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Pyruvate, a Sacrificial Antioxidant

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Abbreviations:
ATP, adenosine triphosphate; NAD^+: nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide (reduced form); CoA, coenzyme A; GSH, glutathione; GSSG, glutathione disulfide; LDH, lactate dehydrogenase enzyme; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substance; tbHP, *tert-tutyl* hydroperoxide; TCA, tricarboxylic acid

Table of Contents

Abstract

Pyruvate is an important small molecule that is generated from glycolysis and participates in the citric acid cycle in biological systems. It generates adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (reduced form) (NADH), which are essential for cell survival. Recently, pyruvate has become famous for its antioxidant functions. It can non-enzymatically react with hydrogen peroxide and other lipid peroxides to protect cells from attack by reactive oxygen species (ROS). It may also change oxidative status of the cell by increasing the ratio of NADH to nicotinamide adenine dinucleotide (NAD^+) and the ratio of glutathione (GSH) to glutathione disulfide (GSSG). It may prevent ischemia reperfusion injury that mainly caused by free radical attack. Moreover, it has been shown to inhibit the growth of carcinoma cells in rats. The best utilization of its metabolic and antioxidant effects certainly will optimize the clinical treatment. Under experimental conditions, fluorimetric assay is usually used to detect pyruvate.

Introduction

Living organisms obtain energy by carrying out oxidation-reduction reactions. For animals, it means to “burn” the food. The two major processes are glycolysis and the tricarboxylic acid (TCA) cycle. Pyruvate is a "salt" form of pyruvic acid -a 3-carbon molecule derived from glycolysis. The structure of pyruvate is shown as below in figure 1.

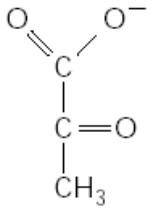


Figure 1: The molecular structure of pyruvate.

As a metabolic substrate, pyruvate may be transformed by pyruvate dehydrogenase to acetyl-CoA and enter the citric acid cycle [1]. Besides the function of being a natural fuel, pyruvate has been identified to be an antioxidant in biological systems. It can scavenge hydrogen peroxide and reduce the toxic effects of ROS [2]. It was reported that pyruvate supplements could protect heart from ischemia reperfusion injury [7]. It may replenish intermediates in citric acid cycle to supply energy, and it may also change the extreme oxidative status during ischemia by increasing GSH level. This paper mainly focuses on pyruvate generation, detection, metabolism and its antioxidative behaviors.

Overview of pyruvate in biological systems---generation, metabolism, and functions.

In biological systems, glycolysis has two phases. The breakdown of the six-carbon glucose into two molecules of the three-carbon pyruvate consists of ten steps. In the preparatory phase of glycolysis, glucose is phosphorylated and converted to glyceraldehyde 3-phosphate. In the payoff phase, glyceraldehyde 3-phosphate undergoes oxidative conversion to generate pyruvate and form some ATP and NADH. The fates of pyruvate are various depending on the availability

of oxygen. Under aerobic conditions, it undergoes citric acid cycle and give off energy. Under anaerobic conditions, it is fermented to generate alcohol in yeast or lactate in some cells and microorganisms (Figure 1) [1].

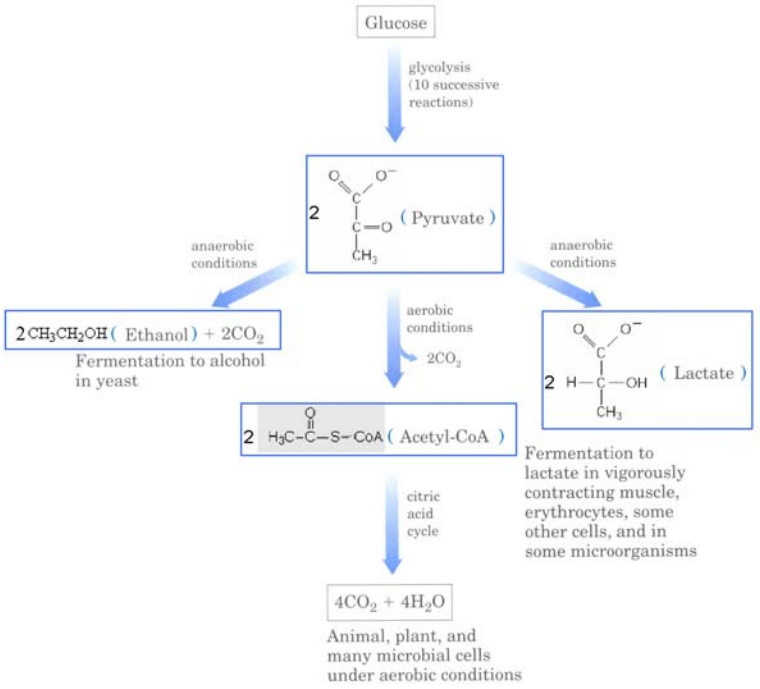


Figure 1: Pyruvate is generated from glycolysis. Depending on various oxygen conditions, there are three possible catabolic fates of the pyruvate formed in glycolysis. Adapted from [1].

Generation of pyruvate:

The bottom line of glycolysis is that one mole of glucose is oxidized to generate two moles of pyruvate (Figure 1). The oxidizing agent is NAD^+, which is in turn reduced to NADH. NAD^+ is a good oxidizing agent for this reaction and the process is exergonic. The mechanism, which has evolved, allows the energy of reaction to be captured as two moles of ATP per mole of glucose. And a lot of energy remains in the product, pyruvate [1].

$$2\ NAD^+ + \text{glucose} + 2\ ADP \rightarrow 2\ \text{pyruvate} + 2\ NADH + 2\ ATP \qquad (1)$$

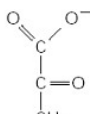
In the catabolic pathways for most amino acids such as tryptophan, alanine, cysteine, serine, glycine and threonine, pyruvate is generated and it will end up with acetyl-CoA [1].

$$\text{Tryptophan} \longrightarrow \text{Alanine} \longrightarrow \text{Pyruvate} \longleftarrow \text{Cysteine} \qquad (2)$$

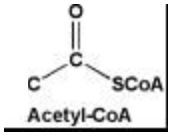
$$\text{Threonine} \longrightarrow \text{Glycine} \longrightarrow \text{Serine} \longrightarrow \text{Pyruvate} \qquad (3)$$

Aerobic and anaerobic metabolism of pyruvate:

In most tissues of higher organisms, pyruvate is further oxidized to acetate that enters the tricarboxylic acid cycle and is oxidized to CO_2 and H_2O under aerobic conditions (reaction 4). Here, the major oxidizing agent is NAD^+ although other oxidizing agents are also needed. The NADH and other reduced coenzymes that result from these oxidations are ultimately re-oxidized by molecular oxygen (through intermediate redox reactions in the electron transport chain). For this reason, this is referred to as aerobic metabolism. These processes depend on complex organelles (mitochondria). Pyruvate dehydrogenase is the enzyme to catalyze the reaction [1]. But, not every type of cell or organism can carry out aerobic respiration and use the ATP obtained from glycolysis for energy.

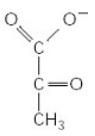

pyruvate

$$+ \text{CoA–SH} + NAD^+ \rightarrow$$

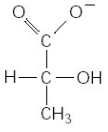


$$+ CO_2 + \text{NADH} + H_2O \qquad (4)$$

For those organisms or tissues that do not carry out aerobic metabolism, there is problem of re-generating the oxidizing agent NAD^+. For mammalian tissues such as muscle and RBC, and for some microorganisms such as lactic acid bacteria, the NADH is used to reduce pyruvate to lactate. This reaction is catalyzed by lactate dehydrogenase [1].

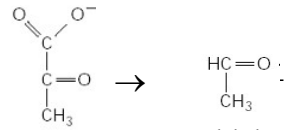


$$+ \text{NADH} + H^+ \rightarrow$$


lactate

$$+ NAD^+ \qquad (5)$$

For other microorganisms the pyruvate can be further oxidized to acetaldehyde, which can be subsequently reduced by NADH to a number of different products depending on the particular organism [1]:

1.Pyruvate decarboxylase:  + CO_2 (6)

pyruvate acetaldehyde

2. Alcohol dehydrogenase:  + NADH →  + NAD^+ (7)

acetaldehyde ethanol

Other functions:

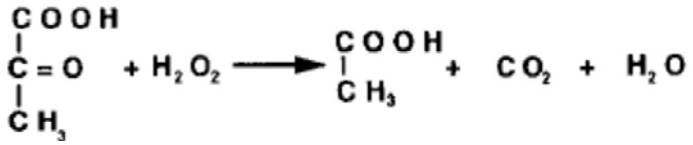
Pyruvate not only is important in the energy production process, but also plays a role in amino acid biosynthesis; it is the precursor of alanine, valine, and leucine [1].

Pyruvate as an antioxidant

Pyruvate is not only a natural energy supplier but also one of the antioxidant nutrients that provide the body with protection against free radicals. Two different mechanisms mediate pyruvate's antioxidant effects [14].

Pyruvate directly neutralizes hydrogen peroxide and lipid peroxides in a nonenzymatic reaction [7, 8, 9, 10].

Researchers found that pyruvate protects cells against damage caused by hydrogen peroxide [2, 4, 5]. This protection has been shown in a range of settings, from test tubes to laboratory animals [2, 5]. It is attributed partly to pyruvate's antioxidant action [6]. Also, pyruvate may help inhibit cancer growth in laboratory animals [5]. It was reported that pyruvate and other α - keto acids directly reduced H_2O_2 to water, then pyruvate acts as an antioxidant to protect cells from oxidative stress [2, 5, 7].



Pyruvate Hydrogen Peroxide Acetate Carbon Dioxide Water (8)

Figure 2: Pyruvate reduces hydrogen peroxide to water. Pyruvate undergoes nonenzymatic oxidative decarboxylation at the carbon one position. (Adapted from [7])

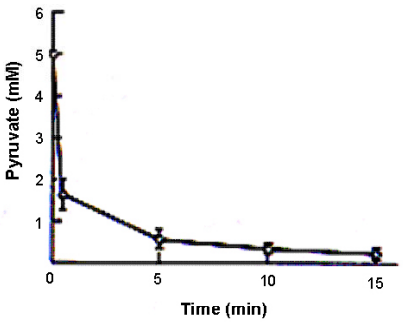


Figure 3: This figure indicates that the reaction of H_2O_2 and pyruvate in RPMI 1640 medium. Pyruvate and H_2O_2 in 1 mL RPMI 1640 were incubated at 37^{o}C. The half-life of the reaction was less than 30 s when both were present initially at 5 mM [5].

If cells in culture are exposed to pyruvate- free medium, pyruvate is constitutively secreted until a plateau is attained. This phenomenon indicates that cells try to maintain equilibrium between extracelluar and intracellular pyruvate. The presence of exogenous catalase enhanced pyruvate accumulation. It was suggested that pyruvate functions as an antioxidant [5].

Moreover, cell survival rate is greatly increased after addition of pyruvate into the cells that are exposed to H_2O_2 [4].

Pyruvate scavenges lipid peroxides to protect cells from oxidative injury [8, 9]. Pyruvate directly scavenged tbHP that led to the generation of hydroxyl and peroxyl radicals [8]. Figure 4 shows that lipid peroxidation caused by tbHP is greatly reduced by application of pyruvate.

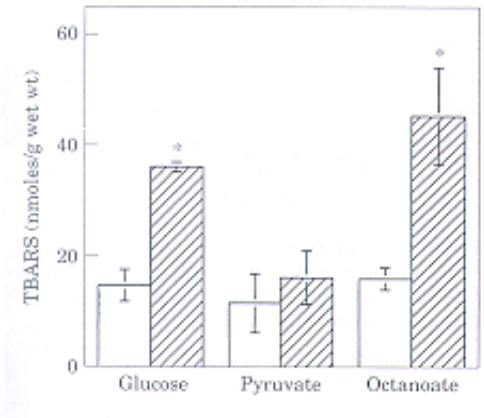


Figure 4: TBARS in the heart tissue serves as an indicator of the extent of membrane lipid peroxidation. Empty bars represent the heart tissue without tbHP, shaded bars represent the heart tissue with tbHP. The extent of lipid peroxidation is greatly reduced in the pyruvate-applied tissue. Adapted from [8].

Indirect antioxidant mechanism of pyruvate:

Pyruvate exerts opposite effects on NADH redox states in the cytosol and mitochondrial matrix. Pyruvate is a cytosolic oxidant; increased pyruvate concentration in this compartment increases NAD^+/NADH via the lactate dehydrogenase equilibrium. In the mitochondria, pyruvate oxidation by pyruvate dehydrogenase and the TCA cycle generates reducing equivalents in the form of NADH for oxidative phosphorylation [12]. Pyruvate carboxylation increases intracelluar citrate content. In the cytosolic compartment, glutamate–pyruvate transaminase catalyzes pyruvate transamination with glutamate and generates α-ketoglutarate, from which citrate is formed [12, 13]. The increase of citrate inhibits phosphofructokinase, which causes the disappearance of NADH [13, 14]. The inhibition of phosphofructokinase diverts glycolytic flux into the hexose monophosphate shunt that is the source of NADPH that may regenerate GSH from oxidized glutathione disulfide (GSSG) [12, 13, 14].

Through these two mechanisms, pyruvate may increase the NADH/NAD^+ and GSH/GSSG ratio in cells or tissue and scavenge some free radicals to protect cells.

Commercial and therapeutic value of pyruvate

Pyruvate has a strong antioxidant activity; it is taken into cell culture medium to protect cells. Dulbeccos Modification of Eagles Medium (DMEM) is a good exmple that contains sodium

pyruvate. It is also commercially produced as daily antioxidant supplement for people. As a ROS scavenger, pyruvate also can increase the ratio of GSH/GSSG. Now, there is no doubt of the function that pyruvate will attenuate the ischemia-reperfusion injury of heart and prevent the lens from intracellular oxidative stress. Pyruvate is a therapeutic agent that has a promising future.

Detection of pyruvate

Fluorimetric assay is often used to detect pyruvate. It is a modified form of the method of Lowry and Passonneau [5]. Pyruvate was converted to lactate by LDH in the presence of NADH. When NAD^+ was converted to NADH, the fluorescence was lost at excitation 340 nm and emission 460 nm [5]. Sometimes, chromatogram was used as a method to detect pyruvate, it was visualized using long wavelength and short wavelength UV and green fluorescence appeared [3]. Moreover, the electrophoresis was used as an electrochemical method to detect pyruvate in human sweat. Among these various methods, fluorimetric assay is the most popular.

Summary

The metabolic and antioxidant functions make pyruvate a very ideal intracellular scavenger of ROS. It participates the energy production and reducing molecule generation. It also reacts with bad guys—hydrogen peroxide and lipid peroxides. Pyruvate has different functions in cytosol and mitochondria. In mitochondria, the place where hydrogen peroxide usually is generated, pyruvate mediates through intracellular citrate to greatly increase the NADH / NAD^+ and GSH / GSSG ratio to protect cells from oxidative damage. Due to the above reasons, pyruvate may be an effective and safe medication to deal with many oxidation-related disease in the near future.

References:

1. Nelson DL, Cox MM. (2000) *Lehninger Principles of Biochemistry.* Third edition**.** Worth publishers.

2. Salahudeen AK, Clark EC, Nath KA. (1991) Hydrogen peroxide-induced renal injury: a protective role for pyruvate *in vitro* and *in vivo. J Clin Invest.* **88:**1886-1893.

3. Que M. (1967) The detection of pyruvate and phosphoenolpyruvate on chromotograms. *J Chromatog*. **34:**122-130.

4. Andrae U, Singh J, Ziegler-Skylakakis K. (1985) Pyruvate and related alpha-ketoacids protect mammalian cells in culture against hydrogen peroxide-induced cytotoxicity. *Toxicology Lett*. **28:** 93-8.

5. O'Donnell-Torney J, Nathan CF, Lanks K, DeBoer CJ, Harpe JD. (1987) Secretion of pyruvate: an antioxidant defense of mammalian cells. *J Exp Med*. **165**: 500-514.

6. Stanko RT, Mullick P, Clarke MR, Contis LC, Janosky JE, Ramasastry SS. (1994) Pyruvate inhibits growth of mammary adenocarcinoma 13762 in rats. *Cancer Res*. **54:**1004-1007.

7. Crestanello JA, Kamelgard J and Whitman GJR. (1995) The cumulative nature of pyruvate's dual mechanism for Myocardial Protection. *J Surg Res*. **59:**198-204.

8. Marcengill MB, Puri S, Puri SK, Mohanram A, Leonova E, Raymond RM, Watts JA. (1995) Antioxidant effects of pyruvate in isolated rat hearts. *J Mol Cell Cardiol.* **27:**2059-2067.

9. Constantopoulos G, Barranger JA. (1984) Nonenzymatic decarboxylation of pyruvate. *Anal Biochem.* **139**:353-358.

11. DeBoer LWV, Bekx PA, Han L, Steinke L. (1993) Pyruvate enhances recovery of rat hearts after ischemia and reperfusion by preventing free radical generation. *Am J Physiol* **265**:H1571-H1576.

12. Mallet RT, Sun J. (1999) Mitochondrial metabolism of pyruvate is required for its enhancement of cardiac function and energetics. *Cardiovasc Res.* **42**:149-161.

13. Garland PB, Randle PJ, Newsholme EA. (1963) Citrate as an intermediary in the inhibition of phosphofructokinase in rat heart muscle by fatty acids, ketone bodies, pyruvate, diabetes, and starvation. *Nature.* **200**:169-170.

14. Mallet RT. (2000) Pyruvate: Metabolic protector of cardiac performance. *Proc Soc Exp Biol Med.* **223**:136-152.