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A luminometric assay for peroxisomal β-oxidation

Effects of fasting and streptozotocin-diabetes on peroxisomal β-oxidation

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1. A luminometric assay for acyl-CoA oxidase activity is described. The assay uses the luminol/microperoxidase system to monitor continuously acyl-CoA-dependent generation of H_2O_2. The assay is rapid, convenient, and lends itself to automation with an LKB 1251 luminometer. The assay is extremely sensitive, requiring at the most 10 μg of liver-homogenate protein per assay. 2. The assay can also be used to measure other oxidases, e.g. glycollate oxidase (EC 1.1.3.15), D-aspartate oxidase (EC 1.4.3.1) and urate oxidase (EC 1.7.3.3), the only modification being substitution of substrates to appropriate concentration. 3. With rat liver homogenates, spectrophotometrically measured rates of palmitoyl-CoA-dependent NAD^+ reduction and acyl-CoA oxidase activity [Hryb & Hogg (1979) Biochem. Biophys. Res. Commun. **87**, 1200–1206] was generally found in good agreement with luminometrically measured acyl-CoA oxidase activity. 4. With liver homogenates from streptozotocin-diabetic rats, however, rates of palmitoyl-CoA-dependent NAD^+ reduction were consistently lower than the corresponding acyl-CoA oxidase activity. This difference was most marked with respect to luminometrically assayed acyl-CoA oxidase activity.

INTRODUCTION

The range of compounds reported to cause induction of hepatic peroxisomal β-oxidation has grown extensively with time (for review see Osmundsen *et al.*, 1987). Induction is generally brought about by exposure to a compound foreign to the body, although diabetes, fasting, or vitamin E deficiency (Reddy *et al.*, 1984) have also been reported to cause induction. Horie *et al.* (1981) reported induction of peroxisomal β-oxidation in diabetic rat liver, as did Snoswell & Fishlock (1980) in diabetic-sheep liver. On the other hand, Kawashima *et al.* (1983) found no evidence of diabetes-dependent induction of peroxisomal β-oxidation in rats. Similarly, the literature contains conflicting reports about effects of fasting on induction of peroxisomal β-oxidation. Some authors have reported a doubling of specific activity (Ishii *et al.*, 1980; Thomassen *et al.*, 1982; Berge *et al.*, 1984). Mannaerts *et al.* (1979) and Slauter & Yamazaki (1984), in contrast, reported only a minor increase in specific activity.

The present investigation was initiated as an attempt to elucidate the situation described above, by using conventional spectrophotometric assays to measure peroxisomal β-oxidative activity in rat liver homogenates. In the course of this investigation it became apparent that results obtained with streptozotocin-diabetic rats by using rates of palmitoyl-CoA-dependent NAD^+ reduction (Lazarow & de Duve, 1976) did not parallel those obtained by using a spectrophotometric assay of acyl-CoA oxidase activity (Hryb & Hogg, 1979). This finding stimulated development of an alternative luminometric assay for peroxisomal β-oxidation.

As the acyl-CoA oxidase is considered the rate-limiting enzyme for peroxisomal β-oxidation (Inestrosa *et al.*, 1979), it was thought valid to use this activity as a measure of peroxisomal β-oxidative activity. Although a fluorimetric acyl-CoA oxidase assay is available (Walusimbi & Harrison, 1983), it was considered worthwhile to develop a reliable luminometric method. Such a method has the potential of being run on an automated instrument without loss of sensitivity relative to a fluorimetric method. Also, smaller assay volumes are often used, and much smaller amounts of sample protein are generally required.

A luminometric assay for glycollate oxidase (EC 1.1.3.15) has been described (Leupold *et al.*, 1985). This assay operates at an extremely alkaline pH, and was not shown to function as an acyl-CoA oxidase assay. The assay described here measures acyl-CoA oxidase activity, as well as other oxidase activities. Because the luminol/microperoxidase system is utilized for H_2O_2 detection, this assay functions at a moderately alkaline pH.

EXPERIMENTAL

Materials

Luminol, microperoxidase (sodium salt, horse heart), CoA (free acid), palmitoyl-CoA, defatted bovine serum albumin, NAD^+ (sodium salt), streptozotocin, L-thyroxine (sodium salt), *p*-hydroxybenzoic acid, 4-aminoantipyrene, DL-α-glycerophosphate and FAD (disodium salt) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Clofibrate (ethyl *p*-chlorophenoxyisobutyrate) was purchased from Fluka A.G., Buchs, Switzerland. Horseradish peroxidase (grade II) was purchased from Boehringer, Mannheim,

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Germany. All other reagents used were of analytical grade, or of the highest purity available.

Animals

Male albino Wistar rats (125–200 g) of a Departmental in-bred strain were used. The animals were fed on a standard pelleted fodder (Møllesentralen A/S, Oslo, Norway), which contained (by wt.) about 24% protein, 2.1% fat, 5% fibre and 64% carbohydrate. The rats were kept at 22 °C, relative humidity being 40–60%, and with the light on between 08:00 and 20:00 h. The animals were killed between 09:00 and 10:00 h.

Rats were rendered diabetic by an intraperitoneal injection of streptozotocin (100 mg/kg body wt.). Animals with a blood glucose concentration of 15 mM or more were classified as diabetic, and were used experimentally after 10–12 days. Hyperthyroid animals were prepared by giving some rats a subcutaneous injection of L-thyroxine (0.5 mg/kg body wt.) daily for 2 days before use. Animals showing at least 2-fold increase in mitochondrial *sn*-glycerol-3-phosphate dehydrogenase (EC 1.1.99.5) activity were judged to be hyperthyroid (Klingenberg, 1970).

Some rats were fed on fodder containing clofibrate (0.5%, w/w) to induce peroxisomal β-oxidation (Lazarow, 1977). Rats were allowed water and fodder *ad lib.* When required, rats were fasted for 23 h before experimental use.

Preparation of rat liver homogenates

Rat liver homogenates (10%, w/v) were prepared in a medium containing 300 mM-mannitol, 0.1 mM-EGTA and 10 mM-Hepes, pH 7.20. The homogenates were centrifuged for 1 min at 1000 *g* in a Sorvall SS-34 rotor. The resulting supernatants were used for all enzyme assays. The supernatants were, when required, stored at −22 °C, and thawed immediately before use. Repeated freezing and thawing of any one sample was avoided.

All samples were assayed within 1 month of preparation, as it was found that extended storage led to progressive loss of acyl-CoA oxidase activity.

Statistical analysis

The significance of differences between sample means was tested by using the Bonferroni modification of the *t* test (Wallenstein *et al.*, 1980).

Assays

Spectrophotometric assays for peroxisomal β-oxidation. Peroxisomal β-oxidation was routinely assayed as palmitoyl-CoA-dependent NAD^+ reduction by using an assay mixture containing 50 mM-potassium phosphate, 0.5 mM-NAD^+, 0.2 mM-CoA, 1 mM-dithiothreitol, 0.005% (w/w) Triton X-100, pH 8.2, and at most 500 μg of protein/ml of assay. The reaction was started by addition of 50 μM-palmitoyl-CoA.

Parallel spectrophotometric measurements of acyl-CoA oxidase activity were also carried out as described by Hryb & Hogg (1979). The cuvette holder was thermostatically maintained at 30 °C.

Luminometric assay for peroxisomal β-oxidation. Luminometric assays for peroxisomal β-oxidation were carried out in an assay mixture containing 100 mM-potassium phosphate, 4 mM-NaN_3, 10 μg of microperoxidase/ml, 20 μM-luminol, 0.5 mg of defatted bovine serum albumin/ml, 0.1 mM-NAD^+ and 5 mM-EDTA, pH 8.50. These assays usually contained 50–500 ng of protein. The reaction was started by addition of 25 μM-palmitoyl-CoA. The total assay volume was 400 μl. Homogenates were diluted 100-fold with ice-cold 100 mM-potassium phosphate buffer, pH 8.5, immediately before use. All samples were kept on ice until cuvettes containing samples in assay buffer could be transferred to the luminometer for assay.

H_2O_2-dependent luminescence was measured in a LKB–Wallac 1251 luminometer, used in the automatic mode. A cuvette containing diluted sample is placed in the sample-changer of the luminometer. On initiation of the assay program, the samples are assayed sequentially. Luminol is injected initially by one dispenser, followed 15 s later by palmitoyl-CoA from a second dispenser. Luminescence was monitored at 10 s intervals after injection of palmitoyl-CoA. Both the integrated and the continuous signal was monitored, routinely up to 50 s after injection of palmitoyl-CoA. The sample compartment of the luminometer was thermostatically maintained at 25 °C.

Quenching of the luminescence signal was not significant, as judged from the signal obtained from a standard amount of H_2O_2 injected into the reaction mixtures, provided that the amount of protein per assay was kept within the range stated above. Calibration curves based on injection of known amounts of H_2O_2 proved most unreliable, because of the instability of H_2O_2 in the very dilute solutions required to provide luminescence signals which did not saturate the luminometer's photomultiplier.

At least three blank samples (not containing protein) were always included with each series of assays. The net luminescence signal was always taken as the sample signal after subtraction of the corresponding blank value. The coefficient of variation between replicate assays was found to be about 10%.

Assay of protein. Proteins were assayed with the Bio-Rad protein assay kit, with freeze-dried γ-globulins as protein standard.

RESULTS

Luminometric assay for acyl-CoA oxidase

Conditions of assay. The conditions described in the Experimental section have been found essential for reliable and reproducible luminometric acyl-CoA oxidase assays. The use of either Tris or potassium phosphate buffer was found to give practically identical results (not shown). The use of a high concentration of buffer was found essential. Reproducibility of assays was markedly enhanced by using 100 mM-potassium phosphate buffer (or 50 mM), as opposed to lower concentrations (25 mM) (results not shown).

The reason for this latter variability may be that the assay is critically dependent on pH, as appears also the NaN_3-dependent inhibition of catalase. This is illustrated by results presented in Fig. 1. These results show that increasing assay pH from 8.00 to 8.50 brings about a several-fold increase in assay sensitivity. The effect of NaN_3 was relatively constant over the range of concentrations used. This effect is presumably due to inhibition of catalase, which otherwise would decompose H_2O_2 as it is generated. A similar effect of NaN_3-

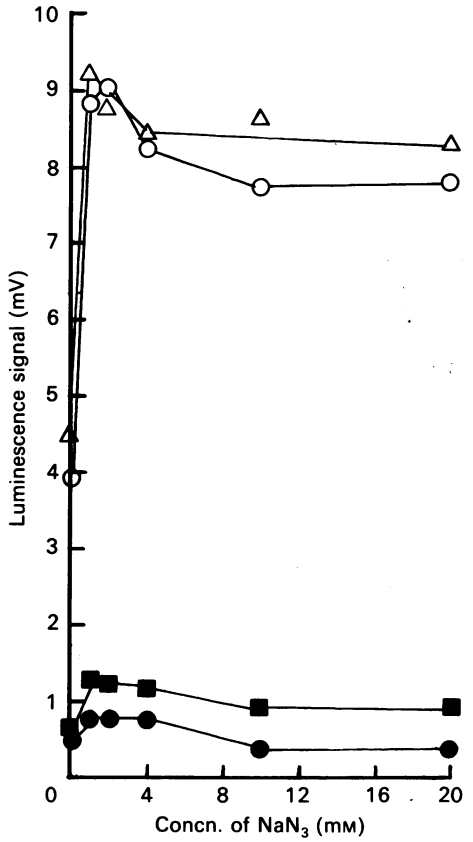

Fig. 1. Effects of pH and various concentrations of NaN_3 on luminometrically assayed acyl-CoA oxidase activity

Acyl-CoA oxidase activity in rat liver homogenates was assayed luminometrically as described in the Experimental section, by using assay mixtures containing the concentrations of NaN_3 shown in the Figure. The experiment was carried out with homogenate from a control rat in reaction mixtures adjusted to pH 7.50 (●), 8.00 (■), 8.50 (○) or 9.00 (△). The assays contained about 3.5 μg of homogenate protein per assay.

dependent inhibition of catalase was demonstrated by Leupold *et al.* (1985).

Increasing the pH to 9.00 did not cause any further improvements of the assay. For routine measurements pH 8.50 was therefore selected. Also, increasing the concentration of NaN_3 beyond 4 mM did not further increase the luminescence. However, this concentration also gave more reproducible luminescence readings, particularly with samples containing low acyl-CoA oxidase activities (results not shown). It was therefore used routinely in all assays.

The amount of microperoxidase in the assay influenced the sensitivity of the assay, as shown by results presented in Fig. 2. A linear relationship between luminescence signal and concentration of microperoxidase was observed up to a concentration of 20 μg/ml of assay. A concentration of 10 μg/ml of assay was routinely used. A somewhat higher concentration could have been used, but would have added significantly to the cost of the assay.

Defatted bovine serum albumin was included in the assay to protect the low concentrations of enzyme protein against possible detergent effects of palmitoyl-CoA. The

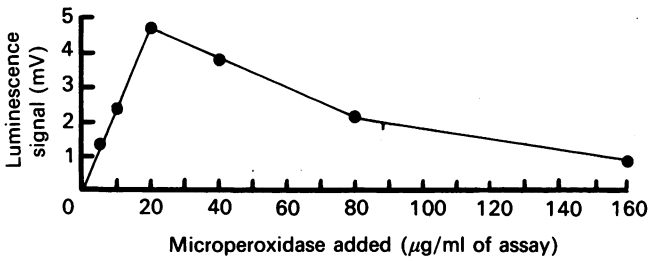

Fig. 2. Effects of various concentrations of microperoxidase on luminometrically measured acyl-CoA oxidase activity

Acyl-CoA oxidase activity in control rat liver homogenate was measured in the presence of the amounts of microperoxidase shown in the Figure. The conditions of assay are otherwise as described in the Experimental section.

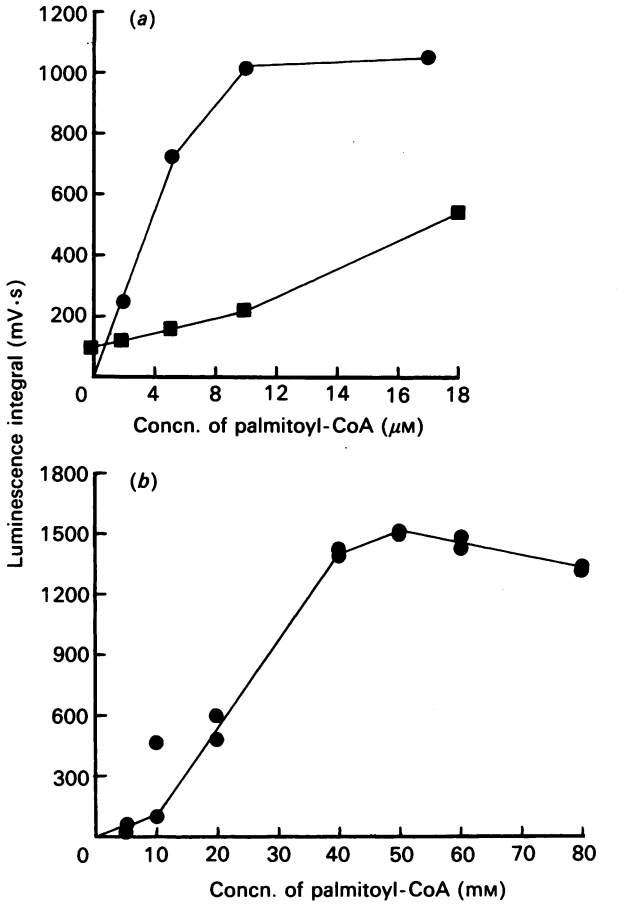

Fig. 3. Effects of defatted bovine serum albumin on luminometrically measured acyl-CoA oxidase activity

Acyl-CoA oxidase activity in control rat liver homogenates was assayed luminometrically (*a*) in the absence of defatted serum albumin from the assay (◕), or included at 0.5 mg per ml of assay (■), with the concentrations of palmitoyl-CoA indicated in the Figure. The experiment was also repeated with 0.5 mg of defatted serum albumin/ml of assay, but with an extended range of palmitoyl-CoA concentrations (*b*). The conditions of assay are otherwise given in the Experimental section.

effect of serum albumin in the assay is illustrated by data presented in Fig. 3, showing that a lag appears in the plot of v against s, presumably owing to binding of palmitoyl-CoA to the albumin molecule. With albumin in the assay, however, the assay can readily accommodate a higher amount of palmitoyl-CoA (Fig. 3). The decrease in luminescence observed with concentrations of palmitoyl-CoA higher than 50 μM is probably due to substrate inhibition (Hovik & Osmundsen, 1987).

Increasing the concentration of luminol in the assay did not alter the performance of the assay (results not shown). NAD^+ was included in the assay to ensure that the reaction product of the acyl-CoA oxidase did not accumulate. Inclusion of NAD^+ had no effect on measured luminescence (results not shown).

Reaction time course and concentration of enzyme protein. The data presented in Fig. 4 show the reaction time courses obtained with different amounts of protein in the incubations; these results demonstrate that the reaction time course was linear with time of measurement. A linear relationship between luminescence signal and amount of protein in the assay suggests that quenching does not significantly interfere with the assay, provided that the amount of homogenate protein in the assay is less than 10 μg (Fig. 5). This was confirmed by measuring luminescence from a standard amount of H_2O_2 injected into the reaction mixture (results not shown).

With homogenates derived from rats treated with clofibrate, acyl-CoA oxidase activity was readily measurable with as little as 1 ng of homogenate protein present in the assay (results not shown).

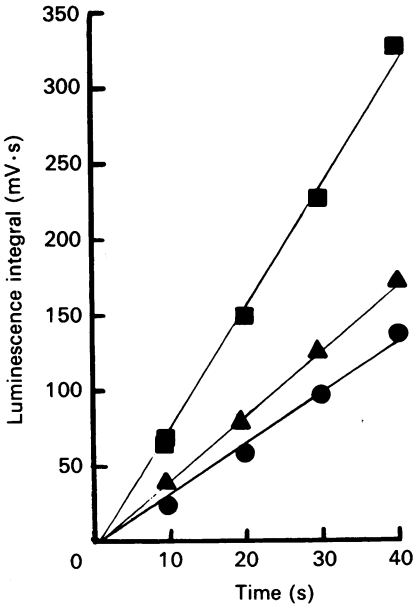

Fig. 4. Time course of luminometrically assayed acyl-CoA oxidase activity

Acyl-CoA oxidase activity in a control rat liver homogenate was assayed with 2.5 μg (●), 5 μg (▲) or 10 μg (■) of homogenate protein per assay. The resulting luminescence integrals were plotted against time of reaction, as indicated in the Figure. Conditions of assay are otherwise given in the Experimental section.

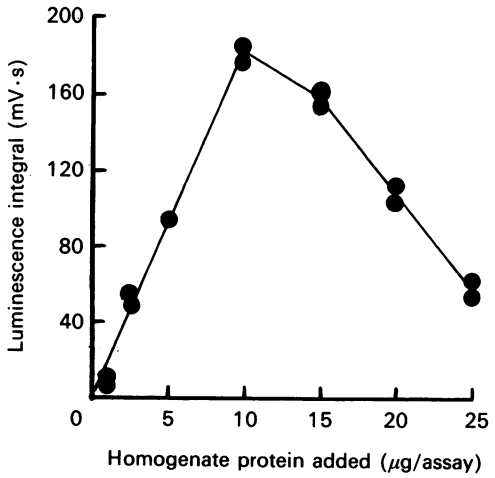

Fig. 5. Effect of concentration of homogenate protein on luminometrically assayed acyl-CoA oxidase activity

Acyl-CoA oxidase activity was measured in a liver homogenate from a control rat, by using the amounts of homogenate protein indicated in the Figure. Conditions of assay are otherwise given in the Experimental section.

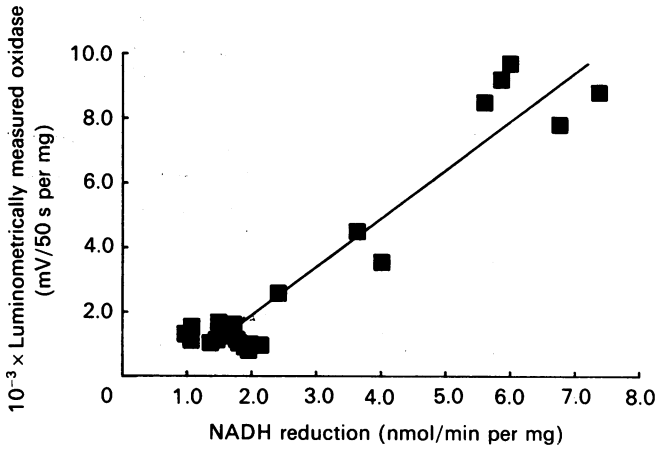

Fig. 6. Correlation between spectrophotometric and luminometric assays of peroxisomal β-oxidation

The correlation between luminometrically measured acyl-CoA oxidase activity and palmitoyl-CoA-dependent NAD^+ reduction was studied in control rat liver homogenates. The correlation curve obtained by assuming a linear relationship between the two parameters is defined by $y = 1.404x - 1.094$, and has a correlation coefficient (r) of 0.94.

Correlation of spectrophotometric and luminometric data. The luminometric assay makes use of the microperoxidase-catalysed oxidation of luminol by H_2O_2. This reaction generates light, the intensity of which is proportional to the amount of H_2O_2 generated (see Roswell & White, 1978). There is, however, no coefficient of proportionality between light intensity (or photomultiplier voltage) and amounts of H_2O_2, hence the need for a calibration curve. Because of the instability of extremely dilute H_2O_2 solutions we were unable to prepare a curve relating the signal (mV) from the photomultiplier directly with amounts of H_2O_2 generated (in terms of, e.g., pmol of H_2O_2/mV).

The correlation curve shown in Fig. 6, however, circumvents this problem. These results show that luminescence-measured acyl-CoA oxidase activity exhibits a very high degree of correlation with rates of palmitoyl-CoA-dependent NAD^+ reduction measured in the same samples ($r = 0.95$). Similarly, a linear relationship was also observed between spectrophotometrically and luminometrically measured rates of acyl-CoA oxidase activity, although the degree of correlation is somewhat poorer between the last two sets of measurements ($r = 0.78$; results not shown). Statistical analysis indicated that this was due to the spectrophotometric acyl-CoA oxidase being more variable than that of palmitoyl-CoA-dependent NAD^+ reduction (results not shown). The reason why the spectrophotometric acyl-CoA oxidase assay appears less precise may be the relatively short linear time interval of the reaction course (see Hryb & Hogg, 1979).

To provide a reliable calibration, it is probably advisable that each group of users prepare their own correlation curve.

Table 1. Glycollate, D-aspartate, urate and acyl-CoA oxidase activities as measured luminometrically in rat liver homogenates

The oxidase activities were assayed in control rat liver homogenates as described in the Experimental section. The assays contained 40 μM-palmitoyl-CoA. 20 mM-glycollate, 10 mM-D-glutamate or 20 nM-urate as appropriate. The tabulated values represent means ± S.D. for four animals. Means denoted by (*) were significantly different from the corresponding control mean ($P < 0.05$).

Enzyme activity	Activity [integral (mV·s)/μg of protein] Control rats	Clofibrate-treated rats
Acyl-CoA oxidase	15 ± 10	160 ± 70*
Glycollate oxidase	220 ± 60	230 ± 40
D-Aspartate oxidase	18 ± 3	33 ± 6*
Urate oxidase	26 ± 11	29 ± 6

Luminometric assay of other oxidases

Glycollate oxidase, D-aspartate oxidase (EC 1.4.3.1) and urate oxidase (EC 1.7.3.3) activities in rat liver homogenates were assayed luminometrically as described for acyl-CoA oxidase, except that the assays respectively included 20 mM-glycollate, 10 mM-D-glutamate or 20 nM-urate. The results obtained are presented in Table 1.

These data demonstrate that it is possible to measure urate oxidase activity also by the luminometric method. Urate was, however, found to be an exceedingly powerful quencher of luminescence, hence the use of the extremely low concentration of urate. The assay should therefore be used with caution with this substrate.

Both glycollate oxidase and D-asparate oxidase were readily detectable, and no evidence of substrate-dependent quenching of luminescence was observed. D-Aspartate oxidase is a presumptive peroxisomal enzyme (Hamilton, 1985). Our data suggest that its activity is increased after clofibrate treatment. A report (Yamada *et al.*, 1988) suggests that D-aspartate oxidase activity has been difficult to detect in rat liver homogenates. The luminometric assay therefore appears to offer a convenient assay for this activity also.

Treatment with clofibrate is shown to bring about the expected increase in acyl-CoA oxidase activity, whereas glycollate oxidase and urate oxidase activities were unaffected.

Effects of fasting, diabetes and/or thyroxine

The results presented in Table 2 demonstrate that streptozotocin-diabetes gave a small, but statistically significant, increase in acyl-CoA oxidase activity. This increase is not apparent as judged from measurements of palmitoyl-CoA-dependent NAD^+ reduction. This activity was lower than the spectrophotometrically measured acyl-CoA oxidase activity, in contrast with findings with any of the other groups.

The use of thyroxine gave here no significant increase in β-oxidative activity. This is probably due to the treatment regime used here, as a mild increase often has been observed (Kramar *et al.*, 1986). When diabetic animals were treated with thyroxine, an activity similar to that observed with streptozotocin-treated rats was found by the luminometric assay, but not with the spectrophotometric assays (Table 2).

Table 2. Effects of fasting, streptozotocin-diabetes and/or thyroxine on peroxisomal β-oxidative activity in rat liver homogenates

Peroxisomal β-oxidation was assayed spectrophotometrically and luminometrically in rat liver homogenates derived from control rats, or rats treated with streptozotocin, or with thyroxine, or with streptozotocin followed by thyroxine, or with clofibrate. The tabulated values represent means ± S.D. for the numbers of animals indicated in parentheses. Experimental details are otherwise given in the Experimental section. Means denoted by (*) were significantly different from the corresponding control mean ($P < 0.05$).

Treatment	NAD^+ reduction (nmol/min per mg of protein)	Acyl-CoA oxidase activity: Spectrophotometric (nmol of H_2O_2/min per mg)	Acyl-CoA oxidase activity: 10^3 × Luminometric (mV/min per mg)
Control (11)	1.4 ± 0.3	1.2 ± 0.3	1.1 ± 0.4
Fasted (11)	1.9 ± 0.3*	1.9 ± 0.7*	1.0 ± 0.4
Streptozotocin (9)	0.6 ± 0.1*	0.9 ± 0.2*	1.7 ± 0.4*
Thyroxine (6)	1.4 ± 0.2	1.3 ± 0.2	1.4 ± 0.2
Streptozotocin + thyroxine (6)	1.4 ± 0.5	0.8 ± 0.3*	1.7 ± 0.1*
Clofibrate (9)	7.5 ± 1.5*	6.1 ± 1.3*	11.5 ± 2.1*

With homogenates from clofibrate-treated animals about 7-fold stimulation was observed by using the spectrophotometric assays. The luminometric assay suggested a 10-fold increase in specific activity. With these homogenates, however, the spectrophotometric assays yielded identical results.

DISCUSSION

We consider the luminometric assay described here to be a convenient means of measuring acyl-CoA oxidase activity in small tissue samples. It can be used for other oxidases, e.g. glycollate oxidase or D-aspartate oxidase (Table 1), and for urate oxidase provided that the problem of urate-dependent quenching is recognized.

The luminometric acyl-CoA oxidase assay provided valuable data in a situation where conventional spectrophotometric assays gave inconsistent results, i.e. with liver homogenates from streptozotocin-diabetic rats. The reason for this is not clear, although it is likely that some other activity induced by this treatment may interfere with the spectrophotometric assays. This outcome may offer one explanation for the discrepancies in the literature with respect to effects of diabetes on induction of peroxisomal β-oxidation.

Although we observed significant differences between the various treatments, these were small compared with effects caused by clofibrate treatment (Table 2). The effects observed also differed depending on the assay used, e.g. fasting gave a small increase as assayed spectrophotometrically, but no effect was detected by the luminometric assay. Streptozotocin treatment similarly gave a decrease in activity with spectrophotometric assays, whereas a significant increase was observed luminometrically. Rendering streptozotocin-diabetic rats hyperthyroid a few days before experimental use evidently eliminates the problem as regards spectrophotometric assays (Table 2).

From this experience it is evident that the spectrophotometric assays, although convenient in use, should not be employed uncritically in every experimental situation.

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REFERENCES

Berge, R. K., Hosoy, L. H. & Farstad, M. N. (1984) Int. J. Biochem. **16**, 403–410
Hamilton, G. A. (1985) Adv. Enzymol. **57**, 85–178
Horie, S., Ishii, H. & Suga, T. (1981) J. Biochem. (Tokyo) **90**, 1691–1696
Hovik, R. & Osmundsen, H. (1987) Biochem. J. **247**, 531–535
Hryb, D. J. & Hogg, J. F. (1979) Biochem. Biophys. Res. Commun. **87**, 1200–1206
Inestrosa, N. C., Bronfman, M. & Leighton, F. (1979) Biochem. J. **182**, 779–788
Ishii, H., Horie, S. & Suga, T. (1980) J. Biochem. (Tokyo) **87**, 1855–1858
Kawashima, Y., Katoh, H. & Kozuka, H. (1983) Biochim. Biophys. Acta **750**, 365–372
Klingenberg. M. (1970) Eur. J. Biochem. **13**, 247–252
Kramar, R., Kremser, K., Hohenegger, M. & Mayer, M. (1986) Enzyme **35**, 27–33
Lazarow, P. B. (1977) Science **197**, 580–581
Lazarow, P. B. & de. Duve, C. (1976) Proc. Natl. Acad. Sci. U.S.A. **73**, 2043–2046
Leupold, C., Volkl, A. & Fahimi, H. D. (1985) Anal. Biochem. **151**, 63–69
Mannaerts, G. P., Debeer, L. J., Thomas, J. & De Schepper, P. J. (1979) J. Biol. Chem. **254**, 4585–4595
Osmundsen, H., Thomassen, M. S. M., Hiltunen, J. K. & Berge, R. K. (1987) Peroxisomes in Biology and Medicine, pp. 1530–1565, Springer, Berlin and Heidelberg
Reddy, J. K., Lalwani, N. D., Dabholkar, A. S., Reddy, M. K. & Quershi, S. A. (1984) Biochem. Int. **114**, 41–49
Roswell, D. F. & White, E. H. (1978) Methods Enzymol. **57**, 409–423
Slauter, R. W. & Yamazaki, R. K. (1984) Arch. Biochem. Biophys. **233**, 197–202
Snoswell, A. M. & Fishlock, R. C. (1980) Proc. Aust. Biochem. Soc. **13**, 38–42
Thomassen, M. S., Christiansen, E. N. & Norum, K. R. (1982) Biochem. J. **206**, 195–202
Wallenstein, S., Zucker, C. L. & Fleiss, J. L. (1980) Circ. Res. **47**, 1–9
Walusimbi, K. M. & Harrison, E. H. (1983) J. Lipid Res. **24**, 1077–1084
Yamada, R., Nagasaki, H., Wakabayashi, Y. & Iwashima, A. (1988) Biochim. Biophys. Acta **965**, 202–205

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