

BP203T BIOCHEMISTRY-THEORY

UNIT-ONE



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UNIT I – Bioenergetics – 08 Hours

Concept of free energy, endergonic and exergonic reaction, Relationship between free energy, enthalpy and entropy; Redox potential.

Energy rich compounds; classification; biological significances of ATP and cyclic AMP

INTRODUCTION:

- Bioenergetics is the part of biochemistry concerned with the energy involved in making and breaking of chemical bonds in the molecules found in biological organisms.
- It can also be defined as the study of energy relationships and energy transformations and transductions in living organisms.
- The ability to harness energy from a variety of metabolic pathways is a property of all living organisms that contains earth science.
- Growth, development, anabolism and catabolism are some of the central processes in the study of biological organisms, because the role of energy is fundamental to such biological processes.
- Life is dependent on energy transformations; living organisms survive because of exchange of energy between living tissues/ cells and the outside environment.
- Some organisms, such as autotrophs, can acquire energy from sunlight (through photosynthesis) without needing to consume nutrients and break them down.^[8] Other organisms, like heterotrophs, must intake nutrients from food to be able to sustain energy by breaking down chemical bonds in nutrients during metabolic processes such as glycolysis and the citric acid cycle.
- In a living organism, chemical bonds are broken and made as part of the exchange and transformation of energy. Energy is available for work (such as mechanical work) or for other processes (such as chemical synthesis and anabolic processes in growth), when weak bonds are broken and stronger bonds are made. The production of stronger bonds allows release of usable energy.
- Adenosine triphosphate (ATP) is the main "energy currency" for organisms; the goal of metabolic and catabolic processes are to synthesize ATP from available starting materials (from the environment), and to break- down ATP (into adenosine diphosphate (ADP) and inorganic phosphate) by utilizing it in biological processes. In a cell, the ratio of ATP to ADP concentrations is known as the "energy charge" of the cell.

- Environmental materials that an organism intakes are generally combined with oxygen to release energy, although some can also be oxidized anaerobically by various organisms.
- The bonds holding the molecules of nutrients together and in particular the bonds holding molecules of free oxygen together are relatively weak compared with the chemical bonds holding carbon dioxide and water together.
- The utilization of these materials is a form of slow combustion because the nutrients are reacted with oxygen (the materials are oxidized slowly enough that the organisms do not actually produce fire).
- The oxidation releases energy because stronger bonds (bonds within water and carbon dioxide) have been formed. This net energy may evolve as heat, which may be used by the organism for other purposes, such as breaking other bonds to do chemistry required for survival.

TYPES OF REACTIONS:

- **EXERGONIC REACTION :**

- It is a spontaneous chemical reaction that releases energy.
- It is thermodynamically favoured, indexed by a negative value of ΔG (Gibbs free energy).
- Over the course of a reaction, energy needs to be put in, and this activation energy drives the reactants from a stable state to a highly energetically unstable transition state to a more stable state that is lower in energy.
- The reactants are usually complex molecules that are broken into simpler products. The entire reaction is usually catabolic.
- The release of energy (specifically of Gibbs free energy) is negative (i.e. $\Delta G < 0$) because the energy of the reactants is higher than that of the products.

- **ENDERGONIC REACTION :**

- It is an anabolic chemical reaction that consumes energy.
 - It is the opposite of an exergonic reaction.
 - It has a positive ΔG , for instance because $\Delta H > 0$, which means that it takes more energy to break the bonds of the reactant than the energy of the products offer, i.e. the products have weaker bonds than the reactants.
 - Thus, endergonic reactions are thermodynamically unfavourable and will not occur on their own at constant temperature. Additionally, endergonic reactions are usually anabolic.
- ✚ **The free energy gained or lost (ΔG) in a reaction can be calculated as follows: $\Delta G = \Delta H - T\Delta S$ where ΔG = Gibbs free energy change, ΔH = enthalpy change, T = temperature (in kelvins), and ΔS = entropy change.**

Examples of major bioenergetic processes:

1. Glycolysis :

- ✓ It is the process of breaking down glucose into pyruvate, producing two molecules of ATP (per 1 molecule of glucose) in the process.
- ✓ When a cell has a higher concentration of ATP than ADP (i.e. has a high energy charge), the cell cannot undergo glycolysis, releasing energy from available glucose to perform biological work.
- ✓ Pyruvate is one product of glycolysis, and can be shuttled into other metabolic pathways (gluconeogenesis, etc.) as needed by the cell.
- ✓ Additionally, glycolysis produces reducing equivalents in the form of NADH (nicotinamide adenine dinucleotide), which will ultimately be used to donate electrons to the electron transport chain.

2. Gluconeogenesis

- ✓ It is the opposite of glycolysis; when the cell's energy charge is low (the concentration of ADP is higher than that of ATP), the cell must synthesize glucose from carbon- containing biomolecules such as proteins, amino acids, fats, pyruvate, etc.

- ✓ For example, proteins can be broken down into amino acids, and these simpler carbon skeletons are used to build/ synthesize glucose.

2. The Citric Acid Cycle:

- ✓ It is a process of cellular respiration in which acetyl coenzyme A, synthesized from pyruvate dehydrogenase, is first reacted with oxaloacetate to yield citrate.
- ✓ The remaining eight reactions produce other carbon-containing metabolites. These metabolites are successively oxidized, and the free energy of oxidation is conserved in the form of the reduced coenzymes FADH_2 and NADH .
- ✓ These reduced electron carriers can then be re-oxidized when they transfer electrons to the electron transport chain.

3. Ketosis :

- ✓ It is a metabolic process whereby ketone bodies are used by the cell for energy (instead of using glucose). Cells often turn to ketosis as a source of energy when glucose levels are low; e.g. during starvation.

4. Oxidative phosphorylation :

- ✓ It is the process where the energy stored in the relatively weak double bonds of O_2 is released in a controlled manner in the electron transport chain.
- ✓ Reducing equivalents such as NADPH , FADH_2 and NADH can be used to donate electrons to a series of redox reactions that take place in electron transport chain complexes.
- ✓ These redox reactions take place in enzyme complexes situated within the mitochondrial membrane.
- ✓ These redox reactions transfer electrons "down" the electron transport chain, which is coupled to the proton motive force.

- ✓ This difference in proton concentration between the mitochondrial matrix and inner membrane space is used to drive ATP synthesis via ATP synthase.
- 5. **Photosynthesis**, another major bioenergetic process, is the metabolic pathway used by plants in which solar energy is used to synthesize glucose from carbon dioxide and water. This reaction takes place in the chloroplast. After glucose is synthesized, the plant cell can undergo photophosphorylation to produce ATP.

FREE-ENERGY RELATIONSHIP :

- In physical organic chemistry, a **free-energy relationship** or **Gibbs energy relation** relates the logarithm of a reaction rate constant or equilibrium constant for one series of reactions with the logarithm of the rate or equilibrium constant for a related series of reactions.
- Free energy relationships establish the extent at which bond formation and bond breakage happen in the transition state of a reaction, and in combination with kinetic isotope experiments a reaction mechanism can be determined.
- Free energy relationships are often used to calculate equilibrium constants since they are experimentally difficult to determine.
- The most common form of free-energy relationships are linear free-energy relationships (LFER).
- The Bronsted catalysis equation describes the relationship between the ionization constant of a series of catalysts and the reaction rate constant for a reaction on which the catalyst operates.
- The Hammett equation predicts the equilibrium constant or reaction rate of a reaction from a *substituent constant* and a *reaction type constant*.
- The Edwards equation relates the nucleophilic power to *polarisability* and *basicity*. The Marcus equation is an example of a quadratic free-energy relationship (QFER).

ENTHALPY:

- Enthalpy is defined as the sum of internal energy of a system and the product of its pressure and volume.
- It is denoted by the symbol E. It is a state function. Units used to express are calorie, BTU, or joules. Below we have given the equation.

Enthalpy Equation:

E = U + PV where,

E is the enthalpy

U is the internal energy of a system

P is the pressure

V is the volume

ENTHALPY CHANGE :

An enthalpy change is defined as the difference between the energy gained by the formation of new chemical bonds and the energy used to break bonds in a chemical reaction at constant pressure. In simple terms, it tells about the amount of heat evolved or absorbed during a reaction. It is denoted as ΔH . It is expressed as follows:

$$\Delta H = \Delta U + P\Delta V$$

Some important terms related to enthalpy:

Enthalpy of reaction – It is defined as the difference between the total enthalpy of the reactants and the total enthalpy of the products in a reaction. It is denoted as ΔH_{RXN}

Enthalpy of formation – Enthalpy of formation is the amount of energy required to produce a compound from its composition of elements. It is denoted as ΔH_f

Enthalpy of combustion – It is the change in enthalpy accomplished when one mole of an element is heated in the presence of excess oxygen under standard conditions. It is denoted as ΔH_c .

Enthalpy of solution – It is defined as the total amount of heat released or absorbed when two substances are put in a solution. It can be either negative or positive. If it is positive it will result in an endothermic reaction. If it is negative it will result in an exothermic reaction. It is denoted as $\Delta H_{\text{solution}}$.

ENTROPY :

Entropy is defined as the measure of the thermal energy of a system per unit temperature which is not available for doing useful work. It is denoted as S. The SI unit for Entropy is Joules per Kelvin. Entropy change at constant temperature is calculated as given below:

$$\Delta S_{\text{system}} = q_{\text{rev}} / T$$

ΔS represents the change in entropy,

q_{rev} represents the reverse of the heat, and

T is the temperature in the Kelvin scale.

PROPERTIES OF ENTROPY:

- Entropy is greater in malleable solids whereas it is lower in brittle and hard substances.
- When gas is dissolved in water the entropy decreases whereas it increases when liquid or solid is dissolved in water.
- With an increase in chemical complexity, the entropy also increases.
- As mass increases entropy increases.

THE RELATIONSHIP BETWEEN ENTHALPY AND ENTROPY:

The relationship between enthalpy and entropy can be seen to calculate the Gibbs free energy. Josiah Willard Gibbs developed Gibbs energy in the 1870s. He termed it as available energy of a system that can be used to do work. It is defined as the sum of the enthalpy of a system and the product of the entropy and temperature of the system. It is denoted as G.

$$G = H + TS$$

Or

$$G = U + PV - TS$$

Where,

U is the internal energy in joules

P is the pressure in Pascal

V is the volume in m³

T is the temperature in Kelvin

S is the entropy in joules/Kelvin

H is the enthalpy in joules

The change in Gibbs energy is given as $\Delta G = \Delta H - T\Delta S$

REDOX POTENTIAL :

- It is also known as oxidation-reduction potential or ORP, is a way of representing the tendency of a chemical substance to lose electrons to an electrode or to acquire electrons from an electrode.
- Therefore, it provides insight into the likelihood of that chemical substance to undergo oxidation or reduction.
- This quantity is often measured in millivolts but it is not uncommon for it to be represented in volts.
- The measurement of redox potential enables quick and relatively easy characterization of the degree of reduction of a chemical reaction.
- Redox potentials also have applications in predicting the stability of the various compounds that play an important role in the regulation of nutrients and the availability of metals in sediment and soils.

ENERGY RICH COMPOUNDS:

- Organisms require energy for various activities like muscle contraction and other cellular movements (Active transport and synthesis of macromolecules).
- All these processes are energetically very demanding and use chemical energy.
- Chemical compounds liberate energy by hydrolysis of some groups which are bound to them by high energy bonds.
- When hydrolyzed products go energetically low (ΔG -ve) • High-energy phosphate compounds .
- Phosphate-containing compounds are considered “high-energy” if they have ΔG° for hydrolysis “more negative than -20 to -25 kJ/mol”.
- High-energy phosphate compounds are not used for long-term energy storage. They are temporary forms of stored energy, and are used to carry energy from one reaction to another.

TYPES OF HIGH ENERGY BONDS. •

They are of five types:

1. **Phosphoanhydrides:** formed b/w two molecules of phosphoric acid.
 - In ATP there are two high energy diphosphate (phosphoanhydride bonds).
 - The third between phosphate and ribose is not much energy rich as it is a phosphate ester bond.
 - ATP serves as principle immediate donor of free energy in most endergonic reactions eg. Active transport, muscle contraction, transmission of nerve impulse.
 - Apart from ATP, GTP (Guanidine triphosphate) is also used as energy source in protein synthesis and gluconeogenesis.

- Also UTP (Uridine triphosphate) and CTP (Cytidine triphosphate) are used as energy sources for metabolism of saccharides and lipids respectively.

1. **Enolphosphatic bond:**

- This bond is energetically very high whose hydrolysis release 61KJ/mole.
- Such kind of bond is present in phosphoenol pyruvate which in turn is formed in breakdown of glucose in glycolysis.

2. **Acyl phosphatic bond:**

- This bond releases 49 KJ/mole of energy on hydrolysis.
- Such kind of bond is in 1-3 bisphosphoglycerate formed in glycolysis.

3. **Guanidine phosphate :**

- It is formed when phosphate is attached to guanidine.
- Releases about 43 KJ/mole of energy on hydrolysis.
- Such kind of bond is present in phosphocreatine (PC). PC is found in muscle cell and acts as reserve of energy in tissues.

4. **Thioester Bond:**

- It is not much high energy containing bond because there is no energy rich phosphate. Such kind of bond is in acetyl Co-A.
- The body is a complex organism, and as such, it takes energy to maintain proper functioning. Adenosine triphosphate (ATP) is the source of energy for use and storage at the cellular level.

- The structure of ATP is a nucleoside triphosphate, consisting of a nitrogenous base (adenine), a ribose sugar, and three serially bonded phosphate groups.
- ATP is commonly referred to as the "energy currency" of the cell, as it provides readily releasable energy in the bond between the second and third phosphate groups.
- In addition to providing energy, breakdown of ATP through hydrolysis serves a broad range of functions in the cell, including signaling and DNA/RNA synthesis.
- The synthesis of ATP utilizes energy obtained from multiple catabolic mechanisms, including cellular respiration, beta-oxidation, and ketosis.
- The majority of ATP synthesis occurs in cellular respiration within the mitochondrial matrix: generating approximately thirty-two molecules of ATP per molecule of glucose that is oxidized.
- ATP is consumed for energy in processes including ion transport, muscle contraction, nerve impulse propagation, substrate phosphorylation, and chemical synthesis.
- These processes, as well as others, create a high demand for ATP. As a result, cells within the human body depend upon the hydrolysis of 100 to 150 moles of ATP per day to ensure proper functioning. In the forthcoming sections, ATP will undergo further evaluation of its role as a crucial molecule in the daily functioning of the cell.

CELLULAR :

- ATP is an excellent energy storage molecule to use as "currency" due to the phosphate groups that link through phosphodiester bonds.
- These bonds are high energy because of the associated electronegative charges exerting a repelling force between the phosphate groups.

- A significant quantity of energy remains stored within the phosphate-phosphate bonds. Through metabolic processes, ATP becomes hydrolyzed into ADP, or further to AMP, and free inorganic phosphate groups.
- The process of ATP hydrolysis to ADP is energetically favorable, yielding a Gibbs free energy of -7.3 cal/mol.
- ATP must continuously undergo replenishment to fuel the ever-working cell. The routine intracellular concentration of ATP is 1 to 10 μ M.
- The enhancement or inhibition of ATP synthase is a common regulatory mechanism. **For example, ATP inhibits phosphofructokinase-1 (PFK1) and pyruvate kinase, two key enzymes in glycolysis, effectively acting as a negative feedback loop to inhibit the breakdown of glucose when there is sufficient cellular ATP.**
- Other systems regulate ATP, such as in the regulatory mechanisms involved in the regulation of ATP synthesis in the heart.
- Novel experiments have demonstrated that ten-second bursts called mitochondrial flashes can disrupt ATP production in the heart.
- During these mitochondrial flashes, the mitochondria release reactive oxygen species and effectively pause ATP synthesis.
- ATP production inhibition occurs during mitochondrial flashes.
- During low demand for energy, when heart muscle cells received sufficient building blocks needed to produce ATP, mitochondrial flashes were observed more frequently.
- Alternatively, when demand for energy is high during rapid heart contraction, mitochondrial flashes occurred less often. These results suggested that during times when substantial amounts of ATP are needed, mitochondrial flashes occur less frequently to allow for continued ATP production.
- Conversely, during times of low energy output, mitochondrial flashes occurred more regularly and inhibited the production of ATP.

FUNCTION:

ATP hydrolysis provides the energy needed for many essential processes in organisms and cells. These include intracellular signaling, DNA and RNA synthesis, Purinergic signaling, synaptic signaling, active transport, and muscle contraction. These topics are not an exhaustive list but include some of the vital roles ATP performs.

ATP IN INTRACELLULAR SIGNALING :

- Signal transduction heavily relies on ATP. ATP can serve as a substrate for kinases, the most numerous ATP- binding protein.
- When a kinase phosphorylates a protein, a signaling cascade can be activated, leading to modulation of diverse intracellular signaling pathways.
- Kinase activity is vital to the cell and, therefore, must be tightly regulated. The presence of the magnesium ion helps regulate kinase activity.
- Regulation is through magnesium ions existing in the cell as a complex with ATP, bound at the phosphate oxygen centers. In addition to kinase activity, ATP can function as a ubiquitous trigger of intracellular messenger release.
- These messengers include hormones, various-enzymes, lipid mediators, neurotransmitters, nitric oxide, growth factors, and reactive oxygen species.
- An example of ATP utilization in intracellular signaling can be observed in ATP acting as a substrate for adenylate cyclase. This process mostly occurs in G-protein coupled receptor signaling pathways. Upon binding to adenylate cyclase, ATP converts to cyclic AMP, which assists in signaling the release of calcium from intracellular stores.
- The cAMP has other roles, including secondary messengers in hormone signaling cascades, activation of protein kinases, and regulating the function of ion channels.

DNA/RNA SYNTHESIS:

DNA and RNA synthesis requires ATP. ATP is one of four nucleotide-triphosphate monomers that is necessary during RNA synthesis. DNA synthesis uses a similar mechanism, except in DNA synthesis, the ATP first becomes transformed by removing an oxygen atom from the sugar to yield deoxyribonucleotide, dATP.[8]

PURINERGIC SIGNALING :

- Purinergic signaling is a form of extracellular paracrine signaling that is mediated by purine nucleotides, including ATP.
- This process commonly entails the activation of purinergic receptors on cells within proximity, thereby transducing signals to regulate intracellular processes.
- ATP is released from vesicular stores and is regulated by IP₃, in addition to other common exocytotic regulatory mechanisms.
- ATP is co-stored and co-released among neurotransmitters, further supporting the notion that ATP is a necessary mediator of purinergic neurotransmission in both sympathetic and parasympathetic nerves.
- ATP can induce several purinergic responses, including control of autonomic functions, neural glia interactions, pain, and control of vessel tone.

NEUROTRANSMISSION :

- The brain is the highest consumer of ATP in the body, consuming approximately twenty-five percent of the total energy available.
- A large amount of energy is spent on maintaining ion concentrations for proper neuronal signaling, as well as on synaptic transmission.

- Synaptic transmission is an energy-demanding process. At the presynaptic terminal, ATP is required for establishing ion gradients that shuttle neurotransmitters into vesicles, and for priming the vesicles for release through exocytosis.
- Neuronal signalling is dependent upon the action potential reaching the presynaptic terminal, signalling the release of the loaded vesicles.
- This process is dependent upon ATP restoring the ion concentration in the axon after each action potential, allowing another signal to occur.
- Active transport is responsible for the resetting of the sodium and potassium ion concentrations to baseline values after an action potential occurs through the Na/K ATPase.
- During this process, one molecule of ATP is hydrolysed , three sodium ions are transported out of the cell, and two potassium ions are transported back into the cell, both of which move against their concentration gradients. Action potentials traveling down the axon initiate vesicular release upon reaching the presynaptic terminal.
- After establishing the ion gradients, the action potentials then propagate down the axon through the depolarization of the axon, sending a signal towards the terminal.
- Neurons will need to hydrolyse nearly one billion molecules of AT to restore the sodium/potassium ion concentration after each cell depolarization.
- Excitatory synapses largely dominate the grey matter of the brain. Vesicles containing glutamate will be released into the synaptic cleft to activate postsynaptic excitatory glutaminergic receptors.

ATP IN MUSCLE CONTRACTION :

- Muscle contraction is a necessary function of everyday life and could not occur without ATP.
- There are three primary roles that ATP performs in the action of muscle contraction.

- The first is through the generation of force against adjoining actin filaments through the cycling of myosin cross-bridges.
- The second is the pumping of calcium ions from the myoplasm across the sarcoplasmic reticulum against their concentration gradients using active transport.
- The third function performed by ATP is the active transport of sodium and potassium ions across the sarcolemma so that calcium ions may be released when the input is received. The hydrolysis of ATP drives each of these processes.

MECHANISM :

Many processes are capable of producing ATP in the body, depending on the current metabolic conditions. ATP production can occur in the presence of oxygen from cellular respiration, beta-oxidation, ketosis, lipid, and protein catabolism, as well as under anaerobic conditions.

CELLULAR RESPIRATION :

- Cellular respiration is the process of catabolizing glucose into acetyl-CoA, producing high energy electron carriers that will be oxidized during oxidative phosphorylation, yielding ATP.
- During glycolysis, the first step of cellular respiration, one molecule of glucose breaks down into two pyruvate molecules.
- During this process, two ATP are produced through substrate phosphorylation by the enzymes PFK1 and pyruvate kinase.
- There is also the production of two reduced NADH electron carrier molecules.
- The pyruvate molecules are then oxidized by the pyruvate dehydrogenase complex, forming an acetyl-CoA molecule.
- The acetyl-CoA molecule is then fully oxidized to yield carbon dioxide and reduced electron carriers in the citric acid cycle.

- On completion of the citric acid cycle, the total yield is two molecules of carbon dioxide, one equivalent of ATP, three molecules of NADH, and one molecule of FADH₂.
- These high energy electron carriers then transfer the electrons to the electron transport chain in which Hydrogen ions (protons) are transferred against their gradient into the inner membrane space from the mitochondrial matrix. ATP molecules are then synthesized as protons moving down the electrochemical gradient power ATP synthase.
- The quantity of ATP produced varies depending on which electron carrier donated the protons. One NADH molecule produces two and a half ATP, whereas one FADH₂ molecule produces one and a half ATP molecules.

BETA-OXIDATION:

Beta-oxidation is another mechanism for ATP synthesis in organisms. During beta-oxidation, fatty acids chains are permanently shortened, yielding Acetyl-CoA molecules. Throughout each cycle of beta-oxidation, the fatty acid is reduced by two carbon lengths, producing one molecule of acetyl-CoA, which can be oxidized in the citric acid cycle, and one molecule each of NADH and FADH₂, which transfer their high energy electron to the transport chain.

KETOSIS :

Ketosis is a reaction that yields ATP through the catabolism of ketone bodies. During ketosis, ketone bodies undergo catabolism to produce energy, generating twenty-two ATP molecules as well as two GTP molecules per acetoacetate molecule that becomes oxidized in the mitochondria.

ANAEROBIC RESPIRATION:

When oxygen is scarce or unavailable during cellular respiration, cells can undergo anaerobic respiration. During anaerobic conditions, there is a buildup of NADH molecules due to the inability to oxidize NADH to NAD⁺, limiting

the actions of GAPDH and glucose consumption. To maintain homeostatic levels of NADH, pyruvate is reduced to lactate, yielding the oxidation of one NADH molecule in a process known as lactic fermentation. In lactic fermentation, the two molecules of NADH that are created in glycolysis are oxidized to maintain the NAD⁺ reservoir. This reaction produces only two molecules of ATP per molecule of glucose.

CLINICAL SIGNIFICANCE :

ATPS ROLE IN PAIN CONTROL

- ATP demonstrates a reduction in acute perioperative pain in clinical studies.
- In these studies, patients received intravenous ATP.
- The intravenous adenosine infusion acts on the A1 adenosine receptor, initiating a signalling cascade that ultimately aids the pain-relieving effects observed in inflammation.
- Studies have shown that adenosine compounds decrease allodynia and hyperalgesia when administered in moderate doses.
- A1 adenosine receptor activation renders effective pain intervention due to delivering a slow onset as well as a long duration of action, potentially lasting for weeks in some cases.

ANESTHESIA :

ATP supplementation produced positive outcomes during anesthesia. Evidence shows that low doses of adenosine reduce neuropathic pain, ischemic pain, and hyperalgesia to a level comparable to morphine. Adenosine also decreased postoperative opioid usage, suggesting a potential long-lasting A1 adenosine receptor activation.

CARDIOLOGY AND SURGERY :

In patients affected by pulmonary hypertension, ATP has demonstrated to be a safe and practical pulmonary vasodilator.[21] Similarly, adenosine and ATP can be employed during surgery to induce hypotension in patients

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