Heterogeneity of Ornithine Decarboxylase during Mouse Colon Carcinogenesis and in Human Colon Tumors

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ABSTRACT

Ornithine decarboxylase (ODC) was separated, using diethylaminoethyl ion-exchange chromatography, into multiple peaks of activity. We investigated the isoforms of ODC during 1,2-dimethylhydrazine-induced colon carcinogenesis and in human colon tumors. ODC in both mouse and human normal-appearing colon mucosa was consistently separated into two active peaks by diethylaminoethyl-Sepharose CL-6B column chromatography. The major peak (Peak I) contained about 75% of the mouse and 72% of the human colon mucosal ODC activity. During and after 10 weekly injections of 1,2-dimethylhydrazine (20 mg/kg, i.p.), colon ODC activity was significantly enhanced with induction of both peaks but with a more significant increase in Peak II. ODC activity in both 1,2-dimethylhydrazine-induced and human colon tumors was significantly higher compared with the normal colon mucosa. The chromatographic profile of tumors showed the predominance of the second peak. Furthermore, the chromatographic profile of ODC after alkaline phosphatase treatment yielded an elution of only one peak coincident with the Peak I and the disappearance of Peak II. The second peak of ODC (the phosphorylated form) may be a specific isoform associated with colon tumorigenesis and tumor growth.

INTRODUCTION

ODC is a rate-limiting enzyme in the biosynthesis of polyamines linked with normal and neoplastic cell proliferation (1). Induction of ODC has been suggested to play an important role in tumor including skin, urinary bladder, stomach, and colon carcinogenesis rodent models (2-6). Luk et al. (7) demonstrated a biphasic induction (initiation and promotion stage) of ODC activity during azoxymethane-induced colon carcinogenesis in rats. Moreover, administration of the specific ODC inhibitor difluoromethylornithine has been shown to reduce the incidence of chemical carcinogen-induced tumors in rats and mice (8-11).

Studies have shown that human colon mucosal levels of ODC activity are lowest in colon mucosa from healthy controls but are increased in normal-appearing mucosa from subjects with colon polyps and from colon cancer patients (9, 12-14). Significantly high levels of ODC activity are found in colonic tumors, and similar results were reported for several rodent carcinogenesis models (7, 9, 12-14).

ODC activity in mouse kidney (15), heart, liver, and thymus in rats (16-18), and in HT29 cell lines (19) has been separated into 2 or more distinctly charged species by DEAE ion-exchange chromatography. These isoforms are similar in size and appear to be caused by a post-translational modification. O'Brien et al. (20) reported the existence of multiple forms of ODC both in mouse epidermis and in dimethylbenzanthracene-initiated/phorbol 12-myristate 13-acetate-promoted epidermal papilloma. These isoforms differed in isoelectric point but not subunit molecular weight by 2-dimensional gel electrophoresis/immunoblotting methods. The detection of such ODC isoforms in the colon was the aim of this study.

MATERIALS AND METHODS

Treatment of Animals. Sixty-six CF1 female mice, 7 weeks old (Charles River Laboratories, Inc., Wilmington, MA), were used in this study. They were housed 5 to a cage and maintained on AIN-76A purified diet (Dyets Inc., Bethlehem, PA) and given water ad libitum. The light-dark cycle was alternated every 12 h. Thirty mice were given a maximum of 10 weekly i.p. injections of DMH (Aldrich Chemical Co., Inc., Milwaukee, WI) in 1 mMEDTA, pH 6.5, at a dose of 20 mg/kg body weight. Control mice were given injections of the EDTA only. Six mice each in both groups were sacrificed at 2, 5, 10, 20, and 30 weeks after the first treatment. Six intact mice were sacrificed just before initiating treatment. The colons of all mice were removed, and the mucosa was scraped to use in the ODC studies (21). Five polypoid tumors that developed in mice administered DMH at Week 30 were used for ODC studies.

Human Specimens. Normal-appearing human colon mucosa and colonic tumors were obtained from the surgically resected colons of colon tumor patients (6 men and 6 women; Department of Surgery, The University of Texas M. D. Anderson Cancer Center). Normal-appearing mucosa was scraped from the colon at a distance of 15 cm or more from the tumor.

Sample Preparations. Mucosal scrapings and tumor tissue were homogenized with 10 volumes of 50 mM Tris-HCl buffer, pH 7.3, containing 5 mM DTT, 0.2 mM PLP, and 0.1 mM EDTA using a polytron homogenizer and centrifuged at 40,000 X g for 30 min at 4°C. Supernatants were used for ODC assay. Prior to DEAE chromatography, the supernatants were adjusted to pH 8.0 by Trizma base, and NaCl was added to a final concentration of 0.1 M.

DEAE Chromatography. ODC isozymes were separated by a modified method of Pereira et al. (17). A DEAE-Sepharose CL-6B column (1.5 x 30 cm; Pharmacia, Uppsala, Sweden) was pre-equilibrated with 50 mM Tris-HCl buffer, pH 8.0, containing 5 mM DTT, 0.05 mM PLP, 0.1 mM EDTA, and 0.1 M NaCl (Buffer A). After application of the supernatant, the column was washed with a 2-column volume of Buffer A and then eluted with a linear salt gradient from 0.1 to 0.25 M NaCl in a total volume of 450 ml. The flow rate was 20 ml/h, and 4 ml fractions were collected.

ODC Assay. Enzymatic activity was measured as the release of [14C]-CO2 from L-[1-14C]ornithine, described previously (21). The standard assay mixture contained in a total volume of 500 ml was aliquot of supernatants (100 ml) and fractions (200 ml), 0.25 or 0.5 mM L-[1-14C]-ornithine (59 mCi/mmol; Amersham Co., Arlington Heights, IL) and final concentrations of 50 mM Tris-HCl (pH 7.3), 0.2 mM L-ornithine, 5 mM DTT, 0.2 mM PLP, and 0.1 mM EDTA. After the incubation for 60 min at 37°C, the reaction was stopped by addition of 200 ml of 30% trichloroacetic acid. The CO2 released was trapped on filter paper impregnated with 200 ml hyamine hydroxide (Du Pont-New England Nuclear, Boston, MA) suspended in a polyethylene well. Radioactivity on the filter paper was counted in Scintiverse II (Fisher Scientific, Houston, TX) with a Packard scintillation counter. Protein was measured by the method of Bradford (22) with the Bio-Rad reagent (Bio-
Rad Laboratories, Richmond, CA), using bovine serum albumin as the protein standard. Statistics. The data obtained were evaluated by Student's t test.

RESULTS

ODC Activity and ODC Isozymes during Carcinogenesis: Tumor Incidence. Colonic tumors were found in 5 of 6 DMH-treated mice sacrificed at Week 30. Five tumors (3 individual tumors and the pooled supernatant of 2 small tumors) were used in further ODC studies.

ODC Activity and ODC Isozyme. ODC activity in colonic mucosa was persistently raised with weekly DMH administration (Table 1). Colonic mucosal ODC activity in DMH-injected mice was markedly increased at Week 2 and then declined. However, the level of activity remained at approximately 3 times that of the controls until the end of the experiment. Pooled supernatants were applied to a DEAE column, and more than 75% of applied activity was recovered. ODC in the colonic mucosa, as previously shown for ODC in mouse kidney (15) was consistently separated into 2 peaks of activity by the DEAE chromatography (Fig. 1). Peak I accounted for approximately 75% of ODC activity recovered from the column, and the rest was found in Peak II, with the ratio of Peak I:II being about 3.0. With normal-appearing mucosa in DMH-treated mice, Peak II accounted for more activity compared with that of normal mucosa, and ratio of Peak I:II was below 2.0 throughout the experiment (Table 1).

ODC activity in DMH-induced colonic tumors was 10-fold higher than in normal mucosa from the EDTA-only-treated mice and 4-fold higher than of normal-appearing mucosa from DMH-treated mice sacrificed at Week 30, respectively (Table 1). The 2 ODC isoforms in colonic tumors were eluted from the DEAE column at the same salt concentrations as those in the normal colonic mucosa. However, the elution profile of ODC in tumors was completely different from that in the normal mucosa, with a predominance of Peak II (Fig. 2).

ODC in Human Colonic Tumors. ODC activity in the normal-appearing mucosa and tumors in human colon is shown in Table 2. ODC activity in colonic tumors was significantly increased, being an average of 1951 ± 486 (S.E.) pmol/h/mg protein, whereas activity of controls averaged 149 ± 28 (S.E.) pmol/h/mg protein in the normal-appearing mucosa (P < 0.05).

Pooled supernatants of the normal-appearing human colonic mucosa and individual supernatants of human colonic tumors subjected to DEAE chromatography and representative elution profiles are shown in Fig. 3. ODC activity in human colonic mucosa and tumors was also separated into 2 isoforms. ODC isoforms in human colon were eluted at slightly higher salt concentrations, compared with those of mouse, but the elution profiles were similar to the mouse. Eluted ODC activity in the normal-appearing human colonic mucosa yielded 71.5% of Peak I and 28.5% of Peak II, whereas 42.6% of Peak I and 57.4% of Peak II were observed in colonic tumors. The ratio of Peak I:Peak II in colonic tumors was significantly lower than that in the normal-appearing mucosa (P < 0.05).

Alkaline Phosphatase Treatment. One-half of the pooled supernatant from 3 human colonic tumors was chromatographed before and the remainder after alkaline phosphatase (Sigma Chemical Co., St. Louis, MO) treatment (3 units/ml of 50 mm Tris-HCl, pH 8.0, containing 1 mm MgCl₂ for 1 h at 25°C). Some decrease in ODC activity was found in alkaline phosphatase-treated supernatant (data not shown). However, only one peak, which coincided with Peak I, was eluted from the column.

Table 1. ODC heterogenicity during DMH-induced carcinogenesis in mice

<table>
<thead>
<tr>
<th>Week</th>
<th>Control Activity (pmol/h/mg protein)</th>
<th>Ratio (I:II)</th>
<th>DMH Activity (pmol/h/mg protein)</th>
<th>Ratio (I:II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>249 ± 45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.15</td>
<td>2343 ± 459&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.67</td>
</tr>
<tr>
<td>2</td>
<td>239 ± 41</td>
<td>2.93</td>
<td>730 ± 139&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.65</td>
</tr>
<tr>
<td>5</td>
<td>239 ± 22</td>
<td>2.74</td>
<td>576 ± 58&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.99</td>
</tr>
<tr>
<td>10</td>
<td>217 ± 28</td>
<td>2.93</td>
<td>642 ± 39&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.18</td>
</tr>
<tr>
<td>20</td>
<td>215 ± 22</td>
<td>2.83</td>
<td>538 ± 76&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.65</td>
</tr>
<tr>
<td>30</td>
<td>210 ± 48</td>
<td>2.48</td>
<td>538 ± 76&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.65</td>
</tr>
</tbody>
</table>

<sup>a</sup> Animals were given a maximum of 10 weekly i.p. injections of DMH (20 mg/kg). The control received the EDTA only.

<sup>b</sup> Mean ± S.E. (n = 6).

<sup>c</sup> P < 0.05 versus control.

<sup>d</sup> Data consist of 3 individual tumors and 1 pool of 2 tumors.

<sup>e</sup> P < 0.01 versus the values at Week 30.

Fig. 1. DEAE-Sepharose CL-6B column chromatography of the ODC in cytoplasmic fractions of CF1 female mouse kidney (A) and colon (B).

Fig. 2. DEAE-Sepharose CL-6B column chromatography of ODC in cytoplasmic fractions from mucosa of vehicle-treated (A) or DMH-treated (B) mice and from DMH-induced colonic tumor (C). Animals were sacrificed at Week 30. Total ODC activity applied on the column was not equivalent among the samples.

Table 2. ODC heterogenicity in human colonic tumors

<table>
<thead>
<tr>
<th>Group&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Activity (pmol/h/mg protein)</th>
<th>Peak I (%)</th>
<th>Peak II (%)</th>
<th>Ratio (I:II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 4)</td>
<td>125 ± 24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.5 ± 2.7</td>
<td>28.5 ± 2.7</td>
<td>2.61 ± 0.38</td>
</tr>
<tr>
<td>Tumor (n = 10)</td>
<td>1951 ± 486&lt;sup&gt;c&lt;/sup&gt;</td>
<td>42.6 ± 4.6</td>
<td>57.4 ± 4.6</td>
<td>0.82 ± 0.12</td>
</tr>
</tbody>
</table>

<sup>a</sup> Four pools of normal-appearing mucosa from 3 to 4 surgically resected colons were examined. Tumors were examined individually.

<sup>b</sup> Mean ± S.E.

<sup>c</sup> P < 0.05 versus normal.
cytoplasmic fractions of human normal-appearing colon mucosa (A) and colon tumors (B and C). Total ODC activity applied on the column was not equivalent among the samples.

When the alkaline-phosphatase-treated supernatant was applied (Fig. 4). Total recovered ODC activity of alkaline phosphatase-treated supernatant from the column was equivalent to about 90% of that from the untreated supernatant, which had 2 peaks of ODC activity.

DISCUSSION

Previous studies in carcinogenesis models have shown that induction of ODC might play an important role in tumor promotion (2–6). Luk et al. (7) demonstrated a biphasic induction of ODC during azoxy methane-induced colon carcinogenesis in rats. In this study, we also found a persistent elevation of colonic mucosal ODC activity in mice treated with the colon carcinogen DMH. However, induction of ODC in DMH-treated mice was not biphasic. Because colonic mucosal ODC activity in normal mouse colon is significantly higher (10 times or more) than that in rat, high basal levels of ODC activity could mask an increase in the later stage of carcinogenesis. In our study, human and DMH-induced mouse colon tumors had significantly higher ODC activity, similar to that reported from other laboratories (7, 9, 12–14).

ODC activity isolated form several different mammalian tissues has been separated into 2 or more distinctly charged species by DEAE ion-exchange chromatography (15–19). In this study, we found colonic mucosal ODC activity in mouse and human tissue to separate similarly into 2 peaks of activity. The major peak (Peak I) accounted for approximately 75% of the ODC activity in mouse colon and 72% of the ODC activity in human colon. Increased colonic mucosal ODC activity during DMH-induced carcino genesis resulted in the induction of both peaks but a larger increase in Peak II. Moreover, in human and DMH-induced mouse colonic tumors, 2 ODC activity peaks were eluted from the DEAE column at the same salt concentrations as that used for normal mousea. Of interest, the chromatographic profile of tumors, with the predominance of Peak II, was quite different from that of normal colonic mucosa. These results suggest that the second isoform of ODC may play an important role in colon carcinogenesis and tumor growth. O’Brien et al. (20) reported that isoelectric point values of ODC in mouse skin papilloma were lower than those in normal skin. Under the chromatographic conditions used in our study, acidic substances, i.e., substances having lower isoelectric points, are eluted late from the column. We could speculate that more acidic ODC might be a specific isoform in colon tumors.

Our findings support the recent observation by Hietala et al. (23) that at least 2 isoforms of ODC can be found in human colon tumors, separable by molecular size and ability to be activated by GTP. In our study, we have shown that at least 2 forms of ODC can be found in both normal rodent and human colon as well as respective colonic tumors. Furthermore, we have extended observation to show that one isoform may predominate ODC activity in colon tumors of mice and humans. How these forms are physiologically regulated or dysregulated in the normal and neoplastic state is as yet unknown. Our study reports that the 2 isoforms, separable by molecular charge, may, in fact, imply operational differences in normal and cancerous tissue.

ODC has been shown to be phosphorylated in vitro by casein kinase at a single serine residue (15, 24, 25). Peng and Richards (15) demonstrated that the first peak can be phosphorylated directly by casein kinase but the later peak cannot, suggesting that the second peak is already phosphorylated. We found that Peak II shifted to Peak I in human colon tumor supernatant treated with alkaline phosphatase, as shown by the DEAE-chromatography profile in Fig. 4. These results indicate that the first peak eluted from DEAE column is an unphosphorylated form of ODC and that the second peak is a phosphorylated form. The physiological role of the phosphorylation is uncertain. The kinetic properties of ODC do not differ between peaks (17, 19). Since the more acidic form of ODC has a longer half-life than the first ionic species (17), phosphorylation may affect the stability of the enzyme.

It is interesting to speculate that the phosphorylation of the tumor-associated isoform may be responsive to kinase activity, with protein kinase C a possible candidate. Mustelin et al. (26) have shown that ODC is covalently linked to T-cell membranes by inositol, and speculated that ODC activation is governed by phosphatidylinositol breakdown (with cleavage of the diacylglycerol portion of the compound). Diacylglycerol figures predominantly in protein kinase C activation, which is now suspected to contribute to enhancement of tumor growth and...
increased proliferative activity (27). And, because the activity of ODC may, in part, be regulated by the degradation of the enzyme, the predominance in cancer of an ODC with a longer half-life may lead to the inappropriate prolongation of the ODC response to normal stimuli (28). In any case, the increase in phosphorylated ODC in colonic tumors is of interest in advancing our understanding of the control of tumor cell proliferation. Further study is needed to clarify the biological role of the increased phosphorylated form of ODC in colonic tumors.

REFERENCES

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