Korean Dairy Technol. 1(2): 10-12, 1981

Immunochemical properties and infant allergenicities of milk.

Fumisaburo TOKITA
College of Agriculture, Shinshu
Nationl University, Japan

I wish to express my thanks to the members of the Division of Dairy Technology in the Korean Society of Animal Sciences for your invaluable present a chance to talk about our recent works on above the theme at your meeting.

As you know, it has been observed that human milk was sometime found its allergic responses to sera of human infant, bovine milk however was also found to reponses more frequently than these with human milk, and then that the responseable substances were chiefly protein in milk.

This report is a summary of our recent investigates on the antigenicity and allergenicity of bovine and human milk protein and its utilization.

1. Milk allergy

In Japan the infants with milk allergy were many time noticed as symptoms of urticaria and secondary lactose intolerance and the like, then the many infants were almost lose their weight, lack of appetite and slightly feverish. It is clearly observed to responsed reaginic antibodies(reagin) in the sera of the infants on the bovine and human milk protein, and these main components were casein, alphalactalbumin and beta-lactoglobulin by Radio Allergo Sorbent Test and its Absorption Test.

2. Immunoelectrophoretic patterns

Precipitation patterns obtained by immunoelectrophoretic analysis of bovine(BM) and human (HM) milk, and bovine(BS) and human(HS) serum diffused against various antisera in agar as shown in Fig. 1. From results 12-20 separated precipitation lines wers confirmed in bovine and human milk, and about 1/2 of the lines were crossreacted between milk and sera. It is apparently indicates from the patterns that there are at least about 20 kinds of antigenic substances in milk, and that nearly half of the substances possessed the common antigenic determinants in bovine milk and serum, and in human milk and serum. The other hand antigenic cross-reaction was slightly positive between bovine and human milk. It is an interesting bearing on the nature on a view point of species specificity of milk.

The antigenic substances in milk were almost. protein, these antigenic activety reduced due to the the denaturation and hydrolysis by way of example heat or enzyme treatment. The reduction and treatment are also intresting phenomenon as state following.

3. Casein

Casein is a most main milk protein, and its antingenicity seems simple, because of the antigenic determinants of it were mostly arranged in its primary structure which is in generally known as the sequential determinant from results of decreasing patterns of the antigenic activity by treatment of enzyme and chemical reagents.

One of the determinant in alpha-si-casein was situated in hexapeptide(thr-thr-met-pro-leu-trp-OH) which is a part of the C-terminal sequence in it.

beta-Casein in bovine and human milk are likeness in these structures each other, and the antigenicity of both also cross-reacted in some high degree. Those finding also interestings things,

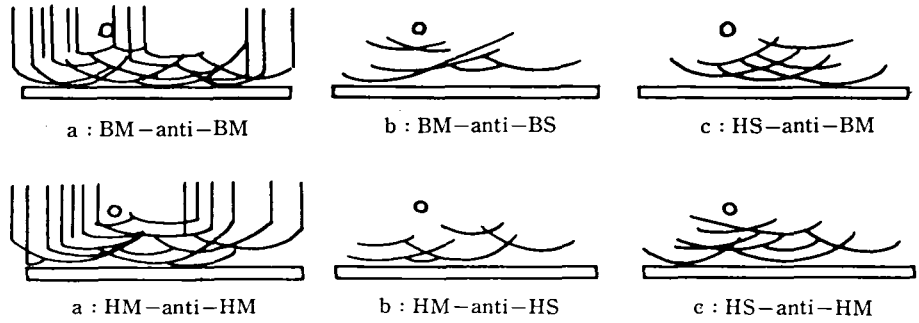

Fig. 1 Diagrams of electrophoretic in agar gel.

and further experiments on the things are being carried out.

4 . alpha-Lactalbumin

alpha-Lactalbumin was a main component in whey protein of bovine and human milk, and those all primary stuctures were determined. From the data 72% of the amino acids in the both similarity, and those situated at some position in the sequences.

It has been also known that the antigenicities of the both alpha-lactalbumin were strongly cross-reacted each other. The cross-reaction was quantitatively analyzed at the latest date by Yamauchi et al., and it is clear that 50~85% of bovine milk was arranged in both sequential and steric conformational side of the molecular.

5 . beta-Lactoglobulin

beta-Lactoglobulin similarly the alpha-lactalbmin was a main component of whey protein in bovine milk, but not it in human milk et al. Then the primary structure of the protein was already clear, and in it free cystein was uncommonly folded as known well.

The antigenicity of this bate-lactoglobulin was arranged only in steric conformational structure which is in commonly called as conformational determinant. That is a singular phenomenon, this interpretation was indicated from the reducing patterns of the antigenic activity during various treatments in our experiments.

The other hand in generally new antigenicity are formed in protein during same various treatments of it. We are called it secondary formed antigenicity. The antigenicity was sometimes formed in beta-lactoglobulin during hydrolysis with proteinase. As shown in Fig. 2 beta-lact-

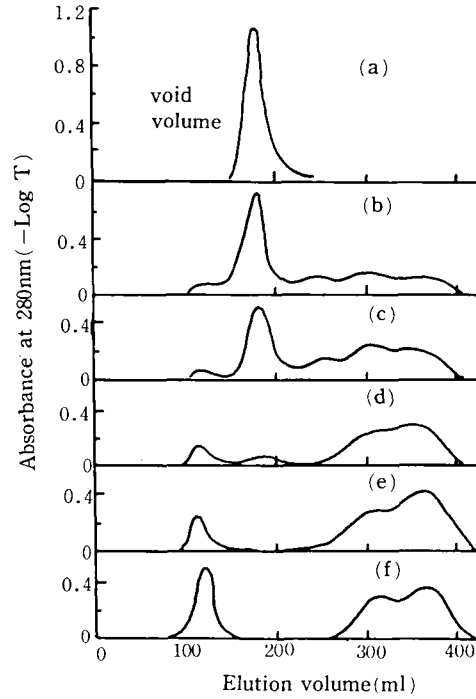

Fig. 2 Elution patterns of β−lactoglobulin hydrolyzed by trypsin on Sephadex G−100 column.
(a) Before hydrolysis. (b) Hydrolyzed for 30 min. (c) Hydrolyzed for 1 hr, (d) Hydrolyzed for 5 hrs, (e) Hydrolyzed for 8 hrs. (f) Hydrolyzed for 24 hrs.
Column : 2.7×70cm, Sample : 3ml, Flow rate : 25 ml hr,
Solvent : Mcilvaing buffer (pH 6.6)

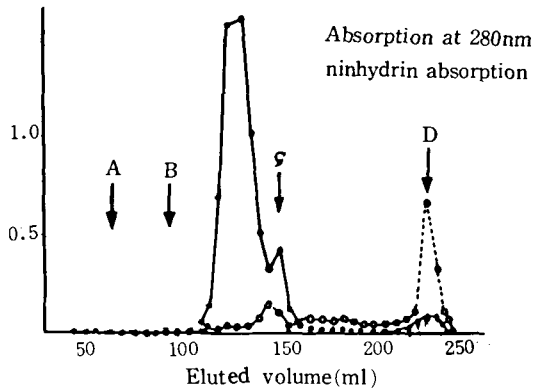

Fig. 3 Gel filtration of casein hydrolysate on Sephadex G−25 column. The amount of casein hydrolysate applied was 50mg. Arrows designate the position of markers.
(A : Blue dextran, B : oxytocin, C : tyrosine, D : tryptophan)

oglobulin was one hand hydrolyzed and the other hand polymerized during hydrolysis by trypsin The polymerized protein contained a secondary formed antigenicity in it differed from the natural antigenicity of beta-lactoglobulin. Its antigenic determinant and allergenicity is being investigated.

It is also clear from our investigation that the another secondary formed antigenicity in beta-lactoglobulin was formed by the Maillard Reaction during heat treatment, its antigenic determinant was situated at the part in bond of together protein with saccharide. Then it is a noteworthy fact that the secondary formed antigenicity was keeped a strong allergenic activity.

6 . Casein hydrolysate

It is extremely important to destroy the milk protein antigenicity for dietetic treatment of infants with milk allergy fed bovine milk formula. Then an enzymatic hydrolysate of bovine casein was studied for molecular size and antigenic properties by Takase et al.

A 10% bovine casein solution(pH 7.0) was hydrolyzed by mixture of pancreation, proteinase of Asp. oryzae and proteinase of L. helveticus at 50℃ for 24 hour. The hydrolysate was on having molecular weights greater than 1,000 2/3 of it free amino acids as shown Fig. 3, no antigenicity and no allergenicity. It is cleary testified by clinical studied in many hospitals that the special modified milk powder, which was prepared with the casein hydrolysate and another nutritive elements, for example saccharide, lipid, mineral, vitamin and others, was a useful foodstuff for the diseased infants with milk allergy.

7 . The immuno chemical studies on milk protein are now beginning, and many questions in this branch are lie in a heap. It is possible that further investigation will show that this branch also can be generalized.