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ASPIRIN HYDROLYZING ESTERASES FROM RAT LIVER CYTOSOL

DONG-HYUN KIM,* YUH-SHYONG YANG and WILLIAM B. JAKOBY†
Laboratory of Biochemistry and Metabolism, National Institute of Diabetes and Digestive and Kidney Diseases, National Insitutes of Health, Bethesda, MD 20892, U.S.A.

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Abstract—Unlike most esterases, which are predominantly bound to the microsomal fraction, the enzymes hydrolyzing acetylsalicylic acid are present in an equal amount in the cytosol. Two soluble isozymes were purified to homogeneity from rat liver and characterized as serine esterases with a M_r of 35,000. Both had the wide substrate spectrum characteristic of enzymes active in detoxication. Both had a very low K_m for acetylsalicylate. Three other cytoplasmic enzymes active with aspirin were observed but these differed in their high M_r (about 220,000) and their lack of reactivity with antibody to one of the homogeneous isozymes.

It is clear that one route of metabolism of acetylsalicylic acid is by way of its hydrolysis to salicylic acid, a reaction catalyzed by a non-specific enzyme that has been referred to as "aspirinase." At least some degree of purification has been attained for such esterase activity although the only homogeneous preparation is of an enzyme, classified as esterase ES4 [1] on the basis of current genetic nomenclature [2], that was obtained after detergent extraction of the microsomal fraction of rat liver [3, 4]. Enzymatic hydrolysis of aspirin has been observed in the intestinal mucosa [5, 6], in liver cytosol [7] and in serum [8–10], each an effective site for detoxication of xenobiotics. These enzymes are all serine esterases that are inhibited by low concentrations of paraoxon [diethyl(4-nitrophenyl)phosphate] [11, 12] and some by bis-(4-nitrophenyl)-phosphate [13]. Whatever the merits of the present system of classification, and in keeping with their probable role in the detoxication process, the carboxylesterases (EC 3.1.1.1) along with the arylesterases (EC 3.1.1.2) appear to have the general properties expected for this function [14], i.e. an affinity for lipophilic compounds, both natural and xenobiotic, that allows a very broad spectrum of substrates. Since homogeneous protein preparations are necessary for substrate assignment, only the microsomal esterase ES4 has been evaluated. That microsomal enzyme catalyzes the hydrolysis of aspirin with a K_m in the millimolar range and at a rate less than 3% of that with 1-naphthylacetate [1].

We have attempted to examine the hydrolysis of aspirin more systematically and have observed five species of such activity in the cytosol of rat liver. Two of these cytosolic enzymes appear to be isozymes that have been prepared here in electrophoretically homogeneous form. Both have a relatively high activity with aspirin and a K_m in the micromolar range for aromatic esters.

* Present address: Kyung-Hee University, College of Pharmacy, Seoul, Korea.
† Correspondence: Dr. William B. Jakoby, NIH, Building 10, Room 9N119, Bethesda, MD 20892.

MATERIALS AND METHODS

Rat livers were obtained frozen in DryIce from Pel-Freez Biologicals (Rogers, AZ) and stored at −70° prior to use. Substrates and inhibitors were purchased at the highest available grade from either the Sigma Chemical Co. (St. Louis, MO) or the Aldrich Chemical Co. (Milwaukee, WI).

Assay Methods. The *standard enzyme assay* for arylesterase activity with aspirin as substrate was carried out in cuvettes of 1 cm light path containing 1 mL of 40 mM Tris–HCl at pH 7.8, 1 mM acetylsalicylic acid, and an appropriate amount of enzyme. Absorbance was followed in a Varian 219 spectrophotometer at 300 nm at 25° for 5 min. Activity was a linear function of time and of enzyme concentration when absorbance changes of less than 0.5 were observed under such conditions. A unit of enzyme is defined as that amount catalyzing the formation of 1 μmol of salicylic acid per min (an absorbance change of 2.84 at 300 nm) under the standard incubation conditions. Specific activity is defined in terms of units of activity per milligram of protein. Protein was determined with the bicinchoninic acid [15] using crystalline bovine serum albumin (Pentax) as standard.

Esterase activity was also determined with other nitrophenyl esters as substrate by following the formation of the free phenol in 96-well microliter plates having a flat well-bottom. In a final volume of 150 μL in each well were included 40 mM Tris–HCl at pH 7.8 and a 1 mM concentration of chromogenic ester; the reaction was started by the addition of an appropriate amount of enzyme. Plates were incubated at 25° for 15 min and were measured at 405 nm (interference filter) with a model FI 309 automated microplate reader (Bio-Tek Instruments, Winooski, VT); the entire plate was measured within 30 sec, and kinetic data could be obtained by measuring all of the wells at 1-min intervals. The reaction was linear with respect to both time and protein concentration when absorbance changes of less than 1.0 were observed. Using microliter plates, the absorbance changes per

micromole under hydrolysis to 4-nitrophenol and 2-nitrophenol were 40.0 and 8.56 respectively.

Esters forming products absorbing in the ultraviolet region were assayed as described for acetylsalicylic acid with the exception that the naphthylacetates were used at a final concentration of 0.25 mM. Under these conditions, the following absorbance changes were found for the hydrolysis of 1 μmol of substrate: 1-naphthylacetate, 2.88 at 313 nm; 2-naphthylacetate, 1.32 at 313 nm; phenylacetate, 1.34 at 271 nm; and 4-acetoxybenzoate, 9.6 at 254 nm. In assays establishing the Hammett plot, 50 mM Bicine at pH 8.0 rather than Tris was used because of the lower spontaneous rate of hydrolysis in Bicine. The following absorbance changes were observed for substrates tested under these conditions: 4-acetoxybenzaldehyde, 19.9 at 330 nm; 4-acetoxyphenylacetic acid, 1.4 at 276 nm; 3-acetoxypyridine, 2.6 at 310 nm; 4-acetylphenylacetate, 11 at 324 nm; and *p*-tolylacetate, 1.7 at 277 nm.

The utilization as substrates of *p*-nitroacetanilide [16], benzyl-DL-argenine 4-nitroanilide [17], and acetylthiocholine and CoA thioesters [18] was examined by the referenced methods. The hydrolysis of methylbutyrate was sought by following the formation of acid in the presence of the pH indicator, bromthymol blue. Incubations were conducted in a total volume of 1 mL containing 1 mM methylbutyrate and 50 μL of enzyme that had been dialyzed overnight against 20 mM Tris–HCl at pH 7.8. After incubation at room temperature for 60 min, 50 μL of a solution of bromthymol blue (0.4 mg/mL in 5 mM Tris–HCl at pH 7.8) was added. Absorbance was measured at 620 nm. This system was linear with respect to acid formation in the range of 50 to 150 nmol; 100 nmol of acid led to a decrease in absorbance of 0.10 at 620 nm.

4-Methylumbelliferylacetate at a final concentration of 12.5 μM was used as a substrate in 25 mM Tris–HCl at pH 7.8; the reaction was followed fluorometrically [19]. Aminopeptidase activity was sought by using fluorescein isothiocyanate-labeled casein as described by Twining [20].

Electrophoresis and activity staining. Homogeneity was assessed by subjecting proteins to electrophoresis under both native and denaturing conditions using a vertical slab gel apparatus (model SE200, Pierce Chemical Co., Rockford, IL) and a group of molecular weight standards (No. 161-0304) prepared by Bio-Rad Laboratories (Richmond, CA). Native proteins were subjected to the discontinuous gel method of Davis [21] using a running time of 5 hr at a constant current of 6 mA in a cold room at 4°. Upon completion, the 8% gel was removed and washed in the cold with 50 mM Tris–HCl at pH 7.8 for 5 min. One column of each sample was used for staining of protein with Coomassie Blue R-250. The remainder of the gel slab was placed on top of a sheet of Whatman No. 1 paper that had been moistened with a solution of 0.25 mM 4-methylumbelliferylacetate in 50 mM Tris–HCl at pH 7.8. After 5 min of contact at room temperature, the presence of fluorescent hydrolysis product was sought by viewing paper and gel under ultraviolet light. Staining for activity could also be accomplished by adding to the gel 5 mL of 0.5 mM 1-naphthylacetate as substrate, and 1% Fast Blue B salt in the same Tris buffer. The latter stain does not require a paper layer [22] but the bands of dye tend to diffuse after 15 min.

The method of Laemmli [23] was used for sodium dodecyl sulfate (SDS)-gel electrophoresis after heating the protein solution at 100° for 90 sec in the presence of 1% SDS and 5% 2-mercaptoethanol. Electrophoresis was conducted at a constant current of 15 mA for 1 hr and at 20 mA for the second hour.

Antibody production. Purified esterase I, 250 μg, was subjected to vertical gel electrophoresis in a non-denaturing gel as outlined above. The section containing the protein was identified by activity staining of small strips of the gel; the area of the stained section was cut out and homogenized with an equal volume of incomplete Freund's adjuvant. New Zealand rabbits were injected intradermally by Gary Thomas (Hazelton Laboratories) with about 55 μg of the esterase divided into four equal doses. The process was repeated at three biweekly intervals. Effectiveness of antibody was tested by Ouchterloni immunodiffusion [24].

Cell fractionation. Fresh livers from three Sprague-Dawley (CD strain, Charles River Laboratories) rats of about 270 g each were perfused with cold 0.25 M sucrose, and homogenized and treated as described by Hess and Brand [25] for the isolation of nuclear, mitochondrial, microsomal and cytosolic fractions.

Enzyme purification. Frozen rat livers, 250 g, were allowed to thaw partially for 20 min, at room temperature, submersed in 700 mL of 20 mM Tris–HCl at pH 7.8. The suspension was homogenized at maximum speed in a Waring blender for two periods of 30 sec each at 4°. All subsequent steps were conducted at 4°. The homogenate was centrifuged at 8,000 *g* for 30 min and the resulting supernatant liquid was centrifuged at 100,000 *g* for 60 min; the residue was discarded (Step 1).

The protein solution was applied to a column (4 × 28 cm) of DEAE-cellulose (Whatman DE52, approximately 200 g) that had been equilibrated with 20 mM Tris–HCl at pH 7.8. After washing the column with 1.6 L of the same buffer, a linear gradient was applied that consisted of 900 mL of the Tris buffer and 900 mL of the buffer supplemented to 0.4 M KCl. Four areas of catalytic activity for aspirin were observed in the elution profile (see Fig. 1), and are labeled as peaks A, B, C, and D respectively. Purification of peak D is presented here, and the data for Step 2 and subsequent steps in Table 1 pertain only to this fraction.

The combined eluates from peak D were treated with 45 g of polyethyleneglycol (PEG 8000, Sigma) which was added to the solution with stirring; stirring was continued overnight. After centrifugation at 37,000 *g* for 1 hr (Step 3), the residue was discarded and the solution was used to charge a second column (2.5 × 16 cm; 50 g) of DEAE-cellulose that had been equilibrated with 20 mM Tris–HCl at pH 7.8. The column was washed with 500 mL of the same buffer and was eluted with 500 mL of a linear gradient of 0 to 0.4 M KCl in the Tris buffer. Collecting fractions of 6.3 mL, fractions 36 through 50 were pooled and concentrated to 25 mL with an Amicon YM 10 filter.

Table 1. Partition of esterase activity for aspirin and 4-nitrophenylacetate (NPA) upon cell fractionation of isotonic extracts of rat liver

Fraction	Total protein (mg)	Total activity (μmol/min) Aspirin pH 6.0	Aspirin pH 7.8	NPA pH 7.8
Homogenate	1150 ± 100	14.1 ± 1.0	8.2 ± 0.7	1420 ± 300
Cytosol	490 ± 80	2.7 ± 0.1	3.7 ± 0.3	70 ± 4
Microsomes	22 ± 12	7.0 ± 0.7	3.4 ± 0.3	940 ± 180
Mitochondria	13 ± 4	1.6 ± 0.2	0.8 ± 0.1	180 ± 40
Nuclei	21 ± 2	0.9 ± 0.2	0.4 ± 0.2	12 ± 3

Values are means ± SD, N = 3.

The resulting solution was dialyzed for a total of 18 hr against one change of two liters of buffer A [10 mM potassium phosphate at pH 6.0 containing 20% (w/v) glycerol] (Step 4).

The protein solution was applied to a column (2.5 × 15 cm) of hydroxylapatite that had been equilibrated with buffer A. After washing with 500 mL of buffer A, activity was eluted with a linear gradient of 250 mL of buffer A and 250 mL of 0.2 M potassium phosphate at pH 6.0 containing 20% glycerol. The active fractions (17 through 36; 5.7 mL/fraction) were pooled and concentrated to about 2 mL by ultrafiltration with a YM 10 membrane (Step 5).

The preparation from Step 5 was applied to a column (1.6 × 85 cm) of Sephacryl S-200 (Superfine) equilibrated with 25 mM Tris–HCl at pH 6.8. Fractions of 1.3 mL were collected while washing with the same buffer. Active fractions (57 through 65) were pooled (Step 6) and were applied to a chromatofocusing column (1.5 × 10 cm) that had been equilibrated with 25 mM Tris–HCl at pH 6.8. The column was eluted with 350 mL of 10-fold diluted Polybuffer 74 at pH 4.2; fractions of 2.7 mL were collected. Two active peaks of activity were separated at this stage (Fig. 2): esterase I (fractions 73 to 79) with peak activity at pH 4.95 (Step 7) and esterase II (fractions 83 to 89) at pH 4.8 (Step 7). The separated enzymes were each pooled and concentrated to about 1 mL each with a YM 10 membrane.

Each of the enzyme solutions was charged in sequence onto a Sephacryl S-200 (Superfine) column that had been equilibrated and was eluted as described for Step 6. In each instance, the enzyme appeared in fractions 57 through 62 (Steps 8 and 9 respectively). The same column was calibrated with standards containing ribonuclease, chymotrypsin A, ovalbumin, bovine serum albumin aldolase, catalase and Blue Dextran (Pharmacia, Piscataway, NJ).

RESULTS

Aspirin hydrolysis. The localization of activity with acetylsalicylate as substrate was sought by fractionation of fresh rat liver extracts by differential centrifugation in isotonic sucrose. Although the preponderance of carboxyesterase activity was expected [26] and found in the microsomal fraction, activity for aspirin at pH 7.8 was distributed evenly between microsomal and cytosolic fractions (Table 1).

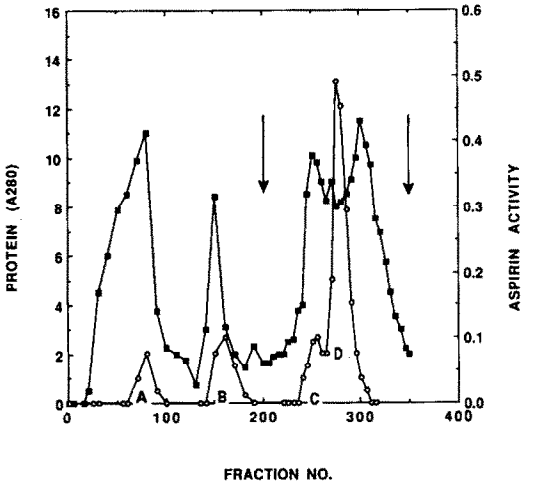

Fig. 1. Elution pattern of rat liver extract on DEAE-cellulose following protein (■) and hydrolysis activity (μmol/min/mL) (○) for acetylsalicylate. Peak D contained the two esterases that were purified further. A linear gradient of KCl from 0 to 0.4 M was arranged between the two arrows; fractions of 23 mL were collected.

When the soluble cell extract was subjected to chromatography on DEAE-cellulose, four distinct groups of hydrolytic activity for aspirin were observed (Fig. 1). The first of these passed through the column and the second, appeared when the column was washed with the equilibration buffer. Two additional peaks of activity were eluted when a salt gradient was applied. The major portion of aspirin-hydrolyzing activity, but not of 4-nitrophenylacetate hydrolysis, was eluted as the last peak, D. The first three peaks represent enzymes that are all estimated as about 220 kD in size based on gel filtration; all three had pH optima for aspirin in the acid range, between pH 5 and 6.5. The fourth and largest peak, D, was a smaller protein, 30 kD, and had optimal activity at pH 7.8. All enzyme activity for aspirin was inhibited completely by preincubation with 0.1 mM paraoxon, an effective inhibitor of serine esterases. The K_m for 4-nitrophenylacetate with the first three peaks was 3, 4 and 1 mM, respectively, whereas the fourth peak of activity had a K_m of 100 μM.

Table 2. Summary of purification of arylesterases I and II

Step	Volume (mL)	Total activity Aspirin (μmol/min)	Total activity NPA* (μmol/min)	Total protein (mg)	Specific activity Aspirin (nmol/min/mg)	Specific activity NPA* (nmol/min/mg)
1. Extract	650	73	4330	15,700	5	270
2. DEAE-cellulose 1	150	21	233	1880	11	125
3. Polyethyleneglycol	205	22	32	492	46	64
4. DEAE-cellulose 2	88	17	20	246	67	79
5. Hydroxylapatite	120	7.9	3.4	14	550	236
6. Sephacryl S-200	11	5.2	3.2	4.3	1230	761
7. Chromatofocusing (I)†	19	1.7	0.9	1.1	1610	820
(II)†	19	1.7	0.8	0.95	1790	840
8. Sephacryl S-200 (I)‡	8	0.95	0.4	0.37	2550	1200
9. Sephacryl S-200 (II)‡	8	1.1	0.6	0.42	2500	1400

* NPA = 4-nitrophenylacetate.

† I and II refer to the two proteins that were separated at this step.

‡ Arylesterases I and II were applied separately to columns of Sephacryl S-200 as represented by steps 8 and 9 respectively.

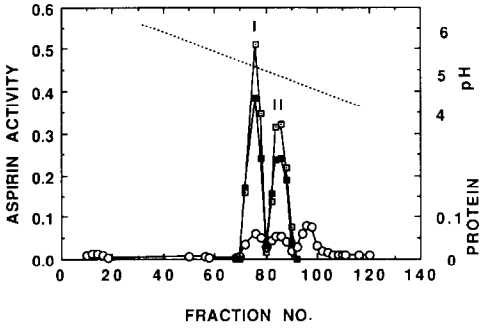

Fig. 2. Elution from a chromatofocusing column leading to separation of esterases I and II. Key: hydrolytic activity for acetylsalicylate (■) and 4-nitrophenylacetate (⊡); protein (○). The dashed line indicates pH of the eluate.

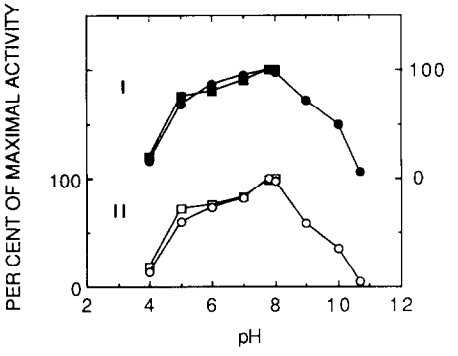

Fig. 3. Activity as a function of pH for esterase I (upper curves) and esterase II (lower curves) using acetylsalicylate (circles) and 4-nitrophenylacetate (squares) as substrates.

Purification. Peak D contained more than two-thirds of the total aspirin-hydrolyzing activity of the cytosol and was selected for purification. The yield of the two purified isoenzymes was only about 3%. Although the poor recovery at the stage of hydroxylapatite and chromatography may be due, in part, to enzyme instability to these matrixes, the major losses throughout purification were the result of draconian choices in the use of only those fractions that were relatively uncontaminated by non-aspirin esterase activity at each step in purification; all fractions were assayed for activity toward aspirin and to 4-nitrophenylacetate so at to be able to make these decisions from elution patterns.

At the stage of chromatofocusing, Step 7 in the purification protocol (Table 2), two esterases were separated eluting at a pI different by 0.15 (Fig. 2). Upon gel filtration (Step 8), each of the esterases was found to be homogeneous by the criteria of polyacrylamide gel electrophoresis and SDS-gel electrophoresis. Activity staining after gel electrophoresis with the acetates of 4-methylumbelliferone or 1-naphthol as substrate disclosed a single band of activity coincident with a single band of protein stained by Coomassie blue. When subjected to Ouchterloni diffusion, a single line of identity was observed when esterase I and esterase II were allowed to diffuse against rabbit antisera prepared against esterase I; the antiserum did not form a line of precipitation with protein from peaks A, B or C.

Properties of the enzymes. Both esterases eluted at the identical volume upon gel filtration using Sephacryl S-200. With a series of globular protein standards and on the assumption of identical shape and partial specific volume, an M_r of 35,000 was calculated for each esterase. The size as determined by SDS-gel electrophoresis, with or without boiling for 90 sec in the presence of 5% 2-mercaptoethanol, was also 35,000, suggesting that these esterases are monomeric proteins. The optimum pH for acetylsalicylate as substrate was at 7.8 (Fig. 3); with 4-nitrophenylacetate as substrate, activity increased to pH 7.8 but the extent of enzyme participation could not be measured reliably because of rapid spontaneous hydrolysis above that pH. Both esterases were relatively stable at pH 6.8 losing about 7 and 10% of their catalytic activity per month when maintained at −20° and 4° respectively.

Table 3. Substrate specificity and apparent kinetic parameters of arylesterases I and II

	Esterase I			Esterase II		
	Specific activity (units/mg)	K'_m (μM)	V_{max} (units/mg)	Specific activity (units/mg)	K'_m (μM)	V_{max} (units/mg)
Acetylsalicylate	2.5	5.9	2.5	2.55	5.9	2.6
4-Acetoxybenzoate	1.3	98	1.3	1.3	110	1.3
2-Nitrophenylacetate	1.5	5.3	1.5	1.9	9.8	1.9
4-Nitrophenylacetate	1.2	8.3	1.2	1.4	4.2	1.4
2-Nitrophenylbutyrate	1.4	22	1.4	1.1	17	1.1
4-Nitrophenylbutyrate	0.50	9.3	0.63	0.59	19	0.57
2-Nitrophenylcaprylate	0.05			0.08		
4-Nitrophenylcaprylate	0.01			0.02		
Phenylacetate	1.9	1.8	1.9	2.1	2.6	2.1
1-Naphthylacetate	1.4	0.43	1.5	1.4	0.78	1.6
2-Naphthylacetate	1.5			1.5		
Acetyl CoA	0.58			0.38		
Butyryl CoA	0.13			0.13		
Palmitoyl CoA	—			0.03		
Methylbutyrate	<0.03			<0.03		
4-Methylumbelliferylacetate	0.69			0.53		

A unit of enzyme is defined as that amount catalyzing the formation of 1 μmol of salicylic acid per min (an absorbance change of 2.84 at 300 nm) under the standard incubation conditions

Substrate range. On the basis of the several model compounds that were tested as substrates, both enzymes displayed entirely similar specificity (Table 3). Both were effective with arylacyl esters but not with alky esters, and were able to hydrolyze thioesters. In each instance, activity was greatest for acetyl esters and decreased in rate with increasing size of the aryl group. All apparent K_m values were in the micromolar range. Consistent with the high rate of aspirin hydrolysis, i.e. a rate approximately the same for aspirin and for 4-nitrophenylacetate, was the relatively high maximum velocity of the other ortho derivatives when compared with their para analogs. The para isomer of aspirin, 4-acetoxybenzoate, was also highly active. Noteworthy for their poor ability as substrates were model amides, 4-nitroacetanilide and benzoyl-DL-argenine-4-nitroanilide, for which hydrolysis was not detected under standard assay conditions. The extremely sensitive fluorescence method [20] that utilizes flourescein isothiocyanate-labeled casein was also negative for activity.

Spontaneous hydrolysis of a number of substituted phenylacetates show the expected relationship in a Hammett plot (Fig. 4): the better the leaving group as based on the σ value, the greater the rate of hydrolysis. In evaluating the effect of enzyme I, however, V_{max} was found to remain constant for the several phenylacetates that were used (Fig. 4) despite differences of two orders of magnitude in K_m. These findings are entirely consistent with the mechanism proposed for serine esterases, in which the rate-limiting step is believed to be the hydrolysis of the acyl enzyme intermediate [26]. The limiting reaction would be the cleavage of acetyl-enzyme I for each of the esters shown in Fig. 1 and, hence, the same V_{max} for each acetyl ester. When the acyl moiety of 4-nitrophenyl ester was changed, the K_m differed by a factor of less than five but V_{max} changed from 1.5 μmol/min/mg of protein for acetate to 5 for propionate and progressively decreased for butyrate (0.8), hexanoate (0.1) and octanoate (0.01).

Inhibition. Incubation of either esterase for 10 min with 0.1 mM paraoxon resulted in total inhibition. Similar incubation with 0.1 mM bis-4-nitrophenyl phosphate yielded 90% inhibition. If, however, the incubation mixtures also contained substrate, i.e. when 2 mM aspirin was presented to the enzyme simultaneously with either inhibitor, protection was complete. The following compounds were without inhibitory effect when present in the standard assay system at the designated concentrations: 2 mM EDTA; 0.25 mM 4-hydroxy-mercuribenzoate; 2 mM 2-mercaptoethanol; 1 mM dithiothreitol; 100 μM pepstatin A; 0.1% (w/v) lysophosphatidylcholine; 1 mM salicylic acid; 40 mM sodium acetate; 1 mM sodium butyrate; 2 mM sodium succinate; and 1 mM barium sulfate, calcium chloride, cadmium sulfate, cobaltous sulfate, cuprous sulfate, magnesium sulfate, manganous chloride, mercuric chloric or zinc sulfate.

DISCUSSION

The carboxyesterases and arylesterases that have been described adequately are those that were first solubilized and then purified from the microsomal fraction of cell extracts. Characterization of the rich variety of these enzymes has yielded recently a clearer view of the number of such esterases available in rat liver microsomes. Attention has been concentrated on the microsomal enzymes because 90% of the total esterases of the cell are in this compartment when model esters are used as substrate [28]. Table 1 confirms this distribution but also discloses that hydrolytic activity for aspirin as substrate is present in equal amounts in the cytosol and microsomes.

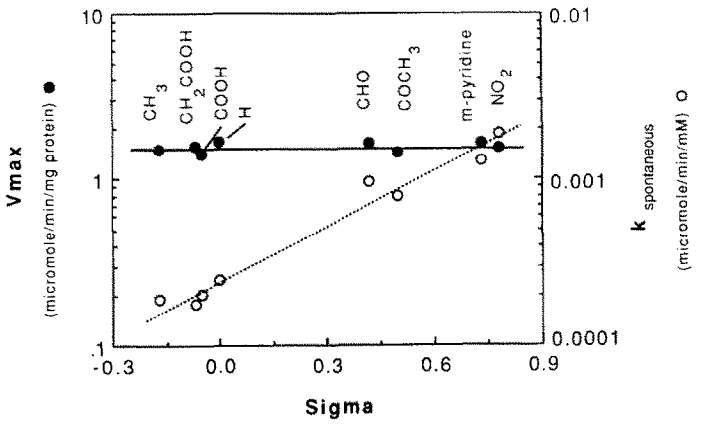

Fig. 4. Effect of substituents on enzymatic (●) and spontaneous (○) hydrolysis of phenylacetates in 50 mM Bicine at pH 8.0. All substitutions are at the 4-position with the exception of pyridine at the 3-position. Values for σ are from Ref. 27.

Prompted by the finding of high cytosolic esterase activity, we set out to characterize it, revealing at least five "soluble" proteins in rat liver capable of hydrolyzing aspirin. Three of these were not purified and were characterized only grossly as of high M_r, relatively high K_m, and acidic pH optimum. Two others, representing two-thirds of the aspirin-hydrolyzing activity of the cytosol, were prepared as electrophoretically homogeneous proteins of similar specificity. The two are probably isozymes since they also have the same M_r, react in an immunologically identical manner with an antibody prepared against one of the isozymes, and differ only by 0.15 in pI.

The purified esterases act optimally at pH 7.8 and have been characterized as to their substrate spectrum with a number of model esters. Unfortunately, comparative data from the purified microsomal enzymes are not available for all of the ortho and para substituted derivatives that are examined here. It is clear, however, that esterase ES4, the homogeneous enzyme prepared from microsomes, hydrolyzed aspirin at only 3% of the rate at which it catalyzed the hydrolysis of 1-naphthylacetate [1], whereas the two substrates were hydrolyzed at near equal rates by both of the presently described soluble isozymes that are being presented. The homogeneous isozymes described here also differ from the homogeneous rat liver esterase ES4 by their M_r (35 kD as compared to 60 kD); by the far lower activity with long chain acylCoA thioesters and glycerol esters than ES4; and by a lower K_m for aspirin that is in the micromolar (Table 3) rather than the millimolar [1] range. A partially purified preparation from guinea pig liver microsomes [7] and a relatively crude preparation from liver cytosol [29] have M_r values of about 55 and 35 kD, respectively, but data for substrate specificity is limited by the presence of contaminating esterases. Exploration of enzymes with activity for aspirin from human liver [6] and intestinal mucosa [6, 30], and from rat intestinal mucosa [31], remains at the stage of unpurified enzymes that may be contaminated by other esterases.

Despite our not having yielded to the temptation, it is difficult to avoid referring to these enzymes as aspirinases since a ready means of distinguishing among the many esterases of overlapping specificity is needed for communication. At present, these enzymes are included under the headings of arlyesterases (EC 3.1.1.2) and carboxyesterase (EC 3.1.1.1), encompassing all of the wide variety of non-specific esterases that exist. Obviously, a more detailed nomenclature is needed.

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