THE OXIDATION OF ACETATE BY LIVER MITOCHONDRIA

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1. Introduction

Acetate produced from ethanol is oxidized to only a very limited extent by the liver [1], even though the enzymes required for activation of acetate to acetyl CoA are present in liver mitochondria in quantities adequate for a substantial rate of oxidation [2]. A number of studies have emphasized the fact that the rate of CO_2 production from acetate and thus, reactions of the citric acid cycle by the liver are markedly suppressed as a result of the mitochondrial oxidation of NADH produced in the cytosol during the oxidation of ethanol [3, 4]. However, these studies do not distinguish between effects on the activation of acetate from those on its further oxidation. The data presented in this communication show that the mitochondrial oxidation of acetate is limited by the rate of recycling of AMP produced during its activation. This limitation is overcome by supporting substrates which generate GTP, but is reinstated by added NADH when it competes with these substrates for oxidation. Links between substrate-linked phosphorylation and the oxidation of long-chain fatty acids by rat liver mitochondria [5], and of acetate oxidation by sheep liver mitochondria have recently been reported [6].

2. Materials and methods

Rat liver mitochondria were prepared as described by Johnson and Lardy [7]. The incubation media contained 50 mM Tris-HCl (pH 7.4), 100 mM KCl, 10 mM $MgCl_2$, 2 mM potassium phosphate (pH 7.4) and 2 mM ATP in a volume of 2.0 ml. Other additions were as listed in tables. The respiration of mitochondria, generally 75% of state 3 [8], was controlled by adding a limiting amount of a highly purified mitochondrial ATPase (specific activities of about 65 μmoles/min/mg protein) prepared by a minor modification of the method of Penefsky [9]. This system, to be reported in detail in a separate communication, simulates closely the situation *in vivo*, i.e., the mitochondria are in a medium of high energy charge with respiration being limited by the supply of ADP. The extramitochondrial concentrations of ATP, ADP and inorganic phosphate are constant during the incubation. Respiration was measured at 30° in a Gilson respirometer. All assays of metabolites were enzymatic [10, 11]. Protein was determined by the Biuret method using bovine serum albumin as a standard. Acetate was determined by acetate kinase, pyruvate kinase and lactic dehydrogenase. Added NADH was assayed as described by Klingenberg [12]. In experiments in which intramitochondrial adenine nucleotides were determined, 0.2 mM ATP and 1 μCi [^{14}C]ATP were in the medium. The methods for separation of mitochondria from the medium and the column elution of adenine nucleotides were as previously described [13]. Enzymes and unlabeled coenzymes were obtained from Sigma Co., and [^{14}C]ATP from New England Nuclear Corporation.

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Table 1
Effect of supporting substrates on acetate consumption.

Other additions	−ΔO (μ atoms)	−ΔAcetate (μmoles)
None	0.85	0.60
Malate	5.01	1.61
Malate plus arsenite	0.39	0.28
Succinate	4.85	1.61
α-Ketoglutarate	5.85	2.57
α-Ketoglutarate plus arsenite	0.25	0.36
Glutamate	5.85	2.57

Liver mitochondria (1.87 mg/ml) were incubated in the basic medium containing 5 μmoles of sodium acetate for 35 min. Supporting substrates were 2.5 mM and sodium arsenite was 2 mM. The results represent the average of duplicate experiments.

Table 2
Correlation of intramitochondrial adenine nucleotide distribution with the effect of malate and α-ketoglutarate on acetate metabolism.

Substrates added	−ΔAcetate (μmoles)	AMP (percent of total)	ADP	ATP
Malate		15	47	38
Malate plus acetate	0.82	39	41	20
α-Ketoglutarate		12	44	43
α-Ketoglutarate plus acetate	2.79	20	45	35

Liver mitochondria (3.0 mg/ml) were incubated in the basic medium with 0.2 mM ATP and 1 μCi [^{14}C]ATP. Respiratory substrates were 5 mM. Aliquots were removed and mitochondria were separated from the suspending medium at 15 and 30 min for determination of adenine nucleotides. Data shown were obtained after 30 min incubation.

3. Results and discussion

The effects of supporting substrates on the consumption of acetate by rat liver mitochondria are summarized in table 1. A small amount of acetate was consumed in the absence of supporting substrate. The moderate utilization in the presence of malate was blocked by the addition of arsenite. Succinate had an effect similar to malate, but α-ketoglutarate and glutamate stimulated the consumption of acetate beyond the effect of malate. The addition of arsenite also abolished the stimulatory effect of α-ketoglutarate on acetate consumption. The presumed underlying mechanism is the substrate-linked phosphorylation of GDP to GTP in the succinic thiokinase reaction which permits a removal of AMP via the GTP–AMP phosphotransferase reaction [14, 15]. Apparently, malate and succinate support acetate oxidation to a limited degree by the same mechanism. Unpublished results of Farrar [16] indicate that, with purified bovine heart acetate thiokinase, AMP is a product inhibitor competitive with acetate, non-competitive with Mg-ATP and uncompetitively with respect to CoASH.

In order to verify this hypothesis, the distribution of intramitochondrial adenine nucleotides was examined with various substrates added (table 2). The stimulation of acetate metabolism by α-ketoglutarate was clearly related to the considerably lower AMP and higher ATP levels when compared to malate plus acetate. These correlations strongly indicate that the rate of removal of AMP is a major determinant in the metabolism of acetate by liver mitochondria.

Table 3
Effect of the transfer of reducing equivalents from extramitochondrial NADH on the metabolism of acetate and glutamate.

Additions									
NADH	Glutamate	Acetate	−ΔNADH	−ΔAcetate	+ΔAspartate (μmoles)	+ΔAcetoacetate	+Δβ-Hydroxybutyrate	$(NAD^+/NADH)_m$	−ΔO (μ atoms)
+	−	−	0.21	−	−	−	−	−	0.22
−	+	−	−	−	2.38	−	−	−	6.59
−	+	+	−	3.23	2.19	0.48	0.35	27.8	8.78
+	+	−	2.06	−	1.50	−	−	−	7.59
+	+	+	1.91	1.83	1.17	0.23	0.30	15.6	8.36

Liver mitochondria (2.3 mg/ml) were incubated for 30 min in the basic medium containing 10 enzyme units each of dialyzed malate dehydrogenase and aspartate aminotransferase. Where indicated, 2.8 μmoles NADH, 5 μmoles glutamate, and 5.8 μmoles acetate were added. The results are the means of duplicate experiments. The mitochondrial $NAD^+/NADH$ ratio was calculated assuming equilibrium ($K_{eq} = 4.39 \times 10^{-2}$) for the β-hydroxybutyrate dehydrogenase reaction.

Table 4
Correlation of intramitochondrial adenine nucleotides with the effect of the malate–aspartate cycle on acetate metabolism.

Other additions						
Glutamate	Malate	NADH	−ΔAcetate (μmoles)	AMP (percent of total)	ADP	ATP
+	–	–	1.95	10	35	55
+	–	+	1.25	25	44	31
–	+	–	0.82	32	33	35

Liver mitochondria (3 mg/ml) were incubated in the basic medium containing 5 mM acetate, 0.2 mM ^{14}C-labeled ATP (1 μCi) and 10 enzyme units of dialyzed malate dehydrogenase and aspartate aminotransferase. Glutamate, malate and NADH, when present, were 5 mM. The distribution of intramitochondrial adenine nucleotides and consumption of acetate were determined at 30 min.

We have previously shown that the effect *in vivo* of ethanol oxidation on metabolic processes of liver mitochondria can be stimulated in a reconstructed system *in vitro* consisting of respiring intact isolated mitochondria with added NADH together with the other requirements for the function of the malate–aspartate cycle [17]. Table 3 summarizes experiments in which the effect of transfer of reducing equivalents from added NADH into the mitochondria on the metabolism of acetate and glutamate was examined. External NADH was negligibly oxidized by liver mitochondria when added with dialyzed malate dehydrogenase and aspartate aminotransferase. In contrast, glutamate supported rapid oxidation of both acetate and extramitochondrial NADH, with its own oxidation being suppressed by both substrates. If acetate, glutamate, and NADH were all present when the malate–aspartate cycle was operational, the reducing equivalents from added NADH competed effectively for oxidation and curtailed the metabolism of both acetate and glutamate. Concomitant with these effects, the intramitochondrial NAD^+/NADH ratio was decreased, and ketone body production from acetate was also decreased. In other experiments (table 4), oxidation of added NADH increased the level of AMP, and suppressed the oxidation of acetate. These findings suggest that a decrease in the availability of NAD^+ is a common mechanism for inhibition of the metabolism of both glutamate and acetate. However, the overriding influence on acetate utilization is most probably at the level of its activation, since ketone body production is also suppressed under conditions which usually promote ketogenesis (i.e., a decreased NAD^+/NADH ratio). The direct effect on acetate activation in turn appears to be due to decreased ability to recycle AMP when flux of carbon through the citric acid cycle is decelerated.

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