REGULATION OF PYRUVATE CARBOXYLATION BY ACETYL-CoA IN RAT LIVER MITOCHONDRIA

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1. Introduction

Acetyl-CoA has been shown to act as allosteric activator for all vertebrate liver pyruvate carboxylases studied thus far [1,2]. Under physiological conditions such as fasting where tissue acetyl-CoA levels are elevated, it is generally assumed that this allosteric activation has a regulatory function for gluconeogenesis from pyruvate in liver and kidney. If this assumption is correct, physiological changes of the acetyl-CoA content in that cellular compartment where the enzyme is located should be within the range of the K_a of pyruvate carboxylase for its activator.

Pyruvate carboxylase is known to be a mitochondrial enzyme [3–5], and in earlier work [6–8] with intact liver mitochondria, we have tried to find a dependence of pyruvate carboxylation on intramitochondrial changes in acetyl-CoA concentrations. However, when mitochondria were incubated with saturating amounts of pyruvate, no such dependence could be detected. On the other hand, a strong correlation between enzyme activity and intramitochondrial ATP:ADP ratios was observed. In this paper, experiments with rat liver mitochondria are described in which the activity of pyruvate carboxylation was limited by the availability of acetyl-CoA. An apparent K_a value of pyruvate carboxylase for acetyl-CoA in intact mitochondria was determined and this value was found to be severalfold higher than the K_a values reported for the isolated enzyme. A few of these results were included in a recent review article [5].

2. Materials and methods

Isolation of mitochondria, incubations and analytical determinations were performed as previously described [7,9]. L-Acetyl-carnitine was a gift of Otsuka Pharmaceutical (Japan).

3. Results

In the presence of bicarbonate, phosphate and ATP, pyruvate is in part oxidized to acetyl-CoA and in part carboxylated to oxaloacetate [6,7]. Upon addition of arsenite, pyruvate oxidation is inhibited; as a result acetyl-CoA levels fall and pyruvate carboxylation was found to be decreased to about the same extent as pyruvate oxidation (fig.1). It has earlier been shown [10,11] that addition of acetyl-CoA producing compounds such as acetyl-carnitine, palmitylcarnitine or octanoate to the arsenite inhibited system resulted in a stimulation of pyruvate carboxylation without changing the rate of pyruvate oxidation. These results indicate that, in the presence of arsenite, pyruvate carboxylation is inhibited mainly by lack of acetyl-CoA. However, measurements of the ATP:ADP ratios (not shown) revealed that the

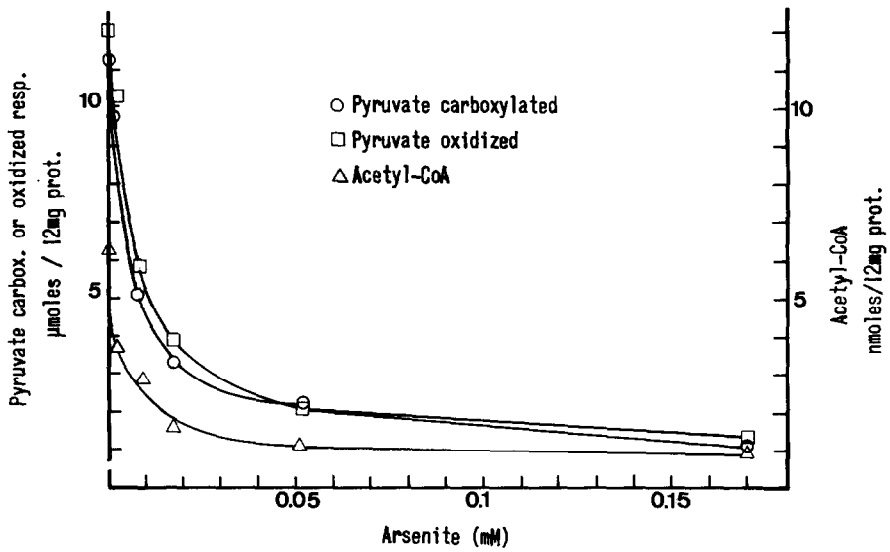


Fig.1. Effects of arsenite on pyruvate oxidation, pyruvate carboxylation and acetyl-CoA levels. The incubation medium contained 4 mM ATP, 10 mM $MgSO_4$, 6.6 mM potassium phosphate buffer, pH 7.4, 10 mM $KHCO_3$, 10 mM potassium pyruvate, 6.6 mM triethanolamine buffer, pH 7.4, 0.5 ml mitochondrial suspension (12 mg protein) and 0.25 M mannitol/0.07 M sucrose solution to make the reaction mixture about isotonic. Arsenite was added as indicated. The final volume was 3 ml, incubation time 10 min. Acetyl-CoA was measured in the pellets after millipore filtration. The values for pyruvate carboxylation represent the sum of the main products malate and citrate which were measured in the filtrates after millipore filtration; pyruvate oxidized is calculated by subtracting the amount of pyruvate carboxylase from the total pyruvate used. Each point represents the average value of duplicate incubations carried out with the same mitochondrial preparation.

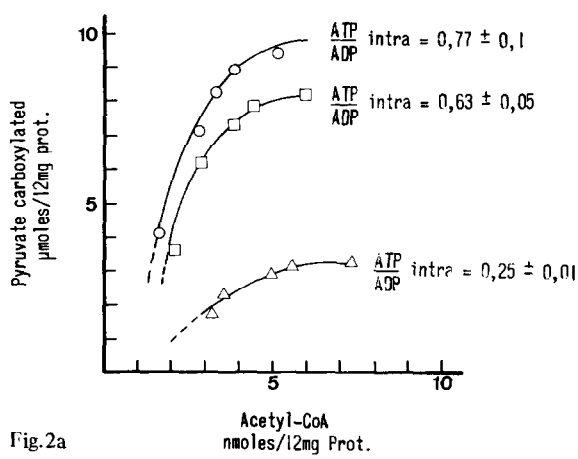


Fig.2a

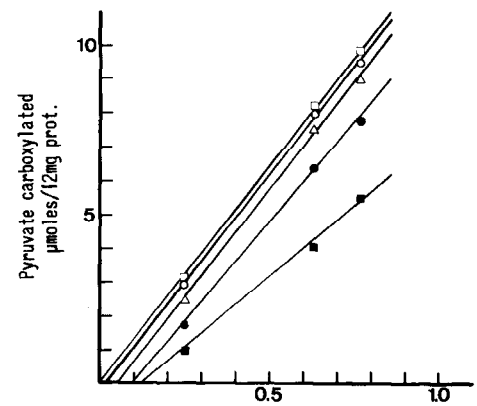


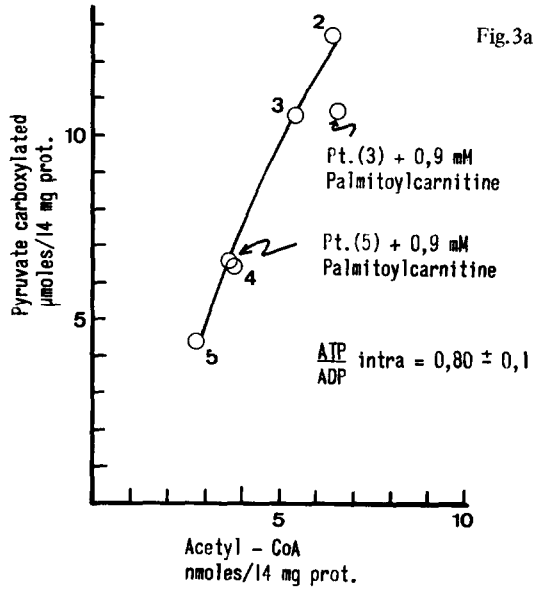
Fig.2b

Fig.2. Effect of varying intramitochondrial acetyl-CoA levels on pyruvate carboxylation. The basic incubation medium and incubation time were the same as in fig.1 except that 10 mM 3-hydroxybutyrate was added to all experiments. Each curve in fig.2a represents a different experiment, and each point shows the average value of triplicate incubations. ATP:ADP ratios are given as means ± standard deviation of all points of each respective curve, i.e., $n = 5$ for each ratio indicated. Adenine nucleotides and acetyl-CoA were measured in the pellet after millipore filtration [9]. The experiment of the lowest curve was obtained in the presence of added creatine kinase and 10 mM creatine. Further additions were from right to left: point 1: 10 mM L-acetylcarnitine, point 2: no addition; point 3: 1.7 μM arsenite, point 4: 3.3 μM arsenite; point 5: 8.3 μM arsenite. Figure 2b is a replot of fig.2a. Each curve represents the result at a constant acetyl-CoA concentration, (□) 6 nmol acetyl-CoA/12 mg protein. (○) 5, (△) 4, (●) 3, (■) 2.

enzyme activity was in part also regulated by changes of this ratio. In order to obtain a meaningful correlation between acetyl-CoA concentrations and pyruvate carboxylase activity, 3-hydroxy-butyrate was added which led to a stabilization of the ATP:ADP ratios. In fig.2a the results of varying acetyl-CoA concentrations on the rate of pyruvate carboxylation at three different ATP:ADP ratios are shown. The acetyl-CoA concentrations were varied on one hand by adding different amounts of arsenite and on the other hand by addition of acetyl-carnitine to the uninhibited control. These results have been replotted in fig.2b and demonstrate the correlation between the ATP:ADP ratio and pyruvate carboxylation at constant acetyl-CoA concentrations. When fatty acids were added to the partially inhibited system (fig.3a,b), the acetyl-CoA increases led to a corresponding increase in enzyme activity only at high arsenite concentrations. When less arsenite was added and therefore more acetyl-CoA was formed from pyruvate, only little or no stimulation of pyruvate carboxylase could be observed upon addition of fatty acid even though the enzyme appeared not yet to be saturated with its allosteric activator.

4. Discussion

The K_a value describing the activation of isolated rat liver pyruvate carboxylase by acetyl-CoA has been reported to be dependent on pH, ionic strength, temperature, concentration of bicarbonate, potassium ions, magnesium ions, $MgATP^{2-}$ and pyruvate (see [2] for references). At saturating pyruvate concentrations, the K_a value at pH 8.0, 25°C and 20 mM KCl is about 20 μM whereas at more physiological conditions, pH 7.3, 37°C and 85 mM KCl the value is about 60 μM [12]. More recently, Scrutton [2] reported a strong dependence of the K_a value on pyruvate concentration, at pH 7.2. At saturating concentrations of pyruvate he observed a K_a value of 50–55 μM whereas at the physiological concentration of 0.1 mM the value increased to about 120 μM. The measurements reported in fig.2 of the present paper were performed at saturating concentrations of pyruvate and it can be calculated on the basis of 0.8 μl water per mg mitochondrial protein [13] that the mitochondrial K_a of acetyl-CoA varies between 210 μM at an ATP:ADP ratio of 0.77 and 290 μM at a nucleotide ratio of 0.25. This dependence of the mitochondrial K_a on the



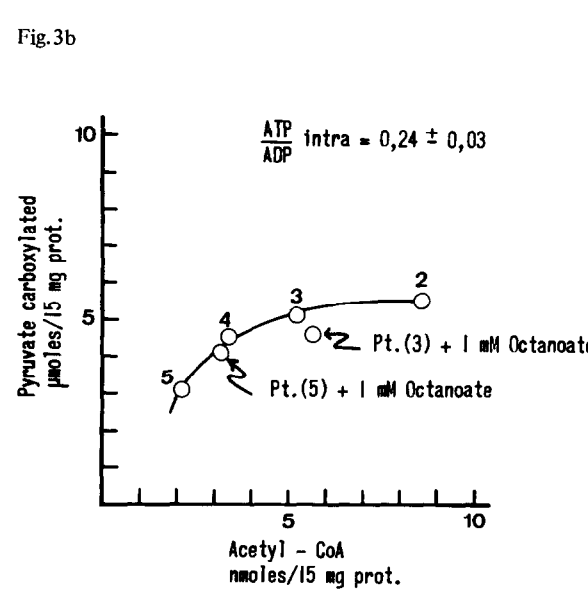


Fig.3. Effect of fatty acids on the correlation between acetyl-CoA levels and pyruvate carboxylation. The incubation mixture and incubation time were the same as in fig.2. Experiment 3b was obtained in the presence of 10 mM creatine and creatine kinase. The additions corresponding to the various points were the same as in fig.2. ATP:ADP ratios are given as means ± standard deviation of all points of each respective curve, i.e., $n = 6$ for each ratio indicated.

ATP:ADP ratio is consistent with the values reported by Seufert et al. [14] who found a positive heterotropic effect of $MgATP^{2-}$ on the binding of acetyl-CoA to the enzyme. It is also apparent that the sensitivity of pyruvate carboxylation towards the ATP:ADP ratio is greater at higher acetyl-CoA concentrations than at the lower values.

It is of interest to note in this context that Barrit et al. [15] have recently found in similar experiments with chicken liver mitochondria a mitochondrial K_a value of 320 μM which is also much higher than the K_a of 13 μM for the isolated enzyme. These mitochondrial experiments were, however, performed in the absence of 3-hydroxybutyrate or other additional substrates and therefore the effects of changes in the ATP:ADP ratio are not known.

The several-fold higher values for the rat liver mitochondrial K_a as compared to the apparent K_a values reported for the isolated enzyme cannot be unequivocally explained. One likely reason is that part of the acetyl-CoA is bound to other proteins in the mitochondria thereby decreasing the free acetyl-CoA concentration (see [15] for extensive discussion). There is, furthermore, increasing evidence for a heterogeneity of the mitochondria in the liver, e.g., it has been postulated that the distribution of acetyl-CoA and pyruvate carboxylase may not be equal in all mitochondria [11]. In fig.3a and b additional evidence for this proposal is presented. It was consistently observed that an elevation of total intramitochondrial acetyl-CoA by the addition of fatty acids only leads to a corresponding activation of pyruvate carboxylation at low but not high acetyl-CoA levels.

The relevance of these results for in vivo conditions is difficult to assess. First, it should be noted that beside the intra-mitochondrial ATP:ADP ratio also other factors known to influence the binding of acetyl-CoA to the isolated enzyme could be critical for the size of the mitochondrial binding constant. In this context it would be especially interesting to see whether Scrutton's findings [2] that the K_a value is about three times higher at physiological pyruvate concentrations than at saturating levels is also valid in mitochondria. As far as the knowledge of acetyl-CoA levels in various compartments of the cell is concerned some progress has recently been made because two new techniques for the separate measurements of cytosolic and mitochondrial acetyl-CoA have become available. Siess and Wieland found by using a modified method of Zuurendonk and Tager [16] that the mitochondrial acetyl-CoA level in isolated liver cells were $\geq$ 860 μM [17] for cells from fasted rats and $\geq$ 500 μM [18] for those from fed rats. The corresponding mitochondrial values by Soboll et al. [13] who used another technique were 1250 μM in perfused livers of fasted rats and 240 μM in those of fed rats. Even though it is too early to draw any definite conclusions it appears likely from the results presented in this paper and from those of the literature that variations in acetyl-CoA concentrations in vivo may play an important regulatory role in the regulation of pyruvate carboxylation.

Acknowledgement

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