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**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**

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# Malondialdehyde (MDA), a lipid oxidation product

By

Suwimol Jetawattana

Department of Radiation Oncology

Free Radical and Radiation Biology

The University of Iowa

Iowa City, IA 52242-1181

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## Abbreviations:

**COX**: Cyclooxygenase; **ELISA**: enzyme-linked immunosorbent assay; **HHT**: 12-hydroxyheptadecatrienoate; **LDL**: low-density lipoprotein; **$M_1A$**: deoxyadenosine; **$M_1C$**: deoxycytidine; **$M_1dG$**: pyrimido[1,2-α]purin-10(3*H*)-one; **$M_1G$**: deoxyguanosine; **MDA**: malondialdehyde; **NMPI**: N-methyl-2-phenylindole; **$N^2$-OPdG**: $N^2$-propanodeoxyguanine; **$PGH_2$**: prostaglandin endoperoxide; **TBA**: thiobarbituric acid; **TBARS**: thiobarbituric acid reactive substance

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## Abstract

Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation; it can also be generated during prostaglandin biosynthesis in cells. MDA reacts with amino groups on proteins and other biomolecules to form a variety of adducts, including adducts with DNA bases that are mutagenic and possibly carcinogenic. Increased levels of lipid peroxidation products, by measurement of MDA, have been associated with various conditions and pathological states of diseases. Therefore, it is important to understand the characteristics and function of MDA as well as the effective techniques of measurement for using MDA as a tool in clinical monitoring of the patient.

## Introduction

Malondialdehyde, MDA, is a highly reactive three carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation [1] and also during arachidonic acid metabolism for the synthesis of prostaglandins [2]. MDA can combine with several functional groups on molecules including proteins, lipoproteins, RNA and DNA [3]. The monitoring of MDA levels in biological materials can be used as an important indicator of lipid peroxidation *in vitro* and *in vivo* for various diseases. This will mainly focus on MDA chemistry, biochemistry, routes of formation, detection, and biological health aspects.

## Routes of formation

Malondialdehyde is a naturally occurring product of lipid peroxidation. Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds, which include reactive carbonyl compounds, including MDA. MDA can be generated during cyclooxygenase (COX) catalysis in human platelets, formation from prostaglandin endoperoxide ($PGH_2$), catalyzed by thromboxane synthase [4] and in liver cells [5] by breakdown of $PGH_2$, **Figures 1 and 2**.

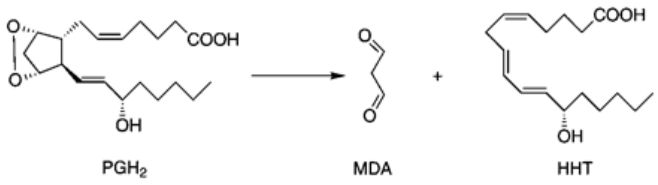



**Figure 1.** Conversion of prostaglandin endoperoxide ($PGH_2$) to 12-hydroxyheptadecatrienoate (HHT) and malondialdehyde (MDA) [5].

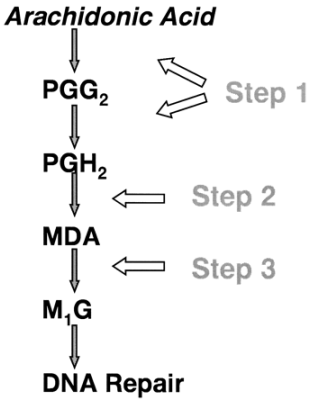



**Figure 2.** Conversion of arachidonic acid to prostaglandin endoperoxides ($PGG_2$) resulting in formation of MDA and potentially $M_1G$ adducts. Step 1 incorporates both oxygenase and peroxidase activities of the cyclooxygenase (COX) enzyme. Step 2 represents breakdown of $PGH_2$ to MDA and hydroxyheptadecatrieonic acid (HHT), which can occur spontaneously or *via* catalysis by thromboxane synthases or other cytochromes P450. Step 3 is one possible mechanism of formation of the $M_1G$ adduct [6].

## Chemical and biological properties

MDA is also called malonaldehyde or *bis*(dimethyl acetal). It has the molecular formula $C_7H_{16}O_4$ and molecular weight 164.2. The boiling point is 183$^oC$, and the freezing point is 130$^oC$[1]. It was suggested that the bulk of MDA in human plasma is bound to protein; this would explain the very low levels of MDA in plasma as measured under standard assay conditions [7].

In addition to biological properties, DNA-protein cross-links are another result of the reaction between DNA and MDA. It has been reported that MDA reacts with DNA bases to form a series of adducts such as deoxyadenosine ($M_1A$), and deoxycytidine ($M_1C$) and deoxyguanosine ($M_1G$) [8]. The major adduct is a pyrimidopurinone, abbreviated $M_1G$ or $M_1dG$. This adduct possesses a blocked Watson-Crick base-pairing region that has been shown to be mutagenic [2, 8]. Another work reported that it is the first endogenous DNA lesion found to be a target of nucleotide excision repair enzymes, which might be a major endogenous DNA adduct that significantly contributes to cancer [9]. Recently, it has been reported that MDA adducts produced in mammalian cells may block enzyme RNA polymeraseII translocation and be subject to removal from DNA by transcription-coupled repair. $M_1dG$ formation results in two

[1] http://www.4adi.com/data/protox/mda51.html; accessed on January 25, 2005.

chemically distinct DNA adducts, **Figure 3.** Both $M_1dG$ and $N^2$-OPdG are highly mutagenic, inducing both frameshifts and base substitution, in bacteria and mammalian cells. Therefore, the efficient repair of MDA adducts is essential for genomic stability [10].

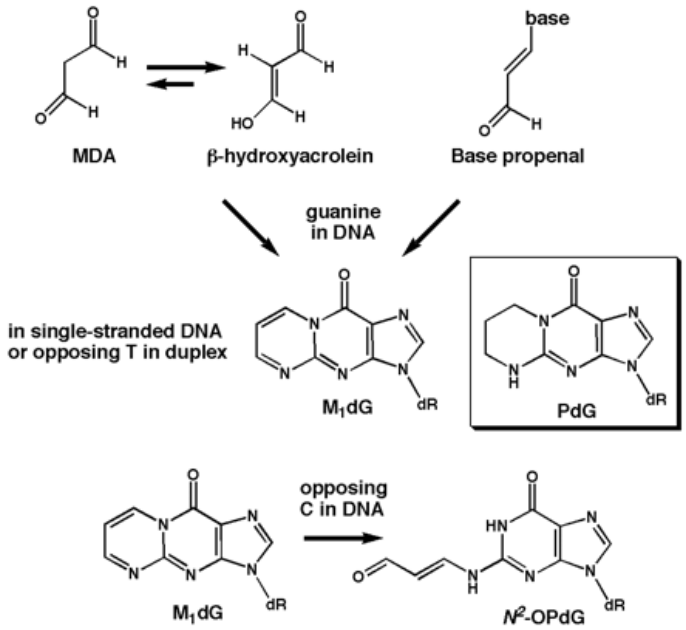



**Figure 3.** Formation of $M_1dG$ and the structures of $M_1dG$ and PdG (A synthetic, saturated analog of $M_1dG$). MDA and its tautomer β-hydroxyacrolein react with guanine in DNA to form the pyrimidopurinone adduct ($M_1dG$). When $M_1dG$ correctly paired with cytosine in duplex DNA, $M_1dG$ undergoes a base-catalyzed conversion to an acyclic structure, $N^2$-OPdG, which is known to be highly mutagenic in bacteria and mammalian cells, inducing both frameshifts and base substitutions [10].

## Detection

Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues. The measurement of MDA, which is the most abundant of lipid peroxidation products, is a convenient and sensitive method for quantitative estimation of lipid peroxide concentration in many types of samples including drugs, food products and biological tissues from human and animal.

The most common method of measuring MDA is based on the reaction with thiobarbituric acid (TBA). The thiobarbituric acid reactive substances (TBARS) assay is the colorimetric method widely used for the detection of lipid peroxidation in biological materials. MDA is formed as a result of lipid peroxidation and reacts with thiobarbituric acid under high temperature (90-100$^{o}$C) and acidic condition. The reaction yields a pink MDA-TBA adduct, the product of 2 mol of TBA plus 1 mol of MDA, **Figure 4**. The colored complex can be extracted into organic solvents such as butanol and measured by fluorometry or spectrophotometry using wavelength 532 nm with an extinction coefficient $\varepsilon_{532} = 1.53 \times 10^5\ M^{-1}\ cm^{-1}$ [11].

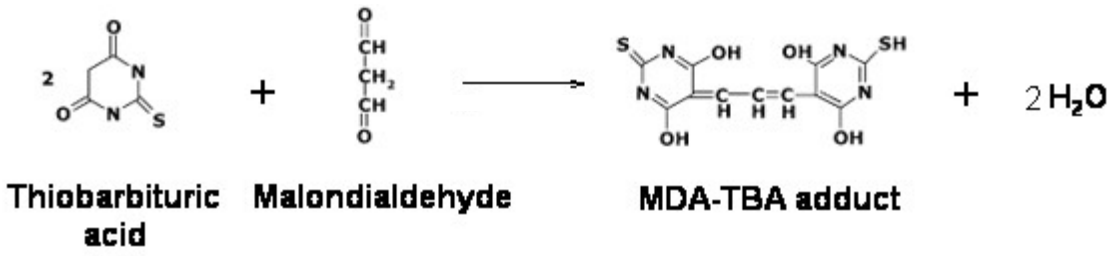
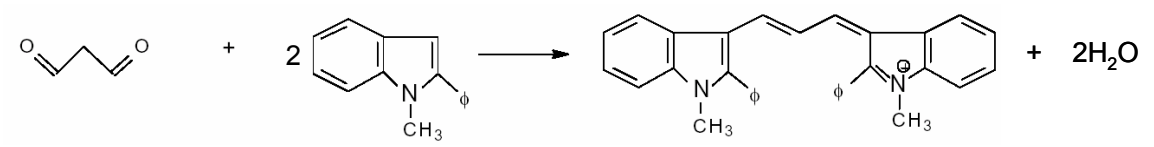



**Figure 4.** Thiobarbituric acid reaction [12].

Another method for the determination of MDA is based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole (NMPI), with MDA at 45°C. One molecule of MDA reacts with 2 molecules of NMPI to yield a stable carbocyanine dye, **Figure 5.** In HCl, the molar extinction coefficient at 586 nm for malondialdehyde is approximately $1.2 \times 10^5\ M^{-1}\ cm^{-1}$ [12].

a.




MDA NMPI carbocyanine dye

b.

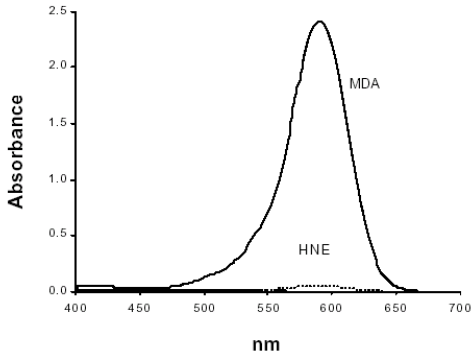



**Figure 5**. a. N-methyl-2-phenylindole (NMPI) reacts with malondialdehyde to form an intensely colored carbocyanine dye with a maximum absorption at 586 nm. b. Absorption spectra obtained from the reaction of NMPI with MDA or HNE in the presence of HCl [12].

Unfortunately the TBA assay is intrinsically non-specific for MDA as TBA also reacts with other compounds that may be present in biological samples and form colored species that can interfere with this assay. However, development of new analytical methods for the isolation and quantification of the MDA-TBA adduct by HPLC and mass spectrometry has been reported to improve the investigation of the TBARS reaction [13, 14].

Besides the TBA assay of MDA and other aldehydes, which has its limitations as a measure of lipid peroxidation *in vivo*, more specific assays to determine whether MDA-modified bases are formed in nucleic acids *in vivo* also had been reported. The monoclonal antibodies specific for malondialdehyde have been developed and characterized for use in sensitive immunoassays to detect MDA-DNA and MDA-RNA adducts by ELISA technique [3], western blot analysis and immunohistochemistry [15,16].

## Biological and health aspects

MDA can be found in most biological samples including foodstuffs, serum, plasma, tissues and urine, as a result of lipid peroxidation. The distribution of plasma lipid peroxides in men and women, determined by MDA, was found to be well approximated by a normal distribution. The median level increased by about 10% between 30 and 70 years of age in both sexes, which may be relevant to the increasing prevalence of atherosclerosis with age [17]. MDA has been reported to be induced in various conditions and chronic disease states such as smoking, hepatitis C infection, and HIV seropositive children and diabetes [18, 19, 20, 21]. It has also been reported that chondrocyte-derived lipid peroxidation product MDA mediates oxidation of cartilage collagens and leads to alteration of biochemical and biophysical properties of cartilage collagen fibrils, making them prone to degradation and initiating the changes observed in aging and osteoarthritis [22]. The determination of MDA-modified LDL was reported as a useful marker for identifying patients related with coronary heart diseases [23].

## Summary

Measurement of MDA has become one of the most widely used indicators for the purpose of estimating oxidative stress effects on lipids. The presence of MDA has been shown to relate to the pathophysiology of various human diseases and the assay of serum or plasma MDA make it an invaluable tool for the clinical management of these patients.

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