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 **BTG 406 – METABOLLIC ENGINEERRING**

 SAILENT FEATURES OF IMPORTANT ANAPLEROTIC REACTIONS INVOLVED IN THE FUNCTIONING OF CITRIC ACID CYCLE (KREBS CYCLE).

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Anaplerotic reactions are chemical reactions that form intermediates of a metabolic pathway. Examples of such are found in the citric acid cycle (TCA cycle).

Introduction to the Krebs cycle

The Krebs cycle (KC, tricarboxylic acid cycle = TCA cycle) is a metabolic pathway localized in the mitochondrial matrix. One should easily deduce that every cell which possesses mitochondria has in physiologic conditions active the TCA cycle. There is one cell population however that lacks mitochondria – the erythrocytes. In the erythrocytes the TCA cycle does not take place. There is one important fact you should notice. The TCA cycle needs aerobic conditions for smooth course (the reason is below – Regulation of the Krebs cycle). The TCA cycle in cells that lack oxygen has limited velocity.

The TCA cycle performs many functions. In simple words the TCA cycle is heart of the energetic metabolism of the cell, i.e. almost all pathways of the energetic metabolism are connected to the TCA cycle. For example the ETC (electron transport chain), gluconeogenesis, transamination, deamination of amino acids or lipogenesis. It is quite obvious that there are both catabolic, and anabolic pathways thus it is not easy to state which one dominates (it is not possible to denote the TCA cycle as either catabolic, or anabolic). This is the reason why it is described as amphibolic pathway (for more details: Subchapter 2/2). Here is the partial list of some functions of the TCA cycle:

1) Oxidation of acetyl residues (supplied as acetyl-CoA (AcCoA))

In the TCA cycle takes place oxidation of acetyl residues (CH3-CO-) to CO2.

This process is source of reducing equivalents these are transferred on cofactors, NAD+ or FAD. This yields reduced forms – NADH and FADH2. NADH and FADH2 are so called reduced coenzymes. Reduced coenzymes enter ETC and their regeneration takes place. This regeneration is loss of reducing equivalents (i.e. electrons). Hence it is called reoxidation.

The reoxidation (regeneration) of reduced coenzymes is the process which connects the TCA cycle with the ETC. The TCA cycle is the main supplier of the reduced coenzymes for the ETC, thus the TCA cycle is very important source of the ATP (regardless fact that one turnover of the TCA cycle produces only one GTP)

2) Many catabolic pathways flow into the TCA cycle

Many catabolic pathways are source of the (1) intermediates of the TCA cycle, (2) pyr, (3) AcCoA. Their fate could be: (1) oxidation to CO2, (2) synthesis of other substances.

3)The TCA cycle provides precursors for many important anabolic pathways

Examples are (1) gluconeogenesis, (2) biosynthesis of tetrapyrroles (hem), (3) synthesis of amino acids (e.g. glutamate – it is the most abundant excitatory neurotransmitter in the brain), (4) source of AcCoA for fatty-acids synthesis.

4) The TCA cycle takes part in excretion of nitrogen

The TCA cycle is connected with urea cycle and glutamate synthesis. Both urea, and glutamate are two main forms used for excretion of amino acids derived nitrogen.

Reactions of the TCA cycle

The overall equation for the TCA cycle is as follows:

CH3-CO~SCoA + 3NAD+ + FAD + GDP + Pi + 2H2O → 2CO2 + 3NADH + FADH2 + GTP

AcCoA provides acetyl residues for the TCA cycle. Majority of it is from (1) the β-oxidation of fatty acids and (2) the pyruvate dehydrogenase reaction. Both these pathways take place in mitochondrial matrix.

Pyruvate dehydrogenase reaction

This reaction is irreversible oxidative decarboxylation of pyruvate. Equation follows:

CH3-CO-COOH + NAD+ + HSCoA → CO2+ NADH + H+ + CH3-CO~SCoA

Connection between pyruvate dehydrogenase reaction and overall equation of the TCA cycle yields an equation that describes complete oxidation of pyruvate:

CH3-CO-COOH + 4NAD+ + FAD + ADP + Pi + 2H2O → 3CO2 + 4NADH + 4H+ + FADH2 + ATP

Individual reactions of the TCA cycle

Oxidation of acetyl residues includes several steps:

1) Acetyl residue (2C) is transferred to oxaloacetate (4C). This reaction is catalysed by the enzyme citrate synthase. Citrate (6C) is generated. This condensation reaction is irreversible, i.e. this is one of the regulatory steps of the TCA cycle.

2) Citrate is isomerised to isocitrate. Isocitrate is generated via aconitate using aconitate-hydratase (aconitase). This step is freely reversible.

3) Isocitrate is oxidised to α-ketoglutarate. This step is catalysed by the enzyme isocitrate dehydrogenase. This reaction is oxidative decarboxylation. This means (1) oxidation of –OH group to keto group (this yields NADH) and (2) one carboxylic group is broken apart (this yields CO2). This reaction is irreversible, i.e. this is one of the regulatory steps of the TCA cycle. This one is however the most important regulatory step of the TCA cycle.

4) α-ketoglutarate is oxidised to succinyl-CoA. This step is catalysed by the enzyme α-ketoglutarate dehydrogenase (multienzyme complex). This reaction is oxidative decarboxylation as well (thus yields CO2 and NADH). Reaction is irreversible, i.e. it is likewise the regulatory step.

5) Conversion of succinyl-CoA to succinate and co-enzym A. This step is catalysed by succinyl-CoA-ligase. This reaction is typical example of substrate fosforylation, i.e. the GTP is produced and is converted to the ATP. This reaction is reversible.

In reactions (1) to (5) acetyl residues is completely oxidised to 2 CO2, oxaloacetate is reduced to succinate. Oxaloacetate is regenerated from succinate during following reactions:

6) Succinate oxidation to fumarate is catalysed by succinate dehydrogenase. This enzyme is an integral protein in the inner mitochondrial membrane. It is part of the ETC – complex II. Succinate dehydrogenase uses FAD. This co-enzyme is reduced thus generating FADH2.

7) Water is added to double bond in fumarate and malate is produced. This step is catalysed by the enzyme fumarate hydratase (fumarase).

8) Malate is oxidised to oxaloacetate. This reaction is catalysed by the enzyme malate dehydrogenase. NADH is produced. This is the last step of the TCA cycle.

Products of the TCA cycle

In one turnover of the TCA cycle (i.e. one acetyl residue is processed) 2 CO2, 3 NADH, 1 FADH2 and 1 GTP are produced.

Carbon dioxide diffuses from mitochondria to blood. At the end CO2 is removed in the lungs. The reduced coenzymes (NADH and FADH2) are substrates for the ETC. The ETC produces the ATP. Overall energetic result of the TCA cycle is 10-12 ATP per one molecule AcCoA.

Replenishing (anaplerotic) reactions

Intermediates of the TCA cycle are in mitochondria in very low concentrations. Their constant regeneration takes place when acetyl residues are oxidised, hence their concentrations are held stable. On the other hand anabolic pathways drain intermediates from the TCA cycle. E.g. succinyl-CoA → hem synthesis, oxaloacetate → gluconeogenesis etc. Nevertheless intermediates could be replenished by so called anaplerotic reactions:

1) Pyruvate is carboxylated to oxaloacetate. This reaction is catalysed by the enzyme pyruvate carboxylase (cofactor is biotin – vitamin B7):

Pyruvate + CO2 + ATP → oxaloacetate + ATP + Pi

2) Carbon skeletons of amino acids are used to produce some intermediates of the TCA cycle (oxaloacetate, α-ketoglutarate, fumarate). For example aspartate could undergo transamination to oxaloacetate. Respectively glutamate to α-ketoglutarate.

3) Propionyl-CoA to succinyl-CoA. This is the way the odd-numbered chain fatty acid can enter the TCA cycle.

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Regulation of the TCA cycle

Regulatory steps of the TCA cycle:

1) Citratesynthase

2) Isocitratedehydrogenase

3) α-ketoglutaratedehydrogenase

Regulatory factors of the TCA cycle:

1) NADH/NAD+ ratio – respiratory control

2) ATP/ADP (AMP) ratio – energetic control

3) Availability of substrates for the TCA cycle – substrate control

1) NADH/NAD+ ratio – respiratory control

The TCA cycle continues to the ETC. Reoxidation of the reduced coenzymes takes place there. In situations when is the ETC slowed, NADH and FADH2 accumulate. It is obvious that NADH/NAD ratio increases and thus α-ketoglutaratedehydrogenase and isocitratedehydrogenase are inhibited.

2) ATP/ADP (AMP) ratio – energetic control

α-ketoglutaratedehydrogenase and isocitratedehydrogenase are inhibited when there is a sufficient amount of energy, i.e. ATP/ADP (AMP) ratio is high. ATP acts as inhibitor, ADP and AMP are activators of those two enzymes.

3) Availability of substrates for the TCA cycle – substrate control

As you know, velocity of the chemical reaction depends among others on the concentration of the reactants and the products. Velocity of the TCA cycle depends on the concentration of the citrate. Activity of the citratesynthase is related to amounts of oxaloacetate and AcCoA that are provided.

Activity of the TCA cycle is interwoven with the availability of the oxygen despite the fact that none of the reactions of the TCA cycle require oxygen. Oxygen is vital for the ETC. Oxygen is the final acceptor of the electrons. In the ETC reoxidation of the NADH → NAD+ and FADH2 → FAD take place. One can easily deduce that reduction of the oxygen supplies for the cell leads to drop of concentrations of the NAD+ and FAD, hence activity of the TCA cycle is decreased.

The expression anaplerotic sequences was a term used in biochemistry by Sir Hans Kornberg (1) to describe a series of enzymatic reactions or pathways that replenish the pools of metabolic intermediates in the TCA cycle. These intermediates are critical for the functioning of the TCA cycle, the primary role of which is the oxidation of acetyl-CoA to carbon dioxide. The pool of TCA cycle intermediates is sufficient to sustain the oxidative carbon flux over a fairly wide range, so that during high energy consumption (e.g. exercise) or during lower energy consumption (e.g. fasting), there is not a large change in the pool size of TCA intermediates (2). However, in several physiological states, there is a large influx (anaplerosis) of 4- and 5-carbon intermediates into the TCA cycle. Because the TCA cycle cannot act as a carbon sink, anaplerosis must be coupled with the exit of intermediates from the cycle via cataplerosis. The importance of anaplerotic reactions for cellular metabolism is thus apparent. However, the coupling of this process with cataplerosis and the roles that both pathways play in the regulation of amino acid, glucose, and fatty acid metabolism have not been emphasized to a sufficient extent.

The terms anaplerosis and cataplerosis describe reciprocal and correlative reactions involved in the function of the TCA cycle. The enzymatic steps in these processes have long been known, but the overall concept of a linkage between anaplerosis and cataplerosis should be underscored, because the balance between these two processes controls the entry and exit of TCA cycle anions. Anaplerotic and cataplerotic reactions are involved in the ultimate disposal of all metabolic intermediates. The metabolic role of anaplerosis and cataplerosis in amino acid metabolism varies with specific organs and is dependent on the nutritional/metabolic status of the individual. During feeding, the intestine is an important site of catabolism of enterally derived amino acids, whereas in the starved state amino acid catabolism occurs primarily in the kidney, liver, and muscle.

The catabolism of amino acids produces gluconeogenic or ketogenic precursors (Table I). The disposal of gluconeogenic anions in the TCA cycle employs anaplerotic and cataplerotic pathways for their terminal oxidation. The only known pathway for the terminal oxidation of leucine is through acetoacetate to acetyl-CoA and subsequent oxidation in the TCA cycle. However, other amino acids also have for their disposal alternate ketogenic pathways for terminal oxidation. Thus, the ketogenic amino acids from proteolysis can be terminally oxidized in muscle, whereas the gluconeogenic amino acids are dependent upon anaplerosis and cataplerosis for conversion to glucose in the liver and kidney before oxidation to CO2 and H2O.

Anaplerosis

The first reaction of the TCA cycle, citrate synthase, catalyzes the condensation of oxalacetate with acetyl-CoA; the oxalacetate is subsequently regenerated by the reactions of the cycle and condenses with another molecule of acetyl-CoA. However, the TCA cycle also functions in biosynthetic processes in which intermediates are removed from the cycle; this necessitates anaplerotic reactions to replenish TCA cycle intermediates to ensure its continued function. Pyruvate carboxylase, which synthesizes oxalacetate from pyruvate in the mitochondrial matrix, is the archetypical anaplerotic enzyme. The activity of this enzyme is high in many tissues (e.g. 10–12 units/g of liver); acetyl-CoA is a positive allosteric regulator of the enzyme. Anaplerosis is obligatory during both gluconeogenesis and lipogenesis when malate (gluconeogenesis) or citrate (lipogenesis) leaves the mitochondria and is further metabolized to form glucose or fatty acids, respectively.

The interplay between anaplerotic and cataplerotic reactions in humans was demonstrated by renal metabolism during total, prolonged starvation (4). Arteriovenous concentration differences of metabolites across the kidneys coupled with urinary nitrogen losses showed that the kidney extracted glutamine and produced urinary ammonium (5). Concurrently, the kidney released glucose into the blood. It was initially recognized that renal ammoniagenesis was related to ketonuria during prolonged starvation when there is an increase in ketogenesis (6). However, it was not generally appreciated that the entry (anaplerosis) and removal (cataplerosis) of intermediates into and out of the TCA cycle as related to renal ammoniagenesis and gluconeogenesis had to be balanced. This fundamental principle is poorly understood and is the foundation of this paper.

During prolonged starvation glutamine is transported from muscle to the kidney where the amino and amide groups are used for ammonia formation. The ammonia released from the renal cells serves to titrate the acidity of the tubular urine created by the disassociation of organic acids, primarily β-hydroxybutyric and acetoacetic acids. For ammonia generation to continue, glutamine undergoes anaplerotic reactions to form α-ketoglutarate that enters the TCA cycle and is sequentially converted to malate that leaves the mitochondria. Malate is oxidized in the cytosol to oxalacetate that is subsequently converted to PEP and then to glucose. Thus, anaplerotic and cataplerotic reactions are essential and balanced during renal ammoniagenesis and gluconeogenesis.

The heightened ketonuria that occurs with ketonemia is related to the need for the kidney to generate glucose during total starvation when renal gluconeogenesis accounts for about 50% of the net glucose synthesis (4, 7). Thus, renal ammoniagenesis and gluconeogenesis are tightly interlocked and dependent upon balanced anaplerotic reactions to replenish the α-ketoglutarate in the TCA cycle and cataplerotic reactions to drain remnant 4-carbon metabolic intermediates from the cycle to synthesize glucose (7). In addition, there is a metabolic bonus when the kidneys excrete urinary ammonium during starvation. The caloric value of protein is greater when amino acid nitrogen is lost in the urine as ammonium rather than urea because it requires four molecules of ATP to generate a molecule of urea via the urea cycle. In addition, energy is required for the synthesis of creatine and uric acid.