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Challenges on Determination of Malondialdehyde in Plant Samples

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Abstract

Malondialdehyde is a marker of lipid peroxidation and redox signaling and is used in many researches in the field of plant and biomedical investigations, possibly due to its simple measurement procedure. However, there are some challenges with its measurements which have been discussed in this communication along with possible solutions.

Keywords

Malondialdehyde, Oxidative stress, Signaling, Biomarker, Lipid peroxidation

Introduction

Malondialdehyde (MDA) is used as a marker of lipid peroxidation and redox signaling in the field of plant physiology and is one of the commonly used biomarkers of oxidative stress in biomedical and animal studies [1-3]. Beside some pitfalls of MDA determination, it is interesting that the number of publications retrieved from Scopus using key words of "malondialdehyde" and "plant*" was increased from 9000 [4] to 13696 records (search date; 8th Aug 2020, Scopus database) with the numbers of 1304, 1574 and 1094 for years 2018, 2019 and 2020, respectively. The corresponding numbers of articles for key word of "malondialdehyde" are 4643, 5254 and 4028, in which 1525, 1579 and 1137 of the articles categorized in the subject area of "medicine", respectively for years 2018, 2019 and 2020. These figures reveal that MDA has an important position in the plant and/or biomedical investigations. The involvement of oxidative stress in many physiological and/or pathophysiological phenomena is a well-accepted subject in both plant and biomedical areas. However, the selection of a reliable biomarker for oxidative stress is still a challenging subject. The characteristics of an ideal biomarker were summarized in a recent paper [5] which most of them are not fulfilled by MDA. The main challenges of using MDA along with possible solutions were highlighted in this communication.

Challenges

MDA is a highly reactive substance produced from different reactions in the biological fluids [6]. Different analytical methods have been used for quantification of MDA in biomedical [6] and plant [4] samples. The methods, their advantages and disadvantages were reviewed in recent works [4,6] in which most of studies employed the spectrophotometric assay after derivatization. MDA is usually quantified using a simple spectrophotometric, spectrofluorometricand/or enzyme linked immunosorbent assay after derivatization with thiobarbituric acid (TBA) at a high temperature (90-100 °C) and in acidic solutions [7-9]. Derivatization at ~ 100 °C increases the possibility of MDA evaporation since its boiling point is 108 °C [8] and this could be a reason for low repeatability of analytical data and using reflux improves of the analytical results [10]. It has been shown that MDA human plasma levels span from 320 nM to 53797 nM for healthy people which is very wide range for normal values [6]. There are so many factors affecting the outcome of MDA measurement using TBA derivatization and spectroscopy of the adduct. The high temperature and low pH conditions are responsible for a part of poor reproducibility and repeatability of MDA assays which are critically reviewed in a previous report [6], a number of possible reasons for poor validation data of MDA measurements along with some possible solutions were also provided. On the other hand, TBA reacts with a number of aldehydes produced during lipid peroxidation which increases

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the complexity of MDA assays. In addition to aldehydes, TBA possesses cross-reactions with other biomolecules such as L-arginine, L-histidine, L-tyrosine, L-cystein, formaldehyde, acetaldehyde, propanal, sucrose, methylamine, aniline, 4-hexylresorcinol, N-methylpyrrole, indole, 4-aminoacetophenone, ethyl p-aminobenzoate, 4,4-sulfonyldianiline, p-nitroaniline, azulene, histamine, melatonin, serotonin, spermidine, amino sugars, collagen, water soluble proteins and glycogen [6].

There is a very similar situation in the biomedical and plant studies regarding the reliability of MDA as a valid biomarker. During last couple of years, a number of communications were published in various biomedical and analytical journals dealing with the shortcomings of MDA and its assay methods in such investigations [4-6,11-16]. In these communications, attempts were made to gather and represent scientific evidences on the non-reliability of the reported MDA data in various biological samples investigated on different diseases and the corresponding control groups. One could find lots of variations in control groups even using a single analytical method [13]. The problems with MDA assay were simply ignored by the research groups as stated by Wade and van Rij [12]. Most of shortcomings of MDA and its assay methods in plant samples are the same as those in biomedical areas and correctly addressed in a recent communication by Morales and Munné-Bosch [4].

Potential Solutions

Some improvements could be achieved by using more suited derivatization reagents [17]. As noticed above, MDA is a highly reactive compound and reacts with lots of existing materials in the biological fluids [6] resulting in many derivatized compounds with similar spectroscopic characteristics. Employing separation techniques (such as chromatography) provide more reliable data for MDA since other derivatives could be separated in the chromatographic column [18,19]. However, the problems associated with high reactivity and chemical stability of MDA along with the variations observed in derivatization step will be remained unresolved [5]. Employing analysis of MDA without derivatization step, as an example with GC-MS, is recommended if the valid data could be obtained [19]. It should be noted that the accuracy and precision of chromatographic methods [17,20] are also relatively out of accepted range recommended by Food and Drug Administration guidelines [21]. Concerning these sets of analytical methods, there are still some concerns on the stability of MDA in mid-term and long-term storage of the samples. Considering technical troubles with derivatization and quantification of MDA, we focused on development of electrochemical sensors for real time analysis of MDA in some biological samples [22-25] and further studies are still ongoing in our and others research groups [26-29]. In the electrochemical methods, no derivatization is required, however one should consider low repeatability of these methods and variations on the electrodes prepared in different batches which limits their practical applications.

Conclusion

Quantification of MDA is still a challenging subject in the field of bioanalysis and further efforts are in demand to provide a fully acceptable and validated analytical method. In addition to the recommendations of Morales and Munné-Bosch, it is recommended that before using an analytical method for determination of any analyte of interest in a given sample, a full validation (or at least partial validation) tests on the quality control samples are critically required. These tests could be found from Food and Drug Administration or International Council on Harmonization guidelines which are readily available from internet. These validation tests are especially recommended for MDA since it is a highly reactive analyte. It is obvious that, ignoring this simple fact will result in some misleading data, waste of time and resources.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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