Isolation and Characterization of a Murine Serum Esterase Which Hydrolyzes a Tumor Promoter, 12-O-Tetradecanoyl Phorbol 13-Acetate*

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An enzyme which catalyzes the following esterase reaction was isolated from mouse serum: 12-O-tetradecanoyl phorbol 13-acetate (TPA) + H₂O → phorbol 13-acetate + tetradecanoic acid. The recovery was 0.18% of total serum protein and 820-fold purification was achieved. The enzyme is composed of a single polypeptide chain with sugar moiety; its molecular weight was estimated to be 77,000. Its sugar content is 18%, the isoelectric point was 4.3, and the α-helix content was 15.3%. The enzyme is stable between pH 5 and 9 under 40 °C; it is insensitive to 2-mercaptoethanol and is not dependent on divalent cations. The optimal pH is around 7.5. The apparent $K_a$ for TPA is $6.6 \times 10^{-7}$ M. The hydrolysis of [3H]TPA is inhibited by phorbol diesters and phorbol 12-myristate, but not by phorbol and phorbol 13-acetate. The enzyme is inhibited to some extent by phosphatidylicholine, cholesterol, and lanosterol, but not by free fatty acids, fatty acid esters of glycerol, cholesterol esters, or cholesterol. The enzyme hydrolyzes ester linkages, but not peptide linkages of synthetic substrates. Esterase inhibitors and serine-reactive reagents affect the activity. Although sera from rodents displayed strong activity, such activity was not detected in human serum. Unlike lipoprotein lipase, the serum enzyme activity was not enhanced by treatment of the animal with heparin. These characteristics and the amino acid composition do not agree with any of the reported characteristics of known serum enzymes with esterase activity.

The phorbol esters, especially 12-O-tetradecanoyl phorbol 13-acetate, are known as the most potent promoters of carcinogenesis in mouse skin (1-5). In addition, they induce a wide variety of biological responses in different tissues and cells. These include stimulation of macromolecular synthesis (6-8), cell proliferation (9), histone phosphorylation (10), phospholipid synthesis (11), and prostaglandin synthesis (12), as well as induction of plasminogen activator (13) and ornithine decarboxylase (14, 15), and loss of surface-associated fibronectin (16). Early attempts to determine the metabolism of phorbol esters were performed to elucidate whether or not metabolic modifications were necessary for promoter activity (17, 18). Metabolism of radioactive TPA has been shown to take place in cultured cells such as mouse L cells (19) and hamster embryo fibroblasts (20), but not in cultured human cells (21). The resulting metabolites, namely phorbol 12-myristate or phorbol 13-acetate, did not have biological activity (21).

Using radioactive phorbol 12,13-dibutyrate, it was found that a variety of cells and tissues have high affinity receptors for biologically active phorbol esters (22-24). Recently, the receptor was shown to be the lipoprotein complex of cellular protein kinase C (25, 26). During a search for the endogenous ligands for the receptor, Shoyab et al. (27) found that mouse liver cytosol contains a protein which inhibits the binding of PDB to its receptor. This protein was purified to homogeneity. It is a hydrophobic glycoprotein consisting of a single polypeptide chain with a molecular weight of 60,000 and an isoelectric point of 5. It was found that the purified material has hydrolase activity which specifically cleaves the ester group on C-12 of phorbol diesters. The resulting phorbol monoester does not have affinity for the cell surface binding site for PDB, and thus, the enzyme seemingly inhibits the binding of PDB. These authors suggested that the hydrolase inhibits the promoter action of TPA in vivo; while the hydrolase activity was demonstrated in hamster skin, guinea pig skin, and rabbit skin, it was not detected in mouse skin, which had been the preferred organ to show the promoter activity of TPA (28). We demonstrate in this report that mouse serum has activity towards TPA. Isolation procedures and characteristics of the enzyme obtained from mouse serum will be described.

**EXPERIMENTAL PROCEDURES**

**Materials**

The chemicals used were obtained from the following sources. Phorbol and its analogues, TPCK, phospholipase A₂ from *Crotalus durissus terrificus* venom, phospholipase C from *Clostridium perfringens*; and heparin were from Sigma. Phosphodiesterase from Russell's viper venom was from Calbiochem-Behring; [3H]phorbol 12-myristate 13-acetate (specific activity, 17.2 or 6.5 Ci/mmol) was from New England Nuclear; and phenylmethanesulfonyl fluoride was from Nakarai Chemical Co., Kyoto. The hydrolyzed starch used for zone electrophoresis was from Jocko Industrial Co., Tokyo. The Affi-Gel blue gel was from Bio-Rad. The phenyl-Sepharose CL-4B and the chromatofocusing kit were from Pharmacia. The molecular weight standards came from Boehringer Mannheim; the agar used for

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*The abbreviations used are: TPA, 12-O-tetradecanoyl phorbol 13-acetate; PA, phorbol 13-acetate; PDB, phorbol 12,13-dibutyrate; TPCK, l-1-tosylamido-2-phenylchloromethyl ketone; BSA, bovine serum albumin; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; [3H]TPA, [20-3H]phorbol 12-myristate 13-acetate; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N',N''-tetraacetic acid.

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Immunoelectrophoresis was from Hoechst. Cholesterol, cholesterol esters, cholestane, lanosterol, and various glycolipid esters all came from Tokyo Kasei Chemical Co., Tokyo, and fatty acids came from Koso Chemical Co., Tokyo. Phosphatidylcholine from a hen egg was kindly provided by Dr. T. Yasuda, Institute of Medical Science, University of Tokyo. Fresh sera from mice, rats, rabbits, guinea pigs, chickens, and fetal calves were obtained from healthy donors of various species. Human sera were from healthy donors of blood type groups A, B, and O. Bovine and fetal calf sera were purchased from Grand Island Biological Co. Leupeptin, antipain, esterastin, and ebelactone were gifts from Dr. T. Aoyagi, Institute of Microbial Chemistry, Tokyo, whose generosity is appreciated. Assays of hydrolase activity of the purified enzyme using synthetic substrates N-benzoyl-l-arginine ethyl ester, N-acetyl-l-tyrosine ethyl ester, and p-nitrophenyl acetate were also performed by Dr. T. Aoyagi as described in Ref. 29.

Analytical Procedures

TPA hydrolase activity was assayed by measuring the conversion of [3H]TPA to a less hydrophobic material. The reaction mixture (0.2 ml) contained 5–10 pmol of [3H]TPA, 0.14 M sodium chloride, 0.01 M sodium phosphate buffer (pH 7.4), 0.02% 0.1% BSA, and the enzyme preparation. The reaction mixture was incubated at 37 °C for 4 h unless otherwise specified. After the incubation, the mixture was shaken vigorously with 0.5 ml of chloroform/methanol (1:1, v/v) (20) and separated into two phases. The upper layer contained PA as the degradation product, while the lower layer contained TPA and a part of PA. An aliquot of each phase was used for the determination of the radioactivity. Another aliquot of the lower phase was treated with performic acid (35). The tryptophan content was measured by the method of Roe (32), using glucose as a standard. Phosphatidylcholine from a chicken egg was obtained from Dr. T. Aoyagi, Institute of Microbial Chemistry, Tokyo, whose generosity is appreciated. Human sera were obtained from Dr. T. Aoyagi, Institute of Microbial Chemistry, Tokyo, whose generosity is appreciated. Assays of hydrolase activity of the purified enzyme using synthetic substrates N-benzoyl-l-arginine ethyl ester, N-acetyl-l-tyrosine ethyl ester, and p-nitrophenyl acetate were also performed by Dr. T. Aoyagi as described in Ref. 29.

RESULTS

Purification of TPA Hydrolase from Mouse Serum—As shown in Table I, sera from rodents had strong activity in hydrolyzing TPA, while bovine, fetal calf, dog, and chicken sera contained only traces of activity.

Table I

<table>
<thead>
<tr>
<th>Source of sera or plasma</th>
<th>TPA hydrolase activity (pmol/h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>100 ± 16</td>
</tr>
<tr>
<td>Mouse pretreated with heparin*</td>
<td>90 ± 14</td>
</tr>
<tr>
<td>Rat</td>
<td>76</td>
</tr>
<tr>
<td>Rabbit</td>
<td>117</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>45</td>
</tr>
<tr>
<td>Bovine</td>
<td>5.6 ± 0.17</td>
</tr>
<tr>
<td>Fetal calf</td>
<td>2.2</td>
</tr>
<tr>
<td>Chicken</td>
<td>3.1</td>
</tr>
<tr>
<td>Dog</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Mouse (blood types A, B, and O) Negligible

* Mice were injected intravenously with 100 μg of heparin dissolved in 0.2 ml of PBS. After 5 min, blood was collected by heart puncture and plasma was separated.

Enzyme Purification

All operations were carried out at 0–4 °C unless otherwise specified. Male C57/He mice approximately 2 months old were bled by heart puncture. The blood was kept at 4 °C to separate the serum. The serum was stored at −60 °C until it was used.

Step 1: Ammonium Sulfate Fractionation—The pooled serum (40 ml) was diluted with an equal volume of phosphate-buffered saline (PBS, 0.14 M sodium chloride, 0.01 M sodium phosphate buffer (pH 7.4)) and was placed in a flask cooled in an ice-water bath. To this solution, 0.5 ml of saturated ammonium sulfate solution was added gradually. The mixture was allowed to stand at 0 °C for 1 h and centrifuged for 20 min at 7000 × g. The supernatant was transferred to another flask. To raise the ammonium sulfate saturation from 55 to 80%, 86 ml of saturated ammonium sulfate was added to the supernatant. The mixture was left standing at 0 °C for 1 h and centrifuged at 7000 × g for 20 min. The resulting precipitate was dissolved in a minimal volume of PBS and was dialyzed against buffer for zone electrophoresis. The enzyme activity was recovered almost exclusively in this fraction.

Step 2: Starch Gel Zone Electrophoresis—The material obtained from step 1 was fractionated by electrophoresis through a starch block (10 × 10 × 1.5 cm) soaked with sodium diethylbarbiturate buffer (0.075 M (pH 8.6)). About 500 mg of protein were used at one time. Electrophoresis was performed at 4 °C at 30 mA for 24 h. The starch block was cut into sections 1 cm in width, and each portion of starch gel was eluted with 15 ml of PBS. Aliquots of each fraction were used for determinations of the hydrolase activity and of protein concentration. The enzyme activity coincided with the major protein peak (albumin fraction). The fractions with the enzyme activity were pooled, concentrated, and dialyzed against PBS.

Step 3: Affi-Gel Blue Column Chromatography—The dialysate from step 2 was applied to a column (2.2 × 21.5 cm) of Affi-Gel blue equilibrated with PBS. The column was eluted with PBS until absorbance at 280 nm became negligible and then with 2 M sodium chloride buffer with 0.01 M sodium phosphate buffer (pH 7.4). An aliquot of each fraction was used for determination of the hydrolase activity. The activity was associated with the nonabsorbed materials. The fractions containing the activity were pooled and rechromatographed two times on the same column to remove albumin completely. The resulting fraction was dialyzed against PBS.

Step 4: Phenyl-Sepharose CL-4B Column Chromatography—The dialysate from step 3 was applied to a column (1.5 × 10 cm) of phenyl-Sepharose CL-4B equilibrated with PBS at room temperature and eluted with PBS until absorbance at 280 nm became negligible. The column was then eluted with water. An aliquot of each fraction was used for determination of the hydrolase activity. The activity was associated with the nonabsorbed materials. The fractions containing the activity were pooled and stored at −20 °C. This material was used as the purified enzyme preparation.
sera had only limited activity. Human serum did not have appreciable activity. There was not much difference between the activity of post-heparin mouse plasma and that of the control mouse serum. The enzyme was purified from mouse serum (Table II). The enzyme activity was associated almost exclusively with the albumin fraction when the serum protein was fractionated by ammonium sulfate precipitation or by zone electrophoresis. The enzyme was then separated from albumin by utilizing the affinity of albumin for Affi-Gel blue. Due to this procedure, the total activity of the enzyme increased to about 2.8 times the original activity. The increase was fractionated by ammonium sulfate precipitation or by exclusively with the albumin fraction when the serum protein control mouse serum. The enzyme was purified from mouse serum (Table...purified material shows a single glycoprotein band, and TPA co-migrated with PA on TLC (Fig. 3).

**Properties of the Purified TPA Hydrolase**—The product of the degradation of [H]TPA by the purified enzyme was analyzed by TLC. The reaction mixture after the incubation was shaken with chloroform/methanol (1:1, v/v), and it separated into two layers. While TPA was found only in the lower chloroform layer, the degradation product was distributed in both layers. The degradation product in both layers co-migrated with PA on TLC (Fig. 3). Therefore, the enzyme seems to catalyze the esterase reaction as follows.

**TABLE II**

<table>
<thead>
<tr>
<th>Purification steps</th>
<th>Total protein (mg)</th>
<th>Specific activity (pmol/h/mg)</th>
<th>Purification fold</th>
<th>Total activity (pmol/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole serum (40 ml)</td>
<td>2879 ± 107</td>
<td>0.774 ± 0.12</td>
<td>1.6</td>
<td>2214 ± 261</td>
</tr>
<tr>
<td>Step 1</td>
<td>1070 ± 106</td>
<td>1.93 ± 0.38</td>
<td>2.6 ± 0.9</td>
<td>2020 ± 201</td>
</tr>
<tr>
<td>Step 2</td>
<td>514 ± 74</td>
<td>5.49 ± 2.45</td>
<td>7.8 ± 4.4</td>
<td>2641 ± 853</td>
</tr>
<tr>
<td>Step 3</td>
<td>35 ± 8</td>
<td>184 ± 18</td>
<td>243 ± 17</td>
<td>6276 ± 820</td>
</tr>
<tr>
<td>Step 4</td>
<td>5.1 ± 0.7</td>
<td>635 ± 98</td>
<td>820 ± 1</td>
<td>3307 ± 941</td>
</tr>
</tbody>
</table>

* Total protein × specific activity.

**Fig. 1.** Polyacrylamide gel electrophoresis of the materials obtained from each purification step. Gel 1, whole serum (72 µg of protein); gel 2, material resulting from step 1 (50 µg); gel 3, material resulting from step 2 (12 µg); gel 4, material resulting from step 3 (8 µg); gels 5 and 6, material resulting from step 4 (3 and 5 µg, respectively). Gels 1-5 were stained with Coomassie brilliant blue and gel 6 with periodic acid-Schiff reagent.

**Fig. 2.** Association of TPA hydrolase activity with the purified material after polyacrylamide gel electrophoresis. The step 4-purified material (0.2 µg of protein) was electrophoresed through 7.5% polyacrylamide gel. After electrophoresis, the gels were cut into 5-mm sections. The TPA hydrolase activity retained in each slice was determined as described. The activity was expressed in terms of radioactivity in the upper layer after partition. The arrow indicates the position of the material detected by Coomassie staining.

**Fig. 3.** Thin layer chromatography of the material recovered after incubation of [H]TPA with mouse serum. [H]TPA (5 pmol) was incubated with 10 µl of mouse serum in 0.2 ml of PBS containing 0.1% BSA at 37°C for 18 h. The reaction mixture was then partitioned as described, and aliquots of each layer were spotted on a silica gel plate. After development with methylene chloride/acetonitrile (3:1, v/v), the plate was divided into areas of 1-cm width. Each area was scraped off the plate, and the radioactivity was determined. A, the results obtained with the upper layer; B, those with the lower layer. Arrows indicate positions of migration of phorbol (P), phorbol 12-myristate (PM), PA, and TPA used as standards. They were detected with vanillin-sulfuric acid reagent.

12-O-Tetradecanoyl phorbol 13-acetate + H₂O → phorbol 13-acetate + tetradecanoic acid

The substrate specificity of the enzyme was studied by inhibition of the enzymatic degradation of [H]TPA by nonlabeled TPA analogues. The degradation was effectively inhibited by TPA, by phorbol 12,13-didecanoyl, and by 4-O-methyl TPA, to some extent by PDB and by phorbol 12-myristate, but not by PA or by phorbol (Table III). Since the enzyme hydrolyzes fatty acid esters of phorbol, the effect of various lipids on the enzyme activity was then studied. Free fatty acids (palmitic acid and stearic acid), fatty acid esters of glycerol (monopalmitin, monostearin, tripalmitin, and tristearin), cholesterol esters (cholesterol acetate, myristate, palmitate, stearate, and benzoate), and cholestanoic acid at a concentration of 10 mmol/l incubation, did not inhibit the enzyme, while lanosterol, cholesterol, and phosphatidylcholine inhibited with apparent Ki values of 1.6 × 10⁻⁶, 1.1 × 10⁻⁴, and 3.7 × 10⁻³ M, respectively. These results indicate that the enzyme is not a nonspecific lipase. The inhibition by lipids may reflect their noncompetitive, environmental effect. However, a synthetic substrate for determination of esterase activity, p-nitrophenyl acetate, was effectively hydrolyzed by the purified enzyme at a rate of 45 nmol/µg of enzyme, while the rate of hydrolysis of substrates for peptidases, N-benzylo-L-arginine ethyl ester and...
TABLE III

Inhibition of hydrolysis of [3H]TPA by the purified enzyme with TPA and its analogues

The reaction mixture contained 10 pmol of [3H]TPA, 1 µg of the purified enzyme, and various amounts (1–1000 pmol) of the inhibitor. The inhibitors were dissolved in dimethyl sulfoxide at a concentration of 1 mg/ml and were added to the reaction mixture. Ki values were obtained by plotting the ratio of initial velocity in the absence of inhibitor to that in its presence against the concentration of inhibitor. PM, phorbol 12-myristate; PDD, phorbol 12,13-didecanoate.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Ki (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPA</td>
<td>5.6 × 10^{-5}</td>
</tr>
<tr>
<td>PDD</td>
<td>1.7 × 10^{-5}</td>
</tr>
<tr>
<td>4-O-methyl TPA</td>
<td>1.2 × 10^{-5}</td>
</tr>
<tr>
<td>PDB</td>
<td>1.9 × 10^{-6}</td>
</tr>
<tr>
<td>PM</td>
<td>1.0 × 10^{-6}</td>
</tr>
<tr>
<td>PA</td>
<td>No inhibition</td>
</tr>
<tr>
<td>Phorbol</td>
<td>No inhibition</td>
</tr>
</tbody>
</table>

TABLE IV

Effect of peptidase inhibitors and esterase inhibitors on hydrolysis of [3H]TPA by the purified enzyme

For the determination of Ki, 10 pmol of [3H]TPA and 1 µg of the purified enzyme were incubated in the presence of various amounts (0.1–10 µg) of the inhibitor. PMSF (phenylmethanesulfonyl fluoride), TPCK, ebelactone, and esterastin were dissolved in dimethyl sulfoxide at a concentration of 10 mg/ml and were added to the reaction mixture.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration</th>
<th>Decrease of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ebelactone</td>
<td>1.6 × 10^{-4}</td>
<td>%</td>
</tr>
<tr>
<td>Esterastin</td>
<td>5.5 × 10^{-6}</td>
<td>%</td>
</tr>
<tr>
<td>Leupeptin</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Antipain</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>PMSF</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>TPCK</td>
<td>1</td>
<td>8</td>
</tr>
</tbody>
</table>

FIG. 5. Immunoelectrophoresis of the purified enzyme. Samples were electrophoresed through 2% agar containing 0.05 M barbiturate buffer (pH 8.8) and 0.1% sodium azide at 20 mA for 2 h. Gels 1 and 2, mouse serum (8 µl); gel 3, the purified enzyme (1.6 µg); gel 4, mouse serum (8 µl and the purified enzyme (1.6 µg)). After electrophoresis, antiserum was applied. Bands A and C, rabbit antiserum raised against the purified enzyme; bands B and D, that against mouse serum; band E, mixture of the above two antisera. The plate was left overnight at 4 °C, then washed and stained with Coomassie brilliant blue.

N-acetyl-L-tyrosine ethyl ester, were only 0.11 and 0.32 nmol/µg of enzyme, respectively. The effects of various inhibitors of esterases or proteases were then tested. As shown in Table IV, two esterase inhibitors, ebelactone and esterastin, strongly inhibited the TPA-hydrolyzing activity of the enzyme. Inhibitors of serine proteases, for example phenylmethanesulfonyl fluoride and TPCK, also inhibited the activity. Other protease inhibitors such as leupeptin and antipain had no effect. These results suggest that the purified enzyme is an esterase which has serine residues at its active sites. On the other hand, it was confirmed that [3H]TPA is not hydrolyzed by any of the tested commercial preparations of phospholipase A2, phospholipase C, or phosphodiesterase (data not shown).

The hydrolysis of [3H]TPA catalyzed by the purified enzyme preparation showed linearity up to 6 h under the assay condition. The optimal pH of the reaction is 7.5 in both sodium acetate and sodium phosphate buffer systems. For kinetic analysis, [3H]TPA at various concentrations (from 37.3 nM to 2.45 µM) was incubated with 1 µg of the purified enzyme at 37 °C for 3 h. The apparent Ki value for TPA was 6.6 × 10^{-7} M. The preparation could be stored at −20 °C for at least 4 months without appreciable loss of activity. The...
activity is stable under 40 °C and between pH 5 and pH 9. After incubation of the enzyme for 30 min (50 °C at pH 7.4 or at pH 4.5; 37 °C at pH 10.0), the activity decreased to 20% of the original. Addition of ethanol, methanol, acetone, or dimethyl sulfoxide to 1% of the reaction mixture did not affect the activity. On the other hand, the enzyme was inactivated by 0.1% SDS or Triton X-100. The activity was not affected by 2-mercaptoethanol or dithiothreitol. EDTA or EGTA had no effect. Divalent cations such as Mg²⁺, Ca²⁺, Mn²⁺, and Ni²⁺ had no effect, while CoCl₂, ZnCl₂, and SnCl₂ at 10 mM decreased the activity by 59, 78, and 97%, respectively.

The purified enzyme showed a single band after electrophoresis through polyacrylamide gel containing 0.1% SDS. Its molecular weight was estimated to be 77,000 using molecular weight standard proteins (Fig. 4). Pretreatment of the material with 2-mercaptoethanol did not alter the electrophoretic pattern. These results indicate that the enzyme is composed of a single polypeptide chain. The composition of the purified preparation shows that it is a glycoprotein with a sugar content of about 15% (Table V). Its α-helix content was determined by CD to be 15.3%. Chromatofocusing of the α-labeled preparation resulted in a symmetrical peak of radioactivity; its pl was determined to be 4.3. Immunoelectrophoresis shown in Fig. 5 shows a single precipitin line between the position of albumin and that of the α-globulin region.

DISCUSSION

There have been discrepancies in preceding reports concerning the product of degradation of TPA and its analogues by the mouse liver enzyme. Berry et al. (42) reported hydrolysis of the ester linkage at C-13 of phorbol, while Shoyab et al. (27) indicated hydrolysis on C-12. O'Brien and Saladin (45) state that the enzyme which hydrolyzes TPA is different from that which hydrolyzes phorbol 12,13-didecanoate. The mouse serum enzyme described here is similar to that from mouse liver cytosol described by Shoyab et al. (27) with regard to nature of TPA attack and sensitivity to inhibitors of serine proteases, but shows considerable differences from the latter with respect to molecular weight (77,000 and 60,000) and pl value (4.3 and 5). It is conceivable that the hydrolyse in liver cytosol is converted to a larger molecule when it is secreted from the cells.

Since a substantial amount (0.18%) of the total serum protein was recovered in the purified preparation of TPA hydrolase, the possibility was considered that the esterase activity may reside on one of the previously characterized major serum enzymes, including lipases, proteases, or complement components. It has been reported that lipoprotein lipase is released into circulation from the surface of fat cells after intravenous injection of heparin (44). However, there was no increase of TPA hydrolase activity in post-heparin mouse plasma, compared to the control serum. The substrate specificity also indicates that it is not a nonspecific lipase. Molecular weight, amino acid composition, pl value, the single polypeptide structure, and insensitivity to protease inhibitors do not match any of the characteristics of lipoprotein lipases (44), complement components, enzymes of the plasminogen system, or other proteases with esterase activity. It is likely, therefore, that TPA hydrolase activity is not due to any of the known serum enzymes. Absence of the activity in human serum supports the novelty of the enzyme.

The physiological roles of this hydrolase and its relationship to tumor promotion are presently unknown. Shoyab et al. (28) speculated that the liver cytosolic ester hydrolase protects tissues from the tumor-promoting activity of TPA, because the enzyme was not detected in mouse skin, which is the preferential organ to study the promoter activity of TPA, whereas skin from other species and other organs of the mouse are rich in the enzyme. On the other hand, others have reported that TPA is rapidly cleaved even in mouse skin (17, 42) and that TPA can act as a promoter in hamster skin (45, 46) and mouse liver (47). The serum TPA hydrolase may take part in the rapid clearance of the tumor promoter from various tissues, including mouse skin, although it cannot be concluded that this enzyme is responsible for the low sensitivity of various tissues other than mouse skin to the promoter action of TPA. However, it can be said at least that the presence of this enzyme in various sera should be taken into consideration in experiments concerning the action of TPA on cells cultured in vitro in serum-containing media.

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Isolation and characterization of a murine serum esterase which hydrolyzes a tumor promoter, 12-O-tetradecanoyl phorbol 13-acetate.

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