Elevated Activities and Properties of Arylsulfatases A and B and B-variant in Human Lung Tumors

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ABSTRACT

The activities of arylsulfatases A and B were determined in human primary and secondary tumor tissues (total, 53 cases) of various histological types. Significantly higher activities of these sulfatases were found in almost all the primary lung carcinomas as compared to their corresponding uninvolved tissues. No significant correlation was demonstrated between the enzyme activities and histological figures (stroma amounts, etc.). Lung adenocarcinoma and squamous cell carcinoma showed the presence of an additional arylsulfatase component (B1) which was not detected in normal human lung. The tumor arylsulfatase B1 had an isoelectric point (pI) of 6.7 and was clearly distinguished from arylsulfatase A (pI 4.9) and arylsulfatase B (pI 9.1 to 9.2) in normal lung and lung tumor. The tumor B1 enzyme was demonstrated to be most probably an isoenzyme of arylsulfatase B, since this unusual enzyme was indistinguishable from arylsulfatase B in terms of Ag+ inhibition; its kinetic parameters of Km for p-nitrophenyl sulfate, which was 2.9 mM with B1; optimum pH of 6.3 for B1; heat stability; and substrate specificity for three synthetic and two physiological substrates.

INTRODUCTION

Three types of arylsulfatases, A, B, and C (arylsulfate-sulfotransferase, EC 3.1.6.1), are distinguished from one another in terms of substrate specificity, response to inorganic inhibitors, pH optimum, subcellular localization, and solubility (for review of these sulfatases, for example, see Ref. 21). Arylsulfatases A and B are localized mainly in lysosomes and can be easily obtained in a soluble form, whereas arylsulfatase C is present mainly in microsomal membranes. Arylsulfatase A hydrolyzes the sulfate ester of cerebroside sulfate (15) and ascorbic acid 2-sulfate (20), whereas arylsulfatase B acts on N-acetylgalactosamine 4-sulfate (10, 25).

Increased activities of arylsulfatases A and B in urine of bladder tumor patients (3, 19) and arylsulfatase B in colorectal carcinomas (17) have been documented. The activity of arylsulfatases was either elevated or depressed in a tissue-specific manner in visceral neoplasms (7, 16).

We previously demonstrated increased glycosaminoglycans in human lung carcinomas of 3 histological types (11, 12) and an elevated level of cerebroside sulfate in lung adenocarcinoma (29). The present study was undertaken to examine if these increases of sulfated complex carbohydrates in lung carcinoma could result from diminished activity of arylsulfatases. Reductions of these enzymes occur with hereditary sulfatidosis (for a review, see Ref. 6) and mucopolysaccharidosis (for a review, see Ref. 14). Contrary to expectation, the activities of arylsulfatases A and B in lung neoplasm were significantly higher than those in normal tissue from the same patients. Furthermore, separation of arylsulfatases from the neoplasm revealed an additional component other than arylsulfatases A and B.

MATERIALS AND METHODS

Chemicals. Potassium salts of p-nitrophenyl sulfate and p-nitrophenyl sulfate were purchased from Nakarai Chemical Co., Kyoto, Japan; 4-methyl umbelliferyl sulfate was from Eastman Kodak Co., Rochester, N. Y.; L-ascorbic acid 2-sulfate was from Sigma Chemical Co., St. Louis, Mo. Other chemicals were of analytical grade. UDP-N-acetylgalactosamine 4-sulfate, and UDP-N-acetylgalactosamine were generously donated by Dr. Sakaru Suzuki, Faculty of Science, Nagoya University, Nagoya, Japan, and Dr. Akira Yoshida, Department of Biochemical Genetics, City of Hope National Medical Center, Duarte, Calif., respectively. L-Tyrosine O-sulfate was synthesized by the method of Tallan et al. (24) as modified by Dodgson et al. (4).

Tumors. Human lung tissue obtained at surgery was quickly separated into tumor which was macroscopically nonnecrotic and the macroscopically normal portions, and it was stored immediately at −80°C until use. All the tissues were subjected to histopathological examination and characterized as described previously (29). Pathological characterizations included histological type, degree of differentiation, necrosis, stroma, and inflammation. When inflammation was observed, the tissue frequently was infiltrated with lymphocytes and, in a few cases, with plasma cells, macrophages, or neutrophils. The enzyme levels were examined in relation to histological findings (Chart 2).

Preparation of Enzymes. All operations were carried out at 2-4°C. The tissue was homogenized for 3 min with 9 volumes of 0.25 M sucrose by a Brinkmann homogenizer followed by centrifugation at 500 × g for 10 min. The supernatant was directly assayed for arylsulfatase activity or subjected to the enzyme separation. The 500 × g supernatant was sonicated twice for 1 min with a Kontes sonifier and centrifuged for 20 min at 20,000 × g. The supernatant obtained was adjusted to a final concentration of 50% by the addition of crystalline ammonium sulfate. After centrifugation, the precipitate was dialyzed against 10 mM Tris-HCl, pH 8.0, overnight. The dialysate was separated into arylsulfatases A and B on a DEAE-cellulose (DE-52; Whatman Co.) column equilibrated previously.


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with 10 mM Tris-HCl, pH 8.0. Fractionation was also performed with a Model HLC-803 high-speed liquid chromatograph (Toyo Soda Manufacturing Co., Tokyo, Japan) equipped with an IEX-540DEAE column (15 cm x 7.5 mm, inside diameter) packed with DEAE-2000SW (a DEAE derivative coupled with a synthetic polymer carrier) and a 280 nm UV monitor. The pressure applied to the column was 50 kg/sq cm, and the flow rate was 60 ml/hr. Arylsulfatase B was eluted with 10 mM Tris-HCl buffer, pH 8.0, and then a density gradient from 0 to 0.5 M NaCl in the same buffer was applied to obtain a more acidic form. An aliquot from 3-ml fractions was assayed for arylsulfatase activity. Similar separation was achieved by either DEAE-cellulose or IEX-540DEAE columns. Therefore, subsequent fractionations were performed with the latter column due to its rapid processing.

**Isoelectrofocusing.** The enzyme was electrophoretically focused in a column (100 ml) containing 1% LKB Ampholine carrier solution (pH 3.5 to 10) with a stepwise sucrose gradient at 500 V for 50 hr according to the method of Vesterberg (26). Fractions of 2 ml were collected at a flow rate of 48 ml/hr and assayed for their arylsulfatase activity. The enzyme-containing peaks were concentrated by ultrafiltration.

**Enzyme Assay.** Arylsulfatase A and B activities were assayed separately according to the method of Baum et al. (1) with a slight modification. The incubation mixture contained 5 mM p-nitrophenol sulfate, 2.0 mg pyrophosphate; 0.8 mg NaCl; 2 mg Triton X-100; 2.24 mg sodium acetate buffer, pH 5.0; and enzyme in a final volume of 0.4 ml for arylsulfatase A. The incubation mixture contained 2.5 mM p-nitrophenol sulfate, 5 mM barium acetate; 1 mg Triton X-100; 0.25 mM sodium acetate buffer, pH 6.0; and enzyme in a final volume of 0.2 ml for arylsulfatase B. The reaction mixtures were treated identically, and the amount of p-nitrophenol liberated was assayed at 515 nm. One unit refers to 1 nmol of p-nitrophenol liberated per hr.

The sulfatase reaction was carried out according to the method of Tsuji et al. (25) when UDP-N-acetylgalactosamine 4-sulfate was the substrate. The reaction product was chromatographed on Whatman No. 3MM paper with isobutyric acid:water; 5.0; and enzyme in a final volume of 0.4 ml for arylsulfatase A.

The incubation mixture contained 25 mM p-nitrophenolate sulfate, 5 mM barium acetate; 1 mg Triton X-100; 0.25 mM sodium acetate buffer, pH 6.0; and enzyme in a final volume of 0.2 ml for arylsulfatase B. The reaction mixtures were treated identically, and the amount of p-nitrophenol liberated was assayed at 515 nm. One unit refers to 1 nmol of p-nitrophenol liberated per hr.

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**RESULTS**

**Activities of Arylsulfatases A and B in Individual Lung Tumors and Normal Lung.** In Chart 1, arylsulfatase activities in various human lung tumors (classified into histological types) are compared with values in the corresponding control lungs. The arylsulfatase A-specific method was highly specific for arylsulfatase A, while the arylsulfatase B-specific method was less specific, as has been described previously (1). Arylsulfatase B activity which was determined on an unfractonated enzyme specimen, therefore, might include some arylsulfatase A activity.

Although the activity varied between tumors, the arylsulfatase A and B activities in all the tumors examined were elevated when compared to those in the normal tissues from the same individuals irrespective of histological types, with the exception of arylsulfatase A in Tumor 103 and arylsulfatase B in Tumor 510. The increased activity of arylsulfatase B showed parallel increases of arylsulfatase A activity with exception of some adenocarcinomas. Among the benign lung tumors available, hamartoma showed a elevation of both arylsulfatases A and B activities. The level of these enzymes in benign lung tumors did not appear significantly elevated compared to normal lung tissue. The mean specific activities of arylsulfatases A and B were highest in adenocarcinoma followed by squamous cell carcinoma (Table 1). Lung tumors which had metastasized to the liver (Tumors 101 and 122) showed the same activity as did primary lung tumors. On the other hand, arylsulfatase A activity in secondary lung tumors which metastasized from other tissues to lung, such as in osteosarcoma, thymus carcinoma, leiomyosarcoma, and rectal carcinoma, was much lower than in lung adenocarcinoma and squamous cell carcinoma and was similar to control values in the latter 2 carcinoma groups. The activity level of the adjacent normal lung tissue from cancer patients appears to be a real property of the normal lung, since the activities of arylsulfatases A and B from lung tissue of non-cancer patients were also low.

An increased level of arylsulfatase activity is present in the urine of the patients with bladder inflammation (19). Since tumor tissue might be inflamed, the arylsulfatase activity was examined with respect to inflammation and other histological characteristics. The activity level of arylsulfatases A and B in tumor and normal tissues could not be correlated with the degree of inflammation, differentiation, or stroma figures (Chart 2).

**Fractionation of Arylsulfatases by Anion Exchange Agent.** The arylsulfatases from tissue samples were analyzed for their ionic properties by DEAE column chromatography. Arylsulfatases of normal human lung were separated into 2 components, termed Peaks B and A as shown in Chart 3. The elution profile of the enzymes was consistent with that of arylsulfatases B and A, respectively (27). Peak B, which was not retained on the anion exchange agent, showed only arylsulfatase B activity using the assay for arylsulfatase B and no activity using the assay for arylsulfatase A and was not inhibited by Ag+. Therefore, the enzyme present in Peak B possessed the characteristics of arylsulfatase B. Peak A, which was eluted by NaCl gradient, had arylsulfatase A activity as well as some “aryl- sulfatase B” activity.

On the other hand, the carcinoma tissues had an additional enzyme designated as Peak B, in Chart 3, b and c, in addition to arylsulfatases B and A. Although the amount of B, varied from one tumor to another, this arylsulfatase component consistently appeared in adenocarcinoma, squamous cell carci-
### Statistical analysis of arylsulfatase A and B activities in human lung tumors

<table>
<thead>
<tr>
<th>Histological type</th>
<th>Arylsulfatase A (units/mg protein)</th>
<th>Arylsulfatase B (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>42.5 ± 8.1 (23)</td>
<td>32.3 ± 3.6 (13)</td>
</tr>
<tr>
<td>Control</td>
<td>13.9 ± 3.7 (14)</td>
<td>19.9 ± 3.5 (12)</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>31.2 ± 4.2 (18)</td>
<td>37.0 ± 3.3 (21)</td>
</tr>
<tr>
<td>Control</td>
<td>10.0 ± 4.1 (17)</td>
<td>19.0 ± 3.5 (12)</td>
</tr>
<tr>
<td>Undifferentiated carcinoma</td>
<td>26.5 ± 2.0 (4)</td>
<td>47.7. ± 35.6 (4)</td>
</tr>
<tr>
<td>Control</td>
<td>26.1 ± 20.9 (2)</td>
<td>15.2 ± 34.1 (2)</td>
</tr>
<tr>
<td>Benign tumors</td>
<td>17.2 ± 1.0 (4)</td>
<td>42.1 ± 9.6 (2)</td>
</tr>
<tr>
<td>Control</td>
<td>30.8 (1)</td>
<td>29.9 (1)</td>
</tr>
<tr>
<td>Secondary tumors metastasized to lung</td>
<td>8.7 ± 3.0 (4)</td>
<td>28.5 ± 35.0 (2)</td>
</tr>
</tbody>
</table>

*a* Mean ± S.E.

*b* *p* < 0.05 (t test; lung adenocarcinoma versus the corresponding normal tissue).

*c* Numbers in parentheses, number of tumors.

*d* *p* < 0.001 (t test; lung squamous cell carcinoma versus the corresponding normal tissue).

*e* *p* < 0.01 (t test; lung squamous cell carcinoma versus the corresponding normal tissue).

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**Chart 1.** Specific activities of arylsulfatases A and B in individual lung tumors and normal lung. Values on the right, enzyme activities of individual tumors; values on the left, activities of their corresponding normal tissues; middle column, individual patient number; ----, arylsulfatase A-specific activity; -------, arylsulfatase B-specific activity. *, not determined because of lack of material; **, lung tumors which metastasized to liver. In undifferentiated carcinomas, L indicates large-cell carcinoma, and S indicates small-cell carcinoma. In other primary tumors are Nos. 111 and 412, hamartoma; No. 201, fibromatosis; No. 506, adenoma. Secondary tumors which metastasized to lung from tumors of other tissues are No. 204, osteosarcoma; No. 208, thymus carcinoma; No. 507, rectum adenocarcinoma; No. 508, leiomyosarcoma.

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**Chart 2.** Arylsulfatase B specific activity (ordinate) with respect to histological figures. P, poorly differentiated carcinoma; M, moderately well-differentiated carcinoma; W, well-differentiated carcinoma. , squamous cell carcinoma; , adenocarcinoma; , undifferentiated carcinoma; CH, benign tumor; , , + + +, + + +, extent; O, normal tissue.
Arylsulfatases in Human Lung Tumors

Substrate Specificity of Arylsulfatases. The 3 arylsulfatase components of lung tumor tissue had the same specific activity with 4-methyl umbelliferyl sulfate as a substrate (Table 2). Tyrosine sulfate and ascorbic acid 2-sulfate, known substrates for arylsulfatase A, were cleaved only by the enzyme in Peak A. UDP-N-acetylglucosamine 4-sulfate, a known arylsulfatase B substrate, was cleaved preferentially by Peak B and B1 enzymes. These confirm the assignment of arylsulfatase A to Peak A and arylsulfatase B to Peaks B and B1.

On the basis of the results, it seems that Peak B, arylsulfatase component, present in human lung tumor but absent from normal lung, is most probably a variant form of arylsulfatase B.

DISCUSSION

These results demonstrate that the arylsulfatases of lung carcinoma consist of 3 components in contrast to 2 components in normal human lung tissue, which is consistent with previous observations on normal lung (27). The additional component present in the tumors was demonstrated probably to be a variant of arylsulfatase B. Multiple forms of arylsulfatase B have been noted in ox brain (2), human skin fibroblasts (22), and human brain (23). Wasserman and Austen (28) demonstrated that rat lung arylsulfatase B has a distinctly lower pi (6.4) than that of the human lung. This lower value was obtained with cells possessing a single arylsulfatase as in eosinophils, or in cells that also contain arylsulfatase A as in mast cells and basophil leukemia tumor. Properties of rat arylsulfatase B (28) and a minor variant, termed Bm (pi 7.0), of human brain arylsulfatase B (23) are very similar to the arylsulfatase B1 of human lung tumor. It is conceivable that a portion of arylsulfatase B present in normal lung is replaced by the B1 enzyme due to extracellular control occurring during the course of lung tumor development.

The present study demonstrated that the activities of arylsulfatases A and B were increased in almost all the cases of lung carcinomas compared to the activities from the corresponding control tissues. Morgan et al. (16) reported similar results with the mixed arylsulfatases in autopsied lung neoplasm of undefined histological type. The elevated activities were statistically significant in the former 2 histological types. Although the number of cases of tumor and its control that were examined was not large, the activity of arylsulfatase A in benign lung tumors and secondary tumors metastasized to lung was nearly in the normal range. The activity of lung tumors metastasized to liver was at the same level as that of primary lung tumors. Therefore, the elevation of arylsulfatase A seems to be characteristic for lung carcinoma. Increased activity of arylsulfatases in lung tumor is probably due to a general increase in lysosomal enzymes, because the activity of β-glucuronidase and β-N-acetylgalactosaminidase was also increased significantly in human lung tumors.

Normal and neoplastic tissues consist of heterogeneous cell populations and the histological features in individual tissues vary from one to another which may result in a widespread variation of the enzyme activity values. However, since the activity values of

Heat Stability Studies on Tumor Enzymes. Peak A arylsulfatase was rapidly inactivated at temperatures above 45°, while Peak B, as well as Peak B arylsulfatase were relatively stable at temperatures below 55° (Chart 7).

pH Optimum of Arylsulfatases. The pH optimum of Peak A arylsulfatase from adenocarcinoma (Chart 6), squamous cell carcinoma, and normal lung was 5.2 to 5.4. The pH optimum of Peak B displayed a relatively broad range with a peak near 6.2. Peak B, arylsulfatase from carcinoma had a pH optimum of 6.3.
the arylsulfatases did not correlate directly with any of the histological changes concomitant with neoplasm, the altered enzyme levels in the tumor tissues must indeed represent neoplastic changes.

Elevations of arylsulfatase activities, however, do not appear to be general for all neoplasms in different tissues. The activities of mixed arylsulfatase in human liver and kidney neoplasms were decreased (16). Differences could not be observed in breast cancer (7) in comparison to normal tissues, although the activities in colon (7, 16, 17), stomach, and skin cancers (7) were increased.
Each enzyme activity was determined using an arylsulfatase B-specific method, and those of Peak B enzyme (C, 93 units each) and Peak B, enzyme (x, 125 units each) were determined using an arylsulfatase B-specific method.

It is conceivable that the increased concentrations of glycosaminoglycans in lung carcinomas are due to the increased activity of their synthetic enzyme, cerebroside sulfate.

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REFERENCES

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