Peroxisomal Dihydroxyacetone Phosphate Acyltransferase

EFFECT OF ACETALDEHYDE ON THE INTACT AND SOLUBILIZED ACTIVITY*

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Rick T. Dobrowsky and Lawrence M. Ballas

From the Department of Anatomy, Physiological Sciences and Radiology and the Department of Biochemistry, North Carolina State University School of Veterinary Medicine, Raleigh, North Carolina 27606

The peroxisomal enzyme dihydroxyacetone phosphate (DHAP) acyltransferase shows a differential response to acetaldehyde. Employing whole peroxisomes, the enzyme displays a 130-400% stimulation of activity when assayed in the presence of 10-250 mM acetaldehyde. Following taurocholate solubilization of the enzyme, the response to 0.25 mM acetaldehyde is one of almost total inhibition. This inhibition of the taurocholate-solubilized enzyme is not observed at acetaldehyde concentrations below 200 mM. The stimulation of DHAP acyltransferase by acetaldehyde is solely a response of the peroxisomal enzyme as evidenced by its insensitivity to N-ethylmaleimide and 5 mM glycerol 3-phosphate. Furthermore, microsomal dihydroxyacetone phosphate acyltransferase activity is inhibited at all acetaldehyde concentrations. The activation of membrane-bound DHAP acyltransferase by acetaldehyde appears to be specific for this enzyme in comparison to several other peroxisomal and microsomal enzymes. The specificity of activation and differential response of the peroxisomal enzyme to acetaldehyde indicates that the microenvironment of the peroxisomal membrane is important for normal enzymatic function of this enzyme.

The properties of dihydroxyacetone phosphate (DHAP) acyltransferase have been characterized in several reports (1-3). The enzyme is membrane bound and is present in mitochondrial (2, 4), microsomal (5-7), and peroxisomal (8-10) membrane fractions of mammalian liver. The enzyme, catalyzing the acylation of dihydroxyacetone phosphate, is considered an obligate step in the formation of ether-linked lipids (11, 12) although its contribution to overall hepatic triacylglycerol and phospholipid biosynthesis is in dispute (2,6,13).

NEM, N-ethylmaleimide.

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Materials and Methods

Carrier-free [32P] and [3H]glycerol were products of ICN Radiochemicals. Acdethaldehyde was obtained from Aldrich. Palmitoyl-CoA was purchased from P-L Biochemicals. Fatty acid-poor bovine serum albumin, dihydroxyacetone, dihydroxyacetone phosphate (lithium salt), Percoll, glycerol 3-phosphate, and taurocholate were from Sigma. All other reagents were of the highest quality obtainable. [32P]DHAP and [3H]glycerol 3-phosphate were synthesized enzymatically as previously described (6). [3P] contamination was measured by toluene isobutyl alcohol (11, 12). Extraction of ammonium molybdate complexed 32P and was routinely <2% (14). Acyltrihydroxyacetone phosphate was chemically synthesized as described (15).

Female Hartley guinea pigs, 20-24 days old, were fasted overnight and killed by decapitation. The livers were quickly excised and immersed in ice-cold 0.25 M sucrose, 5 mM Tris-HCl in 0.1% ethanol pH 7.4 buffer and weighed. A light mitochondrial fraction was then prepared by the method of Baudhuin (16). A peroxisome-enriched fraction was obtained by isopycnic centrifugation on 27% Percoll gradients at 50,000 x g for 30 min in an SW-27 swinging bucket rotor (17). One-mL fractions were collected from the bottom of the gradient and the peroxisome-enriched fractions located by measuring latent catalase activity (2). These fractions were pooled, diluted 5-10-fold with ammonium bicarbonate buffer, and resedimented at 250,000 x g for 30 min (in 7). The resulting pellet was then resuspended with buffer to a final concentration of 2-4 mg/mL. Fractions were stored at -80 °C and thawed once. Microsomes were isolated from guinea pig liver homogenates at 100,000 x g for 60 min.

Purity of the peroxisomal enriched fraction was assayed by measuring various marker enzymes. The activities of glucose-6-phosphatase (14) and DHAP acyltransferase in the presence of 5 mM glycerol 3-phosphate (2) or N-ethylmaleimide (7) were employed to determine microsomal contamination. Previous reports have localized the inhibition of DHAP acyltransferase by these compounds to the microsomal compartment (2, 6, 7). Succinate dehydrogenase (18) and glutamate dehydrogenase (19) were used as a measure of mitochondrial activity. Lysosomol contamination was assayed by the acidic and alkaline hydrolysis of β-glycerophosphate (20). Purity calculations were performed as published by Fujiki et al. (21). Routine preparations contained <1% lysosomal contamination and 2-3% and 9-12% mitochondrial and microsomal contamination, respectively.

DHAP acyltransferase activity was assayed by measuring the amount of chloride-form-soluble radioactivity produced under the following assay conditions. In a final volume of 0.2 ml the assay contained 1 mM [32P]DHAP (2.5 x 106 cpm/nmol), 40 mM NaF, 8 mM MgCl2, 1.25 mg/ml bovine serum albumin, 50 μM palmitoyl-CoA,
Acetaldehyde and Dihydroxyacetone Phosphate Acyltransferase

and 50 mM Tris-HCl, pH 7.4. The reaction was initiated by the addition of 2–20 \mu g of peroxisomal protein and incubated 10 min at 37 °C. Radiolabeled lipid was extracted by the method of Bligh and Dyer (22). The addition of acetaldehyde to an assay was performed immediately prior to thermal equilibrium without changing the reagent concentrations stated above.

Glycerol 3-phosphate acyltransferase (6), diacylglycerol acyltransferase (2), ethanol acyltransferase (23), glucose-6-phosphatase (14), NEM-sensitive DHAP acyltransferase (6), peroxisomal acyl-DHAP oxidoreductase (5), catalase (2), and the peroxisomal enzymes of \( \beta \)-oxidation (24) were assayed in the presence or absence of 0.25 M acetaldehyde.

Experiments utilizing the bile acid taurocholate to solubilize membrane-associated DHAP acyltransferase activity were performed on 0.66–1 mg peroxisomal protein/ml incubation mixture for 20 minutes at 0 °C. The detergent supplemented protein was then subjected to ultracentrifugation at 100,000 \( \times \) g for 60 min to separate solubilized enzyme from particulate protein (3). The supernatant was carefully drawn off and the remaining pellet resuspended in 100–200 \( \mu l \) of 0.25 M sucrose, 5 mM Tris-HCl, pH 7.4. DHAP acyltransferase activity was then measured on each fraction as described.

Protein was determined by the method of Bradford (25) using bovine serum albumin as the standard. All enzyme activities were proportional with protein and time.

RESULTS AND DISCUSSION

The measurement of peroxisomal DHAP acyltransferase activity in the presence of acetaldehyde concentrations less than 10 mM showed no stimulation of DHAP acyltransferase activity above control levels. Further titration resulted in a linear increase in the specific activity of the enzyme at acetaldehyde concentrations from 10 to 300 mM producing a 4-fold increase in DHAP acyltransferase activity measured at pH 7.4 (Fig. 1). Peroxisomal DHAP acyltransferase has also been reported to show an optimum activity at pH 5.5 (2). However, the stimulatory effect of acetaldehyde was not as great at this lower pH (data not shown).

Since DHAP acyltransferase activity is also associated with microsomes (1, 2, 6) experiments were conducted to investigate whether or not the observed stimulation by acetaldehyde was due in whole or in part to microsomal contamination of the peroxisomes. Titration of microsomal DHAP acyltransferase activity with acetaldehyde up to a concentration of 500 mM resulted in no stimulation of enzyme activity (Fig. 1). In fact, the microsomal enzyme was inhibited 85% at all concentrations of the aldehyde above 10 mM. Furthermore, vesicles showing a 4-fold stimulation in DHAP acyltransferase activity with the addition of 0.25 M acetaldehyde also exhibited insensitivity to N-ethylmaleimide and 5 mM glycerol 3-phosphate, indicating that the stimulation was due solely to the peroxisomal enzyme.

In experiments utilizing the bile acid taurocholate to solubilize the membrane-associated enzyme, a similar stimulation of activity was observed. Taurocholate concentrations up to 0.8% resulted in a stimulation of DHAP acyltransferase activity over untreated controls with maximal stimulation (3–4-fold) occurring at a detergent concentration of 0.4%. The effect of acetaldehyde on the degree of solubilization of the enzyme at increasing taurocholate concentrations was then investigated.

Fig. 2 shows the effect of 0.25 M acetaldehyde on enzyme preparations incubated with increasing concentrations of taurocholate. Evident from the data is an almost total inhibition of supernatant-derived enzyme assayed in the presence of 0.25 M acetaldehyde. Incubations of taurocholate-solubilized enzyme with acetaldehyde at concentrations less than 0.2 M showed no effect on the activity of the enzyme (data not shown). However, a 91% inhibition was observed at an acetaldehyde concentration of 0.25 M, which was in agreement with the 95–99% inhibition of DHAP acyltransferase activity present in Fig. 2.

Fig. 3 depicts residual detergent-treated particulate-associated DHAP acyltransferase showing an increase in activity in the presence of 0.25 M acetaldehyde up to taurocholate concentrations of 0.4%. This indicated that an acetaldehyde-
sensitive site was still membrane-bound. As the taurocholate concentration was increased, a loss of stimulation of residual membrane-associated DHAP acyltransferase activity occurred whether acetaldehyde was present or not. The similar decrease observed with the particulate-associated activity above 0.4% taurocholate and in the absence of acetaldehyde indicated that acetaldehyde was not inhibiting the residual membrane-associated activity. Enzyme preparations subjected to an incubation in 0.25 M sucrose, 5 mM Tris-HCl, pH 7.4, for 20 min at 0 °C, with supernatant and particulate fractions isolated as described, served as controls. In these preparations soluble enzyme activity was minimal, whereas particulate-associated activity showed a 235% increase in response to 0.25 M acetaldehyde.

The specificity of the stimulatory effect of 0.25 M acetaldehyde for peroxisomal DHAP acyltransferase was investigated by assaying several peroxisomal and microsomal enzymes in the presence of acetaldehyde. Table I summarizes the results of these experiments. Apparent was a lack of stimulation by acetaldehyde of any of the enzymes tested. In most cases 0.25 M acetaldehyde severely inhibited enzyme activity with the exception of the latent activity of glucose-6-phosphatase, diacylglycerol acyltransferase, and peroxisomal acylcldihydroxyacetone phosphate oxidoreductase activity. These results suggest that a unique site exists on or near the peroxisomal DHAP acyltransferase enzyme which allows for the specific stimulation of this protein by acetaldehyde.

Previous data (10) have shown that the pH optimum of DHAP acyltransferase shifts from pH 5.5 to pH 7.4 following detergent solubilization. It was suggested that this shift may result from a change of the enzyme to a less hydrophobic microenvironment in the membrane. Further extending this supposition we propose that the differential response of the enzyme to acetaldehyde is dependent on the microenvironment of the enzyme. When the enzyme is membrane-bound, acetaldehyde may either promote substrate transport, resulting in stimulation of the enzyme, or bind on or near the enzyme to proteins or lipids, which affects the catalytic rate. The inhibition of soluble enzyme activity by acetaldehyde following taurocholate solubilization favors the second possibility as an explanation for the observed response to acetaldehyde. Additionally, the response of the particulate-associated enzyme following either a control incubation or a taurocholate treatment suggests that surrounding lipids and proteins are an important factor in determining the response of the enzyme to 0.25 M acetaldehyde. As the membrane is increasingly disrupted with increasing detergent concentrations, the degree of stimulation by acetaldehyde diminishes and is finally abolished (Fig. 3).

The binding site for acetaldehyde on the enzyme is not known. It may be proximal to the active site of DHAP acyltransferase since, at taurocholate concentrations exceeding 0.4%, isolated particulate fractions lost the stimulatory response to acetaldehyde but still expressed a significant portion of the control activity. However, this lack of stimulation by acetaldehyde on the detergent-treated particulate fraction at high taurocholate concentrations may be the result of residual taurocholate binding to these pelleted membrane fragments blocking an acetaldehyde-binding site(s).

A recent report (26) has described the formation of a 2-methylimidazol-4-one adduct from acetaldehyde and an N-terminal valine of hemoglobin via Schiff base formation. Stubbs and Hajra (10) have demonstrated a lack of Schiff base formation in the catalytic site of peroxisomal DHAP acyltransferase. As such, it is unlikely that acetaldehyde is binding to a free amino group at the active site to exert its stimulatory effect. Additionally, it has been suggested that the microenvironment of a reactive amino group is a factor in the production of stable acetaldehyde adducts (29). This suggests that the differential response of DHAP acyltransferase to acetaldehyde seen in the intact peroxisomes versus the taurocholate-solubilized membranes may result from the ability of the aldehyde to form a stable inhibitory adduct directly with the solubilized enzyme but not with intact peroxisomal DHAP acyltransferase.

Stubbs and Smith (27) proposed that the alteration of membrane lipids and their associated enzymes can influence the activities and transport properties of membrane proteins. Recent reports (27–29) have also proposed that ethanol could selectively alter lipid microenvironments in the vicinity of hepatic plasma membrane proteins. Acetaldehyde, a product of hepatic ethanol metabolism, may exert similar membrane perturbations at a subcellular organelle(s) when in sufficient cytosolic concentrations, as in chronic alcohol consumption, resulting in increased peroxisomal DHAP acyltransferase activity.

Evidence indicates that DHAP acyltransferase may be oriented with a catalytic site facing toward the peroxisomal lumen (30). The effect of acetaldehyde on the integrity of the peroxisomal membrane is not known. No evidence of membrane disruption was indicated in the presence of 0.25 M acetaldehyde. Treatment of whole peroxisomal membranes with high levels of acetaldehyde did not solubilize any of the DHAP acyltransferase activity (data not shown). If the enzyme was being released to the supernatant one would not detect activity due to acetaldehyde inhibition of the solubilized activity (Fig. 2). However, one would expect less activity in the remaining pellet. All the DHAP acyltransferase activity was recovered in the pellet after acetaldehyde treatment.

Although dihydroxyacetone phosphate may permeate the membrane no evidence exists for free permeation of palmityl coenzyme A. It may be that aldehyde and keto-containing metabolites could reorient the peroxisomal enzyme to accept substrate more efficiently. Evidence, although indirect, suggests that possibility. Compounds that have a stimulating effect on non-detergent-treated peroxisomal DHAP acyltransferase activity include NEM (10), and acetaldehyde (this paper). Dihydroxyacetone phosphate itself (3) stimulates activity of the enzyme as indicated by breaks in the kinetic curve of untreated peroxisomes but not detergent-treated

**TABLE I**

| Enzyme                          | Source       | Disrupted activity* | Intact activity % |
|---------------------------------|--------------|---------------------|------------------|
| Diacylglycerol acyltransferase  | Microsomes   | ND*                 | 6                |
| Ethanol acyltransferase         | Microsomes   | 90                  | 57               |
| Glucose-6-phosphatase           | Microsomes   | 3                   | 3                |
| Glycerol-3-phosphate acyltransferase | Microsomes   | ND*                 | 99               |
| β-Oxidation                     | Peroxisomes  | 90                  | 100              |
| Catalase                        | Peroxisomes  | 44                  | 100              |
| Acyl-DHAP oxidoreductase        | Peroxisomes  | ND*                 | 10               |

* Membranes were treated with 0.1% Triton X-100 for 10 min at 0 °C to obtain full expression of latent enzyme activities.

ND, not determined. Full expression of enzyme activities are obtained in the absence of detergent employing intact membrane preparations.
Acetaldehyde and Dihydroxyacetone Phosphate Acyltransferase

preparations. Whether this peroxisomal enzyme activity is sensitive to other more physiological aldehyde and keto-containing metabolites is presently being investigated.

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