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Salivary-type Hyperamylasemia in Primary Lung Cancer: Observation of a Possible Precursor of the Salivary-type Isoamylase

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Abstract—*Although recent studies by column chromatography and electrophoresis showed that elevated amylase activity in the body fluids and tumor extracts in patients with primary lung cancer was mainly in the salivary-type isoamylase, neither mechanism nor nature of elevation of amylase have been clarified yet. This study was undertaken in order to make clear the nature of amylase elevated in the body fluids and tumor extracts of patients with primary lung cancer. In addition, amylase contained in the extracts of normal lungs and diseased lungs of patients who had died of various diseases was also studied. Our results revealed that amylase elevated in the body fluids and tumor extracts in patients with primary lung cancer was the salivary-type isoamylase and, moreover, that small amounts of salivary-type isoamylase in normal lung tissues and large amounts in diseased lung tissues were also contained. These facts suggested that salivary-type isoamylase was physiologically contained in normal lung tissues and might be activated through pathological processes, such as inflammation, circulatory disturbance or tumor formation. Two peculiar isoamylases with cathodic mobility on polyacrylamide gel electrophoresis were found. One of them was so unstable that it was converted to the stable salivary-type isoamylase, suggesting the precursor of human salivary isoamylase.*

INTRODUCTION

IT IS well-known that serum and urinary amylase levels elevate not only in pancreas and salivary gland disorders, but also in other disorders [1, 2], especially pulmonary diseases such as primary lung cancer, pulmonary tuberculosis and pneumonia [3–6]. Recent studies of electrophoresis and column chromatography revealed that elevated amylase activity in the body fluids and tumor extracts of the patient with primary lung cancer was salivary-type isoamylase [7, 8]. These results suggested ectopic production of salivary-type isoamylase by tumor cells. Moreover, unusual amylase that seemed to be produced by tumor cells was also reported [9].

The present study was undertaken in an attempt to reveal the nature of amylase contained or produced in the lung tissues, especially in the lung tumor tissues.

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MATERIALS AND METHODS

Human saliva, pancreatic juice, serum, urine, pleural effusion, ascites and homogenates of human lung and liver were used as materials. Saliva was collected sublingually from normal persons and centrifuged (15000 **g**, 30 min, 4°C) to remove particulate materials. Pancreatic juice was obtained by intubation of Dreilling's double lumen tube into the duodenum. Urine and serum were obtained before breakfast from normal persons and patients with mumps, pancreatitis, parotidectomy, pancreatectomy and primary lung cancer. Fifty-six lung and 11 liver tissues of 31 patients who had died of various diseases were obtained within 3 hr of death at autopsy. These patients exhibited various cancers (13 cases), including 4 cases of lung cancer, respiratory disorders (4 cases), liver cirrhosis (2 cases), heart diseases (2 cases), collagen diseases (2 cases), leukemia and malignant lymphoma, (one case each) and others (6 cases). These tissues were washed with

distilled water and homogenized at 4°C in 3 volumes of sucrose solution (0.25 mol/l) containing $CaCl_2$ (20 mmol/l) in Potter–Elvenhjem homogenizer with a teflon pestle. The homogenates were centrifuged (105000 *g*, 60 min) at 4°C and the supernates were used for following determinations. In addition to serum, urine, pleural effusion, ascites and primary lung tumor, metastatic left axillary and mesenteric lymph nodes from patients of primary lung cancer, accompanied by hyperamylasemia and hyperamylasuria, were used for the determination of amylase activity and isoamylase analysis.

Amylase activity was measured by a chromogenic method [10] using blue-dyed starch polymer (Pharmacia). Results were expressed as Somogyi units/100 ml. The normal range of serum amylase activity is 53–159 Somogyi units/100 ml (mean ±2 S.D. of 100 normal persons, 16–50 yr old). A value of more than 186 Somogyi units/100 ml (mean +3 S.D.) was considered to be hyperamylasemia. Protein contents in the tissues were determined by the method of Lowry *et al.* [11], and specific amylase activity was expressed as Somogyi units/g of protein.

Amylase isoenzyme separation was performed by horizontal sheet electrophoresis using 5% polyacrylamide gel of 1 mm thick. The discontinuous buffer system was used, gel buffer being 0.19 mol Tris [tris(hydroxymethyl) aminomethane], pH 8.8, and electrophoresis was carried out at 4°C for 2.5 hr at 0.8 mA/cm gel plate. After electrophoresis, the amylase isoenzyme was identified using the starch–iodine reaction.

RESULTS

Amylase isoenzyme patterns of the serum of normal persons and patients with pancreatitis, mumps, parotidectomy and pancreatectomy, pancreatic juice and saliva are shown in Fig. 1.

Serum amylase of a normal person could be separated into 2 major components (Amylases 1 and 3) and 2–3 minor ones (Amylases 2, 4 and 5). Amylases 1, 2 and 4 are consistent with the amylase isoenzymes of pancreatic juice and serum of the patients with pancreatitis and parotidectomy. On the other hand, Amylases 3, 5 and 7 are consistent with those of saliva and serum of patients with mumps and pancreatectomy. Accordingly, the former group are called the pancreatic-type isoamylase and the latter the salivary-type.

Of 55 patients with primary lung cancer, 6 patients (10.9%) were found to be hyperamylasemic (Table 1). Three of adenocarcinomas and two of squamous cell carcinomas were histologically confirmed, while the other one was diagnosed roentgenologically. One patient with primary lung cancer was associated not only with hyperamylasemia and hyperamylasuria, but also high amylase activity in the pleural effusion (12500 Somogyi units/100 ml) and ascites (9800 Somogyi units/100 ml). The amylase activities in the extracts of primary lung tumor and metastatic lesions of left axillary and mesenteric lymph nodes were 2622, 4846 and 1622 Somogyi units/g protein respectively.

Table 1. Frequency of hyperamylasemia in primary lung cancer

	Cases	Hyperamylasemia*
Adenocarcinoma	21	3(14.2%)
Squamous cell Ca	17	2(11.7%)
Undifferentiated	6	0(0%)
Roentgenologically	11	1(9.1%)
Total	55	6(10.9%)

*Hyperamylasemia > 186 Somogyi units/100 ml.

The amylase isoenzyme patterns in these materials showed the predominance of salivary-type isoamylase except urine (Fig. 2). In pleural effusion and ascites, several components with faster migration toward the anode side were observed (Amylases 5, 7 and 9).

In the primary lung tumor and metastatic liver nodule of another patient with primary lung cancer, the peculiar isoamylase with cathodic mobility was found (Fig. 3). This peculiar isoamylase was not found in serum, urine and pleural effusion, but in the tumor tissues. In the metastatic liver module, the salivary-type isoamylase was not recognized, although both salivary-type and peculiar isoamylases appeared in the primary lung tumor. In this case, the amylase activities in the tissues of normal lung, normal liver, primary lung tumor and metastatic liver nodule were 49.6, 25.6, 895.0 and 870.0 Somogyi units/g of protein respectively.

In an attempt to determine the amylase activity in the lung tissues, 56 samples from 46 patients who had died of various diseases were studied. The amylase activity in 45 diseased tissues was 562 ± 138 Somogyi units/g protein (mean ± SE), while that of 11 normal lung tissues was 63 ± 9.7 Somogyi units/g protein, almost the same as that of 11 liver tissues (Table 2).

Amylase isoenzyme patterns of these lung tissues showed the predominance of salivary-type isoamylase not only in the cases with high amylase activity, but also with low amylase

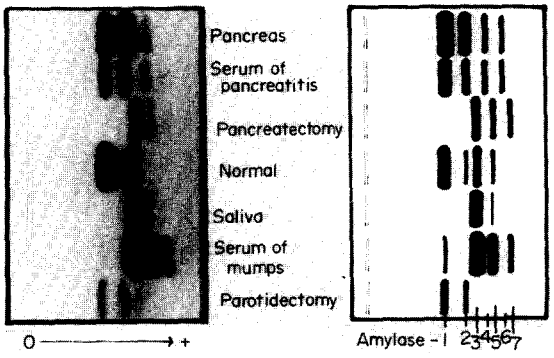

Fig. 1. Amylase isoenzymes of tissue extracts of pancreas and salivary gland, and serum of normal person and patients with pancreatitis, mumps, pancreatectomy and parotidectomy. Amylase isoenzymes of pancreas extract and serum of patients with pancreatitis and parotidectomy show the same mobilities as Amylases 1, 2 and 4. Amylases 3 and 5 are consistent with the amylase isoenzymes of salivary gland extracts and the serum of patients with mumps and pancreatectomy.

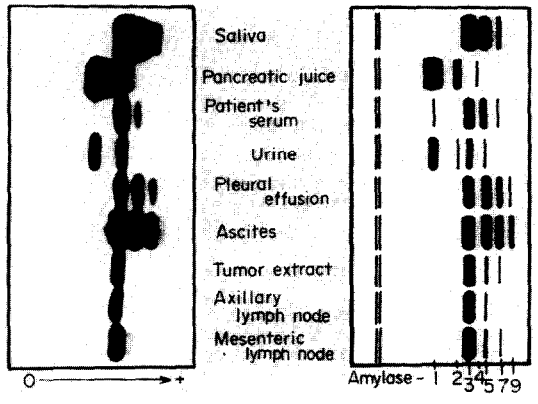

Fig. 2. Amylase isoenzymes in serum, urine, pleural effusion, ascites and tumor extracts of primary lung lesion and metastatic lesions of patients with primary lung cancer associated with hyperamylasemia. Salivary-type isoamylase is the major component in all these materials except urine.

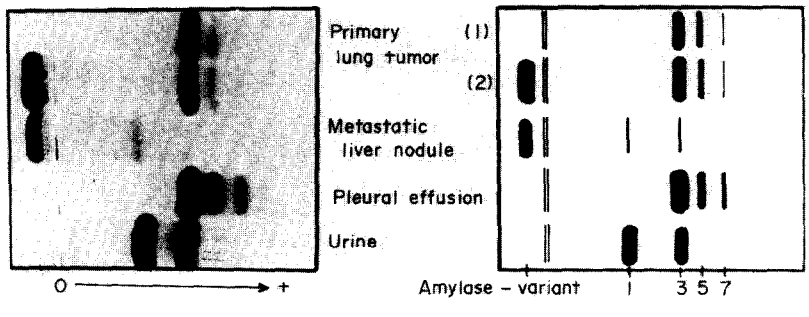

Fig. 3. Amylase isoenzymes in extracts of primary lung tumor (2)*, metastatic liver nodule, pleural effusion and urine of a patient with primary lung cancer. Only the salivary-type isomylase is usually found in extracts of most primary lung tumors* (1).

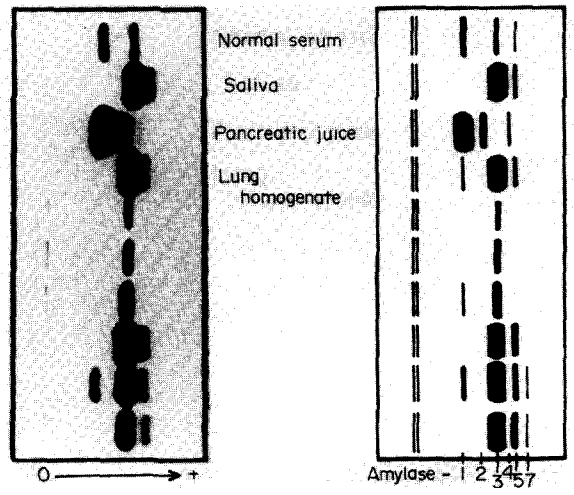

Fig. 4. Amylase isoenzymes in the extracts of lung tissues of the patients who died of various diseases. In all cases, salivary-type isoamylase is predominant.

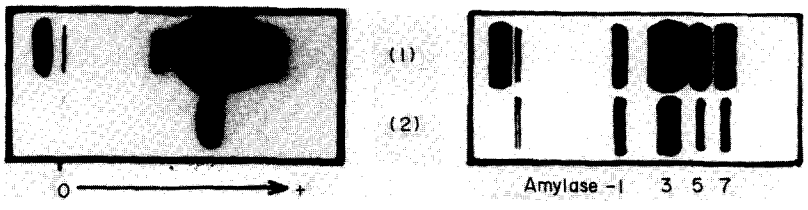

Fig. 5. Amylase isoenzymes in the tumor extract of a patient with vinyl chloride monomer poisoning (1). *The cathodally migrating isoamylase* (1) *was converted to the salivary-type isoamylase by storing* (2).

Table 2. Amylase activity in the homogenates of lung tissues

Diseased tissues	562 ± 138*
Normal tissues	63 ± 9.7
Liver tissues	62 ± 20

*Mean ± S.E. Somogyi units/g protein.

activity (Fig. 4). In addition, a peculiar isoamylase with the same cathodic mobility as described above in a case of primary lung cancer was found in the diseased lung tissues of the patient who had died of liver hemangiosarcoma resulting from vinyl chloride monomer poisoning (Fig. 5–1). This cathodally migrating isoamylase was so unstable that it was rapidly converted to the relatively stable salivary type isoamylase through gel electrophoresis after storage (Fig. 5–2).

DISCUSSION

It has been reported that some lung disorders, such as lung cancer, pulmonary tuberculosis and pneumonia, were associated with hyperamylasemia [3–6]. Since the first report of a case of primary lung cancer associated with remarkably high activity in serum, urine and tumor extracts [12], the possibility that tumor cells might have produced amylase have been suspected. However, neither the mechanism nor the nature of elevated amylase activity was clarified.

Amman *et al.* [7], by column chromatography, and Otsuki *et al.* (8), by agar gel electrophoresis, demonstrated that the mobility pattern of amylase in the serum, urine, pleural effusion and tumor extracts of the patient with primary lung cancer resembled salivary amylase. Our results by polyacrylamide gel electrophoresis confirmed that amylase isoenzyme in the body fluids and tumor extracts of primary lung cancer associated with hyperamylasemia resembled salivary-type amylase, and that the chief amylase activity in both tissues of normal and diseased lungs was in the salivary-type isoamylase, although the amylase activity in the latter was remarkably higher than the former. These observations support earlier demonstrations that normal lung tissues contain salivary-type isoamylase [7, 8]. In addition, the peculiar isoamylases with cathodic mobility were found both in the primary lung tumor and metastatic liver nodule from the patient with primary lung cancer, and in diseased lung tissue from the patient with vinyl chloride monomer poisoning. The finding of these two peculiar isoamylases with cathodic mobility on polyacrylamide gel electrophoresis indicated that this amylase was ectopically produced in the primary and metastatic tumor tissues. Although these two peculiar isoamylases had the same electrophoretic mobility, the difference between the two was that the former was as stable as salivary-type amylase, while the latter was so unstable that it was rapidly converted to the salivary-type isoamylase through gel electrophoresis after storage. The similar, cathodally migrating isoamylases on polyacrylamide gel electrophoresis were found in the saliva and pancreas tissues by Karn *et al.* [13]. These isoamylases were also unstable, converted to the stable isoamylase and identified to be quasi-stable precursors of human amylase isoenzyme. In such a sense, the peculiar isoamylase found by us might also be a quasi-stable precursor of salivary-type amylase.

In the case of primary lung cancer, the cathodally migrating isoamylase was also found in the metastatic liver nodule, where high activity of salivary-type isoamylase was not identified. From these results it is reasonable to conclude that the peculiar amylase was ectopically produced by tumor cells.

These cathodally migrating isoamylases may be bound with high molecular substances such as immunoglobulin, which has been recognized as macroamylase. However, the electrophoretic pattern of macroamylase on electrophoresis is quite different from these cathodally migrating isoamylases since the isoamylase pattern of macroamylase on electrophoresis shows the broad band with tailing between the β- and γ-globulin zones [14, 15]. Moreover, the direct test by column chromatography negated the presence of macroamylase. But there may be no doubt that these cathodally migrating isoamylases resulted from a change of amylase structure of amylase-binding substance.

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