γ-GLUTAMYL TRANSFERASE: A SECRETORY ENZYME

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1. Introduction

Many functions have been suggested for the membrane-bound γ-glutamyl transferase of renal and other transmural transport tissues. These suggested functions have included transport and secretion in general [1], peptide elongation [2], and amino acid and peptide transport [3]. Recently the suggested function in amino acid transport has been revived as part of a 'γ-glutamyl cycle' [4]. As has been pointed out, the evidence does not support such a function [5]. We have recently observed and reported* that human breast cyst fluids and colostral milk contain very high levels of the γ-glutamyl transferase and are now reporting the initial characterization of the activity in those exudates.

2. Methods and materials

Breast cyst fluids from 22 patients were collected by syringe and refrigerated until analyses. The samples were assayed for γ-glutamyl transferase [6], aminopeptidase M [7], alkaline phosphatase [8], and for adenine phosphoribosyl transferase (APRT) [9]. Activity units are μmol substrate hydrolyzed per min per liter of fluid. Diazo Blue B was used for the development of color in the first three assays. Most of the reagents were obtained from Sigma. Total protein was determined by the method of Lowry et al. [10]. Samples of human milk, synovial fluid, cerebrospinal fluid, and plasma were assayed as above. Substrate (acceptor) utilization by the transferase was determined with 0.005 M solutions of the amino acids and peptides and the activity was determined in each sample in the presence and absence of acceptors.

Electrophoretic studies were patterned after those of Fritsche and Adam-Park [8] and were at 250 V, 5–10 mA for 45 min. The transferase was located by the same reaction used in the assay of activity.

3. Results and discussion

3.1. *Enzymatic analyses*

The analyses of breast cyst fluids are summarized in table 1. All cyst fluids had a very high level of γ-glutamyl transferase (roughly comparable to that of kidney tissue per gram) and only an occasional sample had significant levels of alkaline phosphatase, aminopeptidase M or APRT. The γ-glutamyl transferase activity was not removed from the fluids by centrifugation for 1 hr at 105 000 g but was precipitated by ammonium sulfate at 50% saturation; thus it is or is associated with a soluble globulin.

Other fluids were examined for the three activities

Table 1
Enzymatic analyses of human breast cyst fluids

Activity	Positive samples	Range of activity, u/l
Transferase	22	498–6900
APRTase	5	0.004–0.016
Alkaline phosphatase	4	10–100
Aminopeptidase	1	14

Twenty-two samples were analyzed. The protein content ranged from 1.8 to 4.1 g/dl.

* Wiesemann, M. L., Binkley, F. and Groth, D. P. Proc. Southeastern Cancer Res. Assoc. 1974.

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Table 2
Enzymatic analyses of human milk

	Total protein g/dl	Aminopeptidase u/l	Alk. Phosphatase u/l	Transferase u/l
Colostral	2.2–8.5	39–1140	0–96	490–6300
10 weeks	1.17	0	0	134
20 weeks	0.8	0	0	40

Seven samples of colostral milk and single samples of post partum milk were tested.

(excluding APRT). With the methods used in these studies, plasma of patients with breast cysts had little or no γ-glutamyl transferase, aminopeptidase M, or alkaline phosphatase activities and, therefore, the activities in the cyst fluids cannot be derived from blood as a simple filtrate. Cerebrospinal fluids and synovial fluids were found to have little or no activity with the three assays. Human colostral milk was found to have similar high levels of the γ-glutamyl transferase but it also had significant levels of aminopeptidase and alkaline phosphatase activity (table 2). In a single linear study, the aminopeptidase and phosphatase activity essentially disappeared during lactation but significant transferase activity was present 20 weeks post partum.

3.2. *Specificity of acceptors*

The renal enzyme utilizes the amino acids glutamine, methionine, and arginine and various peptides as acceptors of the γ-glutamyl grouping [11]. The purified enzyme has no activity in the absence of acceptors [6] and the activity in cruder preparations is presumed to be due to a transfer to proteins. The specificity of the milk and cyst fluid enzymes appears to be distinct from that of the renal enzyme (table 3) since methionine and arginine were inactive and the highest activities were found with peptides as acceptors. The endogenous activity ranged between 10% and 25% of that with glycylglycine as the acceptor and was proportional to the amount of protein present in the diluted sample, i.e. the sample with the highest activity and requiring the most dilution had the lowest endogenous activity.

3.3. *Characterization of the transferase*

In fig. 1 the electrophoretic behavior of the colostral milk and cyst fluid transferases are compared. The cyst fluid transferase had a larger charge/mass ratio than did the milk transferase; the transferases of renal tissue are sialo glycoproteins and the electrophoretic difference here may be due to the content of sialic acids.

Table 3
Relative transferase activity with assorted acceptors

Acceptor	Fluid 1 (6838 u/l)	Fluid 2 (1170 u/l)	Fluid 3 (490 u/l)	Milk (713 u/l)
None	0.10	0.16	0.25	0.14
Glutamine	0.52	0.31	0.47	0.31
Aspgly	0.75	0.67	0.75	0.51
Leugly	0.62	0.63	0.65	0.39

Three cyst fluids and one colostral milk were tested with various potential acceptors including the following list of inactive compounds: L-methionine, L-arginine, glycyl-L-leucine, L-isoleucyl-L-leucine, L-lysyl-L-glutamic acid, and glycylglycylglycine. The cyst fluids had activities of 6830, 1170 and 490 u/l with glycylglycine as the acceptor and had a protein content of 2.5, 2.2 and 2.1 g/dl, respectively. The colostral milk had an activity of 713 u/l and a protein content of 1.8 g/dl. The activity with glycylglycine is normalized at unity.

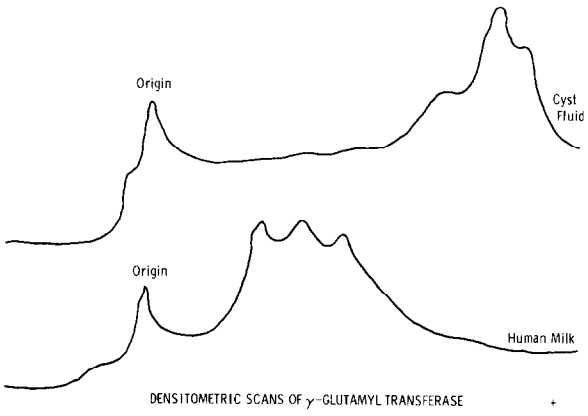

Fig. 1. Densitometric scans of electrophoresis γ-glutamyl transferases of human breast cyst fluid and colostral milk. 0.3 μl samples were electrophoresed at 250 V and 5–10 ma for 45 min at pH 8.8 on Sepraphore III cellulose acetate strips in a Beckman Microzone system. The γ-glutamyl transferase was located by incubating the strips in 1.25 mM γ-glutamyl-β-naphthylamide and 2 mM glycylglycine at pH 8.2 for 30 min at 37°C followed by a 5 min incubation in a solution of Diazo Blue B, 5 mg per ml in acetic acid and water (15:85).

The finding of the γ-glutamyl transferases in such large amounts in exudates raises the question of the function of the membrane bound transferases as found in renal, intestinal, and other transmural transport tissues. Subcellular fractionation of renal tissues has shown that the activity is not associated with alkaline phosphatase or aminopeptidase M, recognized brush border membrane markers [11], but is in smooth membrane vesicles unique to such specialized epitheleal cells. The activity may be used as a measure of the contamination of renal plasma or brush border membranes by what probably are vesicles from a specialized golgi-type organelle.** The presence of the activity in such organelles is consistent with a function in the endo- and/or exocytotic transport of proteins. A heretofore unexplainable association of the activity with glomerular basement membrane fractions possibly indicates a function in the kidney similar to that in cyst fluids and milk. †

Acknowledgements

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** Binkley, F., Mendicino, J. F. and Leibach, F. H. Unpublished observations.

† Binkley, F. Unpublished observations.