Science Education Collection

The ATP Bioluminescence Assay

URL: https://www.jove.com/science-education/5653

Abstract

In fireflies, the luciferase enzyme converts a compound called luciferin into oxyluciferin, and produces light or "luminescence" as a result. This reaction requires energy derived from ATP in order to proceed, so researchers have exploited the luciferase-luciferin interaction to gauge ATP levels in cells. Given ATP's role as the cell's currency of energy, the ATP bioluminescence assay can provide insight into cellular metabolism and overall cell health.

In this video, JoVE discusses cellular respiration, specifically reviewing how glucose metabolism results in ATP production. This is followed by principles behind the ATP bioluminescence assay and a generalized protocol for this technique. Finally, a survey of how researchers are currently using the ATP bioluminescence assay to evaluate cell viability in a variety of experimental conditions.

Transcript

The ATP bioluminescence assay is a common technique used to quantify ATP levels and detect living, metabolically active cells. ATP or adenosine triphosphate is the primary source of energy for all living organisms, and by "all" we mean ALL. At the cellular level, ATP is generated through a set of metabolic processes called cellular respiration.

Today, we'll briefly discuss the pathways involved in cellular respiration. Next, we'll introduce the principles behind the ATP bioluminescence assay, and go through a step-by-step protocol for performing this method. Finally, we'll see how scientists are applying this technique in their current research.

Lets begin by introducing cellular respiration. This phenomenon involves several metabolic processes, but we'll focus on the one dealing with glucose metabolism.

In the cytoplasm, the glycolysis pathway converts glucose to pyruvate, and in the process generates two ATP molecules. Pyruvate is transported into the mitochondria, where it is converted to acetyl-coenzyme A—a process that also generates carbon dioxide. While still in the mitochondria, acetyl-coenzyme A then enters the tricarboxylic acid or TCA cycle, during which carbon dioxide is again generated, as are the high-energy molecules of NADH and FADH2. These molecules ultimately "carry" electrons into the electron transport chain or ETC.

Within the ETC, electrons are sequentially transferred between different protein complexes in the inner mitochondrial membrane, before converting oxygen to water. During this process, protons are "pumped" into the intermembrane space of mitochondria. ATP is actually produced when these protons enter back into the mitochondrial matrix as they pass through a protein called ATP synthase. Together, the TCA cycle and ETC result in the synthesis of 36 ATP molecules. Breakdown of other nutrient molecules, such as fats and proteins, can also feed into the TCA cycle and ETC, leading to ATP production.

Now that we know how cells generate ATP, let's learn about the principles behind the ATP bioluminescence assay, which is commonly used to measure intracellular levels of this molecule.

Structurally, ATP has an adenine base, a ribose sugar, and three phosphate groups—the latter of which are connected by high-energy bonds. These bonds release energy when broken, and the ATP bioluminescence assay capitalizes on this energy.

Basically, this assay requires the luciferin compound, which is obtained from "glowing" organisms like fireflies, and its corresponding catalyst enzyme called luciferase. In the presence of oxygen, luciferase derives energy from ATP and converts luciferin into oxyluciferin. Byproducts of this reaction are pyrophosphate, which is two phosphate groups obtained from ATP—converting it to adenosine monophosphate or AMP—carbon dioxide, and light or luminescence. Luminescence is read by a luminometer, a machine that quantifies light emission. Since the amount of luminescence produced is directly proportional to the amount of ATP, this serves a good indicator of cell viability and metabolism.

Now that you understand the principles behind the ATP bioluminescence assay, let's outline a general protocol.

First, cells are seeded in a 96-well plate containing culture media. Cells are plated at various densities in triplicate, to account for density-dependent variation. Since the outer-most wells are not surrounded by other wells on all four sides, the temperature and evaporation rate in these wells may be variable. Therefore, cells are not plated in the outer wells, and instead they are filled with water to avoid plate-wide evaporation and temperature variation that may affect the reaction. Plates are then incubated overnight at 37°C to allow the cells to adhere to the culture plates.

Then, the media is removed, luciferase and luciferin are added to each well, and the plate is placed on a shaker for 5–15 minutes to facilitate the reaction. Next, a portion of the mixture from each well is transferred to a white 96-well plate; white plates are often used as they reflect light upwards, allowing for more accurate luminescence readings. In addition, bubbles should be avoided, as they could interfere with subsequent analyses. As the luminescence signal can decrease over time, the plate is read within 10–12 minutes on a luminometer.

To analyze the luminometer results, an average luminescence value is calculated from wells with the same cell density. By comparing luminescence data collected in this manner from both healthy control samples and treated cells, researchers can evaluate the effects of a specific treatment on viability and metabolism—specifically by looking for decreased luminescence in the experimental group.

Now that you've seen how to perform an ATP bioluminescence assay, let's discuss its research applications.

Scientists are always trying to develop new antivirals that don't harm or kill host cells. In this study, mammalian cells were seeded in a multiwell plate and infected with a specific virus. Various antiviral compounds were added to these samples, and log concentration-response curves were generated to calculate the effective concentration fifty or EC50. EC50 is the concentration of compound at which cell viability is 50 percent. This is a commonly used parameter to assess a compound's cytotoxicity.

ATP levels can also yield clues about mitochondrial activity under various conditions. Here, the ATP bioluminescence assay was performed on preparations of mitochondria derived from rodent liver and muscle cells, which helped researchers assess the extent of mitochondrial function in normal tissues. Importantly, this protocol could be extended to provide a way to examine mitochondrial function in disease states.

Scientists are also using this assay to investigate potential cancer treatments in *in vivo* systems. In this example, human tumor cells were modified to express luciferase and injected into the brains of living mice. After the tumor cells became established in these animals, they were treated with an anti-cancer drug. A subsequent *in vivo* ATP bioluminescence assay revealed that tumor cells in drug-exposed mice had lower ATP levels.

You've just watched JoVE's introduction to the ATP bioluminescence assay. You should now be familiar with the cellular respiration pathways and the protocol used to measure ATP, which is the end product of these pathways. The ATP bioluminescence assay serves as an excellent screening tool for cell biologists interested in studying the effect of physiological and pathological factors on cell metabolism and viability. As always, thanks for watching!