members shall not be eligible for the Award. Race, sex or age shall not enter into the selection of the Award recipient.

c. Have made a meritorious contribution in the field of milk, food or environmental sanitation, to the public health and welfare of a county, counties, district, state or federal government with the United States or Canada.
d. Have completed the achievements and contributions on which the nomination is based during the seven-year period immediately preceding January 1, of the year in which the Award is to be made.

2. *Additional Criteria*

a. Co-workers are eligible for nominations if both have contributed equally to the work on which the nomination is based and each independently meets the other qualifications for nomination.
b. Where co-workers are selected to receive the Award, each shall receive a certificate and share equally in the cash accompanying the Award.
c. No person who has received, or shared in receipt of the Award, shall be eligible for renomination for this Award.

Nominations

Nominations of candidates for the Sanitarians Award may be submitted by the Affiliate Associations of the IAMFES, or by any member of the Association in good standing except members of the Executive Board, members of the Committee on Recognition and Awards, and employees of the