The Antiproteolytic Activity in Extracts of Human Tissue (33756)

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The presence of a heat-labile, nondialyzable trypsin inhibitor in the 90,000*g* supernatant fraction of human astrocytoma tissue was reported in 1967 (1). By virtue of its heat lability, the astrocytoma inhibitor is not identical to the basic and acidic pancreatic trypsin inhibitors (2, 3), the trypsin inhibitor secreted in the colostrum (4), and the inter-α-globulin (5) and α_2-globulin (6) trypsin inhibitors found in serum. With the availability of pathological human and adjacent normal tissue, it was possible to confirm earlier results and to extend the studies to other malignant conditions.

This communication reports the presence of nondialyzable, heat-labile, inhibitors of tryptic and chymotryptic acitvity in the 27,000*g* supernatant fraction of extracts of mixed astrocytic and oligodendrogliocytic glioma, solid and papillary adenocarcinoma of the ovary, normal breast and adjacent infiltrating duct carcinoma, and normal and adjacent neoplastic colon.

Materials and Methods. Normal and adjacent neoplastic human tissue was obtained at frozen section biopsy or immediately after surgery. The tissue was homogenized with 9 vol (w/v) of 0.32 *M* cold sucrose solution with a Virtis homogenizer for 30 sec., after which time the homogenates were centrifuged for 30 min. at 27,000*g*. The supernatant fractions obtained therefrom were stored frozen in aliquots until used. Twice crystallized trypsin (T) (Nutritional Biochemical Corporation, Cleveland, Ohio) and thrice crystallized chymotrypsin (CT) (Worthington Biochemical Corporation, Freehold, New Jersey) were taken up in 1 m*M* HCl at a concentration of 1 mg/ml as standard solutions. The standard solutions of proteases were diluted 10-fold with phosphate buffer, pH 7.6, before use in assays for proteolytic activity. To 10 μl of T or CT were added various aliquots of extracts of normal and tumor tissue and sufficient 0.1 *M* phosphate buffer, pH 7.6, to a final volume of 0.5 ml. After standing at room temperature for 10 min., 1.5 ml of 1.33% Hammersten casein (Mann Research Laboratories, Inc., New York, N. Y.) was added and the mixture was incubated at 37° in a shaking water bath for 1-hr. The reaction was stopped by the addition of 4 ml of 5% TCA. Zero time samples were similarly run. The increase in meq of tyrosine in the TCA filtrates was determined by the procedure of Lowry *et al.* (7) as a measure of proteolysis.

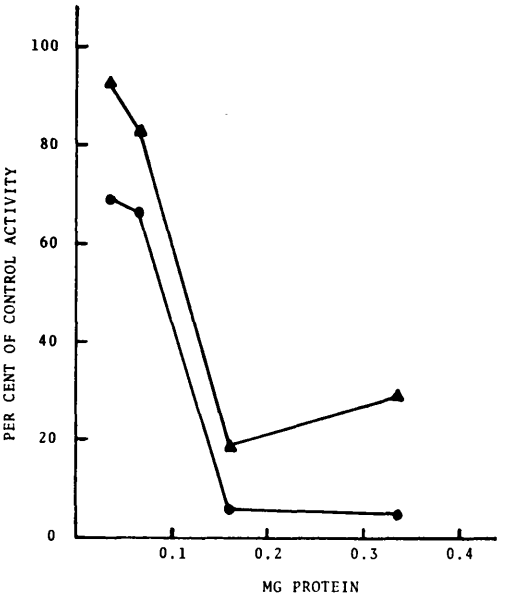

FIG. 1. Inhibition of the tryptic and chymotryptic hydrolysis of casein by the 27,000*g* supernatant fraction of mixed astrocytic and oligodendrogliocytic glioma tissue: (●), T; (▲), CT.

Results. The inhibition of the tryptic and chymotryptic hydrolysis of casein by 27,000 *g* supernatant fractions of extracts of normal and adjacent neoplastic tissue of several patients is given in Fig. 1–4. It may be seen that the extracts of the normal and malignant tissues exert a considerable inhibition of proteolytic activity against both proteases.

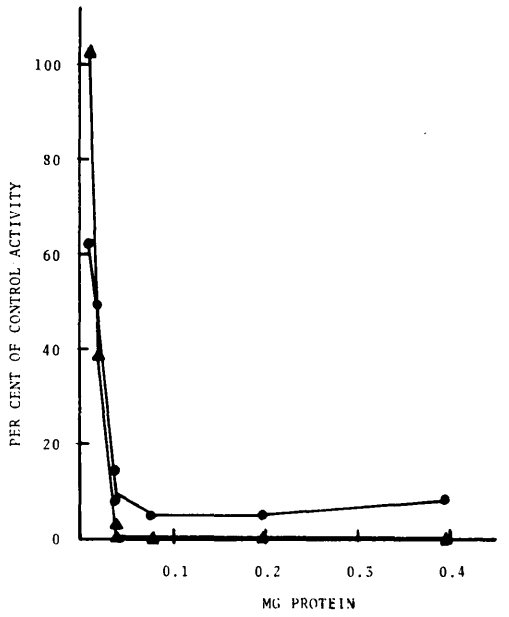

FIG. 2. Inhibition of the tryptic and chymotryptic hydrolysis of of casein by the 27,000*g* supernatant fraction of solid and papillary ovarian adenocarcinoma tissue: (●), T; (▲), CT.

The concentration of inhibitor in ovarian extracts is particularly high. Table I indicates that the inhibitors from brain, breast, ovary, and colon are essentially nondialyzable. All the extracts, with one exception (normal bowel) lose significant activity upon heating for 10 min at 60°, and lose almost all inhibitory activity upon heating for 10 min at 100° (Table II), indicating that the inhibitors from each of the organs are heat labile.

Discussion. The roles of only several of the naturally-occurring inhibitors of proteolytic activity studied to date have been recognized. Thus, the basic inhibitor of mol. wt. 6513 derived from pancreas, lung, and parotid gland serves to inhibit the activity of kallikrein and the subsequent production of the vasoactive peptides (8). The acidic inhibitor of mol. wt. 6155 found in the acinar cells of the pancreas (9), may prevent pancreatitis by inhibiting trypsin within the pancreas. The inhibitor isolated from human and other mammalian colostrum is believed to lower tryptic activity in the intestines of infants thereby permitting the absorption of undigested, active antibodies transmitted to the

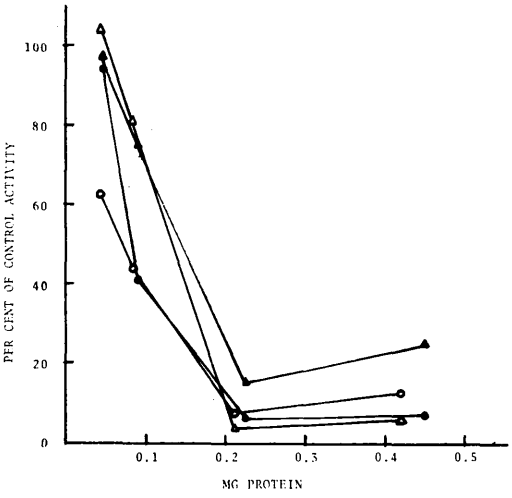

FIG. 3. Inhibition of the tryptic and chymotryptic hydrolysis of casein by the 27,000*g* supernatant fraction of normal and adjacent neoplastic colon tissue of D. F.: (○), T, normal bowel; (●), T, C.A. of the colon; (Δ), CT, normal bowel; (▲), CT, C.A. of the colon.

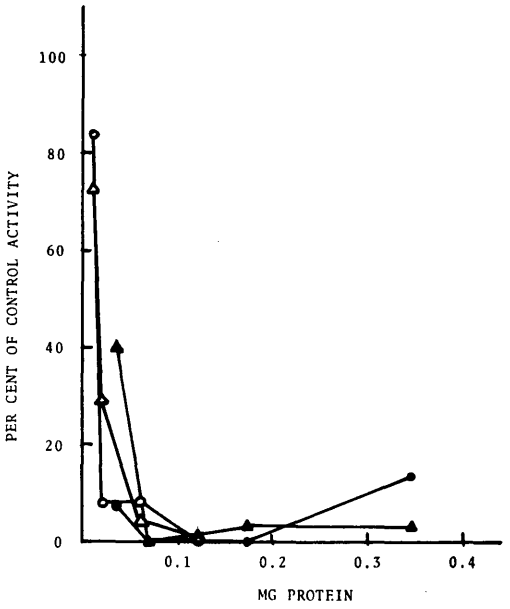

FIG. 4. Inhibition of the tryptic and chymotryptic hydrolysis of casein by the 27,000*g* supernatant fraction of normal and adjacent neoplastic tissue of S.D.: (○), T, normal breast; (●), T, C.A. of the breast; (Δ), CT, normal breast; (▲), CT, C.A. of the breast.

TABLE I. The Effect of Dialysis on the Antiproteolytic Activity of Extracts of Human Tissue.[a]

Additions to T or CT	Protein (μg)	Tyrosine liberated by T (meq; $\times 10^4$)	% of control activity	Tyrosine liberated by CT (meq; $\times 10^4$)	% of control activity
None		6.12	100	8.00	100
Glioma, undialyzed	155	0.82	13.4	3.30	41.3
dialyzed	155	0.29	4.8	1.90	23.8
Ovarian carcinoma, undialyzed	78.5	0.26	4.3	0	0
dialyzed	78.5	0.32	5.3	0.47	5.9
L. C. normal breast, undialyzed	45.0	0.45	7.4	1.10	13.8
dialyzed	45.0	0.44	7.2	1.80	22.5
L. C. malignant breast, undialyzed	106.5	1.68	27.5	0.35	4.4
dialyzed	106.5	2.07	33.8	1.56	19.5
D. F. normal bowel, undialyzed	82.3	2.04	33.4	0.29	3.6
dialyzed	82.3	1.08	17.7	2.00	25.0
D. F. malignant bowel, undialyzed	222	3.00	49.1	0.44	5.5
dialyzed	222	1.26	20.6	1.21	15.1

[a] To 1-μg aliquots of T or CT were added the indicated quantities of 27,000g supernatant fractions of tissue extracts or dialyzed extracts; 1% casein in 0.1 M phosphate buffer, pH 7.6, served as substrate.

TABLE II. The Effect of Heating on the Inhibition of the Tryptic and Chymotryptic Hydrolysis of Casein by Extracts of Human Tissue.

Additions to T or CT and temp (°)	Protein (μg)	Tyrosine liberated by T (meq; $\times 10^4$)	% of control activity	Tyrosine liberated by CT (meq; $\times 10^4$)	% of control activity
None		7.12	100	4.67	100
Glioma, unheated	77.5	3.29	46.2	2.65	56.8
60	77.5	4.9	68.9	5.04	108
100	77.5	5.82	81.7	4.32	92.5
Ovarian carcinoma, unheated	98.2	0.34	4.8	0	0
60	98.2	1.53	21.5	0.24	5.1
100	98.2	6.15	86.4	2.56	54.8
S. H., $breast_N$,[a] unheated	41.3	3.62	50.8	0.83	17.8
60	41.3	5.32	74.7	3.97	85.1
100	41.3	6.35	89.2	4.90	105
$breast_M$,[a] unheated	56.5	3.24	45.5	1.50	32.2
60	56.5	5.5	77.4	4.90	105
100	56.5	6.53	91.7	4.80	103
D. F., $bowel_N$, unheated	105.6	1.82	25.6	3.27	70.0
60	105.6	4.68	65.8	3.88	83.2
100	105.6	4.70	66.0	3.39	72.8
$bowel_M$, unheated	111.2	3.50	49.2	1.44	30.9
60	111.2	5.75	80.8	3.83	82.0
100	111.2	6.06	85.0	3.30	70.8

[a] N = normal; M = malignant.

infants through the colostrum (4, 10). Whereas the function of the low molecular weight peptides has been clarified, that of the larger proteins, such as those in blood, *eg.*, alpha$_1$-globulin (11), alpha$_1$*X*-glycoproteins (12, 13), inter-alpha-globulin (5), alpha$_2$-globulin (6), proteinase A inhibitor (14), and C′-1 esterase inhibitor (15), which inhibit a wide variety of proteases is much more obscure. They may function, in part, to regulate the clotting process.

The inhibitors reported herein in normal and neoplastic tissue, by virtue of their heat lability and nondialyzability, differ from the low molecular weight pancreatic and colostrum inhibitors, and from such heat-stable large proteins of the blood as the inter-alpha-globulin (5) and the alpha$_2$-globulin (6). It is apparent that two different trypsin inhibitors are produced in breast tissue. The heat-stable colostrum inhibitor is produced for secretion in lactating females. The heat-labile inhibitor, found in older women who are not lactating, may serve an as yet unknown function. It is possible that the inhibitor found in intestinal tissue may protect the lining of the intestine from digestion by the pancreatic juices. However, the roles of the ovarian and brain inhibitors remain to be determined. Since the Kunitz and Northrop inhibitor inhibits cathepsin D (16), it is tempting to suggest that trypsin inhibitors may regulate not only the activity of the secreted endopeptidases, but also that of the intracellular proteases.

Summary. The occurrence of soluble inhibitors of tryptic and chymotryptic activity in extracts of human mixed astrocytic and oligodendrogliocytic glioma, solid and papillary ovarian adenocarcinoma, normal and adjacent infiltrating duct carcinoma of the breast, and normal and infiltrating carcinoma of the cecum are reported. The inhibitors are nondialyzable and heat labile. On the basis of such properties, the inhibitors differ from the Northrop and Kunitz inhibitor, the Kazal inhibitor, the colostrum inhibitor, and the inter-alpha-and alpha$_2$-globulins found in mammalian tissue.

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