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Understanding HNE : Chemistry, Biochemistry and Detection

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Abbreviations Used

GSH-glutathione
GST- glutathione-S-tranferase
HIV – Human Immunodeficiency Virus
HNE – 4-hydroxy-2-nonenal
HPLC – High performance liquid chromatography
LIF – Laser-induced fluorescence
MEKC – micellar electrokinetic chromatography
PUFA – polyunsaturated fatty acids
UV – Ultraviolet light

Outline

Abstract

In the presence of free radicals, lipids are peroxidized. One of the most cytotoxic products of lipid peroxidation is 4-hydroxy-2-nonenal (HNE). HNE has been studied extensively for its potential links to several neurodegenerative diseases such as Alzheimer's Disease and Parkinson Disease. HNE has been is a very reactive molecule that is capable of forming complexes with proteins, DNA, lipids and finally with antioxidants. As HNE modifies various biomolecules, it alters their function, causing harmful effects *in vivo*. Although HNE is very dangerous, it can be quenched by carosine and other molecules. There are several ways of detecting the amount of HNE in the system including immunohistochemistry and Electrospray Ionization Tandem Mass Spectrometry and HPLC.

Introduction

The presence of 4-hydroxy-2-nonenal (HNE) was first discovered in 1964 by Schaustein *et al* [1,2]. Although it was first thought to be hydroxyl-octenal, HNE began to be studied as one of the major products of polyunsaturated fatty acid (PUFA) autoxidation. A significant increase in HNE study was spurred when studies of hydroxyalkenals in the late 1970s led to the discovery that HNE was the most cytotoxic aldehyde [3,4].

HNE is formed *in vivo* when a free radical reacts with PUFA in lipid bilayers. The toxicity of HNE is due to its ability to react with a number of biological molecules. High levels of HNE have been discovered in HIV patients suffering from encephalitis [1], and in patients with Alzheimer's Disease [5]. HNE plays a role in so many biological processes that it becomes necessary to understand the fundamental properties and reactivity of this molecule. This paper will investigate the formation, chemistry, biochemistry and methods of detecting HNE.

Chemistry

The HNE molecule belongs to a family called 4-hydroxyalkenals. These molecules have three functional groups that can participate in chemical reactions: the hydroxyl group, the aldehyde and the carbon-carbon double bond shown in **Figure 1**. The HNE molecule is an electrophile and the α,β-unsaturated carbonyl group can accept electrons in Michael additions [6]. The molecular formula of HNE is $C_9H_{16}O_2$ and its molecular weight is156.22. It is a colorless liquid and soluble in lipids and most organic solvents [4].

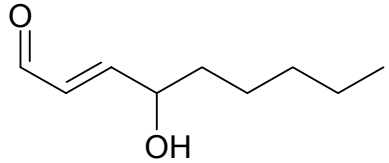

Figure 0. The highly reactive nature of HNE (shown here) is due to its three functional groups.

Formation

In nature, HNE is one of the products of oxygen radicals reacting with when ω-6 PUFA. The mechanisms for this reaction were suggested by Pryor *et al.* [7] and Carini *et al*. [8]. When an ω-6 PUFA (such as arachiodonic or linoleic acid) is peroxidized by oxygen radicals, a lipid hydroperoxide is formed. Lipid hydroperoxides can participate in Fenton reactions (in the presence of iron) creating a radical species. This radical can undergo β-cleavage (also known as a Hock cleavage in the graphic) producing the highly toxic HNE. This mechanism is shown in detail in **Figure 2.**

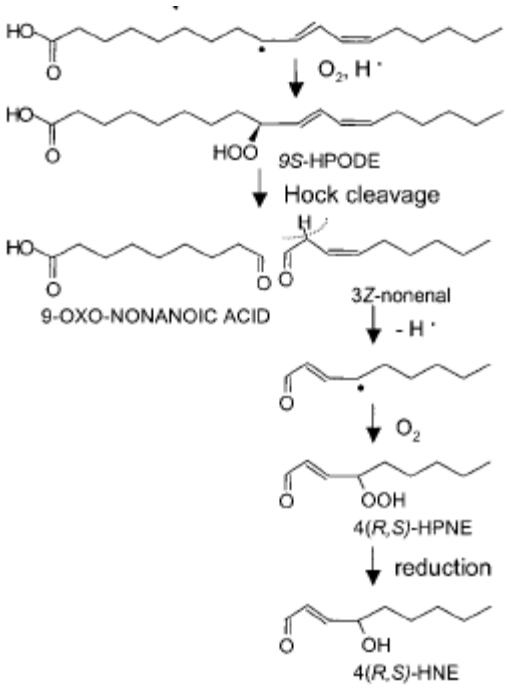

Figure 2. The reactions leading to the formation of HNE [8].

Synthetic preparation of HNE is performed in several ways. One method of synthesizing HNE is to react propynal with diethylacetal and then treating that product with $LiAlH_4$ at -20°C. This process gives a molar yield of 53% HNE. Newer methods using furan have been discovered which give HNE yields as high as 71% [9], however, it is difficult selectively produce a specific stereoisomer due to the chiral center on the fourth carbon [4].

Biochemistry

Biochemical reactions involving HNE reactions are vast, for HNE covalently binds to and thereby modifies many biological molecules. The complex chemistry of HNE has many

consequences in living systems. The mechanisms of four biochemical reactions involving HNE will be discussed: protein adducts, modification of DNA bases, enzyme activity, ascorbate binding.

The adduct formed when HNE binds to proteins can cause the protein to malfunction in the body [10]. Proteins are particularly at risk of reacting with HNE because HNE readily reacts with imidazole groups on histidine residues, thiol groups on cysteine residues and amine groups on lysine residues. Michael addition is the mechanism of reaction. The resulting molecule is a cyclic hemiacetal as shown in **Figure 3.** Of the three amino acid residues, cysteine is most reactive with HNE, then histidine and lysine is the least reactive [8]. These adducts are very stable and usually do not undergo any subsequent chemical reactions. When HNE and lysine react it creates a pyrrole complex which can form cross-linking adducts that fluoresce [11].

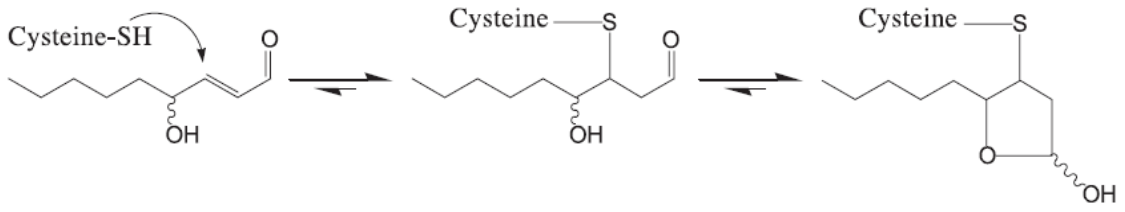

Figure 3. The thiol group on cysteine donates an electron to undergo Michael addition with HNE [7].

Nuclear DNA can also be affected by the presence of HNE. Nuclear DNA is protected by histones which contain 30-40% arginine and lysine and [12] which can form adducts with HNE. These lysine-HNE adducts alter the function of the residue, leaving the DNA with reduced protection from oxidative and other damaging radicals [13]. Deoxynucleotides can also react with HNE in the presence of oxygen to form long-chain adducts with damaging consequences [14]. These consequences include DNA recombination, base substitutions and frameshift mutations.

HNE naturally reacts with glutathione (GSH), although the enzymatically catalyzed reaction is hundreds of times faster [3]. The reaction of HNE with GSH is shown in **Figure 4** and its product is a stable cyclic hemiacetal similar to the adduct HNE forms with cysteine [17]. There are a number of glutathione-S-transferases (GSTs) in biological systems which catalyze the reduction of HNE by adding GSH covalently [15,16]. Carnosine, an β-alanyl-L-histidine-dipeptide, has also been studied for its natural ability to quench HNE giving two Michael addition products [17]. It did not reduce HNE as efficiently as GSH, however, it holds potential for attenuating the negative effects of HNE *in vivo*.

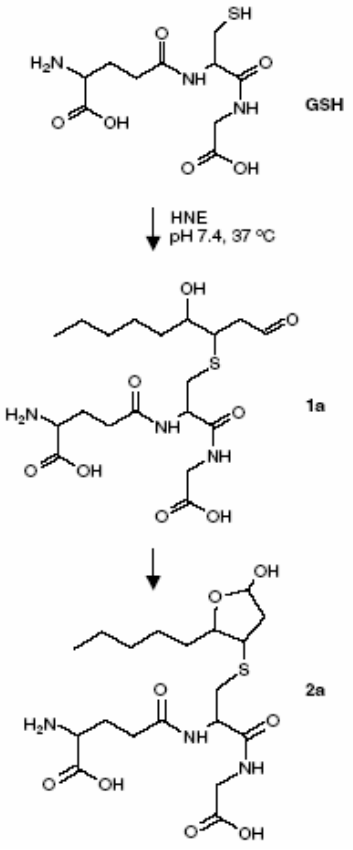

Figure 4. Glutathione (GSH) reacts with HNE at biological conditions to produce and intermediate which cyclizes to produce a cyclic hemiacetal.

Ascorbate is also susceptible to reactions with HNE. The ascorbyl-HNE conjugate (shown in **Figure 5**) is formed when ascorbic acid acts as a Michael Donor the electrophilic HNE. This process involves the addition of two electrons to form ascorbyl-HNE conjugate [18]. Notice that the carbon-carbon double bonds are key the Michael addition reaction. Ascorbate and HNE form a stable adduct which has two immediate consequences. One consequence is that the highly reactive HNE molecule is no longer able to damage other biomolecules, however, a secondary consequence is that an ascorbate molecule which could have been recycled to stop another oxidation process is now irreparably modified.

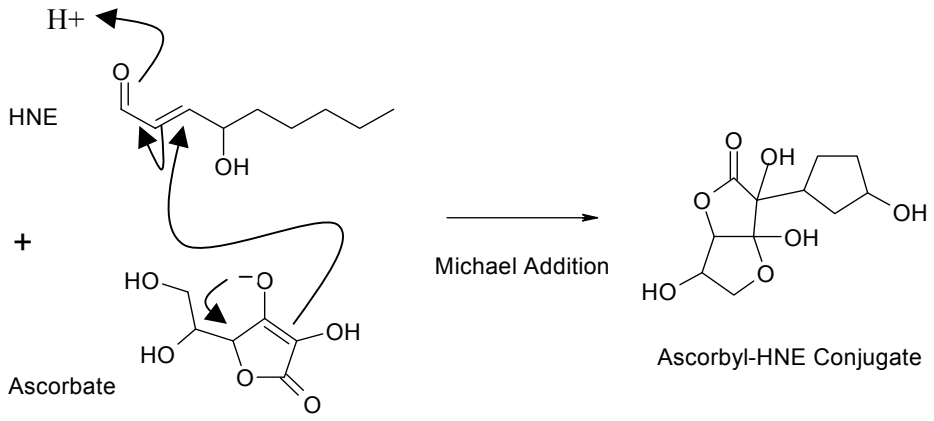

Figure 5. The HNE-ascorbyl conjugate is the result electron donation from ascorbate and the cyclization of the molecule [15].

Detection

There are several ways to detect the presence of HNE including gas chromatography (GC), high performance liquid chromatography (HPLC), immunohistochemistry, and mass spectroscopy. In HPLC testing, samples are extracted using a solvent such as ethyl acetate and HNE is quantified by UV detection at 220 nm. This method works best when HNE concentrations are greater than 2μM [4]. Immunohistochemistry is often used to identify HNE-protein adducts [19]. In this method, an organism, such as a rabbit, is used to produce an antibody to the modified protein adduct [20]. That antibody is then used stain HNE-protein adducts by binding to them in a given sample. This method is an accepted method of adduct quantification although certain antibodies have their own limits of specificity [21]. Mass spectrometry is also used to detect HNE in samples. Some laboratories have employed a gas chromatograph coupled with a mass spectrometer to make measurements of HNE reacting with dinitro-phenylhydrazones in samples [22]. Electrospray ionization techniques are also used to quantify HNE-protein adducts with the aid of mass spectrometry [23, 24].

One of the newest methods of detecting HNE in low concentrations is micellar electrokinetic chromatography (MEKC) separation and laser-induced fluorescence detection (LIF) [25]. This method can detect HNE in nanoliter volumes without sample purification or extraction. There is no need to purify or extract the samples because this method is based on creating micelles to solubilize samples. Laser induced fluorescence detection is one of the most common ways to quantify products of MEKC and uses the fluorescent agent dansylhydrazine (5-(dimethylamino)naphthalene-1-sulphonehydrazine, DNSH).

Conclusion

Much is known about the chemistry and biochemistry of HNE, however there is much more to learn. The presence of HNE is both an indicator of oxidative damage and a sign that forewarns of potential damage of other biological molecules. Many disease states are associated with increases in HNE and its biological adducts. The chemistry of HNE adducts is a vast field with many unanswered questions: for proteins, lipids and antioxidants are all potential targets of HNE damage. As technologies advance, it will be possible to detect smaller amounts and make more accurate measurements of HNE which may have applications in medical therapies and diagnostics. This is why it is important to seek more knowledge about HNE.

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