Abstract is involved with. without lactate dehydrogenase anaerobic

Technology, Development

Abstract Lactate dehydrogenase is avital enzyme in the process of anaerobic cellular respiration.

Anaerobiccellular respiration is an important function in plants, animals, and bacteriato produce ATP. Lactate dehydrogenase is found in almost all living cells toserve as a catalyst for anaerobic cellular respiration. Introduction The objective of studying lactate dehydrogenase is to learnabout the structure, function, and importance.

The goal is to become familiarwith the enzyme and the metabolic processes that it is involved with. Withoutlactate dehydrogenase anaerobic cellular respiration would not occur. Thiswould substantially inhibit the production of ATP by the cell. Some bacteriarely solely on anaerobic cellular respiration as the main source of ATP, and withoutlactate dehydrogenase there would be no energy production in the cell. Metabolic Pathway ofLactate Dehydrogenase Cellularrespiration that does not require oxygen is defined as anaerobic cellularrespiration.

A final acceptor is needed at the end of the electron transportchain. The final acceptor in aerobic cellular respiration is oxygen, but inanaerobic cellular respiration the final acceptor is a less-oxidizing compound. Less energy is formed from each oxidized molecule since these molecules have asmaller reduction potential than oxygen. Anaerobic cellular respiration is muchless efficient when compared to aerobic cellular respiration.

Anaerobiccellular respiration functions to produce lactate acid from pyruvate with nooxygen present. Anaerobic cellular respiration is important for

glycolysis. Theaccumulation of pyruvate would slow down ATP production. Anaerobic cellular respirationfunctions to regenerate NAD+ from NADH. In humans, as one exercises, glucose iscompletely broken down which releases carbon atoms as carbon dioxide andhydrogen molecules as water.

This process requires substantial amounts ofoxygen. Energy production will stop at the end of glycolysis if the supply ofoxygen does not meet the demand for oxygen. Energy can still be produced whenthe supply of oxygen does not meet the demand through anaerobic cellularrespiration.

However, this process is less efficient and less ATP is produced. Lactate dehydrogenase makes this process possible. Lactatedehydrogenase is a key enzyme that is involved with anaerobic cellular respiration. As stated above anaerobic cellular respiration is key in the regeneration ofNAD+ from NADH. Lactate dehydrogenase is the main enzyme involved withconverting NADH to NAD+.

Lactate dehydrogenase converts lactate to pyruvic acidand back to lactate as the conversion of NADH to NAD+ is occurring. During glycolysis, the hydrogen atom from glucose is put on NAD+ and forms NADH. These hydrogenatoms are transferred to oxygen to form water when oxygen is available, however, when oxygen is unavailable, the NADH will build up and there is notenough NAD+ to continue producing ATP using glycolysis. Lactate dehydrogenasecombines pyruvate and the built up NADH to form lactic acid and NAD+. This NAD+formed can then be used to complete another cycle of glycolysis, thus producingmore ATP. This process quickly creates more

energy. The Gene Ontology TermsThe biological processes forlactate dehydrogenase according to gene ontology are vast.

The biologicalprocesses of lactate dehydrogenase include: response to hypoxia, carbohydratemetabolic process, lactate metabolic process, pyruvate metabolic process, glycolytic metabolic process, response to nutrient, response to glucose, response to organic cyclic compound, NAD metabolic process, carboxylic acidmetabolic process, response to drug, response to hydrogen peroxide, positiveregulation of apoptotic process, response to estrogen, post-embryonic animalorgan development, response to cAMP, and oxidation-reduction process. Lactatedehydrogenase can be found throughout the cell. According to gene ontologylactate dehydrogenase is found in the following locations in the cell: nucleus, cytoplasm, cytosol, membrane, and integral component of membrane. It has beenseen that LDH has many molecular functions. Some of the molecular functionsare: catalytic activity, lactate dehydrogenase activity, L-lactatedehydrogenase activity, protein binding, oxidoreductase activity, acting on theCH-OH groups of donors, NAD or NADP as an acceptor, kinase binding, identicalprotein binding, cadherin binding, and NAD binding. History of Isolation Human LDH-X was isolated from frozen samples of semen usingaffinity chromatography. When NAD+ is present the LDH-X does not bind toAMP-Sepharose.

The other lactate dehydrogenase isoenzymes will bind toAMP-Sepharose. This is the key point in isolating LDH-X versus the otherisoenzymes. Thefrozen semen samples were thawed and centrifuged at 30, 000 g and

four degreesCelsius for 20 minutes. Approximately 500 milliliters of the seminal fluid wereseparated by ammonium sulfate. The precipitate that was formed was dialyzedagainst a sodium phosphate buffer. The sodium phosphate buffer had a pH of 6. 8. This same buffer was used for all of the chromatography steps.

The temperaturewas kept at 4 degrees Celsius for the entire procedure. In the presence ofNADH, lactate dehydrogenase isoenzymes will bind to the column and are theneluted by the buffer. In the presence of buffer only, lactate dehydrogenaseisoenzymes will also bind to AMP-Sepharose. It was found that if equal volumesof seminal fluid and buffer containing NADH were mixed immediately beforeloading it into the column, enough NADH was still present to allow completebinding of lactate dehydrogenase to the column. AMP-Sepharose was used toseparate LDH-X from the other LDH isoenzymes since LDH-X does not bind toAMP-Sepharose.

Characteristics of theProtein Thelactate dehydrogenase protein contains a disordered portion of approximately 50residues. This disordered region has discontinuous electron density. Thelactate dehydrogenase protein model contains: residues 9-328, 375-567, anacetate molecule, a FAD molecule, and approximately 200 water molecules foreach monomer.

The two monomers are basically identical. The lactate dehydrogenaseprotein is made up of three discontinuous domains: the FAD-binding domain(residues 1–268 and520–571), the cap domain (residues 269–310, 388–425, and 450–519), and themembrane-binding domain (residues 311–

387 and 426–449, residues 329 –376 are in thedisordered region). The FAD-binding domain contains two alpha + betasubdomains.

The first subdomain is made up of three antiparallel beta betastrands surrounded by five alpha helices and is packed closely to the seconddomain. The second subdomain is made up of five parallel beta strandssurrounded by four alpha helices. The cap domain is composed of a large sevenstranded antiparallel beta sheet that is surrounded on both sides by alphahelices. Four alpha helices make up the membrane binding domain.

The largestdifference between these structures is in the membrane-bounding domain. Lactatedehydrogenase is considered to be a part of the FAD-containing family. The maindifference between LDH and other members of the FAD-containing family is themembrane binding domain. In other proteins that are classified in theFAD-containing family, the membrane binding domain is either not present ormuch different. An electropositive surface with five Lys residues and six Argresidues make up the membrane binding domain of lactate dehydrogenase. Theresidues that make up the membrane binding domain are expected to interact withthe negatively charged phospholipid head groups of the membrane. Rather thanbinding to the membrane with hydrophobic forces, lactate dehydrogenase binds tothe membrane with electrostatic forces.

Some other members of theFAD-containing protein family are: vanillyl-alcohol oxidase, p-cresolmethylhydroxylase (PCMH), and UDP-N-acetylenolpyruvyglucosamine (MurB). Theproteins in this family can be found

in both eukaryotes and eubacteria. Characteristics of theGene for Lactate DehydrogenaseThe LDHA gene in humans islocated on chromosome 11p15. 4.

Chromosome 11 is approximately 135 million basepairs and accounts for around 4-4. 5 percent of DNA in the cells. Chromosome 11contains approximately 1, 300-1, 400 genes that give instructions forsynthesizing proteins.

These proteins have a wide array of tasks in the body. The LDHB gene is located on chromosome12p12. 2-p12. 1.

Chromosome 12 is made upof almost 134 million base pairs and accounts for around 4-4. 5 percent of theDNA in cells. Chromosome 12 contains approximately 1, 100-1, 200 genes thatprovide instructions for synthesizing proteins.

These proteins also also have awide array of tasks in the body. The LDHC gene is only expressed in the testesand can be found on chromosome 11p15. 5-p15. 3. The human genome also has severalnon-transcribed LDHA pseudogenes. M subunit mutations have been observed to bedisease causing, H subunit mutations have not been linked to a certain diseasecausing trait.

LDHA mutations have been linked to cause exertionalmyoglobinuria and Fanconi-Bickel Syndrome. There are four genes forlactate dehydrogenase:

LDHA, LDHB, LDHC, and LDHD. LDHA, LDHB, and LDHC are theL-isomers. LDHD is a D-isomer. The L-isomers use and produce L-lactate.

L-lactate is the major enantiomer found in vertebrates. LDHA is called the Msubunit and is mostly found in skeletal muscle. LDHB is called the H subunitand is mostly found in the heart. Five isoenzymes can be formed from the M andH subunits of LDH. The isoenzymes are: LDH-1 (4H), LDH-2 (3H, 1M), LDH-3 (2H, 2M), LDH-4 (1H, 3M), and LDH-5 (5M).

LDH-1 and LDH-5 have the same active siteregion. These isoenzymes are similar in function but have a differentdistribution throughout tissues. Regulation of theEnzyme at Transcriptional and Enzymatic Levels TheLDHA promoter region is well known to contain the consensus sequences for, andbe regulated by, major transcription factors: hypoxia-inducible factor 1 (HIF1)and c-Myc.

Forkhead box protein M1 (FOXM1) and Kruppel-like factor 4 (KLF4) areidentified as transcriptional regulators of LDHA. The regulation of LDHA isvery complex. Complete understanding of how LDHA is regulated is far from beingachieved. It has also been found that LDHA transcription is influenced by otherfactors such as: lactate, cyclic adenosine monophosphate (cAMP), estrogen, ErB2, and heat shock factor. It is highly likely that transcriptionalregulation of LDHA is influenced by many other unknown factors.

Like many otherknown enzymes, the post-transcriptional activity of LDHA is regulated by thephosphorylation and acetylation of amino acid residues.

PGC-1? regulateslactate dehydrogenase at a transcriptional level. By decreasing LDHA mRNAtranscription and the enzymatic activity of pyruvate to lactate conversion, PGC-1? regulates lactate dehydrogenase. Atthe enzymatic level, LDH is regulated by the relative concentrations of itssubstrates. When there is major muscular output this creates an increase ofsubstrates available for the lactate dehydrogenase reaction, causing lactatedehydrogenase to become more active.

The demand for ATP increases when themuscles are forced to produce a large amount of power. This demand causes abuildup of free Pi, AMP, and ADP. The glycolytic flux that occurs due to thisbuildup makes it difficult for certain shuttle enzymes to metabolize pyruvate. Inresponse to increased levels of pyruvate and NADH, the flux through lactatedehydrogenase increases to metabolize pyruvate into lactate.

Conclusion There are many more processes lactate dehydrogenase isbelieved to be involved with. This enzyme will continue to be further studiedin hopes of being targeted for certain disorders. Recent research has shownlactate dehydrogenase to be a therapeutic target for certain types of cancers. This gives hope that lactate dehydrogenase could be a potential target for thetreatment of cancers and cancer associated disorders.

There are vastpharmacological applications to be considered from this research. It can beseen how important lactate dehydrogenase is in the cell.