Preliminary Notes

PN 10069

Synthesis of malonyl-coenzyme A from acetyl-coenzyme A and oxalosuccinate in mitochondria

Previous communications from this laboratory[1-3] pointed to the existence of carboxyltransferase reactions in heart. The direct demonstration of the formation of malonyl-coenzyme A from acetyl-coenzyme A and β-ketocarboxylic acids of the citric acid cycle (oxalosuccinate or oxaloacetate) were, however, unsuccessful, possibly because of side reactions leading to removal of malonyl-coenzyme A. It is the purpose of the present communication to show that malonyl-coenzyme A can indeed be formed from acetyl-coenzyme A and oxalosuccinate. The enzyme preparation used was obtained by sonicating freshly prepared rat-heart sarcosomes in a Mullard sonic disintegrator (60 W; 20 kHz) for three 1-min periods. The heavy particles were removed by centrifugation for 10 min at 12 000 $\times$ g. The supernatant contained about 3 mg of sarcosomal protein per ml and 0.35 ml was used in each experiment.

It can be seen from Fig. 1 that in the absence of added bivalent metal ions and

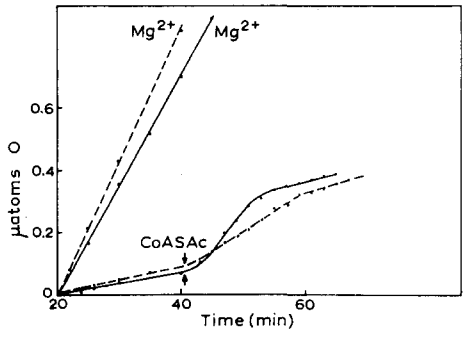

Fig. 1. Influence of acetyl-coenzyme A and magnesium ions on isocitrate oxidation by sarcosomal fragments from rat heart. Solid lines: preparation from two normal rats; broken lines: preparation from two biotin-deficient rats. The reactions were carried out at 25° in small Warburg vessels, containing in 1 ml: 50 mM potassium phosphate buffer (pH 7.4), 90 mM KCl, 5 mM EDTA, 20 mM L$_s$(+)-isocitrate, 1 mM $NADP^+$, 0.1 mM NAD^+, 0.04 mM cytochrome c, 30 μg lewisite (to inhibit the oxidation of α-oxoglutarate, formed during the reaction), 10 mM $MgCl_2$ present only where shown. The reaction was started by the addition of sarcosomal fragments (1.2 mg protein in the experiments with normal rats or 1.1 mg of protein with the biotin-deficient rats) and the flasks attached to differential manometers. Where indicated 0.27 μmole of acetyl-coenzyme A and 1 μmole EDTA were added from the side-arm. All flasks contained in the centre well 10% KOH and a filter paper.

the presence of 5 mM EDTA the rate of isocitrate oxidation is very low, due to the slow removal of oxalosuccinate under the conditions of the experiment. Magnesium strongly stimulates the oxidation of isocitrate. Acetyl-coenzyme A tipped in from

a side-arm of the Warburg vessel also stimulated isocitrate oxidation. In contrast to the effect of Mg^{2+} the effect of acetyl-coenzyme A on isocitrate oxidation is a stoichiometric one; acetyl-coenzyme A cannot be replaced by malonyl-coenzyme A. At the end of the acetyl-coenzyme A response, an accumulation of malonyl-coenzyme A can be expected according tot the following carboxyltransferase reaction:

$$\text{Oxalosuccinate} + \text{acetyl-coenzyme A} \rightleftharpoons \alpha\text{-oxoglutarate} + \text{malonyl-coenzyme A} \quad (1)$$

That malonyl-coenzyme A was formed in the experiment of Fig. 1 was shown by using [^{14}C]acetyl-coenzyme A.

After the stimulating effect of added [^{14}C]acetyl-coenzyme A on isocitrate oxidation had subsided, the reaction was stopped by addition of $HClO_4$. The precipitated protein was removed by centrifugation, the supernatant neutralized with KOH, and the $KClO_4$ removed. The ^{14}C-containing neutral solution was applied to a Dowex-1-formate column and the column washed with water. Subsequent elution of the column with 4 M formic acid caused immediate elution of radioactive material, followed later by the elution of $NADP^+$. The radioactive material was freed from formic acid by ether extraction and applied to a Whatman No. 3 paper. Descending chromatography with isobutyric acid–ammonia–water[4] (66:1:33) yielded a band indistinguishable from malonyl-coenzyme A ($R_F = 0.42$). Elution of this material, followed by saponification, acidification and ether extraction, yielded a non-volatile radioactive acid to which carrier malonic acid was added. Repeated recrystallization from benzene–ether, containing 5% petroleum ether, yielded malonic acid of constant specific radioactivity.

It seems very likely that the mitochondrial biosynthesis of malonyl-coenzyme A through Reaction 1 involves biotin as a coenzyme, since sarcosomal fragments obtained from the hearts of biotin-deficient rats show, in comparison to heart sarcosomal fragments from normal rats, a slow acetyl-coenzyme A-stimulated isocitrate oxidation. It can be calculated from Fig. 1 that the rate of the acetyl-coenzyme A-stimulated reaction in particles from normal rats is 71% of the magnesium-stimulated isocitrate oxidation, whereas in the particles obtained from biotin-deficient rats the rate of the acetyl-coenzyme A-stimulated reaction is only 29% of the magnesium stimulated one. That biotin is likely to be involved in mitochondrial carboxyltransferase reactions is to be expected from the work of SWICK AND WOOD[5] on carboxyltransferase reactions in Propionibacterium.

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[1] W. C. HÜLSMANN, *Biochim. Biophys. Acta*, 58 (1962) 417.
[2] W. C. HÜLSMANN AND C. BENCKHUIJSEN, *Biochem. J.*, 84 (1962) 63P.
[3] W. C. HÜLSMANN, *Biochim. Biophys. Acta*, 62 (1962) 620.
[4] R. BRESSLER AND S. J. WAKIL, *J. Biol. Chem.*, 236 (1961) 1643.
[5] R. W. SWICK AND H. G. WOOD, *Proc. Natl. Acad. Sci. U.S.*, 46 (1960) 28.

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