BOVINE COLOSTRAL γ-GLUTAMYLTRANSFERASE; ITS LOCALIZATION IN SKIM MILK MEMBRANE AND IRRELEVANCE TO SECRETORY IMMUNOGLOBULIN A

Kyoden YASUMOTO, Kimikazu IWAMI, Toru FUSHIKI and Hisateru MITSUDA
Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606, Japan

Received 22 June 1976

1. Introduction

A significant level of γ-glutamyltransferase (EC 2.3.2.2) has been found in epithelial cells of small intestine, kidney and other glands which are relatively abundant in secretory immunoglobulin A(sIgA) [1–3]. Binkely et al. have recently reported that human breast cyst fluid and colostral milk have extremely high levels of γ-glutamyltransferase in a soluble form [4]. Their finding of the enzyme activity in such large amounts in those exudates together with their observation of the enzyme copurification with sIgA led to the suggestion that γ-glutamyltransferase is closely associated with sIgA and probably identical with its secretory component [5]. In the present communication, γ-glutamyltransferase has been obtained from bovine colostral whey by salt fractionation or alternatively by ultracentrifugation. The enzyme activity becomes separated from secretory component and immunoglobulins, and the preparation shows a close similarity with skim milk membrane preparation in the enzymatic composition.

2. Materials and methods

Fresh colostrum from Holstein cow was supplied by a local dairy farm. Fat was removed by centrifugation at 2000 × *g* for 10 min, and casein was precipitated at pH 4.6. The resulting whey was brought to neutral pH and employed as starting material in salt fractionation and ultracentrifugation. Antisera prepared in rabbits against bovine colostrum, IgA and IgM were commercially obtained (Miles Laboratories, Illinois, USA). The sensitivities of the respective antisera were in the range of 1.1 to 1.4 mg protein per ml serum against the corresponding antigen.

γ-Glutamyltransferase activity was determined by using 3.5 mM γ-glutamyl-*p*-nitroanilide and 10 mM glycylglycine in 0.25 M Tris–HCl buffer of pH 8.2, and allowing the reaction to proceed for 10 min at 37°C. Other enzymes assayed by the procedures described in the literatures include 5′-nucleotidase (EC 3.1.3.5) [6], thiol oxidase (EC 1.8.3.2) [7], xanthine oxidase (EC 1.2.3.2) [8] and thiamine pyrophosphatase (EC 3.6.1.6) [6]. Disc electrophoresis with a 7.5% polyacrylamide gel was performed according to the procedure of Davis [9] using 0.05 M Tris–glycine buffer of pH 8.6. Protein was stained with Coomassie Blue in acid methanol. The transferase activity was assayed using a duplicate gel: the gel was cut into segments of 2.5 mm in thickness; the resultant segments were crushed respectively in 0.9 ml of the standard assay medium; and then absorbancy at 410 nm was measured after incubation for 1 h at 37°C. Levels of sIgA were assayed by the single radial immunodiffusion technique [10].

3. Results and discussion

3.1. *γ-Glutamyltransferase activity in Smith's fraction*

The salt fractionation procedure described by Smith [11] was used for preparation of immunoglobulin fractions. In table 1 are given results of typical experiments for the level of γ-glutamyltransferase in the resultant fractions. The enzyme activity was

North-Holland Publishing Company – Amsterdam

Table 1
Distribution of γ-glutamyltransferase among bovine colostrum fractions

Fraction	γ-Glutamyltransferase (units/ml)	Recovery (%)
Principal fractions from colostrum		
Whole colostrum	4.00	100
Defatted colostrum	3.36	84
Casein	0.48	12
Whey	2.50	63 (100)
Smith's fractions from whey		
A (0– 50)	2.45	62 (98)
B (50–100)	0.04	1 (2)
C (0– 25)	0.20	5 (8)
D (25– 40)	1.76	44 (70)

The enzyme activities are expressed in units (μmol *p*-nitroaniline released per min) per ml of colostrum. Values given in parentheses under each Smith's fraction refer to the range of % saturation with ammonium sulfate.

almost completely recovered from the whey as Fraction A and subsequently as Fraction D.

Polyacrylamide gel electrophoresis pattern of Fraction D is shown is fig.1. A single broad band only was located on top of the gel by staining for protein. A maximal transferase activity was detected in the segments corresponding to the same position where the protein peak was located. Since Fraction D is known to be enriched in immunoglobulins apparently free of residual casein and other whey proteins [11,12], the experimental results so far described in this communication are in accord with the suggestion of Binkley and Wiesemann [5] that the enzyme is closely associated with immunoglobulins.

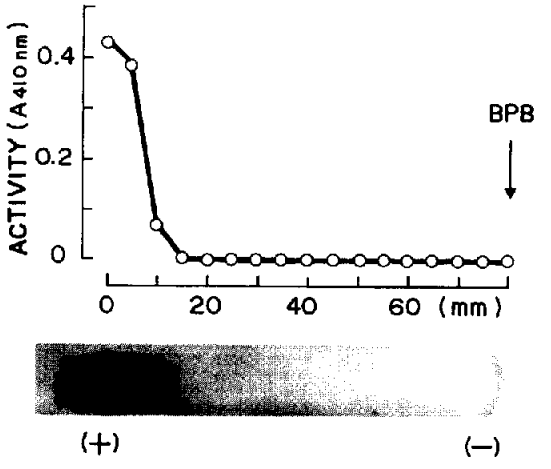

Fig.1. Polyacrylamide gel electrophoresis of Smith's Fraction D. Top curve shows γ-glutamyltransferase activity located in the duplicate gel. Bottom photograph represents the gel stained for protein with Coomassie Blue.

3.2. *Resolution of γ-glutamyltransferase from immunoglobulins and its identification as constituent of skim milk membrane.*

Ultracentrifugation of the colostral whey resulted in a quantitative precipitation and thus almost complete separation of γ-glutamyltransferase from the supernatant immunoglobulins as shown in table 2. This

Table 2
Separation by ultracentrifugation of γ-glutamyltransferase from immunoglobulins in bovine colostral whey

Fraction	γ-Glutamyltransferase (units/ml)	sIgA (%)
Primary whey	2.49	100
Supernatant	0.31	82
1st washing of pellet	0.05	17
2nd washing of pellet	0.03	1
Pellet after 2nd washing	2.08	0

Colostral whey was centrifuged at 105 000 × *g* for 1 h. The resultant pellet was resuspended in 0.1 M Tris–HCl buffer of pH 7.5, and then recovered by ultracentrifugation. Levels of sIgA were evaluated by single radial diffusion with antisera at against bovine IgA, and are expressed in per cent of total sIgA found in primary whey. The enzyme activities are expressed in terms of units per ml of primary whey.

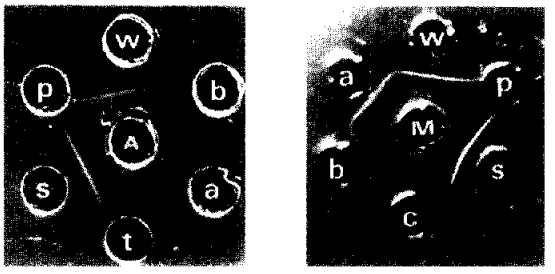

Fig.2. Immunological double diffusion in agar of colostral whey fractions using specific rabbit antiserum against IgA (A) and IgM (M). A 2 μl aliquot of antigen or antiserum was placed in respective well and allowed to stand for 48 hrs at room temperature: *w*, colostral whey; *s*, 105 000 ×*g* supernatant; *a*, 1st washing from the pellet; *b*, 2nd washing; *c*, 3rd washing; *p*, 105 000 × *g* pellet after 3rd washing; and *t*, the same pellet as in *p* but solubilized with 0.25% sodium dodecyl sulfate.

is in striking contrast to the situation found by Binkley et al. [5]. Furthermore, the transferase contained in the 105 000 × *g* pellet was hardly solubilized by means of the reduction and alkylation procedure that is used for dissociating secretory component from sIgA [12] and is thus claimed to be effective for the transferase dissociation from sIgA [5].

Figure 2 shows the results of immunodiffusion analysis of the whey and its fractions with the use of specific antisera for bovine IgA and IgM respectively. The whole whey, the 105 000 × *g* supernatant, and the washings of the 105 000 × *g* pellet respectively provided a single precipitin line with the respective antisera, but washed pellet and its sodium dodecyl sulfate-solubilized preparation gave negative reaction with both antisera. It thus follows that the transferase occurs in a fraction described earlier as 'milk microsomes' or 'lipoprotein particles' [14], while the immunoglobulins including sIgA occur essentially in colostral serum in a soluble form.

In Sepharose 4B gel filtration studies, the transferase as such occurring in whey appeared predominantly in the exclusion peak together with significant level of 5′-nucleotidase. When the 105 000 × *g* pellet from colostral whey was suspended in 0.25 M sucrose, layered on the top of the discontinuous gradient composed of 1.23, 0.635 and 0.335 M sucrose, and then centrifuged for 3 h at 51 000 × *g*, about 80% of the transferase activity originally found in the pellet was detected at 0.335–0.635 M sucrose interface and the remainings at 0.635–1.23 M interface. At 0.335–0.635 M interface was found the transferase (1.74 units/mg protein/min) to be enriched together with 5′-nucleotidase (0.19) and thiol oxidase (0.22) which are recognized as typical plasma membrane and skim milk membrane markers, respectively [6,7]. The fraction however showed very little activity of xanthine oxidase and thiamine pyrophosphatase, both being considered as characteristic markers for fat-globule membrane in bovine milk and for a Golgi apparatus in mammary gland respectively [6,7].

Overall, this investigation can be said to have established that γ-glutamyltransferase of bovine colostrum occurs essentially in skim milk membranes in a bound form, and is not associated with any class of immunoglobulins. It thus by no means follows that the transferase is identical with the secretory component of sIgA. This conclusion has received reinforcement by the latest information provided by Labib et al. that free secretory component and sIgA could be prepared free of the transferase activity [15].

References

[1] Albert, Z., Orlowska, J., Orlowski, M. and Sczewczuk, A. (1964) Acta Histochem. 18, 78–89.
[2] Ross, L., Barker, L., Tate, S. S. and Meister, A. (1973) Proc. Natl. Acad. Sci., USA 70, 2211–2214.
[3] Delap, L. W., Tate, S. S. and Meister, A. (1975) Life Sciences 16, 691–704.
[4] Binkley, F., Wiesemann, M. L., Groth, D. P. and Powell, R. W. (1975) FEBS Lett. 51, 168–170.
[5] Binkley, F. and Wiesemann, M. L. (1976) Life Sciences 17, 1359–1362.
[6] Morre, J. D. (1971) Methods in Enzymology (Jakoby, W. B. ed.), vol. 22 pp. 130–143, Academic Press, New York and London.
[7] Kitchen, B. J. (1974) Biochim. Biophys. Acta 356, 257–269.
[8] Roussos, G. G. (1967) Methods in Enzymology (Grossman, L. and Moldave, K. eds.), Vol. 12 pp. 5–16, Academic Press, New York and London.
[9] Davis, B. J. (1964) Ann. New York Acad. Sci. 121, 404–427.
[10] Mancini, G., Carbonara, O. and Heremans, J. F. (1965) Immunochemistry 2, 235–243.
[11] Smith, E. L. (1946) J. Biol. Chem. 165, 665–676.
[12] Kumar, S. and Mikolajik, E. M. (1975) J. Dairy Sci. 55, 1237–1242.
[13] Cunningham-Rundles, C. and Lamm, M .E. (1975) J. Biol. Chem. 250, 1987–1991.
[14] Bailie, M. J. and Morton, R. K. (1958) Biochem. J. 69, 35–53.
[15] Labib, R. S., Calvanico, N. and Tomasi, Jr., T. B. (1976) Fed. Proc. 35, 272.