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The Double Life of Pyruvate

by

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Abbreviations

ADP- Adensosine diphosphate
ATP – Adenosine triphosphate
LDH – L-lactate dehydrogenase
LOD – L-lactate oxidase
NAD^+ - Oxidized nicotinamide adenine dinucleotide
NADH – Reduced nicotinamide adenine dinucleotide
P*i* – Inorganic phosphate
ROS – Reactive oxygen species
TBARS – Thiobarbituric acid reactive substance
*t*BHP - *Tertiary*-butyl hydroperoxide
TCA – Tricarboxylic acid cycle

Outline

Abstract

Pyruvate is a metabolic component that links glycolysis to the tricarboxlic acid (TCA) cycle. It also functions as a small molecule antioxidant. Through nonenzymatic decarboxylation, pyruvate can scavenge hydrogen peroxide and stop lipid peroxidation. Pyruvate can detoxify intracellular, as well as, extracellular hydrogen peroxide; therefore, cells will secrete this antioxidant until the concentration inside and outside the cell reaches equilibrium. Pyruvate may be detected in samples by a recycling assay that measures the disappearance of NADH or by singlet quenching of photoreduced pyruvate. This small molecule antioxidant is important for the protection of the cell, as well as, metabolic processes.

Introduction

Pyruvate has two distinct functions within the cell. First, it plays a role in cellular metabolism, linking glycolysis to either aerobic or anaerobic respiration. Second, pyruvate acts as a preventative antioxidant. Pyruvate is an α-ketoacid with a methyl function group (**Figure 1).** Pyruvate and other α-ketoacids' ability to scavenge hydrogen peroxide was discovered in 1904 [1,2,3]. Since then, the role of pyruvate as an antioxidant has been greatly studies.

Derivatives of pyruvate are used for industry and research purposes. Ethyl pyruvate, a lipophilic ester derivative of pyruvate, is used as a food additive [4]. Because it is highly soluble in calcium solutions, ethyl pyruvate forms a stable complex with calcium. The stability of this complex is far greater than the stability of pyruvate alone [4]. Sodium pyruvate is often used in cell culture media and for research purposes [1].

This paper will discuss the biochemical formation of pyruvate and its metabolic fates; its role in free radical biology, in particular the effect on hydrogen peroxide and lipid peroxidation; the detection of this molecule; and its medical significance.

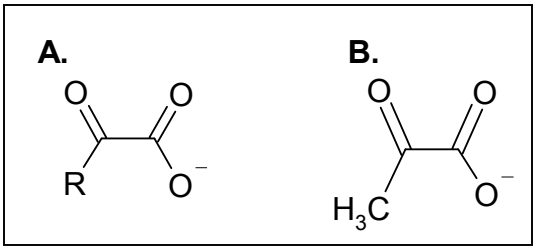

Figure 1. Structure of A. α-ketoacid and B. pyruvate.

Biochemical formation of pyruvate

Pyruvate is a unique component of metabolism in that it may either initiate anaerobic or aerobic pathways [5]. Cellular metabolism leads to the formation of pyruvate *via* glycolysis [5-7]. This process takes place in the cytosol of the cell and is the precursor to the tricarboxylic acid (TCA) cycle, which takes place in the mitochondria [5]. The concentration of pyruvate in the mitochondria may reach 1.5 mM [8].

Glucose is catabolized in a ten-step process to two molecules of pyruvate. Two molecules of NAD^+ are reduced to NADH and a net of two adenosine triphosphates (ATP) are produced through this process (Reaction 1) [5].

$$\text{Glucose} + 2\text{ADP} + 2\ P\textit{i} + 2\ \text{NAD}^+ \rightarrow 2\ \text{Pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\ \text{H}^+ + 2\text{H}_2\text{O} \quad (1)$$

Pyruvate is formed in the last step of glycolysis, in which phosphoenolpyruvate is dephosphorylated into a pyruvate by pyruvate kinase. An adensosine diphosphate (ADP) is then phosphorylated by pyruvate kinase to form a molecule of ATP with a $\Delta G°'$ of -7.5 kcal mol^{-1} [5].

There are three metabolic fates of pyruvate. First, pyruvate may be converted into acetyl-CoA by pyruvate dehydrogenase (reaction 2). This route then leads into the TCA cycle in aerobic oxidation. Pyruvate may also be converted to form lactate, *via* L-lactate dehydrogenase (LDH) (reaction 3), or acetaldehyde, *via* pyruvate decarboxylase (reaction 4), under anaerobic conditions [5].

$$\text{CH}_3\text{-COCOOH} + \text{NAD}^+\ \text{HS-CoA} \rightarrow \text{CoA-S-CO-CH}_3 + \text{CO}_2 + \text{NADH} + \text{H}^+ \quad (2)$$

$$\text{CH}_3\text{-COCOOH} + \text{NADH} + \text{H}^+ \rightarrow \text{CH}_3\text{-CHOH-COO}^- + \text{CO}_2 + \text{NADH} \quad (3)$$

$$\text{CH}_3\text{-COCOOH} \rightarrow \text{CH}_3\text{-COH} + \text{CO}_2 \quad (4)$$

Free Radical Biology

Pyruvate makes a great antioxidant because it does not act as a pro-oxidant [9], and its products are not reactive. Studies have shown that pyruvate is capable of acting as a preventative antioxidant by scavenging hydrogen peroxide [1-4,8,10] and hydroxyl radical [4], and preventing lipid peroxidation [2,9,11]. Also, the use of pyruvate as an antioxidant may prevent metabolically produce reactive oxygen species (ROS). Downstream metabolic pathways of pyruvate, such as oxidative phosphorylation, have been shown to produce ROS [7]. If less

pyruvate is used for metabolism, there may be a decrease in overall free radical production in the cell.

Hydrogen peroxide

Only α-ketoacids, not β-ketoacids, have been found to protect against hydrogen peroxide toxicity. Of the naturally occurring α-ketoacids, pyruvate offers the most protection against hydrogen peroxide [10]. α-Ketoacids are able to scavenge hydrogen peroxide by nonenzymatic oxidative decarboxylation [3,10]. In this reaction a carboxyl group from the α-ketoacid and an oxygen from hydrogen peroxide form carbon dioxide, leaving a water and a carboxylic acid (reaction 5) [3,8]. Specifically, the interaction between pyruvate and hydrogen peroxide yields acetic acid, carbon dioxide and water (reaction 6) [2,4].

$$\text{R-COCOOH} + H_2O_2 \rightarrow \text{R-COOH} + CO_2 + H_2O \quad (5)$$

$$CH_3\text{-COCOOH} + H_2O_2 \rightarrow CH_3\text{-COOH} + CO_2 + H_2O \quad (6)$$

Numerous studies have shown that pyruvate also interacts with hydrogen peroxide extracellularly. **Figure 2** shows that the concentration of pyruvate in the media decreases over time in response the addition of hydrogen peroxide [1]. There is an immediate interaction with hydrogen peroxide that leads to depleted levels of pyruvate. The half-life of the reaction is less than 30 s [1]. Marcengill *et al*. also showed that pyruvate was able to remove *tertiary*-butyl hydroperoxide (*t*BHP) from buffer by reducing it [1].

Lipid peroxidation

Pyruvate has also been shown to have a protective effect against lipid peroxidation through nonenzymatic decarboxylation of pyruvate [11]. Thiobarbituric acid reactive substance (TBARS) levels are an indicator of membrane lipid peroxidation. Marcengill *et al.* showed that in perfused heart tissue, 5 mM pyruvate protected against lipid peroxidation with the addition of

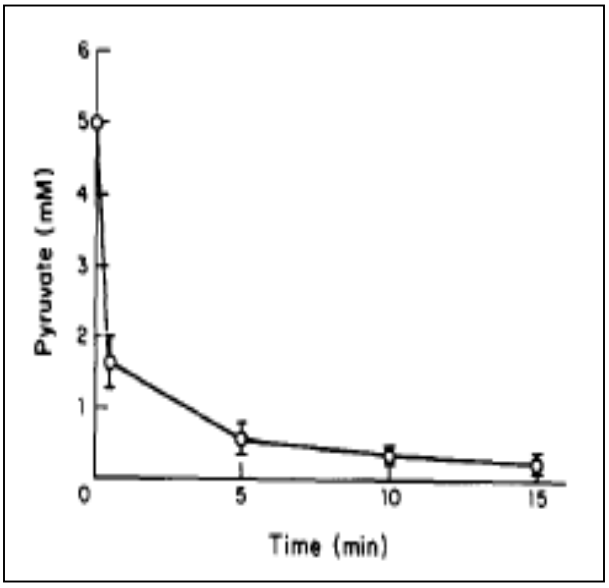

Figure 2. Depletion of pyruvate in the media over time in the presence of 5 μmol H_2O_2 in 1 ml RPMI 1640 media. Pyruvate levels were measured fluorometrically [1].

0.35 mM *t*BHP. This was indicated by the fact that there was no significant difference in TBARS levels between tissue with and without *t*BHP. Glucose and octanoate did not offer this protection, as their TBARS levels increase dramatically with the addition of *t*BHP (**Figure 3**) [2]. Varma *et al.* also noted that when mouse lenses were incubated with xanthine and xanthine oxidase there was a three-fold increase in malonaldehyde, a marker for lipid peroxidation. However, there was no increase when pyruvate was present [9].

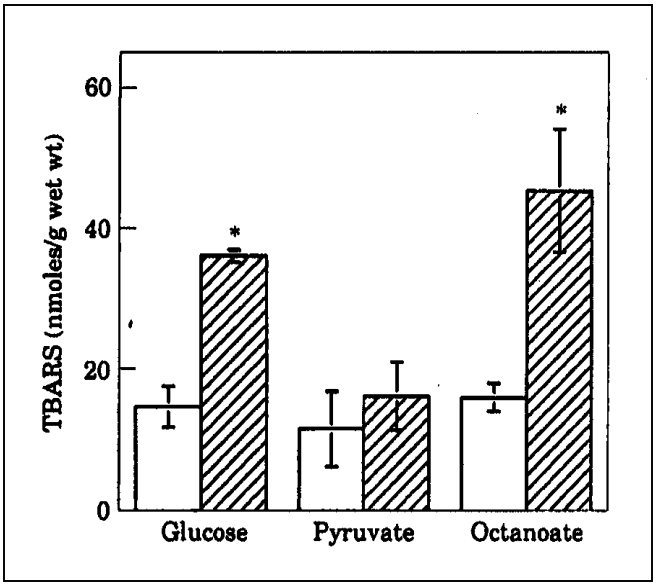

Figure 3. TBARS levels in heart tissue in the absence (open bars) and presence (hashed bars) of 0.35 mM *t*BHP with either 15 mM glucose, 5 mM pyruvate, or 0.5 mM octanoate [2].

Secretion

Hydrogen peroxide can easily diffuse in and out of cells [3]. Because of this, hydrogen peroxide may be made in one cell and then travel to neighboring cells to cause damage. Therefore, the antioxidant ability of pyruvate is important both inside and outside of the cell. Pyruvate's ability to scavenge extracellular hydrogen peroxide makes it a vital component of plasma. Concentrations of pyruvate in human plasma are between 90 and 120 μM [1]. *In vitro* pyruvate levels in serum, which have been reported to contain 1 to 5 mM pyruvate [10], are equally important. O'Donnell-Tormey *et al.* showed that cells in pyruvate deficient media will secrete pyruvate into the media [1]. Pyruvate is transported in and out of the cells by the H^+-monocarboxylate cotransporter [3]. In pyruvate-free medium, intracellular levels drop dramatically until there is equilibrium between the pyruvate concentration inside and outside of the cells [1]. In the first two hours in pyruvate-free media, cells secrete pyruvate at a rapid rate of 9.4 μM $h^{-1}/10^6$ fibroblasts. This rate declines over the next four to ten hours 3.1 μM $h^{-1}/10^6$ fibroblasts until it reaches normal serum levels [1]. This research shows the essential role pyruvate plays even outside of cells.

Detection

Recycling assay

Since pyruvate is present in such a low level, it may be hard to detect. Valero and Garcia-Carmona devised a method to determine the amount of pyruvate present by measuring the disappearance of β-NADH [12]. This is done by recycling L-lactate back into pyruvate by means of the L-lactate oxidase enzyme (LOD). This process reduces oxygen to hydrogen peroxide and has a first order rate constant of 1.8 min^{-1} [12]. Pyruvate then acts with L-lactate

dehydrogenase (LDH) to oxidize NADH to NAD^+ (**Figure 4**). The disappearance in NADH is measured at 340 nm, with an extinction coefficient of 6,270 M^{-1} cm^{-1} [12].

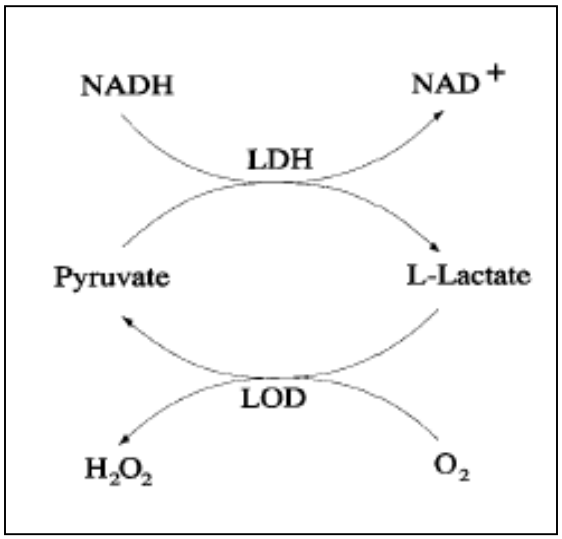

Figure 4. Recycling assay for detection of pyruvate by the disappearance of NADH. Pyruvate is dehydrogenated to form lactate and NAD^+ by LDH. Lactate is then recycled back into pyruvate by LOD [12].

Singlet quenching

Encina and Lissi also developed a method of detecting methyl pyruvate by singlet quenching by hydrogen donors [13]. The photoreduction of pyruvate results in three reactive excited states. The first excited state is seen as the emission band at around 415 nm (**Figure 5**). The change in intensity of the emission band relates to the amount of singlet quenching of the excited substrate by hydrogen donors. Phenols quickly quench the singlet from pyruvate with a rate constant of 2.7 x 10^8 M^{-1} s^{-1} [13]. The extinction coefficient for this is 14.8 M^{-1} cm^{-1} [13].

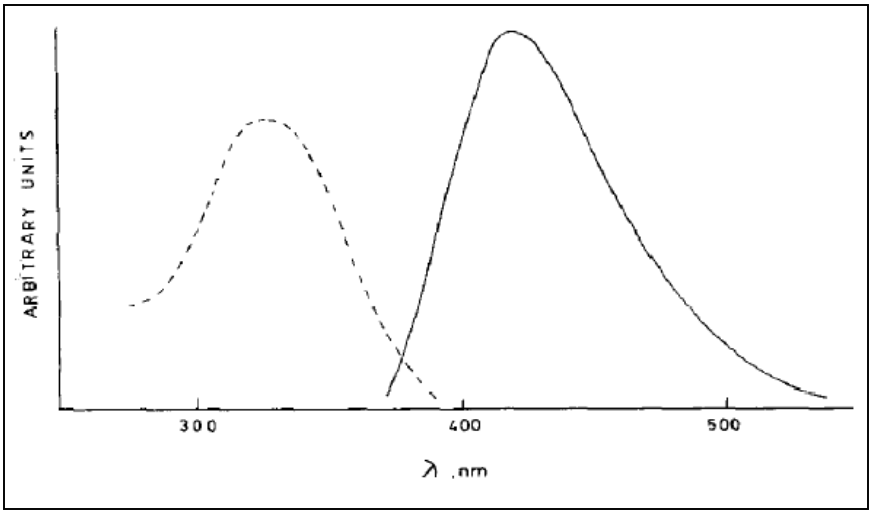

Figure 5. Absorption (----) and emission (—) spectra of methyl pyruvate [13].

Medical importance

Pyruvate has significant contributions to the field of medicine. The antioxidant properties of pyruvate help protect our bodies from everyday damage due to hydrogen peroxide and lipid peroxidation. In addition to this, pyruvate may be given at higher levels in a medical setting to prevent injury. In particular, increased levels of pyruvate have been shown to protect against ischemia-reperfusion injury [2,4]. It has also been shown that pyruvate dehydrogenase is inactivated during ischemia and reperfusion [2]. This would prevent more reactive oxygen species from being formed through metabolism. Studies have also shown that pyruvate can protect against the formation of cataracts by scavenging hydrogen peroxide [7].

Conclusion

Pyruvate is a valuable small molecule antioxidant that is capable of scavenging hydrogen peroxide and stopping lipid peroxidation. Pyruvate's role as an antioxidant has great importance, not only in research, but also in the medical field. However, this molecule also functions as an important component of cellular metabolism that is formed during glycolysis. The biochemical implications of using pyruvate for its antioxidant properties rather than in metabolism not only protect the cell against hydrogen peroxide-mediated toxicity, but also prevents metabolically produced reactive oxygen species.

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