ENZYMIC REDUCTION OF FREE FATTY ALDEHYDES IN BOVINE CARDIAC MUSCLE[1]

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SUMMARY

The reduction of fatty aldehydes to fatty alcohols by a soluble enzyme in bovine cardiac muscle has been demonstrated. The enzymic activity is precipitable between 38-95% saturation with ammonium sulfate. NADPH is the required cofactor for this reduction. The reaction is irreversible under the conditions employed in this assay.

INTRODUCTION

The existence of fatty alcohols has been demonstrated in normal and neoplastic tissues (1,2,6), and these compounds are known to be precursors of glycerol ethers (3-5). Little has been published concerning their metabolism in mammalian tissues.

The interconversion of fatty alcohols, aldehydes, and acids has been recognized by several workers (8,9). In plants and bacteria fatty alcohols and aldehydes arise from the reduction of fatty acids or their corresponding acyl CoA's (10-14). The existence of two different Palmityl CoA reductases in rat brain has been reported (15,16).

Enzymes capable of catalyzing the reduction of various aldehydogenic moieties have been demonstrated in rat brain, liver, intestine, and testes (7,17,18). Similar enzymatic activities have been characterized in bovine brain and rabbit kidney cortex (19,20).

Even though a fatty acyl CoA reductase has been characterized from cardiac muscle (21), little information is available concerning the metabolism of these

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fatty aldehydes. This report presents evidence for the existence of an enzyme involved in the reduction of fatty aldehydes in bovine cardiac muscle.

METHODS

All preparative procedures were carried out at 0-4°C. A 105,000 xg supernatant was prepared from bovine cardiac muscle by a modification of the method outlined by Johnson, et al (21). The homogenization media was 0.25 M sucrose in 0.075 M potassium phosphate buffer (pH 7.5; 5 mM mercaptoethanol). The 105,000 xg supernatant was further fractionated by ammonium sulfate precipitation to yield two fractions: 0-38%, AR-1 and 38-95%, AR-2. Protein concentrations were determined by the method of Biuret (22). All protein fractions were dialyzed for fifteen hours using two changes of the above buffer. Upon assay of the two fractions, greater than 95% of the total enzymic activity observed in the 105,000 xg supernatant was found in AR-2. Fraction AR-2 was routinely diluted to a protein concentration varying between 10-15 mgs per ml and kept frozen in 2-5 ml aliquots at -40°C.

Incubation mixtures described in Table 1 contained the following concentrations of components: (1-^{14}C)-palmitaldehyde (1.56 x 10^6 dpm/μmole), (0.18 mM), NADH, (0.75 mM), NADPH (0.75 mM), and 4 mg of protein in a final volume of 1.0 ml 0.075 M potassium phosphate buffer (pH 7.5; 5 mM mercaptoethanol). The lipid substrates were dispersed with Tween 20 in the phosphate buffer (14). All incubations were carried out in a Dubonoff Metabolic Shaker at 37°C for ten minutes.

The reactions were stopped by the addition of 5 ml Chloroform - Methanol (1:1) containing 20 nanomoles octadecan-2-ol as an internal standard for quantitative gas-liquid chromatographic (GLC) analysis. This method was routinely used for the determination of enzymic activity. In order to verify the enzymic product as hexadecan-1-ol, the lipid extracts (23) from several samples were combined and the alcohols isolated by TLC (6). The fatty alcohols at this stage of purification were further analyzed by combined GC-Mass Spectrometry. The apparatus has been described before (24). In a few instances, the enzymic activity was measured by following the incorporation of radiolabel into hexa-

TABLE 1

Enzymic Reduction of Palmitaldehyde to Hexadecanol

Cofactors	Hexadecanol Formed (nanomoles per ten minutes) Boiled Supernatant	AR-1	AR-2	105,000 xg supernatant
1. NADH:NADPH	0.83; 0.75	2.4; 2.6	12.8; 13.0	14.5; 14.8
2. NADH	0.25; 0.30	1.1; 1.5	2.8; 5.1; 3.7	3.9; 2.9
3. NADPH	0.45; 0.56	3.4; 2.0	14.0; 13.2	13.7; 14.6

1-^{14}C-Palmitaldehyde was incubated with the cofactors and protein fractions listed above under the conditions described in the text. The enzymic product was quantitated by direct GLC analysis.

decan-1-ol. In these cases, 1 μmole carrier hexadecan-1-ol was added to the Chloroform - Methanol. The total lipid extracts (6) were resolved by TLC with Hexane:Chloroform:Methanol (73:25:1.5) as the developing solvent (6). The alcohols were isolated and converted to acetates (25). This derivative was isolated by TLC in the afore-mentioned system and then analyzed by GLC (6); the effluent was collected and radioassayed (21).

Various aliphatic fatty aldehydes were prepared as substrates in the same manner as the palmitaldehyde. These aldehydes ranged in length from 14-18 carbons; the unsaturated cis isomers of oleyl-, linoleyl-, and linolenyl- aldehyde were also included. The assays were carried out in the same manner as described above. Hexadecan-1-ol was used as the internal standard for the GLC assays when heptadecanal, and the 18 carbon aldehydes were substrates.

RESULTS AND DISCUSSION

The data in Table 1 show that palmitaldehyde is reduced to hexadecan-1-ol by a soluble enzyme which precipitates between 38-95% saturation with ammonium sulfate. NADPH is the required reducing cofactor. Under the conditions employed in these assays, the reaction is irreversible, i.e., there is no oxidation of

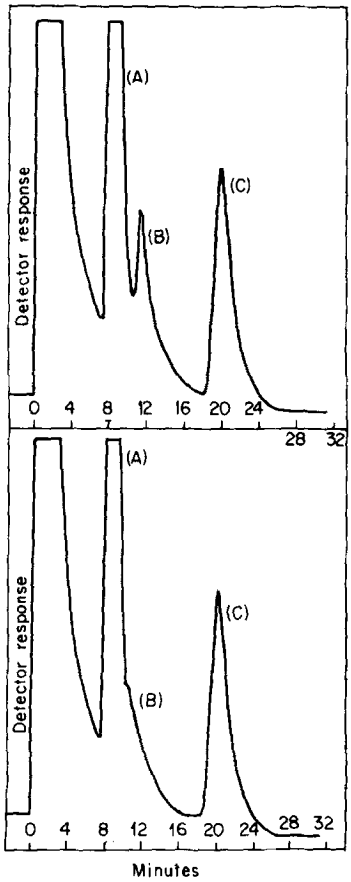

Figure 1. In the upper section is a representative gas-liquid chromatogram obtained by direct GLC analysis of the total lipid extract from reaction mixtures containing palmitaldehyde, NADPH, and 4 mg AR-2. Peak (A) represents the substrate, palmitaldehyde; peak (B) is the product, hexadecan-1-ol; peak (C) is the internal standard, octadecan-2-ol. The lower section is the gas-liquid chromatogram obtained from reaction mixtures containing palmitaldehyde, NADPH, and boiled 105,000 xg supernatant.

hexadecan-1-ol to the corresponding aldehyde with NAD and/or NADP by either the 105,000 xg supernatant or AR-2. The enzymic activity showed a linear increase with the quantity of protein added over a range of 1-10 mgs. Using 4 mgs AR-2, the reaction rate was linear for at least 20 minutes under the normal assay conditions.

Figure 1 is a gas-liquid chromatogram obtained by direct GLC analysis of lipid extracts from the enzyme incubation mixtures. The upper portion is a chromatogram showing the typical results of an assay with 4 mgs AR-2; the lower section shows a trace of the chemical blank using boiled supernatant.

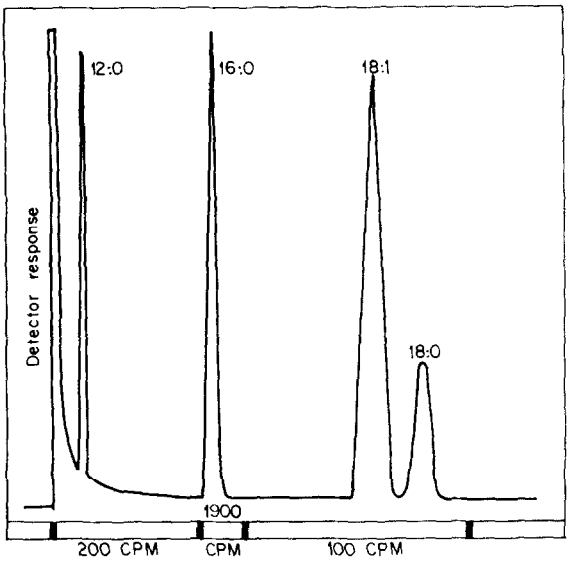

Figure 2. Gas-liquid chromatogram showing the distribution of radioactivity in acetates of fatty alcohols prepared from total lipids isolated from an incubation mixture containing 1-(^{14}C)-palmitaldehyde, NADPH, and 105,000 xg supernatant. 85% of the radioactivity (cpm) was collected as hexadecanyl-acetate. Standard acetates of 12:0, 18:1, and 18:0 fatty alcohols were added to the radioactive sample prior to GLC.

The identity of the product, hexadecan-1-ol, has been verified by its mobility on TLC, both as the free alcohol and as the acetate derivative. Radioassay of the acetates isolated by GLC indicated that greater than 85% of the total ^{14}C - activity applied is associated with the hexadecanyl acetate peak (Fig. 2). Combined GC-Mass Spectrometry of the enzymic product resulted in a fragmentation pattern identical with that of a standard hexadecan-1-ol.

Table 2 compares the relative rates of enzymic activity with various fatty aldehydes as substrates to that obtained with hexadecanal under standard assay conditions. The greatest activity was obtained with the poly-unsaturated 18 carbon aldehydes. Pentadecanal gave the highest activity relative to the other saturated aldehydes.

In experiments where alcohol dehydrogenase, ADH, activity was measured using conditions ideal for liver ADH (26), there was no observable ADH activity. This observation coupled with the irreversibility with hexadecan-1-ol, demonstrate that the enzymic activity measured is not due to the ubiquitous "ADH",

TABLE 2

Relative Activity with Various Fatty Aldehydes

Fatty Aldehyde Used as Substrate	Enzymic Activity Relative to Palmitaldehyde
Tetradecanal	0.6
Pentadecanal	1.5
Palmitaldehyde	1.0
Heptadecanal	0.9
Stearaldehyde	0.6
Oleyl-aldehyde	0.7
Linoleyl-aldehyde	2.0
Linolenyl-aldehyde	2.7

Various fatty aldehydes were incubated under the same conditions as Palmitaldehyde with 4 mgs AR-2 as described in the text. The enzymic products were quantitated by direct GLC analysis.

and that it is primarily responsible for the reduction of long chain aldehydes to their corresponding alcohols.

The in-tandem reaction of an NADH-dependent acyl CoA reductase and an NADPH-requiring aldehyde reductase could be responsible for the formation of fatty alcohols utilized in the biosynthesis of glycerol ethers in bovine cardiac muscle. Based on the apparent lack of specificity of the aldehyde reductase studied here, and the known chain length distribution of glycerol ethers, it is suggested that control of the fatty alcohols utilized in glycerol ether biosynthesis reside at the acyl CoA reductase, which has a high degree of substrate specificity (21).

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