[CANCER RESEARCH 35, 1030-1034, April 1975]

Aminopeptidases and Arylamidases in Normal and Cancer Tissues in Humans[1]

Yoshiyuki Tamura, Michio Niinobe, Teruo Arima, Hiromichi Okuda, and Setsuro Fujii

Department of Enzyme Physiology, Institute for Enzyme Research, School of Medicine, Tokushima University, Tokushima, Japan

SUMMARY

Multiforms of aminopeptidases and arylamidases in normal human liver, stomach, lung, ileum, colon, rectum, and kidney, and cancer tissue from human liver, stomach, and lung were separated by triethylaminoethyl cellulose column chromatography. The aminopeptidases and arylamidases were solubilized from human tissues by treatment with bromelain, and their column chromatograms on triethylaminoethyl-cellulose gave different patterns of multiforms of enzymes in these tissues. The fractions of enzymes separated from various human tissues showed different substrate specificities toward L-leucyl-β-naphthylamide, L-leucinamide, L-methioninamide, L-phenylalaninamide, and L-alaninamide.

The activity of aminopeptidase toward L-leucinamide and of arylamidase toward L-leucyl-β-naphthylamide was higher in human stomach cancer tissue and lower in hepatic cancer tissue than in normal stomach and liver, respectively. In lung cancer tissue, the activity of aminopeptidase toward L-leucinamide was abnormally low, while the activity of arylamidase toward L-leucyl-β-naphthylamide was similar to that in normal lung. The substrate specificities or patterns of the multiforms of these enzymes in cancer tissue from human liver, stomach, and lung were shown to differ from those of normal liver, stomach, and lung, respectively, by triethylaminoethyl cellulose column chromatography.

INTRODUCTION

Various aminopeptidases have been demonstrated in most human tissues, but their physiological functions are unknown. It has been reported that certain enzymes that hydrolyze L-leucyl-β-naphthylamide (arylamidases) are different from leucine aminopeptidase, which cleaves L-leucinamide (1, 5-7, 9-12).

Patterson *et al.* (5) reported the chromatographic separation of leucine aminopeptidase from arylamidase, which hydrolyzed L-leucyl-β-naphthylamide, from Ehrlich-Lettré hyperdiploid ascites tumor cells. They found that the leucine aminopeptidase preparation hydrolyzed L-leucyl-β-naphthylamide 10,000 to 20,000 times slower than did leucinamide.

Previously, we reported (10, 11) that aminopeptidase and arylamidase were easily solubilized from rat liver by bromelain treatment. The solubilized forms of these enzymes were separated by TEAE-cellulose column chromatography into 5 types, which differed in substrate specificities toward L-leucinamide and L-leucyl-β-naphthylamide.

The present paper describes the existence of multiforms of aminopeptidases and arylamidases in solubilized preparations from human tissues such as liver, stomach, lung, ileum, colon, rectum, kidney, and cancer tissues.

MATERIALS AND METHODS

L-Leucinamide was obtained from the Protein Research Foundation, Osaka, Japan; L-leucyl-β-naphthylamide and TEAE-cellulose powder were from Wako Pure Chemical Industries, Ltd., Osaka, Japan; and bromelain was from Shiratori Pharmaceutical Co. Ltd., Chiba, Japan. L-Methioninamide, L-phenylalaninamide, and L-alaninamide were synthesized by the method of Smith and Slonim (8).

Specimens of liver, stomach, lung, ileum, colon, rectum, and kidney, and cancer tissue from liver (hepatocellular carcinoma), stomach (adenocarcinoma), and lung (squamous cell carcinoma) were obtained during surgery or immediately after death and either frozen at −20° or examined immediately.

TEAE-cellulose Column Chromatography. Chromatography was carried out on small columns (2 × 10 cm), as reported previously (11).

Preparation of Solubilized Aminopeptidases and Arylamidases from Various Human Tissues. Human tissues were homogenized in 3 volumes of 10 mM sodium phosphate buffer (pH 7.5) using 10 strokes of a Teflon homogenizer in ice. The homogenates were then sonically extracted at 20 kc for a total of 3 min in periods of 30 sec alternating with cooling periods of 1 min in ice. The sonically extracted suspensions were incubated with bromelain (100 μg/mg protein of the tissue homogenate) in 10 mM sodium phosphate buffer (pH 7.5) at 37° for 30 min. Then the mixture was centrifuged at 105,000 × *g* for 60 min at 4°, and the resultant supernatant solution was used as the preparation of solubilized aminopeptidases and arylamidases.

Enzyme Assay. Aminopeptidase activities were assayed with L-leucinamide, L-methioninamide, L-phenylalaninamide, and L-alaninamide as substrates, and the ammonia produced was estimated by direct colorimetric determination as reported previously (4, 11).

[1] This work was supported in part by a research grant for cancer research from the Ministry of Welfare of Japan.

Received February 13, 1974; accepted December 16, 1974.

Table 1

Aminopeptidase and arylamidase activities of various human tissues

Human tissue homogenates were sonically extracted as described in "Materials and Methods," and the sonically extracted suspensions were used as enzyme preparations.

	L-Leucinamide		L-Leucyl-β-naphthylamide	
	Specific activity[a]	Mean	Specific activity[b]	Mean
Liver	1.15, 1.50, 0.92	1.19	0.89, 1.39, 1.27	1.18
Hepatic cancer (Hepatocellular carcinoma)	0.28, 1.34, 0.42	0.68	0.35, 0.42, 0.51	0.43
Stomach	0.36, 0.35, 0.19 0.43, 0.37, 0.56	0.38	0.41, 0.47, 0.48 0.39, 0.49, 0.36	0.43
Stomach cancer (Adenocarcinoma)	1.43, 0.38, 0.45 1.06, 0.90, 0.60	0.80	0.67, 0.51, 0.46 0.79, 1.09, 0.68	0.70
Lung	0.47, 0.49	0.48	0.60, 0.35	0.48
Lung Cancer (Squamous cell carcinoma)	0.22, 0.35	0.29	0.59, 0.41	0.50
Kidney	1.58		4.54	
Ileum	1.09		3.34	
Colon	0.33		0.66	
Rectum	0.27		0.46	

[a] NH_3 formed (μmoles/hr/mg protein).
[b] β-Naphthylamine formed (μmoles/hr/mg protein).

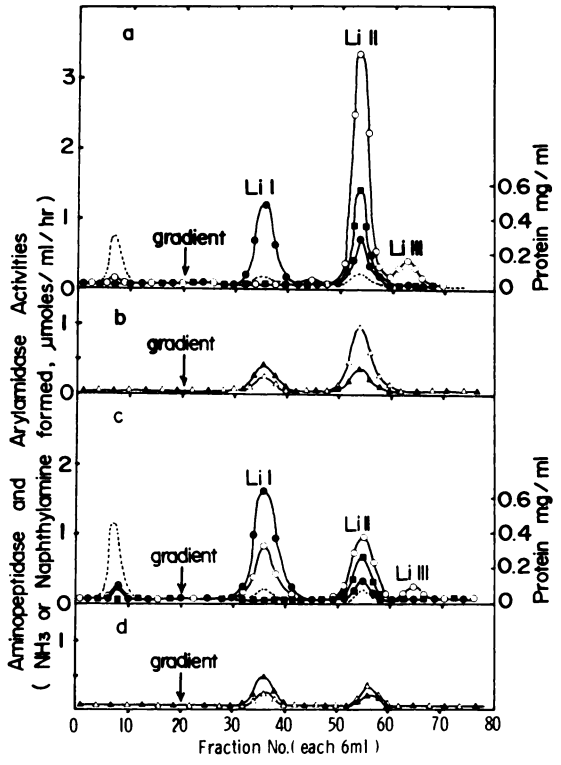

Chart 1. Column chromatograms of aminopeptidases and arylamidases from normal and cancer tissue of human liver on TEAE-cellulose. Chromatography was carried out as described previously (11) with a 2- × 10-cm column. Normal and cancer tissue homogenates were sonically extracted and treated with bromelain as described in "Materials and Methods." The mixtures were centrifuged at 105,000 × *g* for 60 min, and aliquots of the resultant supernatant solutions were applied to columns of Sephadex G-25 equilibrated with 10 mM sodium phosphate buffer (pH 7.5). The columns were eluted with the same buffer. Aliquots of eluate with activity containing 33.5 mg of protein were applied to columns of TEAE-cellulose. *a* and *b*, human liver; *c* and *d*, human hepatic cancer tissue. ●, L-leucinamide; ○, L-leucyl-β-naphthylamide; ■, L-alaninamide; ▲, L-phenylalaninamide; △, L-methioninamide; - - - -, protein.

Arylamidase activities were assayed with L-leucyl-β-naphthylamide as substrate by the method of Goldbarg *et al.* (2).

Protein Determination. Protein concentration was determined by measuring the absorbance at 750 nm by the method of Lowry *et al.* (3), with bovine serum albumin as a standard.

RESULTS

Aminopeptidase and Arylamidase Activities of Various Human Tissues

The activities of aminopeptidase and arylamidase in sonically extracted suspensions of various human tissues were estimated with L-leucinamide or L-leucyl-β-naphthylamide as substrate. Aminopeptidase and arylamidase activities were found in all tissues tested, the activities being especially high in the kidney, liver, and small intestine (Table 1).

The enzyme activities of stomach cancer tissue were higher than those of normal stomach. On the other hand, the activities of liver cancer tissue were lower than were those of normal liver. Lung cancer tissue had higher L-leucinamide activity than did normal lung but had similar L-leucyl-β-naphthylamide activity.

Multiforms of Aminopeptidases and Arylamidases in Human Normal and Cancer Tissues

Liver. As shown in Chart 1, *a* and *b*, the aminopeptidases and arylamidases of normal human liver separated into 3 peaks (Li I, Li II, and Li III) on a TEAE-cellulose column. Peak Li I hydrolyzed L-leucinamide, L-phenylalaninamide, and L-methioninamide but not L-leucyl-β-naphthylamide or L-alaninamide. Peak Li II hydrolyzed L-leucyl-β-naphthylamide preferentially to various L-amino acid amides. Peak Li III hydrolyzed only L-leucyl-β-naphthylamide.

In primary hepatic cancer tissue, the ratio of the activity of L-leucyl-β-naphthylamide to that of L-leucinamide was lower than the ratio in normal liver tissue, and Peak Li I hydrolyzed L-leucyl-β-naphthylamide as well as L-leucinamide, as shown in Chart 1, *c* and *d*.

Cancer tissues from 2 cases of primary hepatic cancer (hepatocellular carcinoma) gave essentially similar column chromatograms of aminopeptidases and arylamidases on TEAE-cellulose.

Stomach. The aminopeptidases and arylamidases of normal stomach separated into 3 peaks (S I, S II, and S III) of activity on a TEAE-cellulose column, as shown in Chart 2, *a* and *b*. Peak S I hydrolyzed L-alaninamide and L-leucinamide more readily than L-leucyl-β-naphthylamide or L-phenylalaninamide. Peaks S II and S III hydrolyzed L-leucyl-β-naphthylamide preferentially to various L-amino acid amides.

On the other hand, the aminopeptidases and arylamidases of stomach cancer tissue separated into 4 peaks (S I, S II, S III, and S IV) on TEAE-cellulose, as shown in Chart 2, *c* and *d*. The activity in Peak S I was higher in cancer tissue than in normal tissue. Peak S IV, with a peak in Tube 38, was found only in cancer tissue; it hydrolyzed L-leucyl-β-naphthylamide and various L-amino acid amides.

Cancer tissues from 4 cases of stomach cancer (adenocarcinomas) gave essentially similar column chromatograms on TEAE-cellulose.

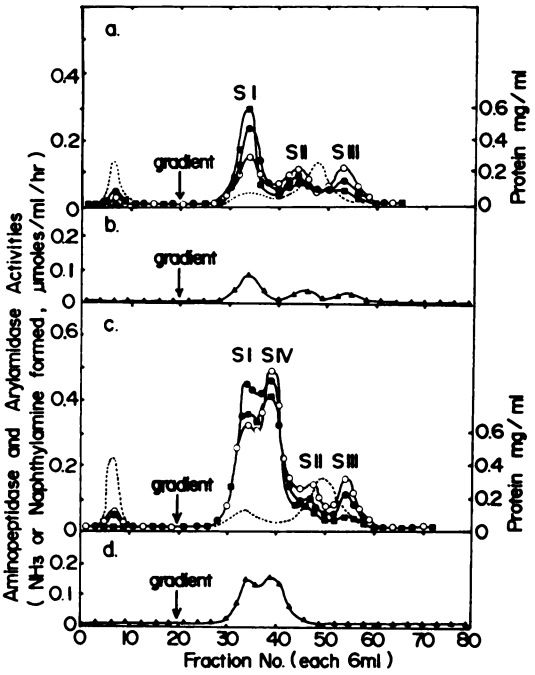

Chart 2. Column chromatograms of aminopeptidases and arylamidases in normal and cancer tissue from human stomach on TEAE-cellulose. Normal and cancer tissues were prepared as described in the legend to Chart 1, and samples containing 33.8 mg of protein were applied to columns. *a* and *b*, human stomach; *c* and *d*, human stomach cancer tissue. ●, L-leucinamide; O, L-leucyl-β-naphthylamide; ■, L-alaninamide; ▲, L-phenylalaninamide; - - - -, protein.

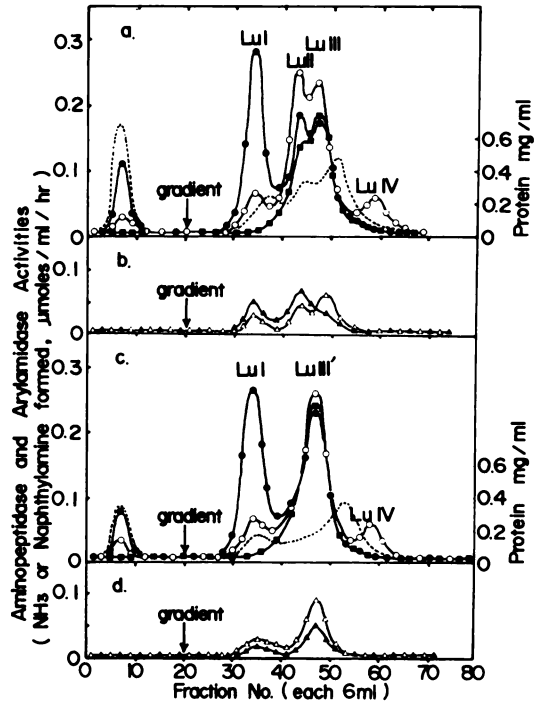

Chart 3. Column chromatograms of aminopeptidases and arylamidases in normal and cancer tissue of human lung on TEAE-cellulose. Normal and cancer tissues were prepared as described in the legend to Chart 1 and samples containing 26.6 mg of protein were applied to the columns. *a* and *b*, human lung; *c* and *d*, human lung cancer tissue. ●, L-leucinamide; O, L-leucyl-β-naphthylamide; ■, L-alaninamide; ▲, L-phenylalaninamide; △, L-methioninamide; - - - -, protein.

Lung. The aminopeptidases and arylamidases of normal lung separated into 4 peaks (Lu I, Lu II, Lu III, and Lu IV) on TEAE-cellulose, as shown in Chart 3, *a* and *b*. Peak Lu I hydrolyzed L-leucinamide preferentially to other L-amino acid amides and L-leucyl-β-naphthylamide. Peaks Lu II and Lu III hydrolyzed L-leucyl-β-naphthylamide, L-leucinamide, and L-alaninamide preferentially to L-methioninamide and L-phenylalaninamide. Peak Lu IV hydrolyzed only L-leucyl-β-naphthylamide.

The enzymes in cancer tissue from human lung separated into 3 peaks (Lu I, Lu III', and Lu IV), as shown in Chart 3, *c* and *d*. The substrate specificity of Peak Lu III in Tube 47 was similar to that of Peak Lu III of normal lung tissue in Tube 49, suggesting that Peak Lu III' corresponds to Peak Lu III.

Cancer tissues from 2 cases of primary lung cancer (squamous cell carcinomas) gave essentially similar column chromatograms on TEAE-cellulose.

Kidney, Ileum, Colon, and Rectum. Human kidney gave 2 peaks of aminopeptidase activities on TEAE-cellulose.

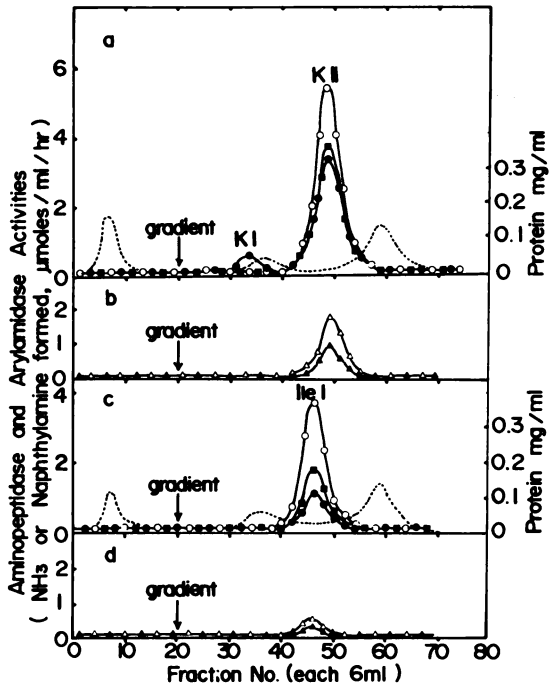

Chart 4. Column chromatograms of aminopeptidases and arylamidases in human kidney and ileum on TEAE-cellulose. Preparations of human kidney and ileum were prepared as described in the legend to Chart 1, and samples containing 24.8 and 18.3 mg of protein, respectively, were applied to the columns. *a* and *b*, kidney; *c* and *d*, ileum. ●, L-leucinamide; O, L-leucyl-β-naphthylamide; ■, L-alaninamide; ▲, L-phenylalaninamide; Δ, L-methioninamide, - - - -, protein.

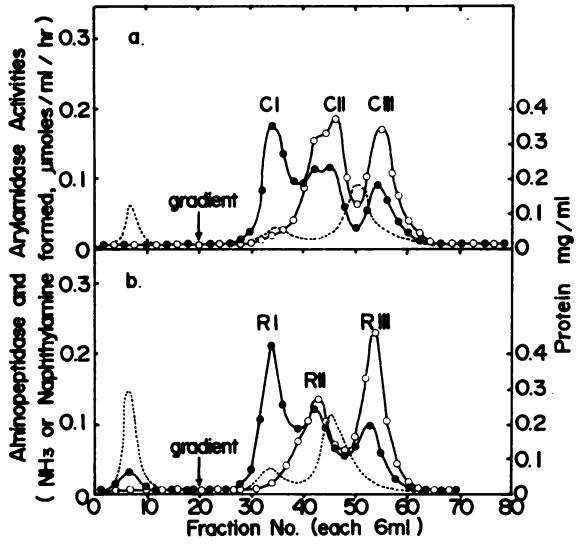

Chart 5. Column chromatograms of aminopeptidases and arylamidases in human colon and rectum on TEAE-cellulose. Preparations of human colon and rectum were prepared as described in the legend to Chart 1, and samples containing 20.4 and 15.0 mg of protein, respectively, were applied to the columns. *a*, colon; *b*, rectum. ●, L-leucinamide; O, L-leucyl-β-naphthylamide; - - - -, protein.

Peak K I hydrolyzed only L-leucinamide, while K II hydrolyzed L-leucyl-β-naphthylamide and various L-amino acid amides, as shown in Chart 4, *a* and *b*. Human ileum gave only 1 peak of aminopeptidase activity on TEAE-cellulose, as shown in Chart 4, *c* and *d*, while human colon and rectum gave 3 peaks (C I, R I, C II, R II, C III, and R III), as shown in Chart 5, *a* and *b*. Peak 1 (C I and R I) hydrolyzed only L-leucinamide, while Peaks 2 and 3 (C II, R II, C III, and R III) hydrolyzed L-leucyl-β-naphthylamide preferentially to L-leucinamide.

DISCUSSION

Column chromatography of aminopeptidases and arylamidases solubilized from various human tissues on TEAE-cellulose demonstrated that in most tissues there are multiforms of these enzymes that differ in substrate specificities toward L-amino acid amides and L-leucyl-β-naphthylamide. It has already been demonstrated that enzymes differing from leucine aminopeptidase hydrolyzed L-leucyl-β-naphthylamide (1, 5–7, 9–12).

The present report demonstrates that various human tissues contain multiforms of aminopeptidases and arylamidases, which differ in substrate specificities toward L-amino acid amides and L-leucyl-β-naphthylamide. It is still uncertain whether these differences in substrate specificity are due to the presence of different enzymes. Clear differences were found between the substrate specificities and chromatographic patterns of aminopeptidases and arylamidases from normal and cancer tissues. Namely, both normal and cancer tissue from human liver gave 3 peaks, but the substrate specificities of the corresponding peaks were quite different. Peak Li I from normal liver tissue hydrolyzed L-leucinamide but not L-leucyl-β-naphthylamide, while Li I from cancer tissue hydrolyzed both substrates. Stomach cancer tissue gave 1 more peak than did normal tissue (Peak S IV) on TEAE-cellulose, as shown in Chart 2; while in the lung, Peak Lu II was found in normal tissue but not cancer tissue, as shown in Chart 3. The above changes in aminopeptidases and arylamidases were found to be of 3 types. One was a change of substrate specificities, as found in hepatic cancer tissue; the 2nd was the appearance of a new chromatographic peak (stomach cancer); and the 3rd was the disappearance of a chromatographic peak (lung cancer).

If these changes are general phenomena in cancer tissues, they may afford a clue to metabolic disturbances in cancer tissues and may also be useful in the diagnosis of human cancer.

REFERENCES

1. Felgenhauer, K., and Glenner, G. G. The Enzymatic Hydrolysis of Amino Acid β-Naphthylamides. II. Partial Purification and Properties of a Particle-bound Cobalt-activated Rat Kidney Aminopeptidase. J. Histochem. Cytochem., *14:* 401–413, 1966.
2. Goldbarg, J. A., Pinder, E. P., and Rutenburg, A. M. Measurement of Activity of Leucine Aminopeptidase in Serum, Urine, Bile and Tissues. Am. J. Clin. Pathol., *32:* 571–575, 1959.
3. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem., *193:* 265–275, 1951.
4. Okuda, H., Yasunaga, T., Matsumoto, K., Tanaka, K., and Fujii, S. New Methods for Determination of Various Plasma Deaminases and

Their Clinical Applications. Proc. Symp. Chem. Physiol. Pathol., *11:* 151-155, 1971.

5. Patterson, E. K., Hsiao, S.-H., and Keppel, A. Studies on Dipeptidases and Aminopeptidases. I. Distinction between Leucine Aminopeptidase and Enzymes That Hydrolyze L-Leucyl-β-napththylamide. J. Biol. Chem., *238:* 3611-3620, 1963.
6. Patterson, E. K., Hsiao, S.-H., Keppel, A., and Sorof, S. Studies on Dipeptidases and Aminopeptidases. II. Zonal Electrophoretic Separation of Rat liver Peptidases. J. Biol. Chem., *240:* 710-716, 1965.
7. Rutenberg, A. M., Smith, E. E., and Kaufman, J. T. The Isolation and Purification of an Aminoacyl Naphthylamidase from Human Liver. J. Cell Biol., *19:* 63, 1963.
8. Smith, E. L., and Slonim, N. B. The Specificity of Leucine Aminopeptidase. J. Biol. Chem., *193:* 265-275, 1951.
9. Suszkiw, J. B., and Brecher, A. S. Brain Aminoacyl Arylamidase. Further Purification of the Soluble Bovine Enzyme and Studies on Substrate Specificity and Possible Active-Site Residues. Biochemistry, *9:* 4008-4017, 1970.
10. Tamura, Y., Niinobe, M., Arima, T., Okuda, H., and Fujii, S. Studies on Leucine Aminopeptidase. Proc. Symp., Chem. Physiol. Pathol., *12:* 19-23, 1972.
11. Tamura, Y., Niinobe, M., Arima, T., Okuda, H., and Fujii, S. Studies on Aminopeptidases in Rat Liver and Plasma. Biochim. Biophys. Acta, *327:* 437-445, 1973.
12. Tjeder, A., and Hofsten, B. The Occurrence of Amino Acid Naphthylamidase Distinct from Leucine Aminopeptidase in *Serratia indica*. Acta Chem. Scand., *21:* 1721-1726, 1964.