This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2001)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2001 Term

Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

The Fine Print:

Because this is a paper written by a beginning student as an assignment, there are no guarantees that everything is absolutely correct and accurate.

In view of the possibility of human error or changes in our knowledge due to continued research, neither the author nor The University of Iowa nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such information. Readers are encouraged to confirm the information contained herein with other sources.

Pyruvate

by

Iman Ahmad

B-180 Medical Laboratories
Free Radical and Radiation Biology Program
The University of Iowa
Iowa City, IA 52242-1181

For 77:222, Spring 2001

22. February 2001

Abbreviations

ATP, adenosine triphosphate
CoAsH, coenzyme A
$FADH_2$, flavin adenine dinucleotide
GPx, glutathione peroxidase
GSH, glutathione
LDH, lactate dehyrogenase enzyme
NAD^+, nicotinamide adenine dinucleotide
$^1O_2$, singlet oxygen
TCA, tricarboxylic acid
MCF7, human breast cancer cells

Table of content ***Page number***

Abstract 2

Introduction 2

Pyruvate formation and metabolism- an overview 3

Pyruvate: intracellular antioxidant 5

Oxidative decarboxylation of pyruvate 7

Pyruvate as therapeutic agent 8

Pyruvate detection 9

Summary 9

References 10

Abstract

During the metabolism of carbohydrates and proteins, the body creates pyruvate. It is formed during digestion, and can also be found in several foods. Pyruvate is the compound, which starts the tricarbocxylic acid cycle (TCA), an energy cycle in the body. Several α-ketoacids, including pyruvate and α-ketoglutarate react non-enzymatically with H_2O_2 and act as "H_2O_2 scavenger" when present in cell culture medium. Pyruvate is an important food source and may exert antioxidant effects. Thus, pyruvate may be useful as an efficient, readily diffusible and specific scavenger of H_2O_2.

Introduction

Pyruvate is a substance that naturally occurs in the body. Pyruvate is the foundation of the TCA cycle. This cycle is the process through which the body converts glycogen to energy. Thus, pyruvate plays a crucial role in this conversion of food to energy. In 1904 Holleman first described the capacity of pyruvate and related α-ketoacids to reduce H_2O_2 to H_2O while these acids undergo non-enzymatic decarboxylation at the one carbon position [14]. Thus, pyruvate appears to

act as an antioxidant. Although the protective action of pyruvate against H_2O_2 damage has been studied in a variety of systems, it seemed there was in fact no available information on the kinetics or significance of this reaction in vitro or in chemical systems. This paper will focus on pyruvate formation, reaction, detection and its potential role as an antioxidant.

Pyruvate formation and metabolism -an overview

Carbohydrates serve as the primary source of energy in the cell, and carbohydrate metabolism is central to all metabolic process, which can be accomplished either anaerobically or aerobically, resulting in the synthesis of ATP. An overview of the process of glucose oxidation appears in Figure 1.

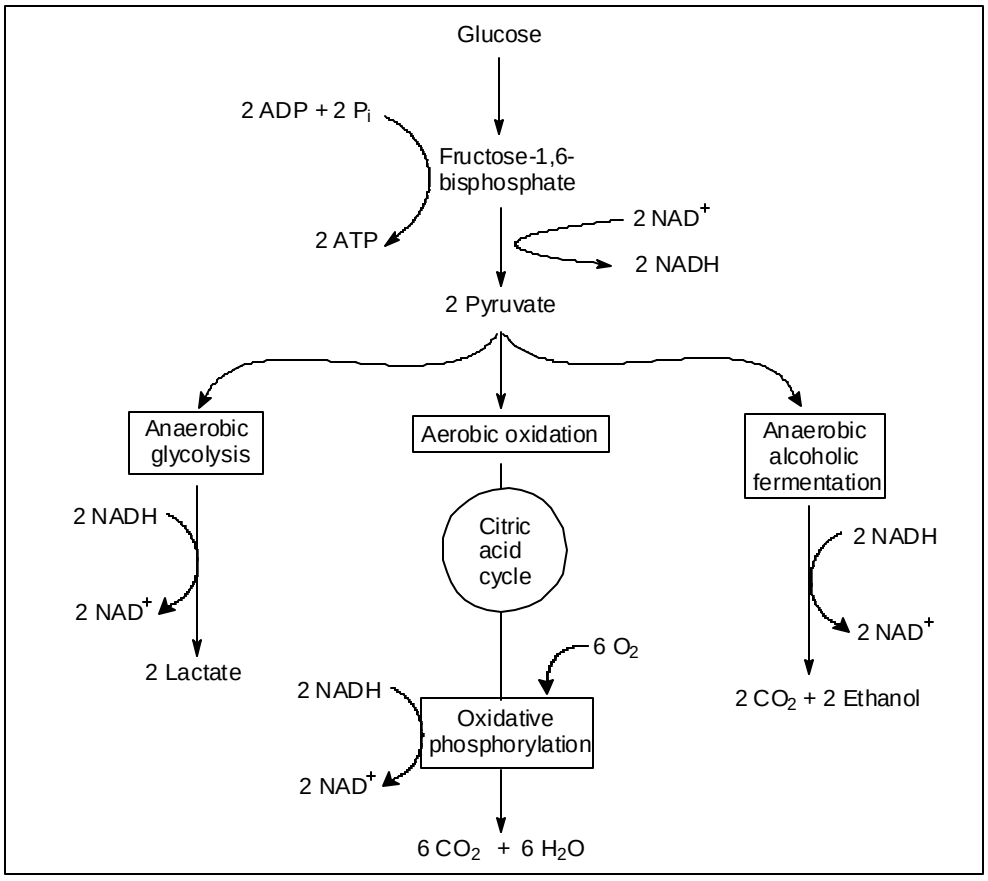
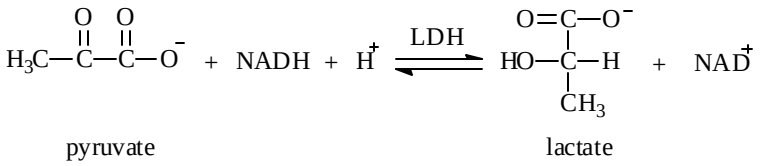
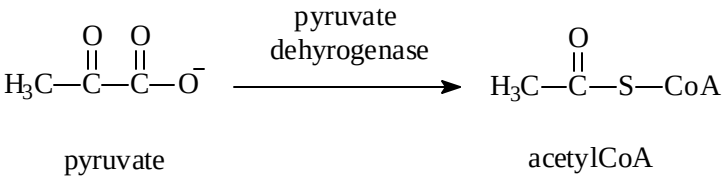


Figure 1. One molecule of glucose is converted to two molecules of pyruvate. Under aerobic conditions, pyruvate is oxidized to CO_2 and H_2O by the TCA cycle. Under anaerobic conditions, lactate is produced, especially in muscle. Alcoholic fermentation occurs in yeast. The NADH produced in the conversion of glucose to pyruvate is reoxidized to NAD^+ in the subsequent reactions of pyruvate. Adapted from[1].

As can be seen, pyruvate is an important junction point in glucose metabolism. Glucose is a six-carbon molecule, and each pyruvate formed is a three-carbon molecule, which can be converted anaerobically to lactate in the cytosol [1].

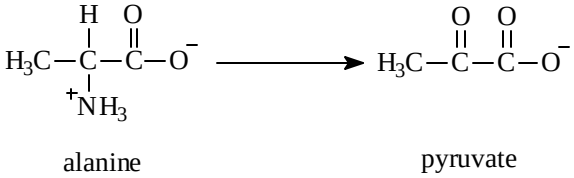


Alternatively, pyruvate can be converted to a metabolite acetyl CoA, which enters into an aerobic process known as TCA cycle [1].



The TCA cycle produces the reducing equivalents NADH and $FADH_2$, which are oxidized in the electron transport chain in a process known as oxidative phosphorylation. All of the steps of aerobic glucose metabolism occur in the mitochondria. In some organisms that can grow anaerobically, such as yeast, pyruvate is converted *via* acetaldehyde into carbon dioxide and ethanol.

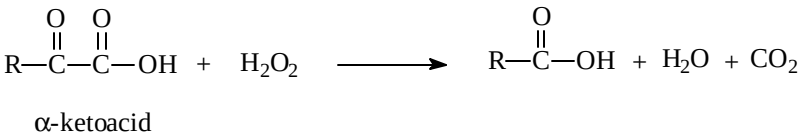
Furthermore, pyruvate can be formed from catabolism of most amino acids in animals except leucine and lysine, in a reaction [1]:



When dietary sources of glucose are not available, and when the liver has exhausted its supply of glycogen, glucose is synthesized from noncarbohydrate precursors by gluconeogenesis. Pyruvate is one of these precursors that can be converted to glucose [1].

Pyruvate: intracellular antioxidant

Pyruvate and other α-ketoacids react rapidly and stoichiometically with H_2O_2 to protect cells from cytolytic effects. Pyruvate is one of the few antioxidants that readily enter cells, making it an ideal cytoplasmic antioxidant [2]. As early as 1904 it was shown that H_2O_2 causes a rapid non-enzymatic and stoichiometric decarboxylation of pyruvate to acetic acid, H_2O and CO_2 [14].



α-ketoacid

It was recently discovered that mammalian cells secrete pyruvate as an antioxidant defense to O_2 radicals [2]. Cells in culture are exposed to marked oxidative stress due to culturing them in 95% air. Toxic reaction products generated include $^1O_2$, $O_2^{\bullet-}$ and H_2O_2. Of these only H_2O_2 has long half-life and accumulates in the medium. The kinetics of pyruvate release showed three phases (Figure 2), a rapid early release followed by a slower phase and finally a plateau phase [2].

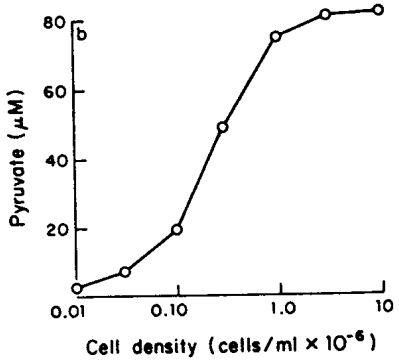


Figure 2. Pyruvate release in relation to cell density. Pyruvate (μM) in medium at 9 h *vs*. initial plating density. Data are means of duplicates from one representative experiment [2].

It was hypothesized that the slowed rate of accumulation of pyruvate as in (Figure 2) represented a loss of pyruvate to a side-reaction, rather than a change in the rate of production or release by the cells. This could be accounted for the exhaustion of another antioxidant in the medium that protects pyruvate from reaction with H_2O_2 during the first early phase of pyruvate secretion. The observation that endogenous catalase enhanced the rate of accumulation of secreted pyruvate

strongly suggests that pyruvate actually function as an extracellular scavenger of H_2O_2 arising in cell culture and supports the previous hypothesis (Figure 3) [2].

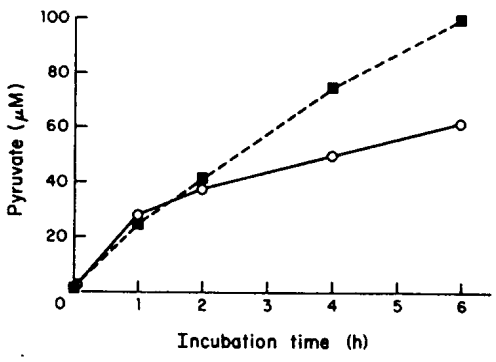


Figure 3. Enhanced pyruvate accumulation in the presence of catalase. Fibroblasts were cultured in the presence (■) or absence (○) of 1,000 Sigma U/ml catalase and medium was collected for fluorometric assay as the times indicated. Means of duplicates from one representative experiment [2].

Extracellular pyruvate was in equilibrium with intracellular stores. Therefore, cells conditioned the extracellular medium with pyruvate at the expense of intracellular pyruvate, until homeostatic levels were reached in both compartments (60-150 μM) [2]. Both O' Donnel-Torney and Eagle's group found that cells plated at low density in the absence of exogenous pyruvate are exposed to the deleterious effects of oxidants for many hours, due to the increased time required by cells to condition the extracellular medium to homeostatic levels of pyruvate [2,4].

Hydrogen peroxide is normally found in every aerobic cell. It is formed intracellularly by mitochondria, endoplasmic reticulum, and peroxisomes, which contains a number of H_2O_2 generating enzymes. This led researchers to wonder whether tissue levels of pyruvate and other α-ketoacids may regulate the net generation of H_2O_2 *in vivo*. Organs, such as the kidney, that generate H_2O_2 in relatively large amounts during their normal metabolism [5], were used to examine whether the acute elevation in α-ketoacids and pyruvate scavenge H_2O_2 *in vivo* by administering maleate to rats and studying the renal generation of H_2O_2 [10]. Maleate administration leads to an increased level of pyruvate and α-ketoglutarate due to the depletion of CoAsH [6]. CoAsH is critical in the metabolism of pyruvate, in that pyruvate reacts with CoAsH to yield acetylCoA, which is an important entry step into the TCA cycle [1]. Also, CoAsH is essential in the metabolism of α-ketoglutarate through the TCA cycle, since CoAsH combines with the α-ketoglutarate to form succinyl-CoA [1].

As a result of increased level of pyruvate and α-ketoglutarate, it was found that urinary concentration of H_2O_2 was decreased as seen in Figure 4 [10]. These findings are particularly striking since the administration of maleate leads to depletion of GSH [7], which with GPx provides one of the main lines of defense against elevation of levels of H_2O_2 in tissues [8]. From these data we find that pyruvate and other α-ketoacids can scavenge H_2O_2 *in vivo* even with the depletion of GSH. Because tissue levels of pyruvate and other α-ketoacids are ranging from 500 μM to 2 mM in accordance with the reported normal liver concentrations of pyruvate in rodents [15], whereas H_2O_2 levels are 100-200 nM as estimated in wild type E.coli [16], Nath's group suggests that α-ketoacids must be considered as part of the intracellular mechanism that regulates the cellular concentrations of H_2O_2 [10].

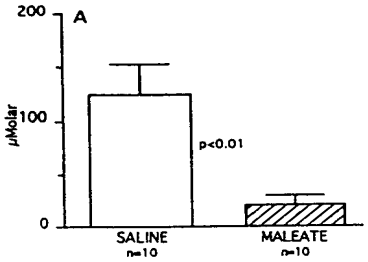


Figure 4. Urinary concentrations of H_2O_2 in rats 2 h after administration of maleate or saline [10].

Several studies have demonstrated that pyruvate protects against H_2O_2-dependent hydroxyl radical-mediated degradation of isolated DNA, and diminishes the amounts of detectable hydroxyl radicals generated by H_2O_2-dependent system [11].

Oxidative decarboxylation of pyruvate

Although the protective action of pyruvate against H_2O_2 damage has been studied in a variety of systems, it seemed as though there was in fact almost no available information on the kinetics or significance of this reaction in vitro or in chemical system. However, Nath's group probed the mechanism underlying the protective effects of pyruvate against H_2O_2 damage. They determined whether CO_2 was produced in pyruvate-treated cells in the presence of menadione, as a source of H_2O_2; the formation of labeled $^{14}CO_2$ would signal oxidative decarboxylation of pyruvate at the carbon-1 position triggered by H_2O_2. As shown in Figure 5, a time-dependent increase in $^{14}CO_2$

was detected in MCF7 cells exposed to [^{14}C] pyruvate and menadione. After 90 min incubation, 716 ± 66 pmol of $^{14}CO_2$ were detected in MCF7 cells exposed to 10 μM [^{14}C]pyruvate and 25 μM menadione. In contrast, cells exposed only to 10 μM [^{14}C]pyruvate produced 78 pmol of $^{14}CO_2$. These data are indicative of the presence of a CO_2-forming reaction in menadione-treated cells exposed to pyruvate [10].

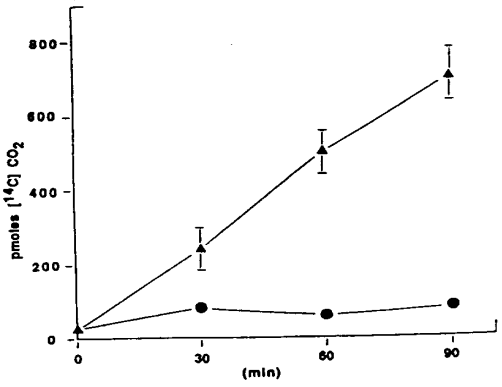


Figure 5. Production of $^{14}CO_2$ in MCF7 cells. MCF7 cells (2×10^5) were incubated for times indicated with [^{14}C]pyruvate (10 μM; 8.8 μCi/μmol) in presence(▲) or absence (●) of menadione (25 mM).

Pyruvate as therapeutic agent

Cataract is one of the major causes of age-dependent visual impairment and blindness. Exposure of animals and humans to active species of oxygen has also been shown to result in cataract formation [12]. Since pyruvate is a natural constituent of cells on tissues and has been proven to act as an antioxidant, it is proposed that it can protect the lens against oxidative damage (Figure 4) [12]. Pyruvate is thus can be used not only for treating cataracts, but also against other disabilities related to acute or chronic oxidative stress. Hence, pyruvate may be useful in the development of therapeutic anticataract agents.

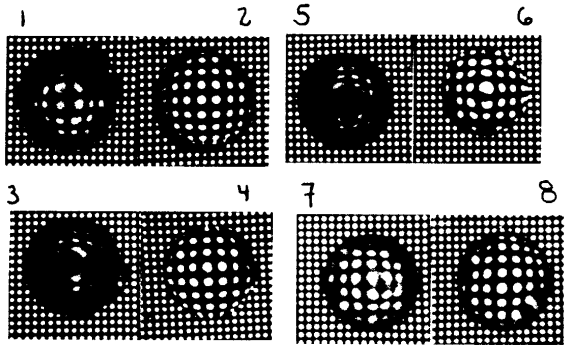
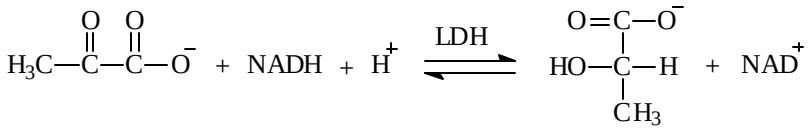


Figure 6. Effects of pyruvate on the transparency of the lens. Contralateral lenses isolated from rats were incubated in medium-199 containing 50 μmol riboflavin/L for a period of 18 h and photographed after putting the petri dishes on a stainless steel grid with a 150 foot-candle transmitted light. Pictures were taken through a microscope with a magnification of 16× using black and white polaroid film. 1,3,5 and 7 indicates lenses incubated without pyruvate; 2,4,6, and 8 indicates lenses incubated with pyruvate (10 mmol/L) [12].

Pyruvate detection

The fluorimetric assay is a method used to measure the intracellular level of pyruvate. The assay was a modification of the method of Lowry and Passonneau [13]. Pyruvate is converted to lactate by LDH in the presence of NADH. The loss of fluorescence (excitation 340 nm, emission 460 nm) as NADH is converted to NAD^+, is followed [2].



pyruvate lactate

Summary

Pyruvate is well suited to act as an intracellular scavenger for H_2O_2 for several reasons. The endpoint of the glycolytic pathway, pyruvate is transported into the mitochondria *via* a carrier on the inner mitochondrial membrane. Once in the mitochondria, pyruvate undergoes oxidative decarboxylation to acetylCoA and enters the TCA cycle. Pyruvate thus links glycolysis to the TCA cycle, which is housed in the mitochondria and is the final common pathway for the oxidation of carbohydrates, lipids and amino acids. H_2O_2 is generated at several cellular sites and is freely diffusible among the cellular compartments. The availability of pyruvate in both cellular compartments thus provides a scavenger for H_2O_2 that has escaped detoxification by GPx and catalase systems. In summary, administration of pyruvate may provide safe and effective therapy for any human diseases related to acute or chronic oxidative stress.

References

1) Campbell MK. (1999) *Biochemistry.* 3rd ed. Saunders college publishing. pp: 454-481.

2) 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.

3) 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.

4) Eagle H, Piez K. (1962) The population-dependent requirement by cultured mammalian cells for metabolites, which they can synthesize. *J Exp Med*. 116-129.

5) Nath KA, Salahudeen AK. (1990) Induction of renal growth and injury in the intact rat kidney by dietary deficiency of antioxidants. *J Clin Invest*. **86**: 1179-1192.

6) Pacanis AT, Rogulski J. (1981) Effects of maleate on the content of CoA and its derivatives in rat kidney mitochondria. *J Biol Chem*. **256**: 13035-13038.

7) Gstraunthaler GW, Pfaller, Kotanko P. (1983) Glutathione depletion and in vitro lipid peroxidation in mercury and maleate induced acute renal failure. *Biochem Pharmacol*. **32**: 2969-2972.

8) Eaton JW. (1991) Catalase and peroxidases and glutathione and hydrogen peroxide: mysteries of bestiary. (Editorial). *J Lab Clin Med*. **118**: 3-4.

9) Boveris A, Chance B. (1973) The mitochondrial generation of hydrogen peroxide. *Biochem J.* **134**: 707-716.

10) Nath KA, Ngo EO, Hebbel RP, Croatt AJ, Zhou B, Nutter LM. (1995) α-ketoacids scavenge H_2O_2 in vitro and in vivo and reduce menadione-induced DNA injury and cytotoxicity. *Cell Physiol*. **37**: c227-c238.

11) Ross WE, Bradley MO. (1981) DNA double-strand breaks in mammalian cells after exposure to intercalating agents. *Biochim Biophy Acta.* **654**:129-134.

12) Varma SD. (1991) Scientific basis for medical therapy of cataracts by antioxidants. *Am J Cli Nutr.* **53**: 335S-345S.

13) Lowry OH, Passonneau JV. (1972) A flexible system of enzymatic analysis. Academic Press. New York. 1-291.

14) Holleman MAF. (1904) Note on the action of oxygenated water on α-ketoacids and 1,2, dicetones. *Recl Trav Chi Pays-Bas Belg.* **23**: 169-172.

15) Veech RL, Lawson JWR, Cornell NW, Krebs HA. (1979) Phosphorylation potential. *J Biol Chem*. **254**: 6538-6547.

16) Conzalez-Flecha B, Demple B. (1995) Metabolic sources of H_2O_2 in aerobically growing E.coli. *J Biol Chem*. **270**: 13681.