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Lipoic Acid*: The Antioxidant Chameleon

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ABBREVIATIONS: (ABAP) 2,2'-azobis(2-amidopropane),(α-LA) α-lipoic acid, (DHAA) dihydroascorbic acid, (DHLA) dihydrolipoic acid, (GC) gas chromatography, (GC-MS) gas chromatography-mass spectroscopy, (GSH) glutathione, (HPLC) high performance liquid chromatography, (HPLC-EC) high performance liquid chromatography-electrochemical detection, (Ki) rate of inhibition, (lipDH) dihydrolipoyl dehyrdogenase, (PMSR) peptide methionine sulfoxide reductase

*The term lipoic acid will be used to denote both forms, α-lipoic acid and dihydrolipoic acid. α-LA refers only to the α-lipoic acid form and DHLA will be used to denote the reduced form.

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ABSTRACT: α-Lipoic acid, and its reduced form DHLA, act as potent antioxidants. α-LA contains a disulfide atom, located in a five-membered ring, and DHLA has a linear form with two thiol groups. They are versatile molecules that are capable of acting as antioxidant, metal chelator and have a synergeristic effect on other antioxidants. Lipoic acid has a many functions in the cell, it is an integral part of several multi-enzyme complexes, a radical scavenger, metal chelator, vitamin C and GSH regenerator and may also inhibit gene transcription. It has a wide variety of treatment possiblities, ranging from heavy metal intoxication to HIV.

INTRODUCTION

α-Lipoic acid has several names; thioctic acid, 1,2-dithiolane-3-penanoic acid, 1,2-dithiolane-3-valeric acid and 6,8-thioctic acid [11]. Lipoic acid is naturally-occuring and is not classified as a vitamin since it is produced by most cells. Endogenous lipoic acid is generally protein-bound as a part of several multienyzyme complexes, such as the pyruvate dehydrogenase complex, α-ketoglutarate dehydrogenase complex and the glycine cleavage system. Mammalian tissues contain 5-25 nmol/g of lipoic acid, but practically all of this is found in the protein-bound form [1]. There is very little free lipoic acid found in the cell unless it has been supplemented.

PHYSICAL AND CHEMICAL CHARACTERISTICS

α-LA contains a five-membered ring, which contains two sulfur atoms and a carboxylic acid group. The molecule has one chiral center, but naturally-occuring α-LA is only found in the R-form. α-LA, when coupled to its different complexes *via* an amide linkage to a lysine, is referred to as a lipoyl group.

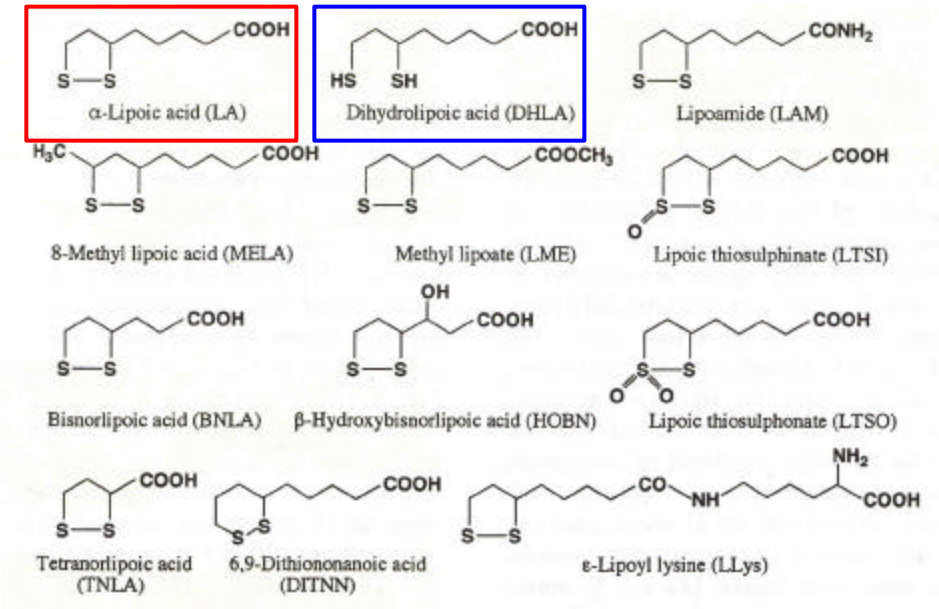

Figure 1. The structure of α-LA, dihydrolipoic acid (its reduced form) and some of its various oxidation products [6].

α-LA is completely insoluble in water, but is soluble in some organic solvents such as methanol and ethyl ether. Other physical and chemical characteristics of both

α-LA and DHLA are listed in Table I. The high pKa for the thiol groups of DHLA indicate that the molecule is a strong nucleophile, even more so than GSH which has a pKa of 9.45.

	a-Lipoic acid	Dihydrolipoic acid
Molecular Weight (g/mol)	206.35	208.35
Melting Point (°C)	60-62 / 43-49*	---
Boiling Point (°C)	---	180
pKa (COOH)	5.3 / 4.76*	4.85
pKa (SH)	---	10.7
Appearance	yellow crystals	Yellow liquid
Chemical Abstracts No.	57828-26-9	7516-48-5
l_{max} **–UV (nm)**	333	---

*Different values reported.

Table I. Characteristics of α-LA and DHLA [3].

α-LA can be reduced to form DHLA. The reduction is done synthetically by $NaBH_4$ and done inside the cell by an enzyme called dihydrolipoyl dehydrogenase (lipDH). LipDH is located in the mitochondria of the cell, and utilizes NADH to provide the electrons for the reduction. Free α-LA is rapidly reduced inside the cell and its reduced form contributes greatly to its antioxidant capabilities. α-LA and DHLA form a two-electron redox couple with a potential of -0.32 V [11].

DHLA, $2H^+$ / α-LA $E°=-0.32$ V **Equation 1**

DETERMINATION

There are many possible methods that can be used for determination of α-LA and DHLA, including the following: microbiological assay, colorimetric assay, GC, GC-MS, HPLC, capillary electrophoresis, enzyme immunoassay and enzyme cycling assay [6]. HPLC-EC is one of the few methods that allow the determination of unbound α-LA and DHLA independently. Most methods determine total lipoic acid, including bound, free, reduced and oxidized form.

b-OXIDATION

The side chain of α-LA containing the carboxylic acid group is prone to β-oxidation. The principal products of this oxidation are bisnorlipoic acid, tetranorlipoic acid, β-hydroxy bisnorlipoic acid and carbon dioxide. These and other β-oxidation products are shown in Figure 1. DHLA causes a reduction in the amount of β-oxidation production, possibly due to the presence of two thiols in the molecule.

ANTIOXIDANT PROPERTIES

Several things need to be considered when evaluating the value of an antioxidant: free radical quenching, ability to chelate metals, interactions with other antioxidants, bioavailability and cell concentration, any effects on gene expression and whether the molecule is located in the membrane or aqueous phase.

Lipoic acid is an inhibitor of xanthine oxidase. A plot of xanthine oxidase activity versus xanthine concentration indicates that lipoic acid works by competing with the substrate. This causes an inhibition of the enzyme since its active site. The inhibition constant (Ki) for this competition is 1.66 (±0.20) mM [1]. It is unknown how much this inhibition contributes to the antioxidant effect of lipoic acid.

α-LA and DHLA can act as antioxidants against a variety of radicals, including $^{\cdot}OH$, $O_2^{\cdot-}$, $LOO^{\cdot}$, $^{\cdot}NO$ and HOCl. It is also known to react with $^1O_2$ and H_2O_2. Table II lists most of the radicals that α-LA and DHLA are known to scavenge.

Oxidant/Radical	Scavenged by a-LA		Scavenged by DHLA	
$O_2^{\cdot-}$	No	---	Yes	($k=3.3\text{-}7.3\times10^5$ $M^{-1}s^{-1}$)
H_2O_2	No	---	No	---
$^{\cdot}OH$	Yes	($k=1.92\text{-}4.71\times10^{10}$ $M^{-1}s^{-1}$)	Yes*	---
HOCl	Yes	---	Yes	---
$CCl_3O_2^{\cdot}$	Yes	($k=1\text{-}1.8\times10^8$ $M^{-1}s^{-1}$)	Yes	($k=2.7\times10^7$ $M^{-1}s^{-1}$)
ONOO-	Yes	---	Yes	---
ABAP	No	---	Yes	---
$LOO^{\cdot}$	No*	---	Yes	---
$^1O_2$	Yes	($k=1\times10^8$ $M^{-1}s^{-1}$)	Yes*	($k=5.7\times10^5$ $M^{-1}s^{-1}$)

*Conflicting results were reported by different scientists.

Table II. Radical species scavenged by α-LA and DHLA[2,11].

Lipoic acid is also able to chelate a variety of transition metals (Table III). DHLA chelates Fe^{3+} more effectively than Fe^{2+}. Metal chelation can lead to antioxidant effects or prooxidant effects. Vitamin C chelates Fe^{2+}, but instead of reducing the negative effects of iron, it increases them. α-LA competes with vitamin C for iron, thereby reducing the prooxidant effects of ascorbate. DHLA chelates Fe^{2+} more efficiently than Fe^{3+}, which causes a reduction in the relative level of Fe^{3+}. In this case it acts as a prooxidant, not an antioxidant.

Metal	a-LA	DHLA
Pb^{2+}	√	√
Cu^{2+}	√	√
Zn^{2+}	√	√
Mn^{2+}	√	
Cd^{2+}	√	
Co^{2+}		√
Hg^{2+}		√
Fe^{3+}/Fe_{2+}	√	√
Ni^{2+}		√

Table III. Transition metals chelated by α-LA and DHLA [2].

One of the most important properties of α-LA is its ability to regenerate vitamin C, GSH and indirectly vitamin E. Vitamin E is an antioxidant that resides in the lipid bilayer of the cell membrane. Its purpose is to stop the chain reaction begun by lipid radicals. Vitamin C then reacts with the α-tocpherol radical, thereby removing the radical from the lipid bilayer and moving it into the cytosol where it can be dealt with by resident enzymes (Figure 2).

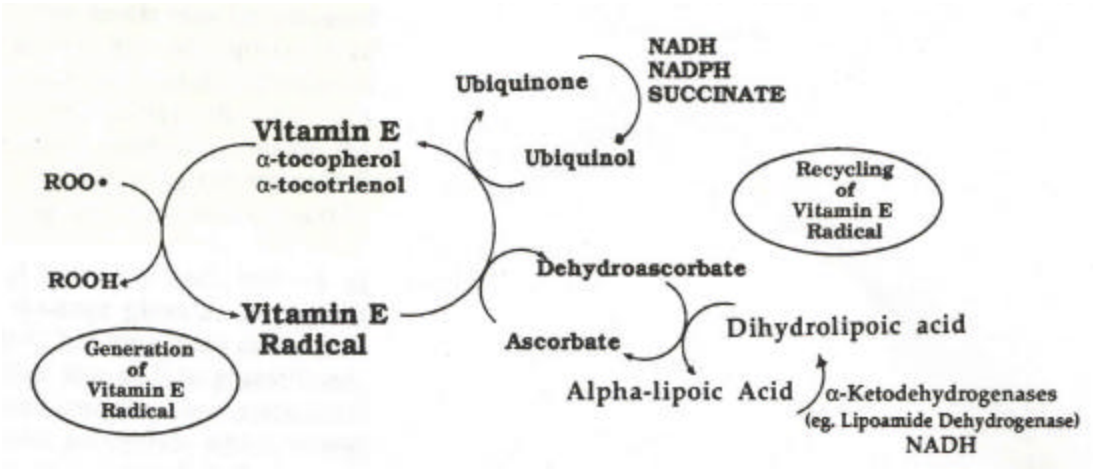

Figure 2. Recycling of vitamin E [10].

$$2 \text{ vit } E^{\bullet} + \text{vit C} \rightarrow 2 \text{ vit E} + \text{DHAA} \qquad \textbf{Equation 2 [2]}$$

GSH can also regenerate vitamin E, forming GSSG which can then be reduced by glutathione reductase back to GSH.

$$2 \text{ vit } E^{\bullet} + 2 \text{ GSH} \rightarrow 2 \text{ vit E} + \text{GSSG} \qquad \textbf{Equation 3 [2]}$$

$$\text{NADPH} + \text{GSSG} \rightarrow \text{NADP}^{+} + 2 \text{ GSH (glutathione reductase)}$$

DHLA is able to regenerate both vitamin C and GSH without the use of enzymes. This allows the indirect regeneration of vitamin E, helping to prevent lipid peroxidation.

$$\text{DHLA} + \text{GSSG} \rightarrow \alpha\text{-LA} + 2 \text{ GSH} \qquad \textbf{Equation 4 [2]}$$

$$\text{DHLA} + \text{DHAA} \rightarrow \alpha\text{-LA} + \text{vit C}$$

GSH is also able to regenerate vitamin C, but DHLA shows greater reactivity towards it (Table IV), so at specific concentrations, DHLA will regenerate vitamin C preferentially.

Reducing Agent	k (M / min)
GSH	32.8
DHLA	875

Table IV. Regeneration rate constants for vitamin C.

DHLA is also known to assist in protein repair. Oxidized methionine residues are repaired by an enzyme called PMSR, which requires reduced thioredoxin to supply

the electrons for the reaction. DHLA can increase the levels of reduced thioredoxin in the cell.

α-LA and DHLA both seem to have several different antioxidant properties, but DHLA also displays some prooxidant characteristics. DHLA increase the relative amount of Fe^{2+} available in the cell by chelating and reducing Fe^{3+} to Fe^{2+}, which can then go on and react with O_2 to form $O_2^{\bullet -}$. DHLA and α-LA can also act as prooxidants in certain cases when the product formed in the radical reaction is more reactive than the original radical.

BIOLOGICAL IMPACT

Lipoic acid has implications in a wide variety of human conditions. It can be used to treat the following disease: alcoholic liver disease, mushroom poisoning, diabetes, glaucoma, radiation injury, Chagas disease, neurodegenerative diseases, ischemia-reperfusion injury, heavy metal poisoning and HIV. The majority of lipoic acid's effect is due to its antioxidant properties and its effect on other antioxidants, but it is also known to affect proteins and enzymes. As mentioned above, α-LA inhibits xanthine oxidase, but it also decreases the activity of NF-κB. NF-κB is a transcription that regulates gene expression of proteins responsible for inflammatory response and also seems to be involved HIV gene replication. The DNA binding activity of NF-κB is augmented by DHLA but decreased by α-LA.

Diabetes produces a vitamin C condition that can be considered a state of "microscurvy". Glucose and vitamin C use the same carriers to enter most cells, so in diabetic patients, the glucose tends to overwhelm the carriers, reducing the amount of vitamin C that makes it into the cells. Lipoic acid can counteract this by acting as an antioxidant in general, regenerating vitamin E and the vitamin C that is available in the cell (Figure 3).

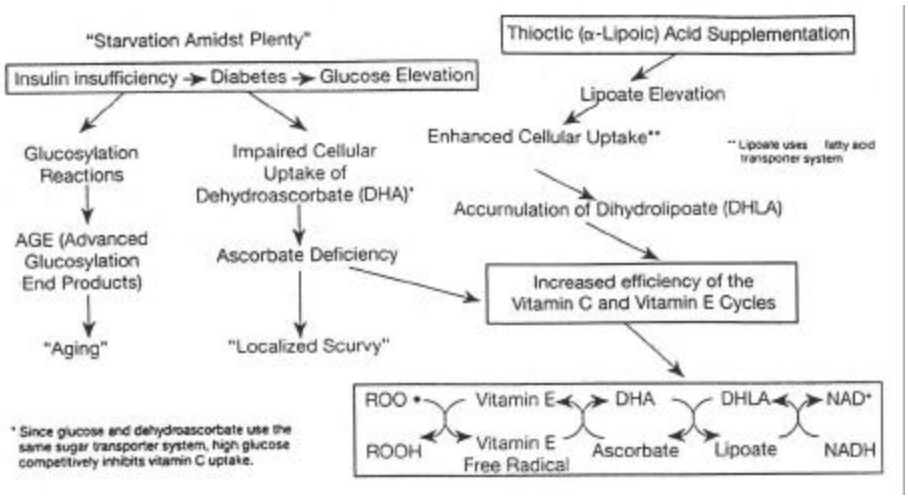

Figure 3. Diagram describing how lipoic acid can be used in diabetes treatment.

It also helps to reduce the prooxidant effect of vitamin C. I t has been shown in animal models that supplementation with lipoic acid can counteract all the effects of scurvy in vitamin C deficient animals [2,11].

CONCLUSION

Lipoic acid is an incredibly diverse antioxidant which is able to function in the membrane or aqueous state, with great promise for treatment of multiple medical problems. It was approved in Germany for the treatment of liver cirrhosis and heavy metal intoxication in 1966 and more recently for use on diabetic neuropathy. There are no known side effects to date and the LD_{50} (the dose which is lethal to 50% of the group) for rats is 400-500 mg / kg. Free radicals are thought to play an important role in the aging process, because of this, keeping a sufficient amount of antioxidants available in the body is important for good health. From the information available at this time, lipoic acid would seem to be a great choice to be part of an antioxidant supplement regime. At this time though, it is not as readily available as other antioxidants such as vitamin C and E, as more research is performed this may change.

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