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

Free Radicals in Biology and Medicine

(77:222, Spring 2003)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2003 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

Liopoic Acid

Benjamin Slane

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

Free Radicals in medicine
77:22
Paper #2
February 27, 2003

Table of Contents

Abstract

Lipoic acid is a powerful well-known antioxidant. It was first isolated in the early 1950s from liver samples. It has been cited as a promoter of liver growth and used in various treatments. Lipoic acid is a molecule containing a 1,2-dithiolane ring and a carboxylic acid group. It is able to directly scavenge hydroxyl radicals within the cell. Lipoic acid is found free in cells, but it is predominately found bound to proteins. These forms of lipoic acid can be best detected and quantified by HPLC. Lipoic acid can also be reduced and play a role in protection against lipid peroxidation. Both isomer forms, R and S, and radiolabeled forms of lipoic acid can be synthesized in the laboratory. Lipoic acid is an important and powerful biological antioxidant that can directly scavenge free radicals and protect cells from oxidative damage.

Introduction

In 1951 Dr. Lester Reed, PhD isolated the first traces of lipoic acid [1]. The first purified sample of lipoic acid was 30 mg of yellow crystals that were extracted from 100 kg of liver residue. The substance was known as α-lipoic acid or ALA. Some scientists believed the substance should be named thioctic acid because it contained two sulfur atoms (*theion* in Greek) and eight carbon atoms (*octo* in Greek). Ultimately, ALA was given the name lipoic acid because of its ability to dissolve in lipids. Scientists knew of lipoic acid even before its isolation. Since the 1930s lipoic acid was recognized as a fundamental chemical required for bacterial growth [2].

Lipoic acid works at the cellular level to help essential substances for metabolism to enter the mitochondria. An increase in the amount of lipoic acid increases the amount of cellular fuel that is burned. This generates a greater energy reserve for the body that is available for growth, tissue repair and muscle development. Many success stories have been reported from the treatment of sick livers with lipoic acid [2]. In 1977 a couple was dying of liver failure after eating poisonous mushrooms. The attending physician was told that they would certainly die and to make it as comfortable as possible for them. However, he had read about a new experimental drug, lipoic acid, which had successfully promoted liver growth in Europe. Within days after administering lipoic acid the couple was back at home in good health with fully functioning and regenerated livers [2]. Since that time lipoic acid has been proposed as preventive agent against a variety of health conditions including aging, diabetes, cancer and cardiovascular disease. This paper will provide an overview of the chemical properties and reactive nature of lipoic acid.

Structure

Lipoic acid is known by a variety of different names. Officially, it is known as lipoic acid or 1,2-dithiolane-3-pentanoic acid. Unofficially, it has been known as α-lipoic acid, 6,8-thioctic acid, 5-(1,2-dithiolan-3-yl)-valeric acid or 5-3-(1,2-dithiolanyl)-pentanoic acid. Lipoic acid is a molecule containing eight carbon atoms, two sulfur atoms, a 1,2-dithiolane ring and a carboxylic acid group (Figure 1) [1].

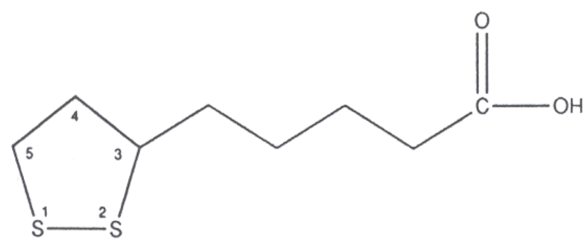


Figure 1. The chemical structure of lipoic acid (carbon 3 of the ring is the chiral center) [1].

The three position of the ring structure is a chiral center (Figure 1). The R and S isomer forms can be produced synthetically and specific rotation values of -107^{o} and $+109^{o}$ have been reported [3]. While both isomers can be synthesized only the R (+) form of lipoic acid occurs naturally [1].

Characterization

Lipoic acid is characterized by its physiochemical constants that are shown in Table 1.

TABLE 1. The physiochemical property constants of lipoic acid [1].

Constant	Value
Molecular Weight	206.35 Da
Melting Point	60-62^{o}C
pK_a	5.3

IR, NMR, and mass spectrometry can be used to characterize lipoic acid. An IR spectrum characteristic of lipoic acid contains bands at 3300-2400, 1690, 1250, and 945 cm^{-1} [1].

A ^{1}H NMR spectrum of lipoic acid in $CDCl_3$ and at 60 MHz shows the following chemical shifts: 1.6 (m,6,3CH_2C), 1.8-2.0 (m,2,CH_2CHS), 2.2-2.6 (m,2,CH_2CO), 2.0-3.3 (t,2,CH_2S), 3.4-3.7 (q,1,CHS), and 10.5 (broad, COOH) [1].

A mass spectrum of lipoic acid contains the following ions: m/z = 81 (100%), 95 (7%), 105 (27%), 123 (67%), 155 (17%), 173 (14%), and 206 (72%) [1].

These modes of detection provide many unique distinguishable characteristics that allow lipoic acid to be accurately identified and characterized.

Protein interactions

A small amount of free lipoic acid is present in cells, the majority of lipoic acid is found tightly bound to proteins. Lipoic acid is bound to protein by an amide linkage to a lysine residue (Figure 2) [1]. This linkage is called a lipoyl group. This group plays a role in many multienzyme complexes such as pyruvate dehydrogenase complex, and the alpha-ketoglutarate dehydrogenase complex. This lipoyl group serves as a flexible arm that carries the substrate from one active site to the other. The lipoyl group can be oxidized and reduced by lipoamide dehydrogenase. The binding of lipoic acid to proteins makes accurate detection and quantification difficult [1].

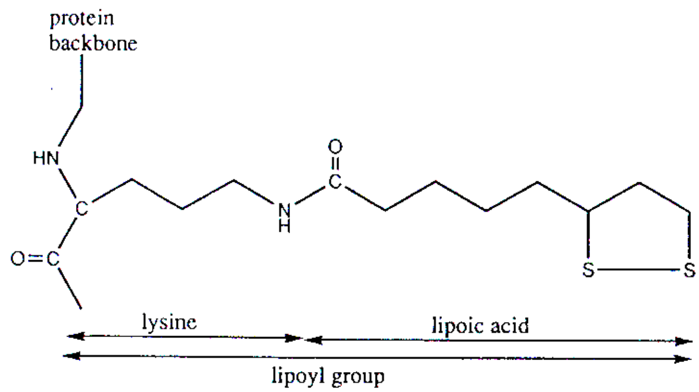


Figure 2. Lipoyl group formed by and amide linkage of lipoic acid to a lysine residue of a protein [1].

Detection

Three different methods, microbiological assays, GC, and HPLC can be used to detect lipoic acid. Microbiological assays were used first to detect lipoic acid. One such assay involved a mutant strain of E.coli that was unable to synthesize lipoic acid. The growth response was used to corolate the amount of lipoic acid present. Biological assays of this type are highly sensitive, difficult to reproduce and labor intensive [1].

As a simpler alternative, GC was introduced to measure lipoic acid content in biological samples. The sample is hydrolyzed by an acid or base and extracted into an organic solvent. This preparation produces a volatile compound that can be detected by GC. However, the preparation of the sample may oxidize dihydrophilic acid, the reduced form of lipoic acid, and remove all free lipids, which may contain free lipoic acid. This may diminish the sensitivity of GC detection. The two previously mentioned detection methods, biological and GC, measure total lipoic acid content. The assays are unable to distinguish between free and protein bound, oxidized and reduced forms of lipoic acid [1].

To provide a more precise detection of lipoic acid HPLC was introduced. Depending on the exact method of HPLC, lipoic acid can be detected down to 0.5 ng. The sample preparation involves cleanup consisting of an enzymatic hydrolysis and extraction with benzene: dichloromethane. Preparing the sample without enzymatic hydrolysis allows for the detection of free lipoic acid only. HPLC is a very relevant assay because it allows for the detection of lipoic acid in human plasma. For instance, after oral administration of 1 g of lipoic acid, HPLC detected lipoic acid levels in human plasma of 400 ng/mL [1]. Currently HPLC is most suitable method to detect lipoic acid. It is the most sensitive and precise method, and it allows for the protein bound and free lipoic acid to be quantified.

Oxidation

Lipoic acid is a powerful antioxidant. Lipoic acid itself is more susceptible to oxidation because of its 1,2-dithiolane-ring structure. This disulfur-containing ring is more easily oxidized than open chained disulfides. When lipoic acid undergoes a one-electron oxidation a relatively stable 1,2-ditholane radical cation is produced. The newly formed cation radical is a weak oxidizing agent. Biologically, the production of this radical cation is significant. Lipoic acid is able to react directly with a hydroxyl radical, one of the most powerful oxidizing agents that exist, to produce the weakly oxidizing 1,2-ditholane radical cation [1]. This new product is a significantly weaker oxidant than the hydroxyl radical. The hydroxyl radical scavenging ability of lipoic acid makes it a very effective and important antioxidant.

$$\text{Lipoic acid} + HO^{\bullet} \longrightarrow \text{1,2-Dithiolanyl radical cation [1]}$$

Lipoic acid can also undergo two electron oxidations. This process yields a thiosulfinate, beta-lipoic acid. This product can be achieved photolytically by oxygen. This can also be done chemically by ammonium persulfate, tert-butyl hydroperoxide, hydrogen peroxide, hypochlorous acid and peracetic acid [1]. In some cases lipoic acid is able to undergo further oxidations. These oxidation products are shown in figure 3.

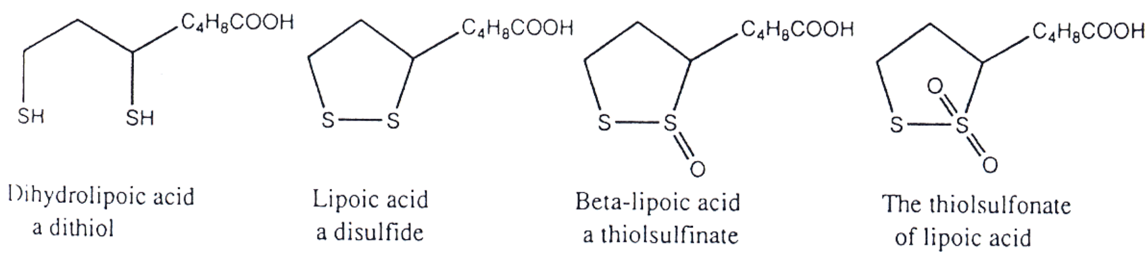


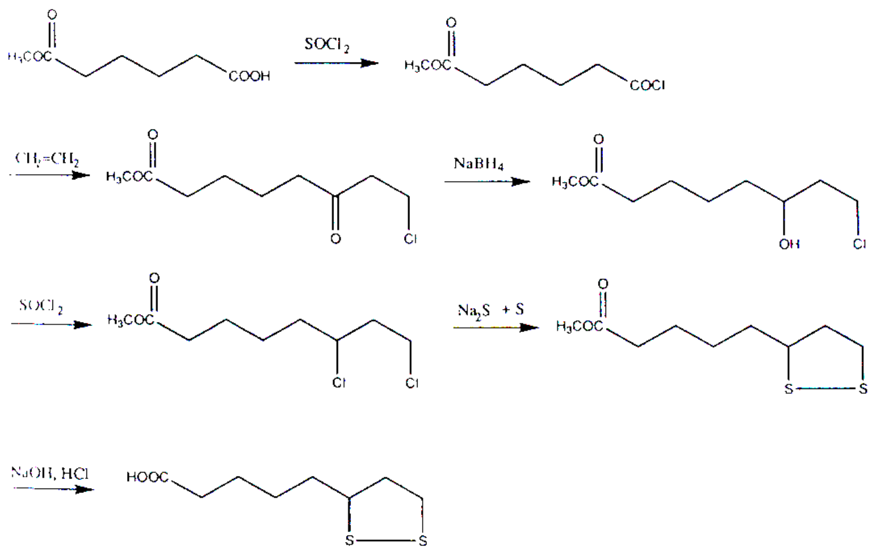
Figure 3. The possible oxidation products of lipoic acid. From left to right each new species is the product of a 2-electron loss [1].

Reduction

Lipoic acid can be reduced to form dihydrolipoic acid (Figure 3). The redox couple of lipoic acid/ dihydrolipoic acid has a low redox potential of -0.294 V (pH 7.1, 22^{o}C) [1]. This low redox potential indicates that lipoic acid needs a good reducing agent to be converted into dihydrolipoic acid. *In vivo* this is most likely done by NADH and/or NADPH. Dihydrolipoic acid generated *in vivo* contains thiolate anions, which are very good nucleophiles. The pK_a of dihydrolipoic acid is 10.7 (at 25^{o}C), which indicates strong basicity of the anions and strong nucleophile capability [1]. Dihydrolipoic acid has been shown to react directly with GSSG. While this reaction proceeds slowly (0.5 mM dihydrolipoic acid reduces 50% of 0.5 mM GSSG in 45 min at 37^{o}C), it has important physiological consequences [1]. When GSSG and dihydrolipoic acid were added in combination, GSH was formed and protection against lipid peroxidation was observed [1].

Synthesis

Initially, synthesis of lipoic acid was hampered by poor yields (8%). In general the synthesis pathway of lipoic acid leads to octanoic acids carrying a halogen, hydroxy, mercapto, or ether groups on carbon 6 or 8. The synthesis pathway has been manipulated and altered many different ways to increase the yield up to 30 percent. R, S and radiolabeled isomers of lipoic acid have also been synthesized. Scheme 1 below shows a basic synthesis pathway of lipoic acid [1].



Scheme 1. Synthesis pathway of lipoic acid discovered and performed by Acker and Wayne [1].

Summary

Lipoic acid is a very powerful antioxidant that can directly scavenge hydroxyl radicals. This compound is gaining momentum in various fields of treatments. It has been proposed to be a possible treatment in liver failure, aging, cancer and cardiovascular disease. It is a bit difficult to precisely detect exact amounts of lipoic acid, but it has been thoroughly characterized and it is synthesized in a variety of ways. Lipoic acid is an essential antioxidant and with further research in this area it may prove even more vital in the battle against human disease.

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