Adenosine Triphosphate Conservation in Metabolic Regulation

RAT LIVER CITRATE CLEAVAGE ENZYME*

(Received for publication, April 28, 1967)

DANIEL E. ATKINSON AND GORDON M. WALTON

From the Biochemistry Division, Department of Chemistry, University of California, Los Angeles, California 90024

SUMMARY

The citrate cleavage enzyme (EC 4.1.3.8) of rat liver is inhibited by adenosine diphosphate, which appears to compete with adenosine triphosphate. This effect may ensure that fatty acids are produced only when the ATP level is high.

The "energy charge" of the adenylyl system, defined as 

\[ \frac{(ATP + \frac{1}{2} ADP)}{(ATP + ADP + AMP)} \]

is proposed as a fundamental metabolic control parameter. Enzymes that utilize ATP and are inhibited by ADP or AMP will yield steep curves of velocity as a function of energy charge (resembling the steep curves of velocity as a function of substrate concentration that are characteristic of many regulatory enzymes) even in the absence of multiple sites and cooperative binding.

The production of acetyl coenzyme A from pyruvate is thought to occur mainly within mitochondria, and CoA esters appear not to pass readily through the mitochondrial membrane. Nevertheless, a number of extramitochondrial metabolic processes, notably fatty acid synthesis, consume large amounts of acetyl-CoA. Thus acetyl-CoA must be generated outside the mitochondrion, and presumably not directly from pyruvate. Sere (1) and Spencer and Lowenstein (2) suggested that this enzyme preparation was obtained from rat liver homogenates (1 ml) contained 200 mM Tris-Cl, pH 7.5, 5 mM MgCl₂, 40 

\[ \mu \text{M CoA-SCoA} \]

+ citrate + HSCoA + acetyl-SCoA + oxalacetate + ADP + P₁, EC 4.1.3.8), and that citrate exported from the mitochondrial serves as substrate. This suggestion has been supported by experimental results of various types, including isotope tracing (3–6) and the variation of the level of the cleavage enzyme with the rate at which fatty acids are synthesized under a variety of conditions (7–10). It thus appears that the reaction catalyzed by the cleavage enzyme is an early step in the synthesis of fats. Since a major role of fat is the storage of potential ATP, the hypothesis that energy metabolism is in general controlled by the ATP-ADP-AMP balance (11, 12) led us to look for effects of AMP or ADP on the cleavage enzyme. The rate of the reaction catalyzed by rat liver citrate cleavage enzyme is a normal Michaelis function of the ATP concentration (Fig. 1), with no suggestion of sigmoid kinetics. The reaction is inhibited by ADP. A plot of reciprocal velocity against the reciprocal of ATP concentration confirmed the suggestion of Fig. 1 that ADP acts as a competitive inhibitor with respect to ATP, increasing the apparent \( K_m \) for ATP but not affecting the maximal velocity. Similar observations were recently reported by Inoue et al. (13).

ADP is a product of the cleavage reaction, and is presumably formed at the enzymatic site that is responsible for ATP binding. Thus some degree of product inhibition might be expected.

*This work was supported in part by United States Public Health Service Grant AM-9863.

---

**FIG. 1.** Rate of the reaction catalyzed by rat liver citrate cleavage enzyme as a function of ATP concentration; effect of ADP. Assay mixtures (1 ml) contained 200 mM Tris-Cl, pH 7.5, 5 mM MgCl₂, 40 

\[ \mu \text{M CoA-SCoA} \]

+ citrate + HSCoA + acetyl-SCoA + oxalacetate + ADP + P₁, EC 4.1.3.8), and that citrate exported from the mitochondrial serves as substrate. This suggestion has been supported by experimental results of various types, including isotope tracing (3–6) and the variation of the level of the cleavage enzyme with the rate at which fatty acids are synthesized under a variety of conditions (7–10). It thus appears that the reaction catalyzed by the cleavage enzyme is an early step in the synthesis of fats. Since a major role of fat is the storage of potential ATP, the hypothesis that energy metabolism is in general controlled by the ATP-ADP-AMP balance (11, 12) led us to look for effects of AMP or ADP on the cleavage enzyme. The rate of the reaction catalyzed by rat liver citrate cleavage enzyme is a normal Michaelis function of the ATP concentration (Fig. 1), with no suggestion of sigmoid kinetics. The reaction is inhibited by ADP. A plot of reciprocal velocity against the reciprocal of ATP concentration confirmed the suggestion of Fig. 1 that ADP acts as a competitive inhibitor with respect to ATP, increasing the apparent \( K_m \) for ATP but not affecting the maximal velocity. Similar observations were recently reported by Inoue et al. (13).

ADP is a product of the cleavage reaction, and is presumably formed at the enzymatic site that is responsible for ATP binding. Thus some degree of product inhibition might be expected.

*This work was supported in part by United States Public Health Service Grant AM-9863.
AMP at levels such that the ratio of ATP to (AMP + ATP)
anhydride-bound phosphate groups per adenosine moiety. In
essentially constant adenylate pool of varying composition, the
0 and 1, and is numerically equal to half the average number of
from the curves of Fig. 3 labeled 6 and 5, and from a similar experiment
in which the sum of adenylate concentrations was 5 mM. These experi-
mental values are normalized to the energy charge scale of the abscissa,
and to a relative rate of 100 at an energy charge of 1.0.

FIG. 2 (upper). Concentrations of AMP, ADP, and ATP as functions
of the energy charge of the adenylate system. Concentrations were
in the legend; thus the agreement in shape between the calculated
curve and the locus of experimental points is significant, but
the agreement with regard to absolute levels is not.)

If the behavior of the cleavage enzyme in the intact cell
resembles the response in vitro illustrated in Figs. 2 and 3, generation
of extramitochondrial acetyl-CoA should occur only when the
energy charge of the cell is high, as seems appropriate for a
reaction leading to the production of storage compounds.

In addition, these results have more general implications.

1. The steep response curves that are desirable for sensitivity
of control have been found, in the cases of most regulatory
enzymes yet studied, to arise from highly cooperative binding of
ligands, with resulting high order kinetics. The behavior of
the cleavage enzyme, in contrast, is consistent with simple
competitive inhibition at a single catalytic site. Thus this
enzyme provides an experimental illustration of the mathe-
matically apparent fact that steep response characteristics may
be achieved without special regulatory sites, cooperative binding,
or even multiple sites, if the control parameter is a concentra-
tion ratio.

2. The affinity of phosphofructokinase for fructose 6-P is
decreased by ATP and enhanced by AMP or ADP (depending
on the species). At least in the case of the yeast enzyme (11),
the affinity depends directly on the AMP:ATP ratio, but the
direction of the response is opposite to that of the citrate
cleavage enzyme. Thus the phosphofructokinase response curve
should be approximately the reciprocal of the broken line in
Fig. 2 and the two curves should both be steep at their inter-
section point. If the cleavage enzyme is in a general way typical
of enzymes regulating the rate of ATP utilization, and phos-
phofructokinase typical of those regulating its regeneration, the
energy charge of the adenylate system would appear to be a
fundamental control parameter, and this charge would be
strongly buffered near the upper end of its range. An equivalent
statement of the hypothesis is that conservation of ATP is a
major feature of metabolic regulation.

3. A troublesome objection to the adenylate control hypothesis
(11, 12) has been that ATP levels in some cases may change
relatively slightly across the nutritional range from adequacy
of substrate to near starvation, although metabolic rates vary
widely. This behavior may seem to argue against direct partici-
pation of the adenylate system in metabolic regulation. An
apparent answer to this objection follows from the preceding
point. The steepness, at intersection, of what may be consid-
ered supply and demand curves for ATP indicates that metabolic
regulation may well be mediated through the ATP system,
even when the ATP concentration varies only within a narrow
range.
Adenosine Triphosphate Conservation in Biosynthetic Regulation

ESCHERICHIA COLI PHOSPHORIBOSYLPYROPHOSPHATE SYNTHASE*

(Received for publication, April 28, 1967)

DANIEL E. ATKINSON AND LANA FALL

From the Biochemistry Division, Department of Chemistry, University of California, Los Angeles, California 90024

SUMMARY

Phosphoribosylpyrophosphate synthase (EC 2.7.6.1) from Escherichia coli is inhibited by adenosine diphosphate and to a much smaller extent by other nucleotides and by tryptophan. We propose (a) that the adenosine diphosphate effect is part of a general adenosine triphosphate conservation system that causes the rate of expenditure of ATP to be sharply curtailed when the energy charge of the cell decreases slightly; and (b) that, as appears to be the case with phosphoribosylpyrophosphate synthase, such ATP conservation control may be superposed on product negative feedback regulation of the same enzyme.

Following its discovery by Umbarger (1) and Yates and Pardee (2), biosynthetic regulation by product feedback inhibition has come to be recognized as a general phenomenon. This type of control seems adequate to account for the relative constancy of the pool levels of metabolic building blocks, such as amino acids and nucleotides, but it cannot be directly responsible for the general depression of biosynthetic activity that accompanies substrate limitation. As an extreme example, when an organism is subsisting on endogenous amino acids (derived from its own proteins) as energy source, it would not seem appropriate for synthesis de novo of amino acids to continue subject only to product feedback control

*This work was supported in part by United States Public Health Service Grant AM 9863.

REFERENCES


*The abbreviation used is: P-ribose-PP, 5-phospho-a-n-ribosylpyrophosphate.

Adenosine Triphosphate Conservation in Metabolic Regulation: RAT LIVER CITRATE CLEAVAGE ENZYME
Daniel E. Atkinson and Gordon M. Walton


Access the most updated version of this article at http://www.jbc.org/content/242/13/3239

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/242/13/3239.full.html#ref-list-1