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Partial purification and characterization of 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine : acetyl-CoA acetyltransferase from rat spleen

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The enzyme 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine : acetyl-CoA acetyltransferase (EC 2.3.1.67) was purified from rat spleen approx. 1500-fold in 1.6% yield. The specific activity of the purified enzyme was 0.317 ± 0.089 μmol/min per mg of protein (mean ± S.D., $n = 6$). The K_m for the substrate acetyl-CoA was 137 ± 13 μM and the pH optimum was about 8. Incubation of the purified enzyme with 1-*O*-[^{3}H]octadecyl-2-lyso-*sn*-glycero-3-phosphocholine followed by electrophoresis resulted in the incorporation of radioactivity into a protein of M_r 29000. The enzyme was most active towards 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine as substrate, 1-palmitoyl-2-lyso-glycero-3-phosphocholine being a poor substrate. In addition, the enzyme preferred acetyl-CoA to palmitoyl-CoA or oleoyl-CoA as substrate.

INTRODUCTION

Paf-acether (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is a biologically active phospholipid with a wide spectrum of actions, including platelet-activating and antihypertensive activities. A number of investigators have demonstrated that paf-acether is released from a variety of tissues and cell types, including basophils, neutrophils, platelets, macrophages, amniotic fluid, urine, saliva, pulmonary alveolar fluid and peripheral blood (for review see Benveniste & Arnoux, 1983). Specific enzymic reactions involved in the biosynthesis of this compound have been documented (Wykle & Snyder, 1976; Wykle *et al.*, 1980; Alonso *et al.*, 1982; Albert & Snyder, 1983). Paf-acether can be synthesized either by the cholinephosphotransferase pathway or by acetylation. The acetylation pathway consists in the addition of an acetyl group to a molecule of lyso-paf-acether, acetyl-CoA being the acetyl donor. This pathway is markedly stimulated in inflammatory cells by agents known to initiate paf-acether synthesis. Only this pathway can be converted from a low-activity form into a high-activity form in human polymorphonuclear leucocytes stimulated with phagocytosable particles (Alonso *et al.*, 1982). The addition of zymosan to these cells induces a fast and transient up-to-10-fold activation in the $V_{max.}$ of the acetylation reaction (Alonso *et al.*, 1982). Rat and mouse peritoneal cells have been reported to exhibit the same activity, associated mainly with adherent mononuclear cells (Ninio *et al.*, 1982).

The process of activation of acetyltransferase in peritoneal macrophages requires Ca^{2+} entry, and Ca^{2+} uptake correlates well with the extent of the enzymic activation (Gomez-Cambronero *et al.*, 1984). It has been reported that Ca^{2+} at micromolar concentrations modulates the acetyltransferase activity by decreasing the K_m for acetyl-CoA. This action seems to be independent of the presence of calmodulin or protein phosphorylation (Gomez-Cambronero *et al.*, 1985*a*). In addition, the enzyme seems to be modulated by reversible phosphorylation through a cyclic AMP-dependent protein kinase (Gomez-Cambronero *et al.*, 1985*b*).

Taking into account that acetyltransferase seems to play an essential role in the regulation of the biosynthesis of paf-acether, we have undertaken the purification of this enzyme from rat spleen. Although acetyltransferase activity has been found in a variety of rat tissues (lung, lymph nodes and thymus), its activity is highest in the spleen (Wykle *et al.*, 1980). Since lymphoid cells do not contain acetyltransferase activity (Jouvin-Marche *et al.*, 1984), all the enzyme activity present in this preparation is accounted for by spleen resident macrophages.

MATERIALS AND METHODS

Materials

1-*O*-Octadecyl-2-lyso-*sn*-glycero-3-phosphocholine was from Bachem Feinchemikalien, Bubendorf, Switzerland. Acetyl-CoA was from Boehringer Mannheim, Mannheim, Germany. [^{3}H]Acetyl-CoA (3.8 Ci/mmol) was from New England Nuclear, Boston, MA, U.S.A. Sodium deoxycholate, 2-mercaptoethanol, leupeptin and lipid standards for t.l.c. were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Triton X-100, soya-bean trypsin inhibitor and thin-layer silica G plates were obtained from Merck, Darmstadt, Germany. DEAE-Sepharose CL-6B, 6-carboxyhexyl-Sepharose 4B and thiopropyl-Sepharose 6B were from Pharmacia, Uppsala, Sweden. Ultrogel AcA-22 was from LKB, Stockholm, Sweden. Products for electrophoresis, reagents for silver staining and reagents for protein determination were from Bio-Rad Laboratories, Richmond, CA, U.S.A. NCS tissue solubilizer, L-lyso-3-

Abbreviations used: paf-acether, platelet-activating factor; lyso-paf-acether, lyso-platelet-activating factor.

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Table 1. Summary of the purification of acetyltransferase from rat spleen

The extract obtained by homogenization of rat spleen was used as starting material. Full experimental details are given in the text. Acetyltransferase activity was assayed with 100 μM-acetyl-CoA under standard conditions. Data represent means ± S.D. for six experiments.

Step	Volume (ml)	Total protein (mg)	Total activity (nmol/min)	Specific activity (nmol/min per mg)	Yield (%)	Purification (fold)
Homogenate	250	3750±150	787±118	0.21±0.04	100	1
Microsomes	33	194±16	312±31	1.60±0.13	39±4	7.6±0.6
Solubilized microsomes	38	182±13	236±17	1.29±0.11	30±2	6.1±0.5
$(NH_4)_2SO_4$ fraction	8	62±10	220±20	3.55±0.60	28±3	16.9±2.9
Ultrogel AcA-22 fraction	65	13±3	174±24	13.4±3.3	22±3	63.7±15.9
DEAE-Sepharose CL-6B fraction	16	1.2±0.2	79.3±15.8	66.1±13.8	10±2	315±66
6-Carboxyhexyl-Sepharose 4B fraction	4	0.10±0.03	20.3±3.3	203±67	2.5±0.4	966±319
Thiopropyl-Sepharose 6B fraction	8	0.04±0.01	12.7±1.5	317±89	1.6±0.2	1512±423

[1-*palmitoyl*-1-^{14}C]phosphatidylcholine (56 mCi/mmol), [1-*O-alkyl*-^{3}H]paf-acether (115 Ci/mmol) and lyso-[1-*O-octadecyl*-^{3}H]paf-acether (80 Ci/mmol) were from Amersham International, Amersham, Bucks., U.K.

Preparation of microsomes

In a typical purification, 40 spleens from normally fed Wistar rats of about 200 g body wt. were homogenized in 12 vol. of ice-cold 0.1 M-Tris/HCl buffer, pH 7.4, containing 0.3 M-sucrose, 0.5 mg of soya-bean trypsin inhibitor/ml and 50 μg of leupeptin/ml (buffer A). Microsomal fractions (referred to below simply as 'microsomes') were isolated as previously described (Gomez-Cambronero *et al.*, 1985*a*) by two-step centrifugation and resuspended at a concentration of about 6 mg of protein/ml in 20 mM-Tris/HCl buffer, pH 7.4, containing 5 mM-2-mercaptoethanol (buffer B). Acetyltransferase activity in this fraction was stable at least for 2 months at −80 °C.

Assay of acetyltransferase activity

The acetylation reaction was carried out in buffer B containing 10 μg of lyso-paf-acether, 0.5 μCi of [^{3}H]-acetyl-CoA and 100 μl (steps 1–4) or 400 μl (steps 5 and 6) of fraction obtained in the purification procedure. This medium was used for detection of enzyme activity in the different fractions of the purification steps only with the purpose of determining the enzyme activity peaks; however, for the assays carried out on the pooled fractions, whose results are expressed as nmol/min per mg of protein, the standard medium was supplemented with 100 μM unlabelled acetyl-CoA. The enzyme reaction was carried out for 15 min at 37 °C and stopped by the addition of 3.7 ml of chloroform/methanol (1:2, v/v) for lipid extraction (Alonso *et al.*, 1982).

Products of the enzyme reaction were separated by t.l.c. After lipid extraction, samples were dried under a stream of N_2, dissolved in 100 μl of chloroform/methanol (9:1, v/v) and applied to silica-gel plates, which were developed with propionic acid/propanol/chloroform/water (2:2:1:1, by vol.). The areas of the plates migrating as synthetic paf-acether were scraped off and counted for radioactivity (Alonso *et al.*, 1982).

Purification procedure

All the procedures were carried out at 4 °C and are summarized in Table 1.

Step 1: solubilization of microsomes. All attempts to solubilize the microsomes by sonication in the absence of detergents were unsuccessful, and for this reason microsomes were incubated with various solubilizing agents and then were centrifuged at 105000 *g* for 1 h at 4 °C. The supernatant from this centrifugation is designated solubilized microsomes and was used for subsequent steps. On the basis of the results obtained with the different detergents employed, the final design for this step was as follows: 5.5 vol. of microsomes at a concentration of about 6 mg of protein/ml in ice-cold buffer B was incubated for 30 min at 4 °C in the presence of 0.4% sodium deoxycholate (final concentration).

Step 2: $(NH_4)_2SO_4$ precipitation. Solubilized microsomes were incubated with $(NH_4)_2SO_4$ at a concentration of 145 g/l for 3 h at 4 °C. The precipitated proteins were collected by centrifugation at 105000 *g* for 5 min, and the supernatant was centrifuged again under the same conditions. The pellet was resuspended in a small amount of buffer B, dialysed against 4 litres of the same buffer for 2 h and then centrifuged at 45000 *g* for 1 h. The supernatant of this last centrifugation (fraction I) was used for the next step.

Step 3: Ultrogel AcA-22 chromatography. Gel filtration of fraction I was carried out on a column (2.6 cm × 100 cm) containing Ultrogel AcA-22 equilibrated with buffer B and eluted in the same buffer. The fraction containing acetyltransferase activity that resulted from this procedure and was used for further purification procedures is designated fraction II.

Step 4: DEAE-Sepharose CL-6B chromatography. Fraction II was applied on a DEAE-Sepharose CL-6B column (1.6 cm × 40 cm) equilibrated with buffer B. The column was washed with 2 column volumes of 70 mM-NaCl in buffer B. The chromatography was then developed with a 220 ml linear gradient of

70–600 mM-NaCl in buffer B. The fractions containing acetyltransferase activity that resulted from this procedure and were further processed are designated fraction III, and after 2–3-fold concentration in a Shandon Speed Vac concentrator, were dialysed against buffer B without 2-mercaptoethanol (buffer C).

Step 5: 6-carboxyhexyl-Sepharose 4B chromatography. After dialysis, fraction III was applied on to a 6-carboxyhexyl-Sepharose 4B column (0.9 cm × 15 cm) previously equilibrated with buffer C. After a washing, the column was eluted with the same buffer containing 50 mM-NaCl until the A_{280} was less than 0.05. The column was eluted with a linear gradient of 50–500 mM-NaCl in buffer C, and the fractions containing the enzyme activity were pooled as fraction IV, concentrated 4-fold in a Speed Vac concentrator and dialysed against buffer C.

Step 6: covalent chromatography with thiopropyl-Sepha·ose 6B. Fraction IV was applied on to a thiopropyl-Sepharose column (0.9 cm × 4 cm) equilibrated in buffer C. It was essential to avoid the presence of reducing agents in this step, since these might interfere with the coupling reaction. In order to remove 2-mercaptoethanol, the preceding step was carried out in buffer C and the sample was extensively dialysed against the same buffer. The column was washed with buffer C until the A_{280} of the eluate was close to zero. Then the column was eluted with buffer C containing 50 mM-2-mercaptoethanol. Owing to the high degree of substitution of Sepharose with activated thiol groups, some residual 2-pyridyl groups remained after the coupling reaction and are released by the eluting buffer as free 2-thiopyridone. Since this compound interferes with the enzymic assay and absorbs at 280 nm, each fraction was extensively dialysed against buffer B and then prepared for the enzymic assay and characterization (fraction V), or was passed through Sephadex G-25 before electrophoresis in the specific radiolabelling experiments.

SDS/polyacrylamide-gel electrophoresis

SDS/polyacrylamide-gel electrophoresis was performed with 12.5%-(w/v)-acrylamide slab gels in a Tris/HCl buffer containing SDS as described by Weber & Osborn (1975). Proteins were silver-stained as described in Bio-Rad Laboratories Bulletin 10898. Standards for M_r determination were: lysozyme (M_r 14400), soya-bean trypsin inhibitor (M_r 21500), carbonic anhydrase (M_r 31000), ovalbumin (M_r 45000), bovine serum albumin (M_r 66200) and phosphorylase *b* (M_r 92500).

Specific radiolabelling with [^{3}H]lyso-paf-acether

A portion of the purified enzyme was concentrated in a Speed Vac concentrator and dialysed against buffer C. Samples (50 μl) containing about 20 μg of protein were incubated at 37 °C for 10 min with 4 μl of lyso-[^{3}H]paf-acether (0.1 μCi), 10 μl of lyso-paf-acether (1 mg/ml) and 10 μl of 0.5 mM-acetyl-CoA. After incubation, the sample was centrifuged at 1000 *g*, and the supernatant was mixed with 1 vol. of sample buffer for SDS/polyacrylamide-gel electrophoresis containing 5 mM-2-mercaptoethanol and 27% (v/v) glycerol. The sample, which was not boiled, was applied to 12.5% polyacrylamide gels and then electrophoresed as described above. After electrophoresis, the gel was washed twice, with a 2 h interval, with 10% (v/v) acetic acid and then cut into 2 mm slices. Each piece of gel was treated with NCS solubilizer/water (9:1, v/v) in a scintillation vial and incubated for 2 h at 50 °C, and, after neutralization with 34 μl of acetic acid/ml of NCS solubilizer, the amount of radioactivity was determined as described (Mato & Marin-Cao, 1979).

In order to ascertain the specificity of the enzyme, in some experiments lyso-[^{3}H]paf-acether was replaced by [^{3}H]acetyl-CoA, lyso[^{14}C]phosphatidylcholine, [^{14}C]phosphatidylcholine or the product of the acetylation reaction, [^{3}H]paf-acether. Controls were carried out in parallel with samples incubated at 4 °C and with albumin or soya-bean trypsin inhibitor in the absence of the enzyme.

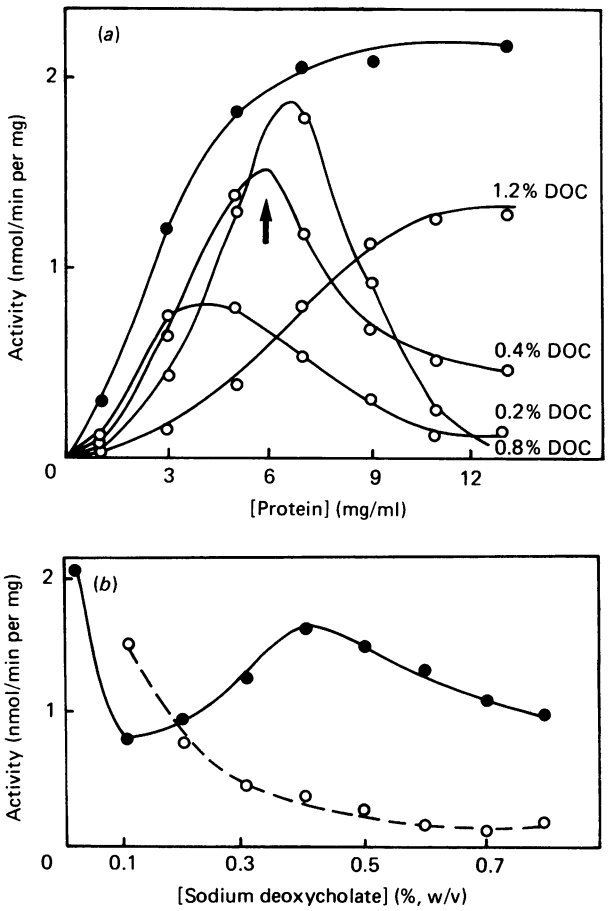

Fig. 1. Effect of different concentrations of deoxycholate on microsomal acetyltransferase activity

Portions (100 μl) of intact microsomes at various protein concentrations were treated with various concentrations of sodium deoxycholate (DOC), in a total volume of 1 ml in buffer B for 30 min at 4 °C (*a*). At the end of this period the acetyltransferase activity was measured in the mixtures as described in the Materials and methods section. ●, Microsomes in the absence of detergent; ○, deoxycholate-treated microsomes. In (*b*) 100 μl portions of intact microsomes (6 mg of protein/ml) were solubilized with different concentrations of sodium deoxycholate in a total volume of 1 ml in buffer B. The mixtures were then centrifuged at 105000 *g* for 1 h and the precipitates were resuspended in 1 ml of the same buffer without detergent. Activity in the supernatants (●) and precipitates (○) was assayed as described in the Materials and methods section.

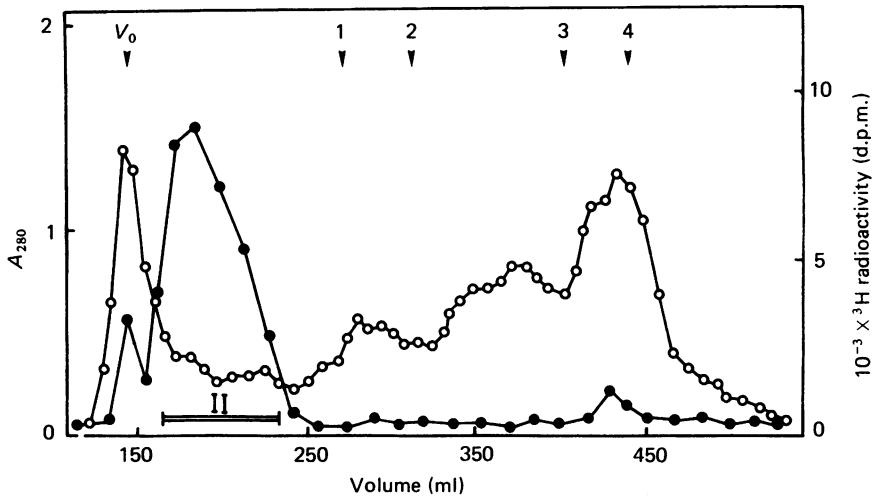

Fig. 2. Ultrogel AcA-22 column chromatography of the $(NH_4)_2SO_4$ fraction of microsomes

The crude fraction I (62 mg of protein) in 8 ml of buffer B was applied to an Ultrogel AcA-22 column (2.6 cm x 100 cm) equilibrated with the same buffer. The column was eluted with the equilibration buffer at a flow rate of 10 ml/h. Fractions (4 ml) were collected and 100 μl samples were assayed for protein (A_{280}, ○) and acetyltransferase activity (as ^3H radioactivity incorporated, ●) as described in the Materials and methods section. The bar indicates the fraction that was used for the following steps (fraction II). Arrows indicate markers used for the calibration of the column: V_0, Dextran 2000; 1, ferritin; 2, IgA; 3, bovine serum albumin; 4, cytochrome *c*.

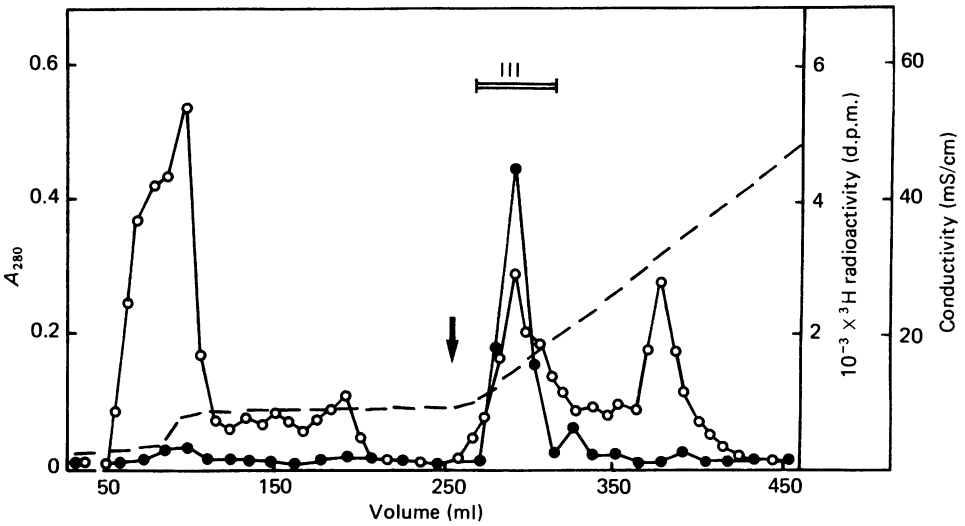

Fig. 3. Elution profile of acetyltransferase on DEAE-Sepharose CL-6B

The sample (65 ml), fraction II, was applied and, after being washed with buffer B containing 70 mM-NaCl, the column was eluted (arrow) with a linear gradient (——) of 70–600 mM-NaCl (220 ml total volume) in the same buffer. Fractions (4 ml) were collected and 100 μl samples were assayed for protein (A_{280}, ○) and acetyltransferase activity (as ^3H radioactivity incorporated, ●) as described in the Materials and methods section. Fractions with acetyltransferase activity were pooled (fraction III), concentrated and dialysed against buffer C.

Other methods

Protein concentration was determined by the method of Lowry *et al.* (1951) and, when the protein concentration was less than 0.5 mg/ml, as described by Bradford (1976); bovine serum albumin was used as standard. To calibrate the Ultrogel AcA-22 column the following markers were used: Dextran 2000 (M_r 2000000), ferritin (M_r 440000), IgA (M_r 150000), bovine serum albumin (M_r 66000) and cytochrome *c* (M_r 13000).

RESULTS AND DISCUSSION

Enzyme source

Experiments were carried out to determine the subcellular distribution of the acetyltransferase activity of spleen and liver in rats and dogs. Most of the activity was recovered in the 105000 *g* pellet (microsomal fraction), and the specific activity in spleen was higher than that of the liver (up to 15-fold). The animal species with the

higher specific acetyltransferase activity was the rat (6.2 nmol/min per mg of protein versus 57 pmol/min per mg of protein in the dog). Hence rat spleen microsomes and microsomal fractions were used as the enzyme source for the purification process.

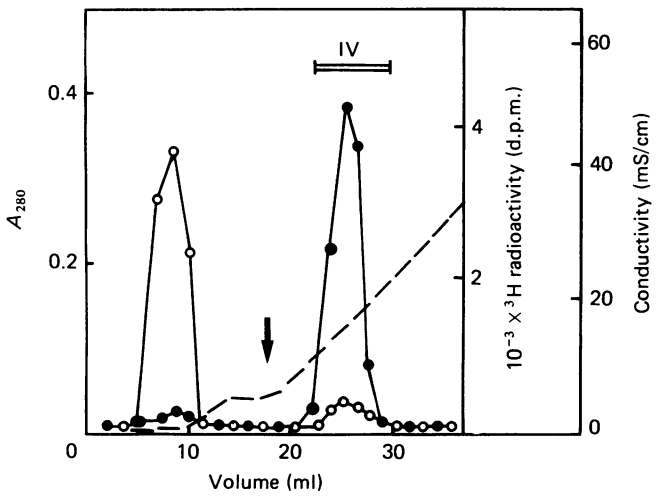

Fig. 4. 6-Carboxyhexyl-Sepharose 4B chromatography of the concentrated fraction obtained after DEAE-Sepharose chromatography

The column (0.9 cm × 15 cm) was equilibrated in buffer C. After application of the sample (16 ml), the column was washed with buffer C containing 50 mM-NaCl. Acetyltransferase was then eluted (arrow) with a linear gradient (——) of 50–500 mM-NaCl (20 ml total volume) in the same buffer. The flow rate was 4 ml/h. Fractions (1 ml) were collected and 0.5 ml samples were assayed for protein (A_{280}, ○) and acetyltransferase activity (as ^{3}H radioactivity incorporated, ●). Fractions with activity were pooled (fraction IV), concentrated and dialysed against buffer C.

Purification procedure

All attempts to solubilize microsomes by sonication in the absence of detergents were unsuccessful, less than 10% of the acetyltransferase activity being found in the supernatant after centrifugation at 105000 *g* for 1 h. Urea (8 M) and SDS (0.1%) inactivated the enzyme. Triton X-100 at 0.6% induced a solubilization of almost 95% of the total activity initially found in microsomes. Deoxycholate was the most effective of the reagents tested and it solubilized nearly 100% of the activity found in the microsomes. Measurement of the acetyltransferase activity in the presence of deoxycholate showed a close dependence on both the amount of protein in the microsomes and the concentration of the detergent. The enzyme was active in a narrow range of protein concentration and had a maximum for 6 mg of protein/ml and 0.4% sodium deoxycholate (Fig. 1*a*, arrow). Hence a compromise protein/detergent ratio of 1.5:1 was chosen. Although 0.8% sodium deoxycholate is more effective than 0.4% sodium deoxycholate, the latter concentration provides the highest specific activity, as demonstrated by measuring the enzyme activity after centrifugation at 105000 *g* (Fig. 1*b*). The fraction obtained after $(NH_4)_2SO_4$ precipitation (fraction I) was chromatographed in a column of Ultrogel AcA-22. Two peaks of activity were separated under these conditions (Fig. 2). The first peak was eluted with the void volume and contained about 10% of the applied activity and a large amount of protein. The second peak was eluted in a volume close to the void volume and corresponded to M_r about 700000–1000000. About 80% of the applied enzymic activity was contained in this peak. The second peak was pooled as fraction II and used for DEAE-Sepharose CL-6B chromatography. A single peak of activity was eluted at about 14 mS/cm conductivity (120 mM-NaCl) (Fig. 3). A minor peak of enzymic activity that was observed when the gradient was discontinued up to 30 mS/cm conductivity was discarded

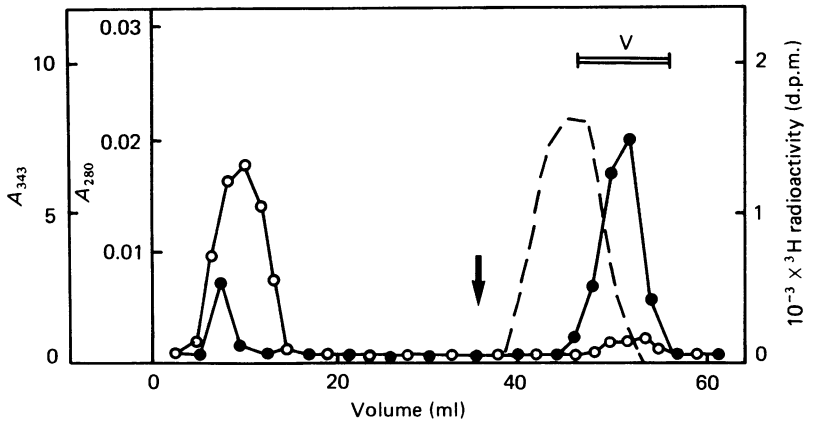

Fig. 5. Thiopropyl-Sepharose 6B chromatography of the concentrated fraction obtained after 6-carboxyhexyl-Sepharose 4B chromatography

The concentrated fraction IV (4 ml) was applied to a thiopropyl-Sepharose column (0.9 cm × 4 cm) equilibrated with buffer C. After the column had been washed with the equilibration buffer, the acetyltransferase was eluted (arrow) with buffer C containing 50 mM-2-mercaptoethanol. Fractions (2 ml) were collected at a flow rate of 2 ml/h. Each fraction was dialysed against buffer B and samples were assayed for protein (A_{280}, ○) and acetyltransferase activity (as ^{3}H radioactivity incorporated, ●). Those fractions absorbing at 343 nm (——) owing to the presence of 2-thiopyridone were corrected for their contribution at 280 nm. The bar indicates the fraction that was used for characterization studies.

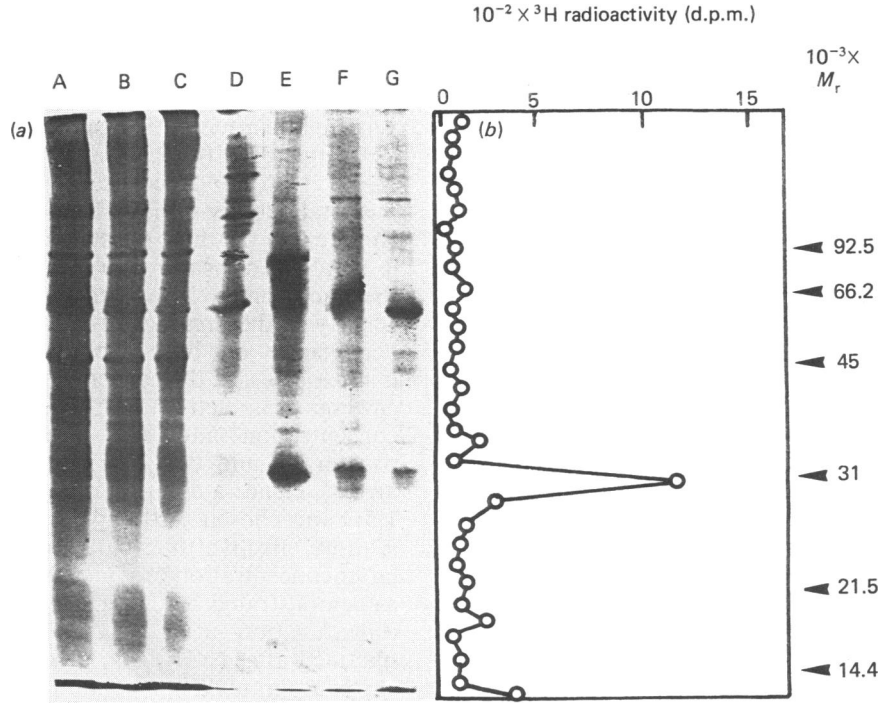

Fig. 6. SDS/polyacrylamide-gel electrophoresis of the different fractions obtained during the purification of microsomal acetyltransferase

Samples from different purification steps were precipitated with 10% (w/v) trichloroacetic acid and mixed with SDS-polyacrylamide-gel electrophoresis buffer containing 2-mercaptoethanol and 27% glycerol. The sample was boiled, except in the specific radiolabelling experiments, and silver-stained after completion of the electrophoresis. (*a*) Lane A, spleen homogenate; lane B, microsomes; lane C, solubilized microsomes; lane D, fraction II in step 3; lane E, fraction III in step 4; lane F, fraction IV in step 5; lane G, fraction V in step 6. ^{3}H radioactivity in (*b*) indicates the binding of the different portions of lane F or G that had been previously incubated with lyso-[^{3}H]paf-acether and acetyl-CoA. See the Materials and Methods section for a more detailed description.

in the purification process. Fraction III was applied on to a 6-carboxyhexyl-Sepharose 4B column. As shown in Fig. 4, acetyltransferase activity was eluted with the fractions whose conductivity was 15 mS/cm, and was pooled as fraction IV for chromatography on a column of thiopropyl-Sepharose 6B. In this last purification step acetyltransferase activity was eluted as a single peak of activity behind the 2-thiopyridone (Fig. 5).

Specific radiolabelling with lyso-[^{3}H]paf-acether

The enzyme purified by the above-mentioned procedure has M_r about 800000, as estimated by gel filtration on Ultrogel AcA-22. However, several bands of smaller M_r were detected after SDS/polyacrylamide-gel electrophoresis of the active fractions eluted from the 6-carboxyhexyl-Sepharose and thiopropyl-Sepharose columns. In order to identify which of these bands contained the acetyltransferase activity, the following experiments were carried out. Portions of fraction IV (Fig. 4) or fraction V (Fig. 5) were incubated with lyso-[^{3}H]paf-acether plus acetyl-CoA. After SDS/polyacrylamide-gel electrophoresis of the mixture, only one protein band contained radioactivity (Fig. 6). This band had M_r about 29000, coincided with one of the proteins detected by silver staining, and contained 1200 d.p.m. of ^{3}H-labelling. This value is taken as 100% for comparison with the labelling obtained with other substances. Incorporation of radioactivity was reduced to 65% when the enzyme was incubated in the presence of [^{3}H]acetyl-CoA and to 70% when the product of the reaction [^{3}H]paf-acether was incubated at 37 °C for 10 min. Incubation at 4 °C resulted in failure to show significant labelling with lyso-[^{3}H]paf-acether (less than 10%). According to these findings, lyso-[^{3}H]paf-acether could be utilized as a specific marker for the identification of the acetyltransferase in SDS/polyacrylamide-gel electrophoresis. In control experiments the enzyme was replaced by albumin or soya-bean trypsin inhibitor, and under these conditions no peak of radioactivity was observed (results not shown). If the substrates were lyso[^{14}C]phosphatidylcholine and acetyl-CoA, the amount of radioactivity incorporated was reduced to 27%, and there was no incorporation when the enzyme was incubated with [^{14}C]phosphatidylcholine and acetyl-CoA.

Partial characterization

The enzyme lyso-paf-acether:acetyl-CoA acetyltransferase has been purified about 1500-fold by this procedure. The enzyme retained its activity after being freeze-dried and was stable for several weeks at 4 °C in the presence of 5 mM-2-mercaptoethanol. Its M_r, as determined by gel filtration on Ultrogel AcA-22, is about 800000. However, several bands of smaller M_r are visible

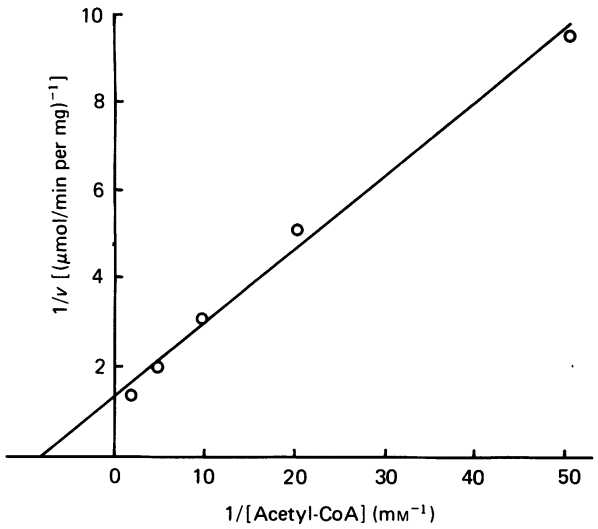

Fig. 7. Double-reciprocal Lineweaver–Burk plot of purified microsomal acetyltransferase

Enzyme activity was assayed in presence of Ca^{2+} in buffer C. Each point represents the mean value of duplicate experiments.

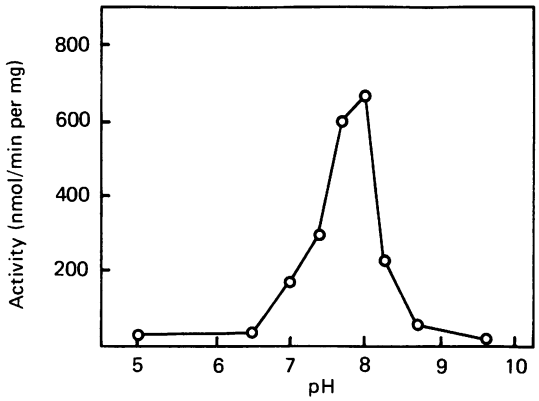

Fig. 8. pH–activity profile for purified microsomal acetyltransferase

Portions of the purified enzyme were incubated with lyso-[^{3}H]-acether and acetyl-CoA as described in the Materials and methods section at various pH values. The pH range 5–7 was obtained with a phosphate buffer and the pH range 7–9 with buffer C.

after SDS/polyacrylamide-gel electrophoresis of the purified enzyme. A single protein with M_r about 29000 incorporated radioactivity after incubation with lyso-[^{3}H]paf-acether. These results suggest that the enzyme is purified as an aggregate of several proteins or aggregates coated with lipids, with M_r about 800000. Affinity-labelling experiments suggest that the protein of M_r 29000 is the acetyltransferase or the catalytic subunit of the enzyme. Whether the other proteins present in the enzyme preparation are contaminants or play any role in modulating acetyltransferase activity remains to be determined. As shown in Fig. 7, the apparent K_m for acetyl-CoA in the presence of Ca^{2+} was 137 ± 12.9 μM, a value that is very similar to our previous data for rat spleen microsomes and to those from other authors (Wykle *et al.*, 1980; Ninio *et al.*, 1982; Gomez-Cambronero *et al.*, 1985*a*). The $V_{max.}$ of the acetylation reaction was 0.82 ± 0.14 μmol/min per mg of protein (mean ± S.D. for three experiments in duplicate), close to that reported to other membrane-bound enzymes of lipid metabolism (Jarvis *et al.*, 1984). The purified enzyme has optimum pH 7.6–8.2 (Fig. 8); this range was repeatedly obtained in a set of five independent preparations of the enzyme and is similar to that reported for the microsomal enzyme, although it differs slightly from the values reported by other authors (Albert & Snyder, 1983).

The enzyme prefers 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine to other lipid substrates. Thus the activity in the presence of 1-palmitoyl-2-lyso-*sn*-glycero-3-phosphocholine was about 12% of that observed with 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine. Similarly, the enzyme also prefers acetyl-CoA to palmitoyl-CoA or oleoyl-CoA. Thus the activities with palmitoyl-CoA and oleoyl-CoA were respectively 5% and 10% of that observed with acetyl-CoA. These results strongly suggest that this enzyme is specific for the synthesis of paf-acether by the acetylation pathway.

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