Isolation of palmitoyl-CoA hydrolases from human blood platelets

Rolf K. BERGE, Leif E. HAGEN and Mikael FARSTAD

Laboratory of Clinical Biochemistry, University of Bergen, N-5016 Haukeland Sykehus, Norway

(Received 8 June 1981/Accepted 26 August 1981)

The palmitoyl-CoA hydrolase activity, which in human blood platelets is mainly localized in the cytosol fraction [Berge, Vollset & Farstad (1980) *Scand. J. Clin. Lab. Invest.* **40**, 271–279], was found to be extremely labile. Inclusion of glycerol or palmitoyl-CoA stabilized the activity during preparation. Gel-filtration studies revealed multiple forms of the enzyme with molecular weights corresponding to about 70 000, 40 000 and 24 000. The relative recovery of the mol.wt.-70 000 form was increased by the presence of 20% (v/v) glycerol or 10 μ M-palmitoyl-CoA. The three enzyme forms are probably unrelated, since they were not interconvertible. The three different species of palmitoyl-CoA hydrolase were purified by DEAE-cellulose and hydroxyapatite chromatography, isoelectric focusing and high-pressure liquid chromatography (h.p.l.c.) to apparent homogeneity. The three enzymes had isoelectric points (pI) of 7.0, 6.1 and 4.9. The corresponding molecular weights were 27 000–33 000, 66 000–72 000 and 45 000–49 000, calculated from h.p.l.c. and Ultrogel AcA-44 chromatography. The apparently purified enzymes were unstable, as most of the activity was lost during purification. The enzyme with an apparent molecular weight of 45 000–49 000 was split into fractions with molecular weights of less than 10 000 by re-chromatography on h.p.l.c. concomitantly with a loss of activity. The stimulation of the activity by the presence of serum albumin seems to depend on the availability of palmitoyl-CoA, as has been reported for other palmitoyl-CoA hydrolases [Berge & Farstad (1979) *Eur. J. Biochem.* **96**, 393–401].

Palmitoyl-CoA hydrolase (EC 3.1.2.2) catalyses the irreversible hydrolysis of long-chain acyl-CoA esters. Human blood platelets have been shown to contain palmitoyl-CoA hydrolase activity (Berge & Farstad, 1978). By density-gradient and differential centrifugation most of the activity was localized to the cytosol fraction (Berge *et al.*, 1980a). However, blood platelets may contain more than one palmitoyl-CoA hydrolase as has been found in other tissues (Kurooka *et al.*, 1972).

Rat liver studies have demonstrated at least two separate and different palmitoyl-CoA hydrolases, one in the mitochondrial matrix fraction and one in the microsomal fraction (Berge & Farstad, 1979a). It has been reported that peroxisomes also contain high acyl-CoA hydrolase activity (Osmundsen *et al.*, 1980). Kurooka *et al.* (1972) have reported a palmitoyl-CoA-hydrolysing enzyme localized to the cytosolic fraction.

Brain from pig seems to contain at least two acyl-CoA-hydrolysing enzymes (Srere *et al.*, 1959),

whereas in bovine and rat brain and rat submaxillary gland the palmitoyl-CoA hydrolase seems to occur in multiple forms (Anderson & Erwin, 1971; Knauer, 1979; Knauer *et al.*, 1980).

We now report that human blood platelets probably contain three separate palmitoyl-CoA hydrolases, which are apparently purified. The hydrolases, both in homogenates and in more purified forms, were quite unstable, and low purification factors were obtained. One of the enzymes seemed to occur in multiple forms, the low-molecular-weight forms being almost inactive.

Experimental

Preparation of platelet homogenates

Platelet-rich plasma (PRP; 2 litres), containing 0.3×10^{12} platelets/litre, in acid citrate/dextrose solution was obtained from the Blood Bank of the University Hospital, Bergen, Norway. The PRP was centrifuged at 1500g for 6 min to remove contaminating erythrocytes and leucocytes. The PRP thus obtained contained about two erythrocytes and leucocytes per 1000 platelets. After centrifuga-

Abbreviation used: h.p.l.c., high-pressure liquid chromatography.

tion of the PRP at 3000g for 15 min, the resulting pellet was washed once with a buffer consisting of 15 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] (pH 7.4), 1.5 mM-MgCl₂, 0.1 mM-dithiothreitol and 0.1 mM-EDTA (buffer A). The final platelet homogenate contained about 7 mg of protein/ml. The platelets were disrupted by freezing and thawing twice, and the resulting whole homogenate was centrifuged in an SS-34 Rotor at 30000g for 20 min. The supernatant, containing about 60–70% of the total palmitoyl-CoA hydrolase activity (Berge *et al.*, 1980a), was used either immediately or after storage for a maximum of 1 week at –70°C.

Preparation of erythrocytes and leucocytes

Erythrocytes were obtained from healthy persons. The cells were washed three times in physiological saline (0.9% NaCl) and made up to a final concentration of 4×10^{12} cells/litre.

Heparinized venous blood (10 i.u./ml) was drawn from non-fasting healthy volunteers and the leucocytes were isolated by sedimentation with dextran (Bøyum, 1968). Contaminating erythrocytes were lysed with NH₄HCl. After washing twice in 10 vol. of 15 mM-phosphate buffer (pH 7.0) in 0.9% saline, the leucocytes were resuspended in 0.155 M-NaCl. Leucocyte counts were done in an electronic particle counter (Cell 920A). The final cell suspension contained about 70% neutrophils.

Chromatography

DEAE-cellulose, hydroxyapatite and Ultrogel AcA-44 chromatography were performed as described previously (Berge & Farstad, 1979b; Berge, 1979).

Isoelectric focusing

The LKB isoelectric-focusing column (model 8101; volume, 110 ml) was used. The pH gradient was created with 3% (w/v) ampholine, pH 3–10, in a sucrose gradient (0–48%, w/v). The focusing was continued for 48 h with a voltage of 150 V at the start and 600 V at the end of the run. Palmitoyl-CoA hydrolase activity and pH (at 4°C) were then measured for each 1 ml fraction.

Polyacrylamide-gel electrophoresis

The procedure used was as described by Berge & Farstad (1979b).

H.p.l.c.

A Waters h.p.l.c. instrument, equipped with a model 6000 A solvent-delivery system, model U6K sample injector, model 440 absorbance detector and a W+W recorder, was employed in these studies. A single Protein Column I-125 column (7.8 mm × 30 cm), pore size 12.5 nm, and designed to separate proteins that have a molecular-weight range

between 2000 and 80000, was equilibrated with 0.05 M-Na₂HPO₄ at pH 7.4 and placed on line with the solvent-delivery system. Appropriate volumes were injected into the column by using a Hamilton microlitre syringe. The proteins were eluted by using a solvent flow of 0.8 ml/min (1380–4150 kPa) at 25°C (unless otherwise stated) and detected at 280 nm.

Determination of molecular weights

The molecular weights of the native hydrolases were determined by gel-filtration chromatography on an Ultrogel AcA-44 column (1.6 cm × 90 cm). The enzymes were eluted at a flow rate of 0.7 ml/min (0.7 ml fractions) by a buffer containing 15 mM-Hepes, pH 7.4, 0.1 mM-dithiothreitol and 0.15 M-KCl (buffer B). The molecular weights were also determined by h.p.l.c. For both methods the elution time/volume or V_e/V_0 was a linear function of the logarithm of the molecular weights of the standard proteins. For the two methods, Blue Dextran was used to determine the void volume, and the standard proteins were serum albumin (mol.wt. 66000), ovalbumin (mol.wt. 45000), trypsinogen (mol.wt. 24000), myoglobin (mol.wt. 17000) and cytochrome *c* (mol.wt. 12500). Reproducible results of V_e/V_0 were obtained whether a complex protein mixture of the standards was used or single standard proteins were used in the presence of Blue Dextran.

Enzyme assays and other analytical methods

The palmitoyl-CoA hydrolase activity was assayed both radiochemically and spectrophotometrically as previously described (Berge & Døssland, 1979), except that 0.15 M-KCl, but no sucrose, was used. The radiochemical assay was based on the release of [1-¹⁴C]palmitate from [1-¹⁴C]palmitoyl-CoA in 30 mM-Hepes buffer (pH 7.4) and 150 mM-KCl. The spectrophotometrical assay was based on the trapping of CoA released from palmitoyl-CoA by 5,5'-dithiobis-(2-nitrobenzoic acid) in the same buffer. One unit of activity is defined as 1 nmol of palmitate or CoA formed/min. In all experiments an amount of enzyme was added to give a rate within the linear range with both time and protein. Substrate concentrations were adjusted to give constant ratios of substrate to protein in each experiment (Berge, 1979; Berge & Farstad, 1979b). All spectrophotometric measurements were performed with a Shimadzu MPS 5000 recording spectrophotometer. Radioactivity was counted in a Packard Tri-Carb model 3385 liquid-scintillation spectrometer.

Protein was determined by using the Folin-Ciocalteu reagent (Lowry *et al.*, 1951).

Reagents

[1-¹⁴C]Palmitoyl-CoA was obtained from New England Nuclear Corp., Boston, MA, U.S.A. Palmitoyl-CoA, Hepes, 5,5'-dithiobis-(2-nitrobenzoic acid) and dithiothreitol were from Sigma Chemical Co., St. Louis, MO, U.S.A. DEAE-cellulose was from Whatman Biochemicals, Maidstone, Kent, U.K., and hydroxyapatite was purchased from Bio-Rad Laboratories, Richmond, CA, U.S.A. Ampholine (pH 3–10) was from LKB, Bromma, Sweden. Proteins used as molecular-weight standards were obtained from Boehringer, Mannheim, Germany. All other reagents were of analytical purity.

Results

Both blood cells from the rat (Kurooka *et al.*, 1972) and blood platelets from man (Berge & Farstad, 1978) contain enzyme(s) that hydrolyse palmitoyl-CoA. The activities of palmitoyl-CoA hydrolase in platelets, erythrocytes and leucocytes were respectively 15–30, 5–8 and 2–3 units per 10⁹ cells. From these activities and the purity of the platelet preparation, it can be calculated that less than 1% of the hydrolase in the platelet suspension was due to contamination.

Fig. 1. Stabilizing effect of palmitoyl-CoA and glycerol on the hydrolase activity of the 30000 g supernatant as a function of storage at 4°C and 25°C

The concentration of glycerol and palmitoyl-CoA was 20% (v/v) and 10 μ M, respectively. Symbols: ●, buffer B; □, buffer B + glycerol; △, buffer B + palmitoyl-CoA.

Stabilization and gel-filtration studies of the palmitoyl-CoA hydrolase activity in human platelets

The presence of substrate (palmitoyl-CoA) stabilized the activity of palmitoyl-CoA hydrolase from both rat brain (Knauer, 1979) and rat submaxillary gland (Knauer *et al.*, 1980). These reports also suggested interconversion of two or more forms of the hydrolase. To study if this also might occur in blood platelets, some studies with platelet homogenates and the 30000 g supernatant were performed. The palmitoyl-CoA hydrolase was sensitive to manipulations as a considerable activity was lost after, e.g., dilution, dialysis, changes of temperature and gel filtration. Fig. 1 shows that the presence of 20% glycerol or 10 μ M-palmitoyl-CoA stabilized the enzyme activity in platelet homogenates at 4°C, but not at 25°C on storage up to 48 h. After 40-fold dilution, 60–70% of the hydrolase activity was lost even in the presence of 20% glycerol. Also dialysis for 6 h resulted in a loss of activity of 50–60%.

Gel filtration of the 30000 g supernatant eluted by buffer B on Ultrogel AcA-44 (see the Experimental section) revealed multiple peaks of palmitoyl-CoA hydrolase activity as shown in Fig. 2(a). The first peak coincided with the void volume and probably represented palmitoyl-CoA hydrolase activity associated with the fatty acid synthetase complex (Kumar, 1973). The following three peaks were eluted in regions consistent with molecular-weights of approx. 70000, 40000 and 24000 respectively. The recovery of the activity was low, approx. 5–10% of that applied. When palmitoyl-CoA (10 μ M) (Fig. 2b) or glycerol (20%, v/v) (Fig. 2c) was included in the elution buffer, the recovery was increased to 50–60% and 15–25% respectively. These stabilizing agents did not produce new peaks of hydrolase activity. The 70000-mol.wt. form seemed, however, either to be generated or stabilized by the presence of the stabilizing agents. In an attempt to increase the recovery of the hydrolase activity during gel filtration, 0.01% (w/v) Triton X-100 was included in the elution buffer (Fig. 2d). The elution pattern was similar to that obtained in the absence of Triton X-100, but the recovery increased to approx. 70%. When a portion of the pooled peak fraction from the column depicted in Fig. 2(b) or 2(c) (fractions 51–56 in both cases) was desalted to remove palmitoyl-CoA (non-protein bound) and glycerol, dialysed against buffer B, concentrated and re-chromatographed in the absence of added glycerol or palmitoyl-CoA, single peaks of hydrolase activity were eluted with mol.wt. 70000 in both cases. When the pooled fractions 63–69 were treated in the same way, single peaks of activity, consistent with mol.wt. 40000, were seen. The recovery of both peaks was in each case 50–60% of that applied. In addition, by exposing the

Fig. 2. Gel filtration of the 30000g supernatant of blood platelet homogenate

A portion (2 ml) of the 30000g supernatant was applied to an Ultrogel AcA-44 column as described in the Experimental section. Fractions (1.8 ml) were collected and assayed for palmitoyl-CoA hydrolase activity (220 nmol of palmitoyl-CoA/mg of protein). (a), Eluted by buffer B. The recovery was 5–10% of that applied. (b), A portion (2 ml) of 30000g supernatant was adjusted with palmitoyl-CoA (10 μ M) and eluted with buffer B + 10 μ M-palmitoyl-CoA. The recovery was 50–60% of that applied. (c), A portion (2 ml) of the 30000g supernatant was adjusted with glycerol to 20% (v/v) and eluted with buffer B + 20% (v/v) glycerol. The recovery was 15–25%. (d), A portion (2 ml) of the 30000g supernatant was adjusted with Triton X-100 to 0.01 (w/v) and eluted with buffer B + 0.01% (w/w) Triton X-100. The recovery was approx 70%. V_0 , void volume. The arrows marked with 1, 2 and 3 correspond to mol.wt. 70000, 40000 and 24000 respectively, on the basis of the molecular weight of the standard proteins serum albumin, ovalbumin and trypsinogen. Hydrolysis of 1 nmol of [^{14} C]-palmitoyl-CoA corresponds to 800 c.p.m. in (a) and to 200 c.p.m. in (b), (c) and (d).

40000-mol.wt. peak depicted in Fig. 2(a) to glycerol or palmitoyl-CoA, no generation of the 70000-mol.wt. form was found after gel filtration. When the 24000-mol.wt. peak was treated as described above,

to remove palmitoyl-CoA and glycerol (Figs. 2b and 2c), or exposed to palmitoyl-CoA and glycerol, no palmitoyl-CoA hydrolase activity was detected after re-chromatography on Ultrogel AcA-44, indicating that this enzyme form was unstable. Thus generation of higher molecular-weight forms of the hydrolase in human blood platelets from lower molecular-weight forms or vice versa by exposure to palmitoyl-CoA or glycerol seemed not to take place as has been reported for a similar enzyme from rat brain (Knauer, 1979).

The different molecular-weight forms of palmitoyl-CoA hydrolase of human blood platelets seem to be unrelated since they are not inter-convertible. Furthermore, the 40000- and 24000-mol.wt. forms always were eluted from the Ultrogel AcA-44 column, regardless of the presence or absence of glycerol, palmitoyl-CoA or Triton X-100.

Purification of palmitoyl-CoA hydrolases from human blood platelets

The different palmitoyl-CoA hydrolase species from human blood platelets seemed to be unrelated enzymes. However, solubilization and purification of the different species to homogeneity could provide further evidence that they are separate enzymes, or that the higher-molecular-weight species is generated by substrate-induced di- or tri-merization of lower molecular-weight species (subunits). Unless otherwise noted, the following steps were carried out at 4°C.

DEAE-cellulose (DE-52) chromatography

The 30000g supernatant from the platelet homogenate was applied to a DEAE-cellulose (DE-52) column (1.5 cm $\times$ 30 cm), equilibrated with buffer A, and the proteins were passed through the column at a flow rate of 1.5 ml/min. The proteins were eluted from the column by using a linear gradient from 0 to 0.5M-KCl in buffer A at the above flow rate. Fractions (5 ml) were collected and absorbance was recorded at 280 nm. The palmitoyl-CoA hydrolase activity was eluted as three peaks (I, II and III), one of the peaks being eluted through the column without binding (Fig. 3).

Hydroxyapatite chromatography

Peak-enzyme-activity fractions 5–15 (enzyme species I), 45–50 (enzyme species II) and 84–87 (enzyme species III) that were eluted from the DEAE-cellulose column (Fig. 3) were separately collected and dialysed for 4 h against a buffer containing 10 mM-KH₂PO₄, 1.5 mM-MgCl₂, 0.1 mM-diethiothreitol and 0.1 mM-EDTA (buffer C). The corresponding diffusates of these three peak-enzyme-activity fractions were applied separately to

Fig. 3. Elution profile of palmitoyl-CoA hydrolase activity from a DEAE-cellulose column
Fractions were collected and assayed for palmitoyl-CoA hydrolysis (■) by using the spectrophotometric assay method. For details see the Results and Discussion sections. Hydrolysis of 1 nmol of palmitoyl-CoA corresponds to a ΔA_{412} of 6×10^{-4} .

Fig. 4. Separate hydroxyapatite chromatography of palmitoyl-CoA hydrolase activity from the DEAE-cellulose chromatogram
(a) represents enzyme peak I, (b) peak II and (c) peak III from Fig. 3. Symbols: ■, palmitoyl-CoA hydrolysis; O, protein measured continuously by percentage transmission at 280 nm.

Fig. 5. Isoelectric focusing of palmitoyl-CoA hydrolase
The separate enzyme preparations (I, II and III) from hydroxyapatite chromatography were separately subjected to isoelectric focusing as described in the Experimental section. (a), Enzyme species I; (b), enzyme species II; (c), enzyme species III. Symbols: ■, palmitoyl-CoA hydrolysis; ●, pH.

a hydroxyapatite column (1.5 cm $\times$ 30 cm) equilibrated with buffer C. The different enzymes were eluted from the column using a linear gradient from 0 to 0.5 M-KH₂PO₄ in buffer C. The palmitoyl-CoA

hydrolase activities were eluted as sharp peaks (Figs. 4a-4c) (enzymes I, II and III) after approx. 96.3 ml (fraction 36), 143 ml (fraction 59) and 112 ml (fraction 38) respectively of buffer. Fractions con-

Fig. 6. Elution of palmitoyl-CoA hydrolase through a protein Column I-125

The amount of protein was about $1.2\mu\text{g}$ in $10\mu\text{l}$ injection volume. (a), Enzyme species II; (c), enzyme species III; (d) enzyme species I. (b) Re-chromatography of enzyme species II (peak from a), $400\mu\text{l}$ injection volume. The flow rate was 0.8 ml/min .

taining the enzymes I, II and III were separately collected, concentrated and dialysed for 3 h against 4 litres of a buffer B.

Isoelectric focusing

This was performed as described in the Experimental section. Fractions containing enzyme species I, II and III from the hydroxyapatite column were separately diluted in the middle three-fifths of the sucrose gradient. After completion of the focusing, fractions were collected and assayed for palmitoyl-CoA hydrolase activity (Figs. 5a–5c). Enzyme I was separated into three different peaks (Fig. 5a) with isoelectric points (pI) at 5.0, 6.2 and 6.9 respectively. Enzyme species II and III were eluted mainly as single peaks with isoelectric points (pI) at 6.1 and 4.9 respectively (Figs. 5b and 5c). Thus the isoelectric points of the latter enzymes are consistent with the pI obtained for the two eluted peaks of enzyme I. This indicates that enzyme species I was contaminated by enzyme species II and III, in spite of the apparent separation after DEAE-cellulose chromatography (Fig. 3).

H.p.l.c.

The palmitoyl-CoA hydrolase activity in fractions 65–70 from enzyme species I (Fig. 5a) and the

peak-enzyme-activity fractions (enzyme species II and III) in Figs. 5(b) and 5(c) were separately collected, concentrated and dialysed for 2 h against buffer C. The dialysis tubing used had a molecular-weight cut-off of approx. 10000. The different diffusates were injected into a Protein Column I-125 and chromatography was performed as described in the Experimental section at 25°C . The palmitoyl-CoA hydrolase activities for the different diffusates were eluted as symmetrical, sharp peaks (Figs. 6a, 6c and 6d). Fractions containing about 75% of the eluted hydrolase activities were collected and stored at -20°C .

Notes on the recovery and stability of the purified enzyme species

The inactivation of the enzyme species during the purification procedures represented an obstacle in the isolation of the three palmitoyl-CoA hydrolases. The enzyme activity was sensitive to manipulations, as considerable activity was lost after dialysis, concentration by Sephadex G-25/poly(ethylene glycol) or by Diaflo and by column desalting after each purification step. About 20% of the activity remained 2 h after dialysis with a pressure at 4 kg/cm^2 at 4°C . Some activity was found on the membrane filter, indicating that some absorption took place at high pressure. Freezing and thawing also inactivated the enzyme(s). In addition, the purified enzymes were less stable at 4°C than at room temperature in low ionic strength. An Ultrogel AcA-44 chromatography step between the hydroxyapatite chromatography and isoelectric focusing could not be used in the purification procedure as about 90% of the activity was lost during elution, dialysis and concentration of the eluted fractions. When the three enzyme species were separately applied to an Ultrogel AcA-44 column and eluted with buffer B, a single peak of hydrolase activity was eluted in regions corresponding to the molecular weights of the respective enzymes added to the column (results not shown). In addition, when the low-molecular-weight enzyme was exposed to palmitoyl-CoA, higher molecular-weight forms could not be eluted from the column, findings in agreement with the results in Fig. 2.

Table 1 outlines the results of a purification. The low purification factor may be explained by the inactivation that took place during each step of purification. For the enzyme species I and III no meaningful purification tables can be presented owing to the very low recovery of the activity after each purification step. However, from 500 mg of platelet protein about 0.1 mg of enzyme I and enzyme III was obtained with a specific activity of respectively 2 and 8 units/mg of protein.

Table 1. *Purification of enzyme II from human blood platelets*

The palmitoyl-CoA hydrolase activity was determined by the radiochemical method at a substrate concentration of approx. 220 nmol of palmitoyl-CoA/mg of protein, adjusted by the addition of serum albumin (see Fig. 8).

Purification step	Volume (ml)	Total protein (mg)	Total activity (units)	Specific activity (units/mg of protein)	Recovery (%)	Purification factor
Platelet homogenate	69	476	2600	5.5	100	1
30 000g supernatant	66	370	2220	6.0	85	1
DEAE-cellulose	43	60	1000	16.7	39	3
Hydroxyapatite	17	37	1400	37.8	54	7
Isoelectric focusing	4	1	90	90	3.5	16
H.p.l.c.	2	0.3	36	120	1.4	22

Criteria of purity and some physicochemical properties

The isolated enzymes were apparently purified on the basis of the following observations. Polyacrylamide-gel electrophoresis at pH 8.9 gave a single major band of each of the three isolated enzyme preparations. The gels were cut into slices (2 mm) and the single protein bands were eluted from the gels after 24 h at 4°C in tubes containing 50 mM-Hepes, pH 7.5, and 0.15 M-KCl. Single symmetrical peaks of a constant specific activity (using protein concentrations calculated from measurements of absorbance at 260 and 280 nm) of the palmitoyl-CoA hydrolase were obtained in the eluted protein bands after gel electrophoresis performed as previously described (Berge & Farstad, 1979b). Fig. 7(a) shows the results for enzyme species II. Migration of enzyme species II (Fig. 7a) indicated that this species is structurally stable and not a result of proteolysis during purification (Fig. 7b).

Single protein peaks, containing palmitoyl-CoA hydrolase activity, were obtained for the isolated enzyme preparations after h.p.l.c. (Fig. 6). This system had a high sensitivity as it permitted analytical separation of samples containing as little as 1 µg of protein. However, when the peak of enzyme species II (Fig. 6a) was re-chromatographed on the h.p.l.c.-column, new peaks of enzyme activity emerged (Fig. 6b), with low hydrolase activity, if any, and with mol.wt. <10 000. In addition, the original hydrolase peak (Fig. 6a) disappeared. That the hydrolase species II and III were of high purity was indicated by a single symmetrical peak of constant specific activity after re-chromatography on an Ultrogel AcA-44 column (results not shown). Re-chromatography of enzyme species I on this column gave no activity. The molecular weights of the hydrolase species were determined by an Ultrogel AcA-44 column (enzymes II and III) and h.p.l.c. On the basis of these results, enzyme species I, II and III had molecular weights in the range 27 000–23 000, 66 000–72 000 and 45 000–49 000 respectively.

Fig. 7. *Elution profile of palmitoyl-CoA hydrolase from polyacrylamide gels*

(a), Purified enzyme species II; (b), enzyme species II eluted from the DEAE-cellulose column (Fig. 1). For details, see the Results and Discussion sections.

Inclusion of serum albumin in the incubation medium stimulated the palmitoyl-CoA hydrolase (enzyme II) activity by as much as 35% (Fig. 8). In addition, the presence of increasing amounts of serum albumin shifted the ascending portion of the velocity–substrate-concentration profile from that of hyperbola to a more sigmoidal form. Thus as the serum albumin concentration was increased, higher concentrations of palmitoyl-CoA were required for maximum activity.

This effect is in agreement with observations on purified long-chain acyl-CoA hydrolases or thioesterases from other tissues (Lin & Smith, 1978; Berge & Farstad, 1979b; Berge, 1979). However, the inhibitory effect of excess substrate (Fig. 8) was less than with previously purified hydrolases and thioesterases.

Fig. 8. Effect of serum albumin on the substrate-concentration-velocity profile of enzyme species II. The enzyme (approx. 1 μ g) was incubated for 3 min at 37°C under standard incubation conditions in a final volume of 250 μ l (for details see Berge & Farstad, 1979b).

Discussion

The isolated palmitoyl-CoA hydrolase species of human blood platelets are unstable, especially enzyme species I and III. Also enzyme species II is unstable as re-chromatography on h.p.l.c. resulted in lower molecular-weight forms without measurable activity. Although higher-molecular-weight forms could not be formed from lower molecular-weight forms, some interconversion of two or more forms cannot be completely ruled out. The concentration of palmitoyl-CoA used in the present experiments may be sufficient for the formation of stable dimer forms of the enzyme in agreement with observations for palmitoyl-CoA hydrolase from other tissues (Knauer, 1979; Knauer *et al.*, 1980). In addition, enzyme I was contaminated with enzymes II and III (Fig. 5) in spite of apparently complete separation of the enzymes by DEAE-cellulose chromatography (Fig. 3). However, generation of higher molecular-weight forms from lower molecular-weight forms in the presence of glycerol or palmitoyl-CoA could not be observed as has been reported for similar enzymes from both brain and submaxillary gland of the rat (Knauer, 1979; Knauer *et al.*, 1980). As all the three forms were eluted also from the Ultrogel AcA-44 column in both the absence and presence of glycerol, palmitoyl-CoA and Triton X-100, it seems likely that enzyme species I, II and III are unrelated enzymes. This is also supported by the finding that all enzyme peaks were found regardless of whether the recovery was 5–10%, 20% or 60% of the amount applied (Figs. 2a–2d). As no intercon-

version seemed to take place in the presence of glycerol or palmitoyl-CoA, the increased relative recovery in the presence of these agents (Figs. 2b and 2c) of the 70 000-mol.wt. form enzyme (enzyme II) might be due to stabilization of this form without significant stabilizing effect on the two other forms.

The effect of serum albumin on the activity of enzyme II seems to be related to the substrate/protein ratio, as increasing palmitoyl-CoA concentration required higher concentrations of serum albumin for maximum activity (Fig. 8). Maximal activity was obtained at a ratio of about 220 nmol of substrate per mg of protein. Interpretation of the kinetics of the palmitoyl-CoA hydrolases is difficult since the substrate, palmitoyl-CoA, forms micelles (Zahler *et al.*, 1968; Lin & Smith, 1978; Berge *et al.*, 1980b). Recently, we have found that in the presence of a few μ g of purified palmitoyl-CoA hydrolase from rat liver, the critical micelle concentration was in the range 14–25 μ M at 37°C (Berge *et al.*, 1981). In the absence of serum albumin, maximal hydrolase activity occurred at palmitoyl-CoA concentrations that were approximately equal to the critical micellar concentrations mentioned above (Fig. 8). This suggests that the monomer form of palmitoyl-CoA is the active substrate also for the hydrolases isolated from the platelets, and that palmitoyl-CoA micelles are inhibitory.

In the presence of serum albumin (Fig. 8) at low palmitoyl-CoA concentration (range 7–15 μ M) the rate of hydrolysis was not proportional to the concentration of substrate, and small increases over the corresponding substrate concentration showed large increases in the rate of hydrolysis. Thus, the sigmoidal kinetics can be explained by the fact that at low substrate concentration the tight binding sites of serum albumin compete effectively for the substrate (monomer form) and when these sites are saturated the substrate becomes available to the enzyme. Furthermore, higher activity was obtained in the presence of serum albumin, indicating that serum albumin prevented micelle formation of palmitoyl-CoA. This is in agreement with the effect of serum albumin on the purified palmitoyl-CoA hydrolases from the mitochondrial matrix and microsomes from the liver of rat (Berge & Farstad, 1979b; Berge, 1979). We have more recently shown that monomeric palmitoyl-CoA not only is the substrate for the enzyme(s), but also has some stabilizing effect on the enzyme(s) (Berge & Farstad, 1979b; Berge, 1979; Berge *et al.*, 1980b; Berge *et al.*, 1981).

We thank K. Halvorsen, M.D., Head of the Blood Bank, The University Hospital (Haukeland Sykehus) for his kind co-operation. The study was supported by the Norwegian Council for Cardiovascular Diseases.

References

- Andersen, A. D. & Erwin, V. G. (1971) *J. Neurochem.* **18**, 1179–1186
- Berge, R. K. (1979) *Biochim. Biophys. Acta* **574**, 321–333
- Berge, R. K. & Døssland, B. (1979) *Biochem. J.* **181**, 119–125
- Berge, R. K. & Farstad, M. (1978) *Scand. J. Clin. Lab. Invest.* **38**, 699–706
- Berge, R. K. & Farstad, M. (1979a) *Eur. J. Biochem.* **95**, 89–97
- Berge, R. K. & Farstad, M. (1979b) *Eur. J. Biochem.* **96**, 393–401
- Berge, R. K., Vollset, S. E. & Farstad, M. (1980a) *Scand. J. Clin. Lab. Invest.* **40**, 271–279
- Berge, R. K., Slinde, E. & Farstad, M. (1980b) *FEBS Lett.* **109**, 194–196
- Berge, R. K., Slinde, E. & Farstad, M. (1981) *Biochim. Biophys. Acta* in the press
- Bøyum, A. (1968) *J. Clin. Lab. Invest.* **21**, Supp. **97**, 1–106
- Knauer, T. E. (1979) *Biochem. J.* **179**, 515–523
- Knauer, T. E., Gurecki, J. J. & Knauer, G. R. (1980) *Biochem. J.* **187**, 269–272
- Kümar, S. (1973) *Biochem. Biophys. Res. Commun.* **53**, 334–341
- Kurooka, S., Hosoki, K. & Yoshimura, Y. (1972) *J. Biochem. (Tokyo)* **71**, 625–634
- Lin, C. Y. & Smith, S. (1978) *J. Biol. Chem.* **253**, 1954–1962
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Osmundsen, H., Neat, C. E. & Borrebaek, B. (1980) *Int. Biochem. in the press*
- Srere, P. A., Seubert, W. & Lynen, F. (1959) *Biochim. Biophys. Acta* **33**, 313–319
- Zahler, W. L., Barden, R. E. & Cleland, W. W. (1968) *Biochim. Biophys. Acta* **164**, 1–11