KINETICS OF REGULATORY ENZYMES: EFFECT OF ADENOSINE TRIPHOSPHATE ON YEAST CITRATE SYNTHASE*

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We have suggested (Hathaway and Atkinson, 1963) that modulation of the kinetic behavior of DPN-specific isocitrate dehydrogenase by AMP is part of a complex control system by which the utilization of acetyl coenzyme A (AcSCoA) for fat production is regulated in response to the momentary energy needs of the cell. This proposal suggested the possibility that the alternative fate of AcSCoA--entry into the citric acid cycle--might be under complementary control. This paper presents evidence that the kinetic properties of the appropriate initial enzyme, citrate synthase [citrate oxaloacetate-lyase (CoA-acetylating) EC 4.1.3.7] are directly modulated by changes in ATP concentration within the physiological range.

Methods--Baker's yeast (Fleischmann) was suspended in half its weight of 0.1 M $NaHCO_3$ and the cells were disrupted in a French press. After centrifugation for 90 minutes at 105,000 x g, the supernatant solution (specific activity, 0.15 μmoles/min/mg protein)was fractionated with ammonium sulfate. The protein that precipitated between 0.40 and 0.62 g of ammonium sulfate per ml contained 90% of the activity at a specific activity between 2 and 3. This material, dissolved in 1.0 mM phosphate,

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pH 8.0, was used for the experiments reported. Activity was assayed photometrically as described by Srere et al. (1963).

Results--The addition of ATP at 5 mM increases the apparent K_m of yeast citrate synthase for AcSCoA from 2 μM to 90 μM. The oxaloacetate concentration was 200 μM in these experiments. The apparent affinity of the enzyme for oxaloacetate does not seem to be affected by ATP. Other nucleoside triphosphates tested--ITP, GTP, CTP, and UTP--all inhibit to a slightly smaller extent than does ATP.

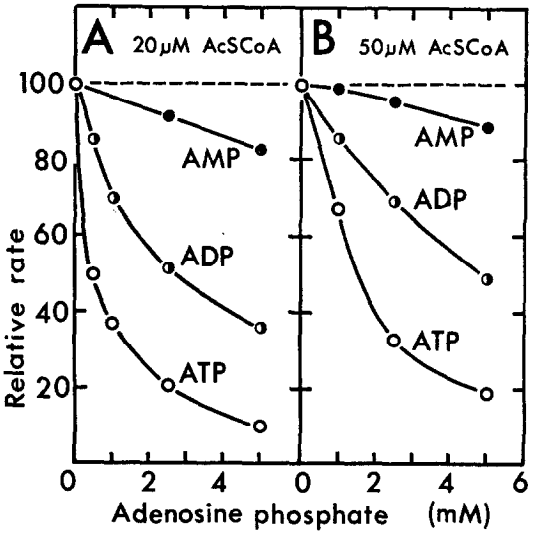

Fig. 1. Effects of ATP, ADP, and AMP on the rate of the reaction catalyzed by citrate synthase. Assays were performed at pH 8.0 in 0.1 M Tris-Cl, with oxaloacetate at 200 μ M. Concentrations of AcSCoA are shown on the figure, and AMP, ADP, or ATP concentration is indicated on the abscissa.

Figure 1 shows enzyme activity, in assay mixtures containing 200 μM oxaloacetate and two levels of AcSCoA, as a function of the concentration of adenosine phosphates added. An increase in the concentration of AcSCoA decreases the relative effectiveness of ATP, as would be expected

if the primary effect of ATP is to decrease the affinity of the enzyme for AcSCoA. Both ADP and AMP are inhibitory, but markedly less so than ATP. Inhibition by these compounds was unexpected, especially since AMP and ATP oppose each other as effectors for yeast phosphofructokinase (Ramaiah, Hathaway, and Atkinson, 1964). The metabolic consequences of the properties shown in Fig. 1 must depend, however, on the relative, rather than absolute, effects of ATP, ADP, and AMP. The total concentration of adenosine phosphates (AMP + ADP + ATP) is presumably virtually constant in the cell, and the relative activity of the condensing enzyme at a given level of AcSCoA could then vary between the values given by the ATP and the AMP curves of Fig. 1.

Crystalline pig heart citrate synthase (Boehringer) is also inhibited by ATP, but somewhat less strongly than is the enzyme from yeast. As with the yeast enzyme, this inhibition seems to result from a decrease in the affinity of the enzyme for AcSCoA rather than for oxaloacetate.

Preliminary sucrose density gradient centrifugations showed no detectable change in sedimentation behavior of either the yeast or pig heart enzyme in the presence of 0.01 M ATP.

We confirmed the inhibitory effect of palmityl CoA on the pig heart enzyme reported by Tubbs (1963) and by Wieland and Weiss (1963), and found a nearly identical effect on the yeast enzyme. This inhibitor, as reported by Wieland et al. (1964) for the pig heart enzyme, affects the interaction of the enzyme with oxaloacetate rather than with acetyl-CoA.

The kinetic properties of citrate synthase from beef liver are similar to those of the yeast enzyme (Jangaard and Atkinson, unpublished), and ATP inhibition of the enzyme from tobacco leaf has been observed by West and Glaser (1965). Thus the effect is probably general.

Discussion and Summary--On the basis of much recent work, it appears that competition for compounds that occupy branch point positions in metabolism is the cutting edge of metabolic regulation. Such a branch

point metabolite is simultaneously the substrate for two or more enzymes that catalyze initial reactions in two or more metabolic pathways. In such a case, the partitioning of substrate between the competing pathways would be expected to depend primarily on the relative affinities of the enzymes for the substrate. Metabolic regulation appears to function to a large degree through feedback mechanisms that, by altering the effective affinity of one or both of the enzymes involved, control the proportion of the branch point metabolite that enters each pathway. The typical operation of biosynthetic negative feedback controls at the first step unique to the synthesis illustrates this generalization.

Acetyl coenzyme A, with its many metabolic roles, occupies a very important metabolic branch point. In terms of energy metabolism (that is, ignoring its uses as a biosynthetic intermediate), the main control problem involved at the AcSCoA junction is regulation of the relative amounts of the compound that (a) enter the citric acid cycle and are used in the immediate regeneration of ATP and that (b) are converted to storage fats, which represent deferred ATP production.

The properties of citrate synthase reported here appear to supplement the previously proposed (Hathaway and Atkinson, 1963) indirect control of fatty acid production by AMP concentration. When the ratio of ATP to AMP is high, the concentrations of isocitrate and citrate should likewise be high (because the K_m of isocitrate dehydrogenase is high at low concentrations of AMP). This high level of citrate should act to increase the apparent affinity of AcSCoA carboxylase for AcSCoA (Martin and Vagelos, 1962; Waite and Wakil, 1963) and thus to favor utilization of this compound for the production of fat. At the same time, the high ATP/AMP ratio will lead to a low apparent affinity of citrate synthase for AcSCoA, thus decreasing the tendency of AcSCoA to enter the citric acid cycle. If the ratio of ATP to AMP decreases, a progressively larger portion of the AcSCoA should be directed into the citric acid cycle, thus increasing

the rate of regeneration of ATP. These proposed relations are tabulated below:

	ATP/AMP ratio	
	high	low
K_m* of isocitrate dehydrogenase	high	low
citrate concentration	high	low
K_m* of AcSCoA carboxylase for AcSCoA	low	high
AcSCoA--tendency to enter fat production	high	low
K_m* of citrate synthase for AcSCoA	high	low
AcSCoA--tendency to enter citric acid cycle	low	high

* K_m is used loosely to indicate the concentration of substrate required for half-maximal velocity. These values are not true Michaelis constants, because regulatory enzymes usually do not follow conventional first-order Michaelis kinetics.

Many regulatory systems must converge on a metabolic branch point as important as that occupied by AcSCoA. Each biosynthetic pathway in which AcSCoA is used must be independently regulated and, as Utter and co-workers (1964) have shown, the level of AcSCoA itself controls the production of oxaloacetate from pyruvate. Despite these complications, it appears that the interactions discussed here may play a major role in the partitioning of AcSCoA between immediate oxidation and storage as fat.

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