The Metabolic Significance of the Malate-Aspartate Cycle in Heart

By Brian Safer

Under physiological conditions, the energy requirements of the heart are met primarily by the oxidation of fatty acids, glucose, and lactate (1, 2). Initially, during the metabolism of glucose and lactate, the coenzyme NAD is reduced to NADH and pyruvate is formed in the cytosolic compartment of the myocardial cell. Subsequent entry of pyruvate into the mitochondria for oxidative metabolism in the citric acid cycle requires an equivalent oxidation of cytosolic NADH by the mitochondrial electron transport chain, which regenerates NAD. However, direct transfer of the reduced coenzyme NADH to the respiratory chain is prevented by a selective permeability barrier across the inner mitochondrial membrane to NADH as well as other metabolic intermediates (3, 4). Although several indirect shuttle mechanisms have been proposed (5-7), current evidence indicates that the reducing equivalents formed in the reduction of cytosolic NAD to NADH are indirectly transferred via malate to the respiratory chain for oxidation.

The return of oxaloacetate thus formed in the mitochondrial compartment to the cytosolic compartment, which completes the malate-aspartate cycle, appears to be limited by a selective impermeability of the inner mitochondrial membrane and the low intramitochondrial concentration of oxaloacetate. Instead, oxaloacetate is transaminated with glutamate in the mitochondrial compartment to form α-ketoglutarate and aspartate. Aspartate and α-ketoglutarate are able to cross the inner mitochondrial membrane, and, by the reverse transamination, oxaloacetate and glutamate are regenerated in the cytosolic compartment to complete the cycle (5, 6, 14). Distinct cytosolic and mitochondrial isoenzymes of malate dehydrogenase and aspartate aminotransferase catalyze these reactions (15).

Role of Metabolic Anion Exchange Carriers in the Malate-Aspartate Cycle

Indirect calculations based on levels of oxidized and reduced metabolites in several tissues (16-18) suggest that, in the heart, the mitochondrial pyridine nucleotide pool is also maintained at a level considerably more reduced than that of the cytosol (the ratio of NADH to NAD is 10-100-fold higher in the mitochondria). Therefore, transfer of cytosolic reducing equivalents to the respiratory chain is energetically unfavorable and cannot occur by free diffusion of the metabolic anions of the malate-

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1 The term cytosolic compartment designates the intracellular space exclusive of the water-permeable space enclosed by the inner mitochondrial membrane.

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The malate-aspartate cycle. NADH formed in the cytosolic compartment of the cell is oxidized by the reduction of oxaloacetate (OAA) to malate (MAL). Malate then indirectly transfers these reducing equivalents into the mitochondrial compartment where oxaloacetate and NADH are regenerated. For steady-state operation of the malate-aspartate cycle, oxaloacetate must be continuously returned to the cytosolic compartment. Because of its low concentration in the mitochondria and the relative impermeability of the mitochondrial membrane to oxaloacetate, this transfer is also indirectly accomplished by transamination with glutamate (GLU) to form α-ketoglutarate (α-KG) and aspartate (ASP). These substances are then transferred to the cytosolic compartment where the reverse transamination regenerates oxaloacetate and glutamate. Distinct mitochondrial and cytosolic isoenzymes catalyze these reactions. A specific electroneutral carrier system catalyzes the equal and opposite exchange of malate and α-ketoglutarate (I). The aspartate-glutamate antiport system (II), in contrast, appears to be electrogenic. By directly utilizing the free energy of the electrochemical gradient established by electron transport, cytosolic NADH can be indirectly transferred into the mitochondrial compartment against a NADH-NAD potential gradient.

LAC = lactate, PYR = pyruvate, and TRIOSE-P = glyceraldehyde-3-P and α-glycerophosphate.

Aspartate cycle across the inner mitochondrial membrane. It has been demonstrated, however, that such anion exchange is mediated by specific carriers, some of which may be energy linked (14, 19–21). In coupled mitochondria, transfer of electrons in the respiratory chain results in the production of a small pH gradient (≤ 0.5 pH units, alkaline inside) and an electrical potential gradient (≤ 250 mv, negative inside) across the inner membrane. Recent evidence suggests that the anion exchange carriers can directly utilize these potential gradients to drive the malate-aspartate cycle (19, 22–26).

During the steady-state operation of the malate aspartate cycle, malate is exchanged stoichiometrically for α-ketoglutarate across the inner mitochondrial membrane by a specific electroneutral antiport system (27) according to the net chemical gradient. It had been proposed that this malate-α-ketoglutarate exchange carrier (labeled I in Fig. 1) is driven by the pH gradient across the inner mitochondrial membrane (19). However, although this mechanism is feasible in the liver, it cannot be the primary energy source for the malate-aspartate cycle in the myocardium, since the activity of the malate-phosphate exchange carrier (also required for utilization of the pH gradient) is low in heart.
The primary energy source for the malate-aspartate cycle appears to be the aspartate-glutamate antiport system. In contrast to the electroneutral malate-α-ketoglutarate antiport system, exchange of glutamate and aspartate appears to be electrogenic due to the carrier-mediated cotransport of one proton per glutamate molecule. Thus, a net transfer of charge accompanies this exchange, which permits direct utilization by the aspartate-glutamate exchange carrier (labeled II in Fig. 1) of the potential gradient established by the outward-directed proton pump that is coupled to electron transport (24–26). Expenditure of energy to maintain the electrochemical gradient across the inner mitochondrial membrane during electron transfer may therefore provide the energy required for uphill transfer of reducing equivalents by the malate-aspartate cycle against an NADH-NAD potential gradient (29). In addition, since this carrier can only exchange intramitochondrial aspartate for extramitochondrial glutamate (24–26, 30, 31), transfer of reducing equivalents is permitted only from the cytosolic to the mitochondrial compartment.

INTERACTION OF THE MALATE-ASPARTATE AND CITRIC ACID CYCLES AND NONUNIFORM CITRIC ACID CYCLE FLUX

Through shared metabolic intermediates, the malate-aspartate cycle interacts directly with the citric acid cycle in the region of citrate synthetase and α-ketoglutarate dehydrogenase. As a result, the flux of substrates through different portions of the citric acid cycle may not be equal but is modulated by the relative rates of reaction at two metabolic branch points indicated by the asterisks in Figure 2 (12, 13). The two key enzymes which determine the extent of nonuniform flux throughout the citric acid cycle are α-ketoglutarate dehydrogenase and mitochondrial aspartate aminotransferase.

Entry of α-ketoglutarate into the second half of the citric acid cycle (labeled CAC II in Fig. 2) or diversion into the malate-aspartate cycle appears to be regulated by the flux through α-ketoglutarate dehydrogenase. The activity of α-ketoglutarate dehydrogenase is primarily regulated by the succinyl-CoA-CoA and NADH-NAD ratios (32, 33); an increased ratio results in inhibition. The succinyl-CoA-CoA ratio is indirectly regulated by the ATP-ADP ratio via substrate-level phosphorylation (at succinate thiokinase). The NADH-NAD ratio generally reflects the rate of electron transport. Therefore, partition of α-ketoglutarate flux into the citric acid cycle or the malate-aspartate cycle in response to the energy state of the myocardium is directed by the activity of α-ketoglutarate dehydrogenase (9, 32).

Similarly, oxaloacetate can either enter the first half of the citric acid cycle (labeled CAC I in Fig. 2) or bypass it by undergoing transamination with glutamate. Flux through a second enzyme, aspartate aminotransferase, appears to indirectly regulate this partition of oxaloacetate. Flux through this enzyme is dependent on the availability of both oxaloacetate and glutamate. The energy-linked glutamate-aspartate exchange carrier, by regulating transfer of glutamate (and aspartate) across the inner mitochondrial membrane, may therefore be primarily responsible for determining whether oxaloacetate bypasses the first part of the citric acid cycle. The rate of glutamate transfer by this carrier is also dependent on the ratio of the concentrations of glutamate and aspartate in the cytosol (9, 30, 34). Thus, the metabolic fate of oxaloacetate is determined not only by reactions of the citric acid cycle in the mitochondrial compartment but also by the cytosolic levels of key metabolites shared by the malate-aspartate cycle.

Control by these two enzymes, α-ketoglutarate dehydrogenase and aspartate aminotransferase (via the aspartate-glutamate carrier), allows flux in span I and span II of the citric acid cycle to be transiently dissimilar. As subsequently discussed, such transient disruptions of uniform flux are absolutely required for rapid alterations of myocardial citric acid cycle intermediate levels.

ROLE OF THE MALATE-ASPARTATE CYCLE IN THE COORDINATION OF GLYCOLYTIC AND CITRIC ACID FLUXES

In addition to providing a mechanism for the oxidation of cytosolic NADH, the malate-aspartate cycle is required to coordinate mitochondrial and cytosolic metabolism in the heart. Under a wide variety of metabolic conditions, balanced glycolytic and citric acid cycle fluxes allow more efficient oxidative metabolism of carbohydrate fuels. Direct feedback inhibition of glycolysis at phosphofructokinase by citrate and ATP has generally been accepted as the primary mechanism for this control (35, 36). Although this mechanism is supported by kinetic studies of isolated enzymes and changes in the tissue levels of metabolic intermediates following changes in flux (37), any direct regulation of...
Interactions of the malate-aspartate cycle, the glycolytic pathway, and the citric acid cycle which allow indirect oxidation of cytosolic NADH, rapid alteration of citric acid cycle intermediate levels, and fine coordination of cytosolic and mitochondrial energy metabolism. The malate-a-ketoglutarate and aspartate-glutamate exchange carriers, labeled I and II, respectively, are located in the inner mitochondrial membrane. Two key metabolic branch points at citrate synthetase and a-ketoglutarate dehydrogenase are identified by asterisks. By partitioning total metabolic flux between the malate-aspartate and citric acid cycles, transiently dissimilar fluxes are allowed in the two functionally distinct spans of the citric acid cycle (13) designated in the figure as CAC I and CAC II. The nonuniform citric acid cycle flux in the heart permitted by these two spans is required for rapid changes in citric acid cycle intermediate levels. A-i indicate key enzymes in these pathways, (a) = glyceraldehyde-3-phosphate dehydrogenase, (b) = lactate dehydrogenase, (c) = malate dehydrogenase, (d) = aspartate aminotransferase, (e) = citrate synthetase, (f) = aconitase, (g) = isocitrate dehydrogenase, (h) = a-ketoglutarate dehydrogenase, and (i) = alanine aminotransferase. LAC = lactate, PYR = pyruvate, MAL = malate, OAA = oxaloacetate, a-KG = a-ketoglutarate, GLU = glutamate, ASP = aspartate, ALA = alanine, ISOCIT = isocitrate, CIT = citrate, AcCoA = acetyl coenzyme A, and TRIOSE-P = glyceraldehyde-3-P and a-glycerophosphate.

MECHANISM FOR THE ALTERATION OF CITRIC ACID CYCLE INTERMEDIATE LEVELS IN THE HEART

In addition to being required for changes in cytosolic citrate levels, rapid changes in total citric acid cycle intermediate levels in the heart appear to require interaction of the citric acid and malate aspartate cycles. Increased tissue levels of citrate cannot be the direct result of an increased rate of acetyl-CoA entry into the citric acid cycle, since two molecules of carbon dioxide are formed per cycle turnover and additional oxaloacetate is not generated. Enzymes required for the carboxylation of pyruvate or acetate are also absent in the heart (13, 40). Although tissue levels of total citric acid cycle intermediates and aspartate appear to hav...
an inverse relationship to one another, transamination of aspartate cannot directly increase the size of the citric acid cycle intermediate pool, since α-ketoglutarate is removed as glutamate in this reaction. However, the coupled transamination of aspartate and glutamate by (1) aspartate aminotransferase of the malate-aspartate cycle and (2) alanine aminotransferase is able to form oxaloacetate in the cytosolic compartment from two metabolic intermediates that are not directly part of the citric acid cycle intermediate pool (12, 13, 41):

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\begin{align*}
\text{(1) Aspartate} + \alpha\text{-Ketoglutarate} & \leftrightarrow \text{Oxaloacetate} + \text{Glutamate} \\
\text{(2) Glutamate} + \text{Pyruvate} & \rightarrow \text{Alanine} + \alpha\text{-Ketoglutarate} \\
\text{SUM} & \text{Aspartate} + \text{Pyruvate} \rightarrow \text{Oxaloacetate} + \text{Alanine}
\end{align*}
\]

For example, provision of glucose to a substrate-deficient heart (12) leads to an increase in the total citric acid cycle intermediate pool by the following interaction of the metabolic pathways presented in Figure 2. An increased cytosolic NADH-NAD ratio, resulting from greater glycolytic flux, leads to the rapid reduction of cytosolic oxaloacetate to malate. This first step results in a displacement of the equilibrium at cytosolic aspartate aminotransferase; by increasing cytosolic glutamate, this displacement is in turn transmitted to alanine aminotransferase. This coupled transamination is responsible for a net gain in the total tissue citric acid cycle intermediate pool.

Initially, however, the rate of oxaloacetate formation is severely limited by the availability of cytosolic α-ketoglutarate required for transamination with aspartate. Additional α-ketoglutarate is provided by an increased rate of production in the first span of the citric acid cycle relative to its rate of entry into the second span. As the result of (1) greater availability of acetyl-CoA secondary to increased glycolytic flux and (2) limiting mitochondrial glutamate, the increased rate of mitochondrial oxaloacetate formation is primarily partitioned toward formation of α-ketoglutarate in the first span of the citric acid cycle. However, because α-ketoglutarate is exchanged stoichiometrically for malate across the inner mitochondrial membrane and both the intramitochondrial NADH-NAD and ATP-ADP ratios increase (thereby inhibiting α-ketoglutarate dehydrogenase), this increased mitochondrial α-ketoglutarate production is primarily diverted to the cytosol. Thus, flux in span I of the citric acid cycle becomes transiently greater than that in span II.

α-Ketoglutarate transferred to the cytosol in this manner can then be directly utilized for transamination with aspartate. Alternatively, α-ketoglutarate can be reduced to isocitrate by NADP-linked isocitrate dehydrogenase, with citrate then being formed via aconitase. In this manner, both the total tissue content and the intracellular distribution of citric acid cycle intermediates can be altered. Once equilibrium is reestablished at cytosolic aspartate aminotransferase, the tissue level of citric acid cycle intermediates stabilizes to a new steady-state level and fluxes in the citric acid and malate-aspartate cycles become uniform. Since all of these enzymes present in the cytosolic compartment are fully reversible, changes in the oxidation-reduction state of cytosolic pyridine nucleotides and the metabolic substrate supply can increase or decrease the tissue levels of citric acid cycle intermediates in this manner.

**SIGNIFICANCE OF THE MALATE-ASPARTATE CYCLE**

The central role of the malate-aspartate cycle in myocardial energy metabolism raises a number of important questions as to its role in the pathogenesis of and the compensatory response to a number of disorders of myocardial function. It has been demonstrated, for example, that generalized inhibition of the malate-aspartate cycle can produce idiopathic lactic acidosis (8, 42, 43). Such inhibition in the heart can also have profound direct and indirect effects on contractility, since both excitation-contraction coupling and the malate-aspartate cycle appear to be inhibited by acidosis (44-49). By decreasing the oxidative metabolism of glucose, which requires the malate-aspartate cycle, localized acidosis can also contribute to contractile failure and affect survival of the ischemic myocardium. Although such suggestions are highly speculative at the present time, further research in these areas appears to be warranted.

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