# Metabolism of acetyl-CoA by isolated peroxisomal fractions: formation of acetate and acetoacetyl-CoA

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**Abstract** Liver peroxisomal fractions, isolated from rats treated with clofibrate, were shown to hydrolyze added [1-$^{14}$C]acetyl-CoA to free [1-$^{14}$C]acetate. [1-$^{14}$C]Acetyl-CoA was, however, also converted to [$^{14}$C]acetoacetyl-CoA. This reaction was inhibited by added ATP and by solubilization of the peroxisomes. The effect of ATP on synthesis of [$^{14}$C]acetoacetyl-CoA was likely due to ATP-dependent stimulation of acetyl-CoA hydrolase (EC 3.1.2.1) activity. The inhibitory effect due to solubilizing conditions of incubation remains unexplained. During peroxisomal $\beta$-oxidation of [1-$^{14}$C]palmitoyl-CoA, [1-$^{14}$C]acetyl-CoA, [1-$^{14}$C]acetate, and [$^{14}$C]acetoacetyl-CoA were shown to be produced. Possible metabolic implications of peroxisomal acetoacetyl-CoA synthesis are discussed.—**Hovik, R., B. Brodal, K. Bartlett, and H. Osmundsen.** Metabolism of acetyl-CoA by isolated peroxisomal fractions: formation of acetate and acetoacetyl-CoA. *J. Lipid Res.* 1991. **32:** 993–999.

**Supplementary key words** ATP • acetate • palmitoyl-CoA

The products of peroxisomal fatty acid $\beta$-oxidation are generally considered to be chain-shortened fatty acids and acetyl-CoA (for review see refs. 1, 2). We have previously reported that isolated peroxisomal fractions can generate acetyl-CoA from exogenous free fatty acids, provided that CoA and ATP are included in the incubations (3). Peroxisomal fractions contain acyl-CoA hydrolase (EC 3.1.2.2) activities (4, 5), including an acetyl-CoA hydrolase (EC 3.1.2.1) activity. Furthermore, acetate may also be a product of peroxisomal metabolism in isolated rat hepatocytes (6).

In the present study we investigate the metabolism of exogenous acetyl-CoA by isolated peroxisomal fractions. We demonstrate that acetyl-CoA is stable when present at low concentrations ($<50$ $\mu$M), but not at a concentration of 100 $\mu$M. Added acetyl-CoA was in part hydrolyzed forming free acetate, and partly converted to acetoacetyl-CoA. The balance between these two metabolic fates was markedly influenced by ATP and by detergents. We also demonstrate that acetyl-CoA, acetate, and acetoacetyl-CoA are formed during peroxisomal $\beta$-oxidation of palmitoyl-CoA.

## EXPERIMENTAL

Acetyl-CoA, CoA, palmitoyl-CoA, NAD$^+$, FAD, DTT, ATP (disodium salt), and bovine serum albumin (essentially fatty acid-free) were purchased from Sigma Chemical Co., St. Louis, MO. Diketene was obtained from Tokyo Kasei, Tokyo, Japan. Triton X-100 was purchased from Fluka AG, Buchs, Switzerland. Acetoacetyl-CoA was prepared from diketene as described by Simon and Shemin (7). All other reagents were of analytical grade or of highest available purity.

[1-$^{14}$C]Acetyl-CoA (2.1 GBq/mmol), [1-$^{14}$C]palmitic acid (2.1 GBq/mmol), and [1-$^{14}$C]sodium acetate (1.9 GBq/mmol) were purchased from Amersham International PLC., U.K.

[1-$^{14}$C]Palmitoyl-CoA was synthesized as described previously (8).

### Isolation of rat liver peroxisomal fractions

Male, albino Wistar rats (about 200 g body wt) were treated with clofibrate (0.5% (w/w)) for 10 days prior to experimental use. Rat liver peroxisomal fractions were isolated in self-generated Percoll gradients as described previously (9, 10). The isolated fractions were used immediately after isolation.

### Peroxisomal incubations

Peroxisomal fractions were incubated using non-solubilizing (iso-osmotic) conditions, or solubilizing (hypotonic) conditions of incubation, as described previously (11). Non-solubilizing incubations contained 130 mM KCl, 20 mM HEPES, 200 $\mu$M CoA, 100 $\mu$M NAD$^+$, 0.5 mM DTT, 20 $\mu$M FAD, and 2 mg bovine serum albumin/ml, pH 7.20. Solubilizing conditions were obtained using an incubation medium containing 30 mM KPi, 1 mM DTT, 200 $\mu$M CoA, 20 $\mu$M FAD, 0.005% (w/v)

Abbreviations: HPLC, high performance liquid chromatography.

Triton X-100, pH 7.20. The incubations contained 0.5–2 mg of protein per ml of incubation. When included, ATP was added as an equimolar mixture of ATP and $MgCl_2$. [1-$^{14}$C]Acetyl-CoA was used with a specific radioactivity of 180 kBq/nmol; [1-$^{14}$C]palmitoyl-CoA was used with a specific radioactivity of 0.6 kBq/nmol.

These two sets of conditions of incubation were chosen to investigate whether any of the observed effects were dependent on the presence of apparently intact peroxisomes. Also, the conditions chosen were identical to those used previously when comparing properties of peroxisomal $\beta$-oxidation in solubilized and non-solubilized peroxisomes (3, 10, 11). The concentration of Triton X-100 used in some of these experiments has been shown to solubilize all catalase in peroxisomes incubated even with isoosmotic conditions (11).

After incubation at 37°C, samples were quenched by the addition of ice-cold $HClO_4$ to a final concentration of 5% (w/v), and centrifuged briefly to sediment denatured proteins. All procedures were carried out at 0–3°C.

### HPLC-analysis of peroxisomal incubations

HPLC analysis was carried out using an RP-18 column from Brown-Lee Labs, Santa Clara, CA. Mobile phase conditions were based on those described by Bartlett et al. (2), and contained initially 96% KPi buffer, pH 5.5 and 4% (v/v) acetonitrile. This composition was maintained for the initial 5 min of the run, after which the concentration of acetonitrile was increased linearly to 30% (v/v) over 15 min.

Eluted components were detected by a UV-detector at 280 nm, and radioactive peaks were determined by a Ramona LS radioactivity-detector coupled in series to the UV-detector. On-line integration of UV and radioactivity peaks was carried out by software running on an Apple IIe computer. Eluted peaks of [1-$^{14}$C]acetate and [1-$^{14}$C]acetyl-CoA were identified on the basis of the corresponding radioactive standards. The peak due to [$^{14}$C]acetate was in addition characterized by having no absorbance at 280 nm.

The peak containing [$^{14}$C]acetoacetyl-CoA was identified by incubation of neutralized acid-soluble supernatants (from peroxisomal incubations) with purified thiolase (EC 2.3.1.9) and 0.2 mM CoA, to facilitate the conversion of [$^{14}$C]acetoacetyl-CoA to [1-$^{14}$C]acetyl-CoA (see Fig. 3). This conversion was taken as evidence that the peak of radioactivity thought to be due to [$^{14}$C]acetoacetyl-CoA was indeed due to [$^{14}$C]acetoacetyl-CoA. Treatment of this preparation with alkali resulted in the loss of the peaks due to [1-$^{14}$C]acetyl-CoA and remaining [$^{14}$C]acetoacetyl-CoA, and a corresponding increase in the peak due to [$^{14}$C]acetate.

### Assay of acetyl-CoA

Acetyl-CoA was assayed luminometrically as described earlier (3).

### Spectrophotometric acetyl-CoA hydrolase assay

Acetyl-CoA hydrolase was assayed by following the liberation of CoA from acetyl-CoA, at 324 nm by the reaction with di-(4-pyridyl)-disulfide (12). The reaction was carried out in 25 mM HEPES, pH 7.2, with 0.005% (w/v) Triton X-100 in the assay.

### Assay of protein

Proteins were assayed with the Bio-Rad protein assay kit, with freeze-dried $\gamma$-globulin as protein standard.

## RESULTS

### Acetyl-CoA hydrolase activity

The results presented in **Fig. 1** show that [1-$^{14}$C]acetyl-CoA incubated with isolated peroxisomal fractions remained relatively stable at low concentrations (10 and 50 $\mu$M), while it was metabolized when included at a concentration of 100 $\mu$M. The presence of an acetyl-CoA hydrolase (EC 3.1.2.1) with a high $K_m$ could explain these observations. Although this type of activity can be demonstrated in our isolated peroxisomal fractions ($K_m$ = 400 $\mu$M, $V_m$ = 3 nmol/min per mg of protein), the activity is much lower than, for example, palmitoyl-CoA hydrolase activity ($K_m$ = 1.4 $\mu$M, $V_m$ = 38 nmol/min per mg of protein).

Acetyl-CoA hydrolase was present in peroxisomal fractions from both untreated and clofibrate-treated rats, and

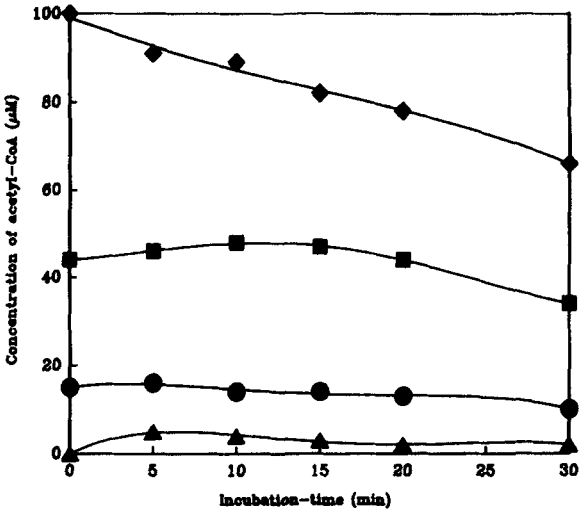


**Fig. 1.** Stability of acetyl-CoA when incubated with isolated peroxisomal fractions using non-solubilizing conditions of incubation. Concentrations of acetyl-CoA remaining after incubation with intact peroxisomes for various times. Incubations were carried out without added acetyl-CoA (▲), and in the presence of 10 $\mu$M (●), 50 $\mu$M (■), and 100 $\mu$M (♦) [1-$^{14}$C]acetyl-CoA. At the various times of incubation the concentrations of acetyl-CoA remaining in the incubations were assayed luminometrically, as described in the Experimental section. The incubation contained 1.2 mg of protein per ml of incubation.

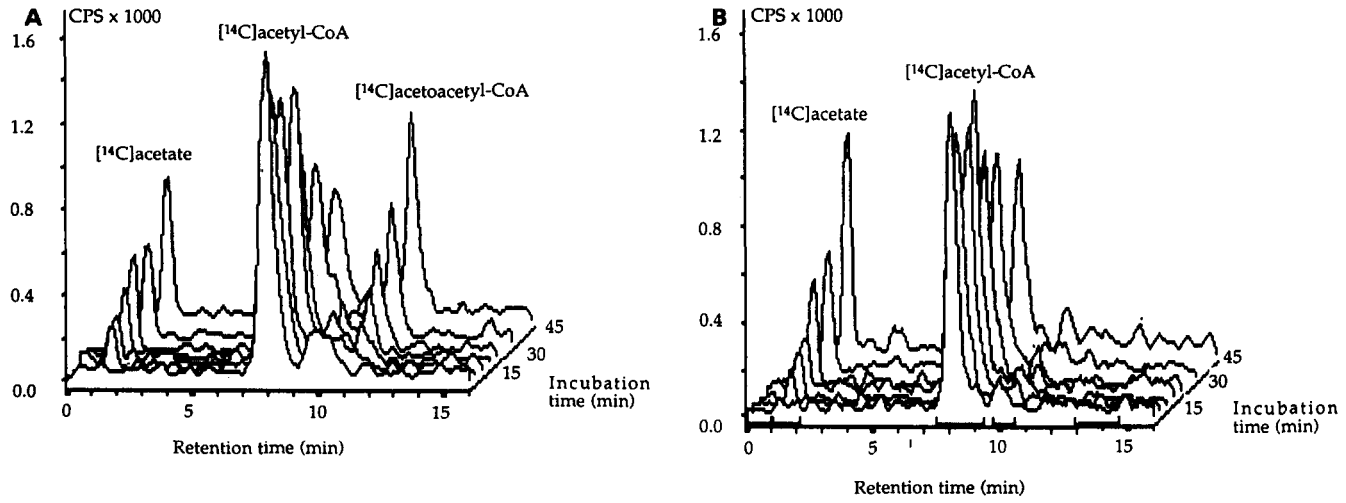


**Fig. 2.** Chromatographic separation of acid-soluble metabolites obtained on incubation of [1-$^{14}$C]acetyl-CoA with isolated peroxisomal fractions. Typical chromatograms showing the accumulation of radioactive acid-soluble metabolites derived from 100 $\mu$M [1-$^{14}$C]acetyl-CoA, using non-solubilizing conditions of incubation, without added ATP (A), and solubilizing conditions of incubations, without added ATP (B). The numbers indicated on the right-hand side (z-axis) represent times of incubation. Each chromatogram is derived from an injection of about 70,000 dpm.

is not markedly increased on clofibrate treatment (results not shown). This is consistent with previous findings as regards acetyl-CoA hydrolase activity in isolated peroxisomal fractions (4).

### Analysis of acid-soluble supernatants with radio-HPLC

To further investigate the metabolic fate of added acetyl-CoA, acid-soluble supernatants derived from peroxisomal incubation with [1-$^{14}$C]acetyl-CoA were analyzed by radio-HPLC.

Results presented in **Fig. 2** show typical chromatograms obtained using non-solubilizing (Fig. 2a), and solubilizing (Fig. 2b) conditions of incubation. As expected, some acetyl-CoA was hydrolyzed to acetate and CoA. Using solubilizing conditions, [$^{14}$C]acetate was the only metabolite observed. With non-solubilizing conditions of incubation, however, a major fraction of the radioactivity emerged with a retention time about 2 min longer than that of acetyl-CoA, which was both radioactive and UV-absorbing at 280 nm. Based on retention time of standard acetoacetyl-CoA, this peak was tentatively identified as acetoacetyl-CoA. The identification was confirmed when we demonstrated that this peak of radioactivity was converted to acetyl-CoA when incubated with a purified preparation of thiolase and CoA (**Fig. 3**). We were able to convert about 50% of the radioactivity in [$^{14}$C]acetoacetyl-CoA to [1-$^{14}$C]acetyl-CoA (Fig. 3). The reason for this may be the powerful inhibitory effect of CoA on the thiolytic cleavage of acetoacetyl-CoA (13, 14). It is also possible that another acyl-CoA ester, with a retention time identical to that of acetoacetyl-CoA, was present. It was, however, not possible to obtain more than one peak of radioactivity in this region of the radio-chromatogram by altering the gradient profile of the mobile phase (results not shown).

In these experiments, a relatively high amount of peroxisomal protein was used (2.6 mg/ml incubation), lead-

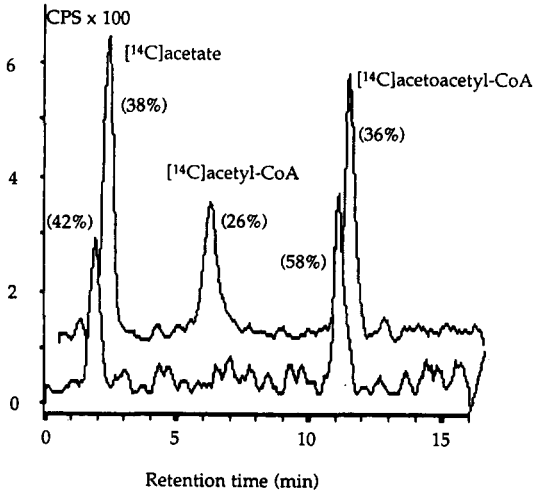


**Fig. 3.** Effects of incubation with thiolase on distribution of peak of radioactivity in radiochromatograms from peroxisomal fractions incubated with [1-$^{14}$C]acetyl-CoA. Typical radiochromatograms showing the effect of exogenous thiolase activity on radioactive metabolites in neutralized acid-soluble supernatants derived from a non-solubilizing incubation, carried out in the absence of ATP. The supernatant was neutralized with $K_2CO_3$/$KHCO_3$ CoA-SH to a final concentration of 0.2 mM and 2 U of purified thiolase was added. Samples (100 $\mu$l) of the reaction mixture were taken at zero time, and after 10 min of incubation at 20°C. The numbers in parentheses represent the amount of radioactivity in the various peaks, expressed as % of total radioactivity. Each chromatogram is derived from an injection of about 20,000 dpm. A control sample incubated in the absence of added thiolase showed no significant change in the distribution of radioactivity in the chromatogram (data not shown).

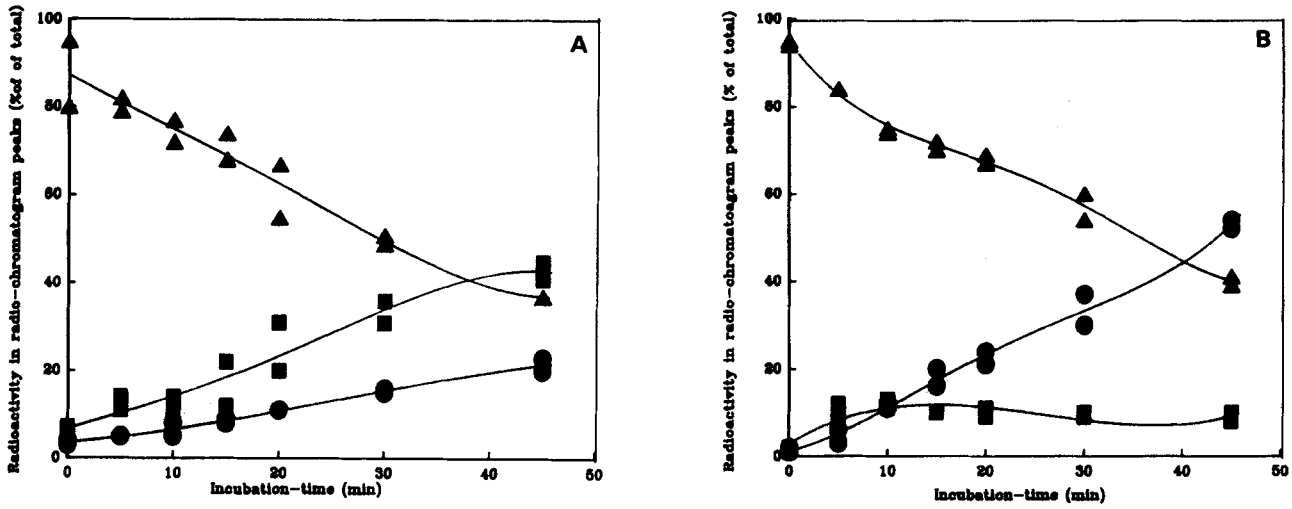


**Fig. 4.** Time-course of accumulation of [$^{14}$C]acetate and [$^{14}$C]acetoacetyl-CoA from [1-$^{14}$C]acetyl-CoA with non-solubilizing peroxisomal incubations. Peroxisomal fractions (0.7 mg of protein/ml of incubation) were incubated under iso-osmotic conditions in the presence of 100 $\mu$M [1-$^{14}$C]acetyl-CoA. Samples (200 $\mu$l) of the incubations were withdrawn at the various times indicated, and quenched by the addition of 200 $\mu$l 5% (w/v) $HClO_4$. After brief centrifugation the resulting supernatants were injected onto the HPLC column. The peaks of radioactivity corresponding to [$^{14}$C]acetate (●), [1-$^{14}$C]acetyl-CoA (▲), and [$^{14}$C]acetoacetyl-CoA (■) were integrated. The radioactivity in the various peaks was expressed as % of total radioactivity. The plotted data represent data from two separate experiments. The incubations were carried out with iso-osmotic conditions of incubation in the absence of added ATP (A), and in the presence of 10 mM-MgATP (B).

ing to complete conversions of [1-$^{14}$C]acetyl-CoA to [$^{14}$C]acetate and [$^{14}$C]acetoacetyl-CoA (Fig. 3). On addition of 2 M KOH to the sample, the peak of radioactivity due to [$^{14}$C]acetoacetyl-CoA disappeared completely. This radioactivity now emerged from the column as [1-$^{14}$C]acetate.

### Effects of ATP on metabolism of [1-$^{14}$C]acetyl-CoA

Inclusion of 10 mM ATP in the incubations effectively inhibited the conversion of [1-$^{14}$C]acetyl-CoA to [$^{14}$C]acetoacetyl-CoA. This is illustrated by the results presented in **Fig. 4,** showing time courses of accumulation of [$^{14}$C]acetate and [$^{14}$C]acetoacetyl-CoA. The rate of appearance of the [$^{14}$C]acetoacetyl-CoA peak was 1.7 nmol/min per mg of protein in the absence of ATP and 0.4 nmol/min per mg of protein in the presence of 10 mM ATP.

From these results it is clear that the formation of [$^{14}$C]acetoacetyl-CoA is strongly inhibited in the presence of 10 mM ATP, the major route of [1-$^{14}$C]acetyl-CoA metabolism now being simple hydrolysis. Using solubilizing conditions of incubation, no [$^{14}$C]acetoacetyl-CoA was formed (Fig. 2 and **Fig. 5**), irrespective of the presence of ATP. Under these conditions [$^{14}$C]acetate was the only product generated.

With non-solubilizing conditions of incubation and 100 $\mu$M [1-$^{14}$C]acetyl-CoA, the rate of disappearance of [1-$^{14}$C]acetyl-CoA was about 2.5 nmol/min per mg of protein in the absence of ATP, and about 1 nmol/min per mg of protein in the presence of 10 mM ATP (data of Fig. 4). These rates have been corrected for non-enzymic spontaneous hydrolysis of [1-$^{14}$C]acetyl-CoA observed in the presence of 10 mM ATP (about 1.1 nmol/min). In the absence of ATP no significant spontaneous hydrolysis of [1-$^{14}$C]acetyl-CoA was observed during 45 min of incubation. Using solubilizing conditions of incubation, the rate of disappearance of [1-$^{14}$C]acetyl-CoA was 0.7 nmol/min per mg of protein, both in the absence and presence of 10 mM ATP. This rate is the same as the rate of acetyl-CoA hydrolysis of 0.66 nmol/min per mg of protein, obtained using the spectrophotometric assay with 100 $\mu$M acetyl-CoA. The rate of hydrolysis can therefore account for the rate of disappearance of [1-$^{14}$C]acetyl-CoA using solubilizing conditions, but not for the rate of disappearance observed in the absence of ATP using non-solubilizing conditions.

ATP has been shown to inhibit acyl-CoA hydrolases in brown adipose tissue (5). The present results, however, show that the acetyl-CoA hydrolase activity in our peroxisomal fractions was not inhibited, but rather activated, by ATP (Fig. 4). To confirm this effect we carried out a spectrophotometric assay of acetyl-CoA hydrolase. The initial rate of hydrolysis from 100 $\mu$M acetyl-CoA was 0.66 nmol/min per mg protein in the absence of ATP, and 6.6 nmol/min per mg protein in the presence of 10 mM ATP. This is analogous to the ATP-dependent stimulation of acetyl-CoA hydrolase described elsewhere (14, 15). The ATP-dependent decrease in synthesis of [$^{14}$C]acetoacetyl-CoA is therefore likely due to stimulation of acetyl-CoA hydrolase activity. Using iso-osmotic conditions of incubation we have also observed that hydrolysis of acetyl-

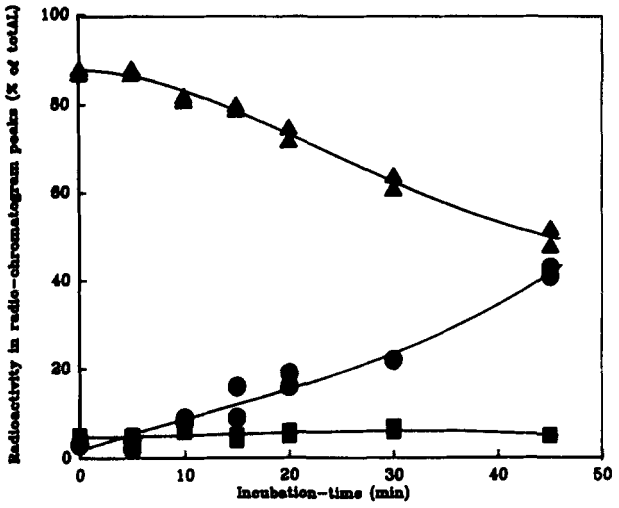


**Fig. 5.** Time course of accumulation of [$^{14}$C]acetate and [$^{14}$C]acetoacetyl-CoA from [1-$^{14}$C]acetyl-CoA incubated with isolated peroxisomal fractions using solubilizing conditions of incubation. Peroxisomal fractions (0.7 mg protein/ml of incubation) were incubated using solubilizing conditions in the presence of 100 $\mu$M [1-$^{14}$C]acetyl-CoA. Samples (200 $\mu$l) of the incubations were withdrawn at the times shown and treated as described in the legend to Fig. 4. The peaks of radioactivity corresponding to [$^{14}$C]acetate (●), [1-$^{14}$C]acetyl-CoA (▲), and [$^{14}$C]acetoacetyl-CoA (■) were integrated. The radioactivity in the various peaks was expressed as % of total radioactivity. The plotted data represent data from two separate experiments. The data were obtained in the absence of added ATP. Identical results, however, were also obtained in the presence of added 10 mMATP (not shown).

CoA is stimulated in the absence of added CoA (results not shown). This is in line with earlier findings regarding the cytosolic acetyl-CoA hydrolase, which is strongly inhibited by CoA (16).

### Effects of concentration of peroxisomal protein, and of concentration of [1-$^{14}$C]acetyl-CoA, on metabolism of [1-$^{14}$C]acetyl-CoA

The extent of conversion of [1-$^{14}$C]acetyl-CoA to [$^{14}$C]acetoacetyl-CoA was found to increase more rapidly with increasing concentration of peroxisomal protein, as compared to extent of conversion to [1-$^{14}$C]acetate (**Fig. 6**). Using a concentration of 100 $\mu$M [1-$^{14}$C]acetyl-CoA the extent of conversion to [1-$^{14}$C]acetate doubled as the concentration of protein was increased from 15 to 150 $\mu$g/ml. The conversion to [$^{14}$C]acetoacetyl-CoA, in contrast, was increased by sevenfold.

Both hydrolysis of [1-$^{14}$C]acetyl-CoA to [1-$^{14}$C]acetate and its metabolism to [$^{14}$C]acetoacetyl-CoA were found to be dependent on the concentration of [1-$^{14}$C]acetyl-CoA in a linear fashion (Fig. 6). Both rates were found approximately linear up to a concentration of 200 $\mu$M [1-$^{14}$C]acetyl-CoA. This suggests also that the reaction synthesizing acetoacetyl-CoA has a high $K_m$ with respect to acetyl-CoA.

### HPLC analysis of the acid-soluble supernatants from peroxisomal incubation of [1-$^{14}$C]palmitoyl-CoA

**Fig. 7** shows a chromatogram of the acid-soluble supernatant derived from a peroxisomal incubation with [1-$^{14}$C]palmitoyl-CoA, using non-solubilizing conditions of incubation in the absence of ATP. As shown, not only [1-$^{14}$C]acetyl-CoA was produced, but also [1-$^{14}$C]acetate and [$^{14}$C]acetoacetyl-CoA.

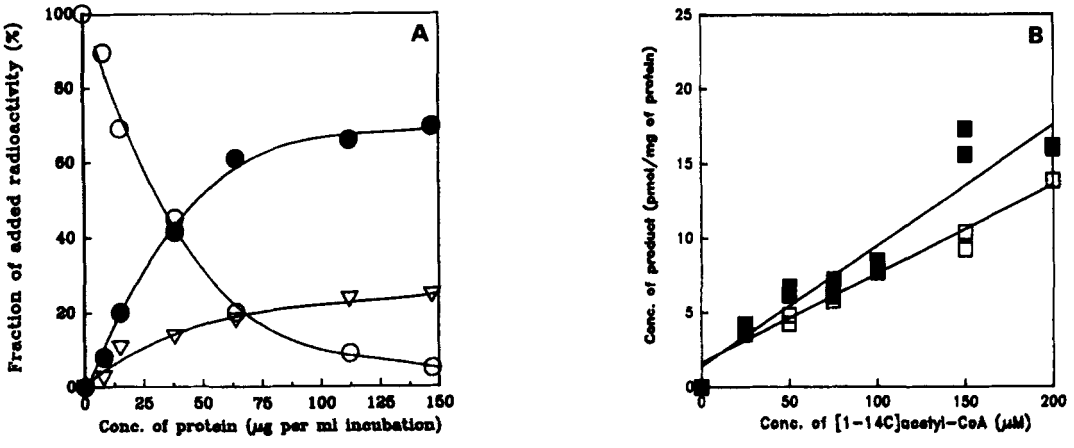


**Fig. 6.** Effects of concentration of peroxisomal protein and of concentration of [1-$^{14}$C]acetyl-CoA on generation of [1-$^{14}$C]acetate and [$^{14}$C]acetoacetyl-CoA by isolated peroxisomal fractions. Isolated peroxisomal fractions were incubated in the presence of 100 $\mu$M [1-$^{14}$C]acetyl-CoA for 30 min, using the different concentrations of protein shown (A). Incubations were carried out using non-solubilizing conditions of incubation, without added ATP. Following quenching with $HClO_4$ (5% v/v), the acid-soluble supernatants were analyzed by radio-HPLC as described in Experimental section. The peaks of radioactivity due to [1-$^{14}$C]acetyl-CoA (○), [1-$^{14}$C]acetate (▽), and [$^{14}$C]acetoacetyl-CoA (●) were integrated, and the radioactivity in each peak is expressed as % of total radioactivity in the peaks. Isolated peroxisomal fractions (1.3 mg of protein/ml) were incubated for 10 min in the presence of the various concentrations of [1-$^{14}$C]acetyl-CoA shown (B). Acid-soluble supernatants were analyzed for [1-$^{14}$C]acetate (□) and [$^{14}$C]acetoacetyl-CoA (■) by radio-HPLC, as described above. The plotted lines represent the least-squares best-fit. The reason these lines do not pass through the origin may be imprecise evaluation of background radioactivity in the chromatograms. Further experimental details are given in the Experimental section.

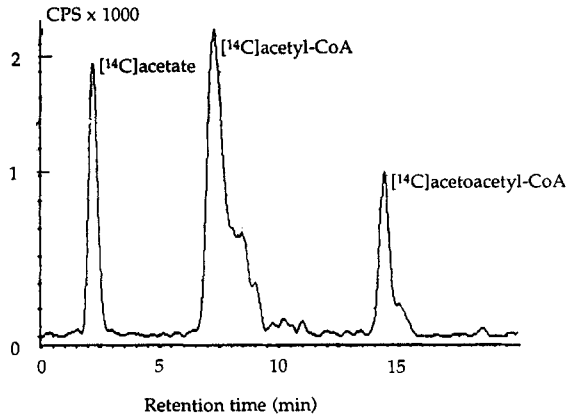


**Fig. 7.** HPLC separation of acid-soluble metabolites from an incubation of [1-$^{14}$C]palmitoyl-CoA with an isolated peroxisomal fraction. A typical chromatograph showing the radioactive metabolites present in the acid-soluble supernatant from a peroxisomal incubation with [1-$^{14}$C]palmitoyl-CoA. Isolated peroxisomal fractions (2.4 mg/ml protein) were incubated with 100 $\mu$M [1-$^{14}$C]palmitoyl-CoA under non-solubilizing conditions, in the absence of ATP, as described in the Experimental section. After 20 min the incubation was quenched with $HClO_4$ (final concentration 5% w/v). After a brief centrifugation a sample of the supernatant was withdrawn for measurement of radioactivity (the acid-soluble radioactivity corresponded to 40% of the total in the incubation). The remaining supernatant was injected onto the RP-18 column and the column was eluted with the gradient described in the Experimental section.

## DISCUSSION

Here we show that peroxisomes can synthesize [$^{14}$C]acetoacetyl-CoA from [1-$^{14}$C]acetyl-CoA. The peroxisomes may also hydrolyze [1-$^{14}$C]acetyl-CoA to [1-$^{14}$C]acetate and CoA. The reaction(s) leading to synthesis of [$^{14}$C]acetoacetyl-CoA is uncertain, although it is possible that reversal of peroxisomal thiolase activity is involved. It has recently been demonstrated that purified peroxisomal thiolase is able to convert acetyl-CoA to acetoacetyl-CoA (17). The permeability of peroxisomal membrane remains to be clearly defined, but it appears permeable to most low molecular weight metabolites (18). It is, therefore, likely that exogenous acetyl-CoA will have access to thiolase present in the peroxisomal matrix. Access to thiolase alone is not, however, the sole criterion for synthesis of [$^{14}$C]acetoacetyl-CoA, as it is abolished by solubilizing conditions of incubation. Thus it may be inferred that formation of [$^{14}$C]acetoacetyl-CoA also requires the presence of "intact" peroxisomes. Loss of structural integrity leads to simple hydrolysis of [1-$^{14}$C]acetyl-CoA. The reason for this may be that intact structural integrity provides a kinetic environment that facilitates synthesis of acetoacetyl-CoA, in spite of the equilibrium constant favoring acetoacetyl-CoA cleavage (19). Thiolase is not inhibited by Triton X-100 at the concentration used in these experiments to solubilize peroxisomal fractions (results not shown).

Although these peroxisomal fractions contain a minor mitochondrial contaminant (9), mitochondrial thiolase is an unlikely mediator of [$^{14}$C]acetoacetyl-CoA synthesis, as mitochondria are impermeable to acyl-CoA esters in general. Further, inhibition of synthesis of [$^{14}$C]acetoacetyl-CoA by solubilizing conditions of incubation (which will expose mitochondrial thiolase activity) (Fig. 4) also suggests that mitochondrial thiolase is not involved.

Leighton and co-workers (6) have shown that acetate was the product of $\beta$-oxidation of dodecanedioic acid, a peroxisomal substrate, by rat hepatocytes (6). Now we show that the hydrolysis of [1-$^{14}$C]acetyl-CoA to [1-$^{14}$C]acetate can occur in the peroxisomes. Thus, all reactions leading to acetate production are peroxisomal.

The observed stimulation of [1-$^{14}$C]acetate production and inhibition of [$^{14}$C]acetoacetyl-CoA formation by ATP is most likely due to ATP-dependent stimulation of the acetyl-CoA hydrolase activity. The cytosolic acetyl-CoA hydrolase has been demonstrated to be an enzyme separate from a mitochondrial acetyl-CoA hydrolase, which is not activated by ATP (16). The effects of ATP observed in our experiments, therefore, suggest that the acetyl-CoA hydrolase of peroxisomes and cytosol have similar properties. Contamination of peroxisomes by cytosolic acetyl-CoA hydrolase is clearly possible. It is, however, also conceivable that the cytosolic enzyme may be peroxisomal. It is particularly striking that the activity of cytosolic acetyl-CoA hydrolase is doubled after injection of a single dose of clofibrate (20), a drug well known to cause proliferation of hepatic peroxisomes (21). Our own findings, however, suggest that the specific activity of acetyl-CoA hydrolase activity in isolated peroxisomal fractions is only marginally increased after treatment with clofibrate (4).

The physiological significance of these findings is not clear. A concentration of acetyl-CoA of 50–100 $\mu$M may be outside the physiological range, although concentrations in this range may be achieved in the peroxisomal matrix during active $\beta$-oxidation. However, [$^{14}$C]acetoacetyl-CoA and [$^{14}$C]acetate, together with [$^{14}$C]acetyl-CoA, were also found to be products of peroxisomal $\beta$-oxidation of [1-$^{14}$C]palmitoyl-CoA. This suggests that both acetate and acetoacetyl-CoA may be formed during peroxisomal $\beta$-oxidation. Sterol and cholesterol biosynthesis has previously been suggested to be partly located in peroxisomes (22). Peroxisomal synthesis of acetoacetyl-CoA for subsequent use in the biosynthesis of sterols is, therefore, one possibility. Horie, Suzuki, and Suga (23) have recently reported evidence of an acetyl-CoA-dependent chain-elongation system in rat liver peroxisomes. Our observations may also be related to this phenomenon.

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