

Substantial reaction between histamine and malondialdehyde: A new observation of carbonyl stress

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Abstract

OBJECTIVES: To explore the mechanism of carbonyl stress-related toxification on neurotransmitter histamine and the potential de-carbonylation function of histamine. **METHODS:** The reaction mixture of malondialdehyde (MDA) and histamine (HA) at pH7.4, 37°C was assayed by high performance liquid chromatography (HPLC), spectrophotometry and spectrofluorometry. The reaction products were assayed by LC/MS. **RESULTS:** In physiological condition, the reaction of MDA and HA yielded two products: a nonfluorescent enamine derivatives and a fluorescent 1, 4-dihydropyridine adducts. The fluorescence maxima of the fluorescent products (Ex. 393nm / Em. 464nm) were similar to those of lipofuscin pigment. The fluorescence intensity of reaction mixture was in direct proportion to the MDA concentration. **CONCLUSIONS:** HA can react with MDA to form stable products, a non-fluorescent enamine (product 1) and a fluorescent 1,4-dihydropyridine (product 2) which are ceroid/lipofuscin-related adducts. The reaction of HA with MDA may reveal toxic effect of unsaturated carbonyls in the brain and may reflect a novel de-carbonylation function of histamine under various pathological conditions.

Abbreviations:

AD - Alzheimer's disease
CNS - central nervous system
DAD - diode array detector
MDA - malondialdehyde
DS - Down's syndrome
HA - histamine
PUFA - polyunsaturated fatty acids
TIC - total ion current chromatogram
TMP - 1,1,3,3-tetramethoxypropane

Introduction

Lipid peroxidation is a complex series of reaction resulting in the oxidative fragmentation of polyunsaturated fatty acids and the production of reactive

and toxic α,β -unsaturated carbonyls. Such toxic carbonyl species have been shown to play a role in the patho-physiology of many acute and chronic diseases, such as neuronal degenerative diseases and diabetes-related vascular and renal failure [1-4]. MDA, as one of the most important intermediates of free radical damages, is a highly reactive unsaturated carbonyl that can target a wide range of primary amine-containing biological components to cause crosslinkages of biomaterials and to result in fluorescent complex. These reactions have been generally regarded as being involved in causing cellular deteriorations as well as in aging-related extracellular alterations, particularly in the formation of ceroid/lipofuscin-related pigments with character-

istic fluorescence [5, 6]. A large amount of data has indicated that reactive carbonyls are a key group of neurotoxic mediators of oxidative stress and glycation resulting in the progression of neurodegenerative diseases [6–8].

Histamine (β -imidazolylethylamine, HA) was discovered primarily as an important inflammatory factor in the pathological situations. Histamine-releasing neurons were found located mainly in the tuberomammillary nucleus of the hypothalamus, receiving a strong innervation. HA has been found later to be an important neuronal mediator involved in various physiological and pathological processes, including neurotransmission, secretion of pituitary hormones, regulation of gastrointestinal and circulatory functions, sleep-wake regulation and inflammatory responses [9–11].

As one of the four most important aminergic systems in the central nervous system (CNS), i.e. the serotonergic, dopaminergic, noradrenergic and histaminergic systems [10], histaminergic pathway was found in rat brain in 1970s [12]. A number of studies demonstrated that the central histaminergic system was substantially impaired by neurodegenerative diseases and aging. Histamine level, analogous to dopamine level, in the brains of patients with Alzheimer's disease (AD) and Down's syndrome (DS) tended to be diminished [13]. Such alteration of brain histamine level in neurodegenerative disease patients suggested the impairment of the histaminergic system in CNS disorders [14,15].

Histamine release was often found enhanced under extreme conditions such as inflammation, oxidative stress, dehydration, hypoglycemia or by other stressors [10]. It has also been reported that derivatives of polyunsaturated fatty acids (PUFA) induced by oxidative free radical damage could trigger mast cell histamine release. On the other hand, such phenomenon was associated with a significant increase of MDA content during stresses [16]. Whether there are some relationship between these two phenomena has not been carefully studied yet.

In this paper, we demonstrate that HA can react with MDA in supra-physiological conditions. These results may suggest a novel function of HA under patho-physiological stresses during neuronal degenerations.

Materials and methods

1. Materials and stock solutions

HA (purity>97.0%) was purchased from Sangon (Shanghai, China). 1,1,3,3-tetramethoxypropane (TMP) was obtained from Fluka Chemie AG (Buchs, Switzerland). The HA stock solution (10mM) was prepared with buffer. 27.8 mg HA was dissolved to 25 ml 0.1M sodium phosphate buffer (pH 7.4). A fresh MDA stock solution (10 mM) was prepared by hydrolyzing 1,1,3,3-tetramethoxypropane (TMP), which was modified according to a method described by Kikugawa et al. [5]. Thus, 0.085 ml (0.5 mmol) TMP was mixed with 1ml 1.0M HCl, and shaken at 40°C for about 2.5 min. After the TMP was fully hydrolyzed, the pH was adjusted to 7.4 with 6M NaOH, and the stock solution was finally made up to 50ml with 0.1M sodium phosphate buffer (pH 7.4). The stock was often checked by measuring absorbance at 267 nm using

ϵ MDA=31500. Other chemicals used were all of analytic grade from Bio-Rad (Shanghai, China).

2. Methods

2.1 Reactions of HA with MDA at different concentrations and pHs

HA (1.0 mM) incubated with MDA at different concentrations in 0.1 M sodium phosphate buffer pH 7.4, at 37°C. Reaction products were diluted 1/2400 in 0.1M sodium phosphate buffer pH 7.4 before spectrofluorometric measurements.

MDA (1.0 mM) incubated with HA at different concentrations in 0.1M sodium phosphate buffer pH 7.4, at 37°C. Reaction products were diluted 1/600 in 0.1M sodium phosphate buffer pH 7.4 before spectrofluorometric measurements.

HA (1.0 mM) was incubated with equi-molar MDA (1.0 mM) in 0.1M acetate buffer pH 4.1, pH 4.8, phosphate buffer pH 5.8, pH 7.4 and carbonate buffer pH 9.2 respectively. Reaction products were diluted 1/600 in corresponding buffer before spectrofluorometric measurements.

Incubated samples at different times were analyzed by HPLC and LC/MS.

2.2 HPLC analysis of the reaction mixture of HA and MDA

HPLC analysis was performed on a reverse-phase C18 analytical column (250 mm \times 4.6 mm, 10 μ m) on a WatersTM Alliance 2690 HPLC system with a model 996 photodiode array detector. The mobile phase consisted of solution A (0.1% trifluoroacetic acid in water) and solution B (acetonitrile, 100%). The flow rate was 0.7 ml/min and the elution gradient was as follows: 0–5min, 0–20% B; 5–10 min, 20–40% B.

2.3 Fluorescence analysis of the reaction mixture of HA and MDA

Fluorescence spectra of reaction products were characterized by utilizing a Perkin-Elmer LS 50 B spectrofluorimeter (Norwalk, USA).

2.4 LC/MS analysis of the reaction products of HA and MDA

The products were characterized by electrospray ionization mass-spectrometry (ESI MS). A Waters HPLC system (Waters Corporation, Milford, MA, USA) was equipped with in-line degasser AF, 600 pump, 600 controller, 2777C sample manager, and Micromass ZQ 4000 electrospray mass spectrometer (Manchester, U.K.). Masslynx 3.4 software (Micromass) was used for data acquisition and processing. An XTerraTM MS C18 column (3.9mm \times 150mm, 5 μ m, Milford, MA, USA) was used. A linear gradient elution of A (water) and B (acetonitrile) was used. The gradient elution was programmed as follows: 0–3 min, 0% B; 3–5 min, 0–15% B; 5–10 min, 15–40% B; 10–15 min, 40–45% B. The solvent flow rate was 1.0ml/min. The injection volume was 20 μ l. 20% eluent was allowed to flow into the mass spectrometry by solvent splitting. The ESI-MS spectra were acquired in the positive ion mode. The conditions of HPLC-MS anal-

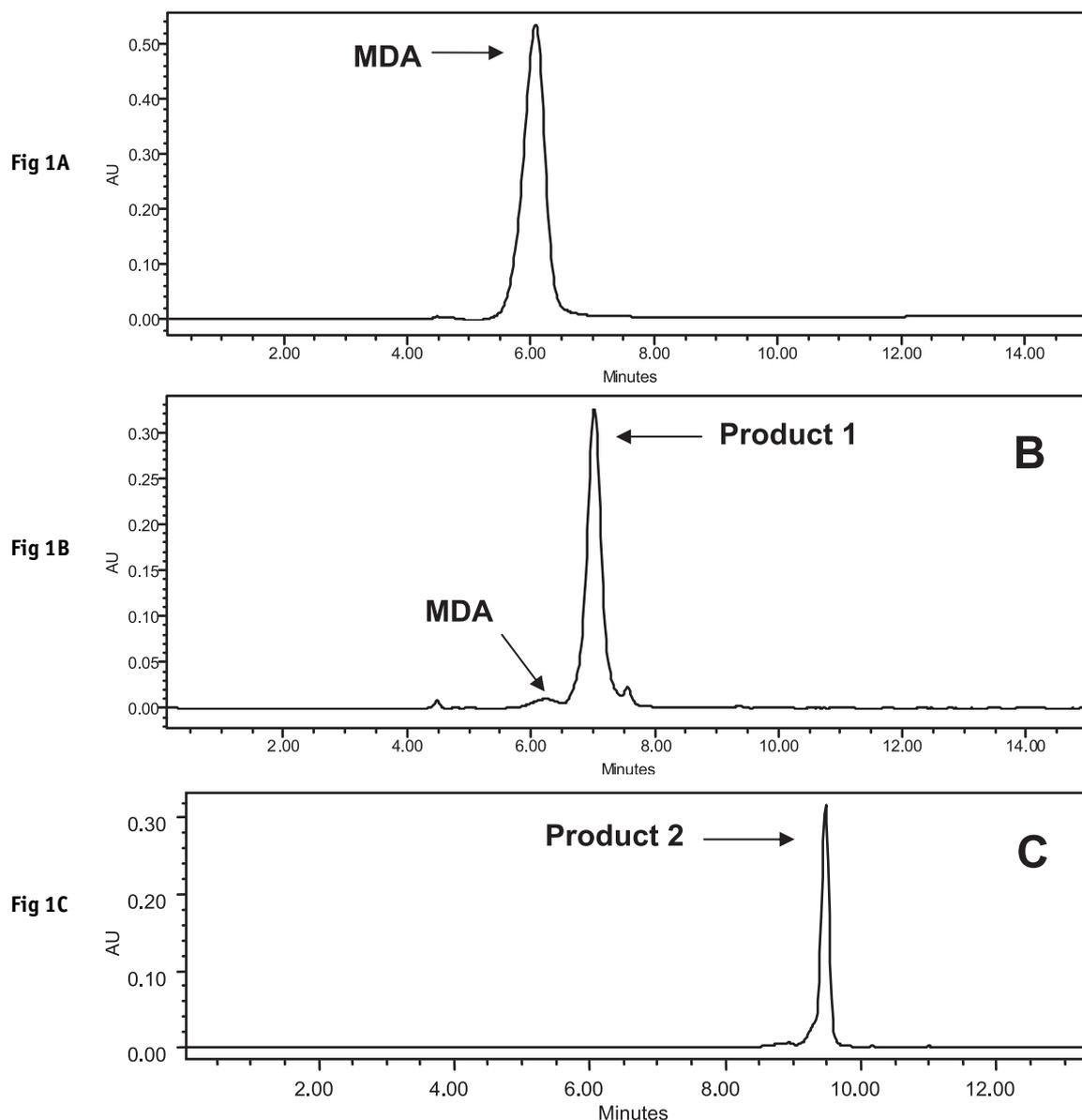


Figure 1. (A, B and C). HPLC analysis of the reaction between HA and MDA. HA (2.0mM) was incubated with MDA (1.0mM) in 0.1M sodium phosphate buffer pH 7.4, 37°C for 48h. Following HPLC separation the absorption of reaction mixture was measured at 243 (Fig.1A), 280 (Fig.1B) and 390nm (Fig.1C) respectively.

ysis were as follows: desolvation gas (N₂), flow rate 350l/h, cone gas 50l/h, desolvation temperature 350°C; the source temperature 105°C, capillary and cone voltages were 4000V and 180V, respectively.

Results

1. HPLC analysis of the HA+MDA reaction

After HA (2.0mM) was incubated with MDA (1.0mM) in 0.1M phosphate buffer pH 7.4, at 37°C for 48h, the reaction mixture was eluted at acidic pH through HPLC and estimated at 243, 280 and 390 nm. As is shown in Fig. 1, the retention time of MDA was seen at 6.2 min (Fig. 1A), while HA has no absorption at testing range of the instrument. One of the new products (product 1) was observed after incubation of HA and MDA with the retention time at 7min (Fig. 1B). The other new product (product 2) was observed with the retention time at

9.5 min (Fig. 1C). The product 1 did not show fluorescence, while the product 2 showed a stable lipofuscin-like blue (393/464 nm) fluorescence (Fig. 2).

The standard absorbance spectrum of MDA showed a peak at 265nm in a neutral condition, while in an acidic condition, its absorption peak was at 243 nm (Fig. 3). The UV-absorption spectrum showed that the maximum of product 1 was at 278.6 nm (Fig. 3). The UV-absorption spectrum of product 2 showed the maxima at 234.6, 261.9 and 390.0 nm (Fig. 3).

2. Effect of HA and MDA concentrations on HA/MDA reactions

The fluorescence yields of 1.0mM HA reacting with MDA in different concentrations are shown in Fig. 4. After 48 h increase, the fluorescence intensity reached a plateau (Fig. 4A) in direct proportion to the MDA concentration (Fig. 4B).

Figure 2. Fluorescence spectra of the product 2 obtained by incubation of HA and MDA for 48h.

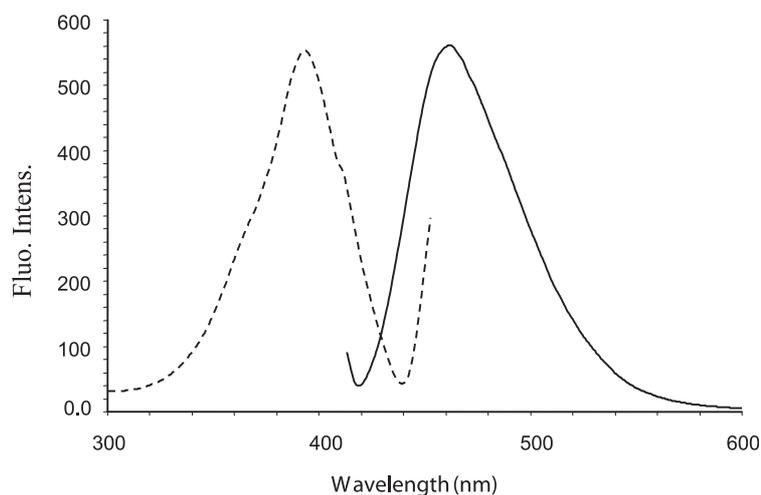
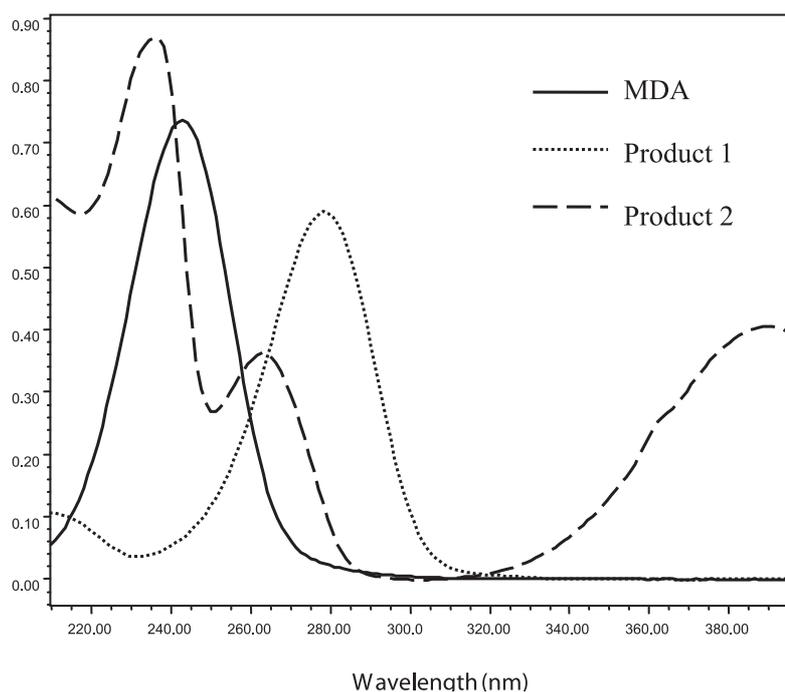


Figure 3. UV-absorption spectrum of HA+MDA products. The UV-absorption spectrum of MDA and HA+MDA products were obtained from HPLC with a model 996 photodiode array detector.



The fluorescence yields of 1.0mM MDA reacting with HA in different concentrations are shown in Fig. 5. The fluorescence intensity is in direct proportion to the HA concentration (Fig. 5A). After 48h, the fluorescence intensity produced from 2.0mM HA was analogous to that from 4.0mM HA (Fig. 5B).

3. pH effect on fluorescence intensity resulting from HA/MDA reaction

The effect of incubation pH on the fluorescence intensity of products of HA (1.0 mM) and MDA (1.0 mM) interaction is given in Fig. 6. The fluorescence intensity in the acidic conditions (pH 4.1, 4.8, 5.8) was greatly higher than that in neutral condition (pH 7.4) (Fig. 6). In basic condition (pH 9.2), the mixture of HA and MDA hardly showed any fluorescence (Fig. 6). The highest fluorescence intensity of the reaction products was at pH 4.8 (Fig. 6).

4. Identification of reaction products by LC/MS

LC/MS was employed to identify the reaction products. While the reaction mixture was incubated for over

2d, a total ion current chromatogram (TIC) (Fig. 7A) in comparison with a diode array detector (DAD) chromatogram showed the peaks of the products were at 2.63min (product 1) and 10.08min (product 2) (Fig. 7B). The mass spectra corresponding to the retention time of the product 1 showed three main peaks: m/z 166 $[MP1+H]^+$, m/z 138 $[MP1-CO+H]^+$ and m/z 95 respectively (Fig. 7C). The mass spectra corresponding to the retention time of product 2 showed three main peaks: m/z 246 $[MP2+H]^+$, m/z 218 $[MP2-CO+H]^+$ and m/z 190 $[MP2-2CO+H]^+$ respectively (Fig. 7D).

Discussion

The discovery of the important patho-physiological functions of histamine has a long history dating back to the beginning of the 20th century. Histamine has been found subsequently to be a chemical mediator in peripheral tissues in allergic diseases, in neuronal degenerative diseases and in sleep-wake regulation [11,17,18]. Since various stressors induce a release of histamine paralleling with MDA formation, we proposed a potential func-

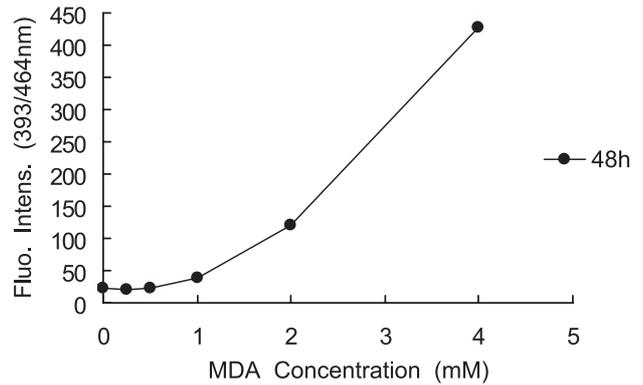
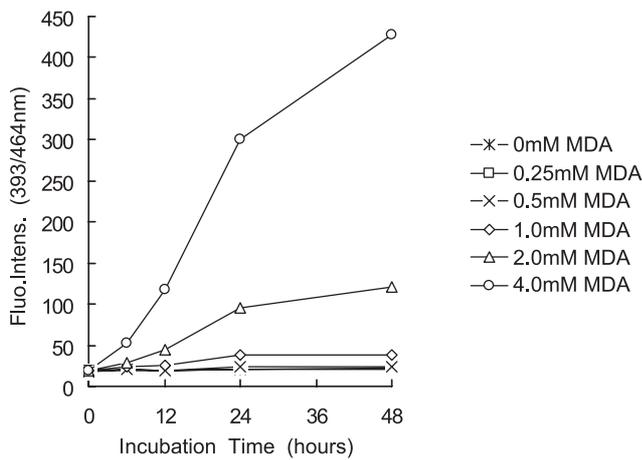


Figure 4 (A left and B right). Increase in fluorescence produced from reaction between HA and MDA. MDA (at indicated concentration) and HA (1.0mM) were incubated in 0.1M phosphate buffer (pH 7.4) solution at 37°C for 48h.

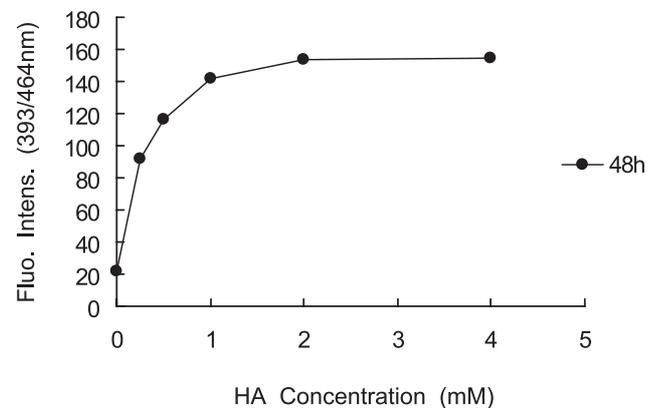
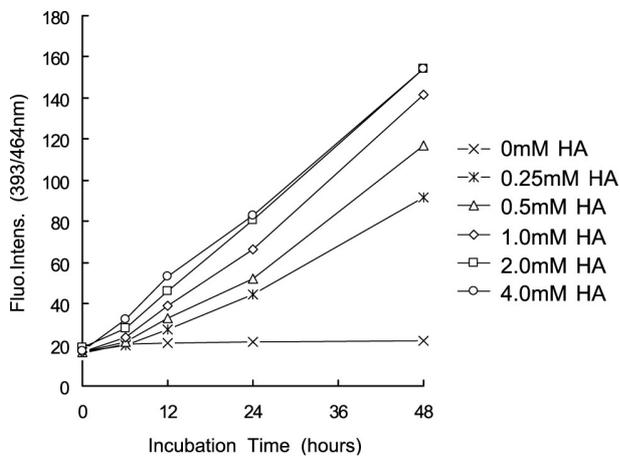


Figure 5 (A left and B right). Increase in fluorescence produced from reaction between HA and MDA. MDA (1.0mM) and HA (at indicated concentration) were incubated in 0.1M phosphate buffer (pH 7.4) solution at 37°C for 48h.

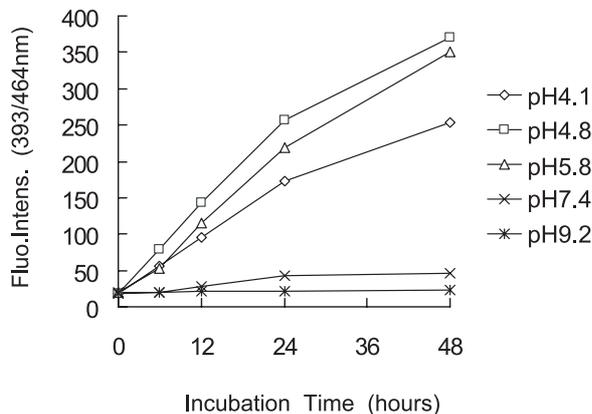


Figure 6. Effect of incubation pH on HA+MDA fluorescence formation. HA and MDA were incubated at 37°C in 0.1M phosphate buffer at pH 7.4 for 48h.

tion of histamine for de-carbonylation against the carbonyl stress-related toxification in crosslinking biological materials.

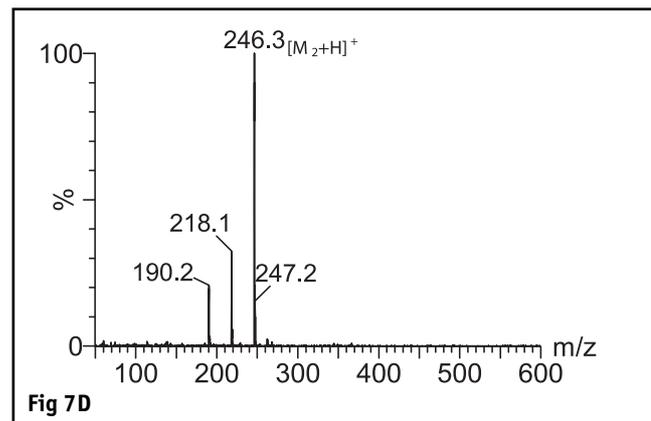
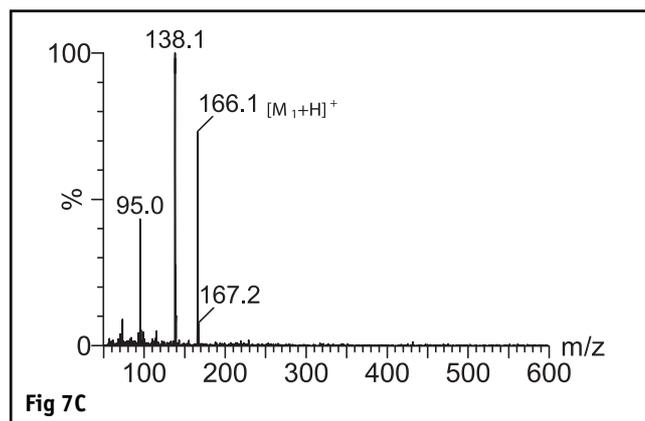
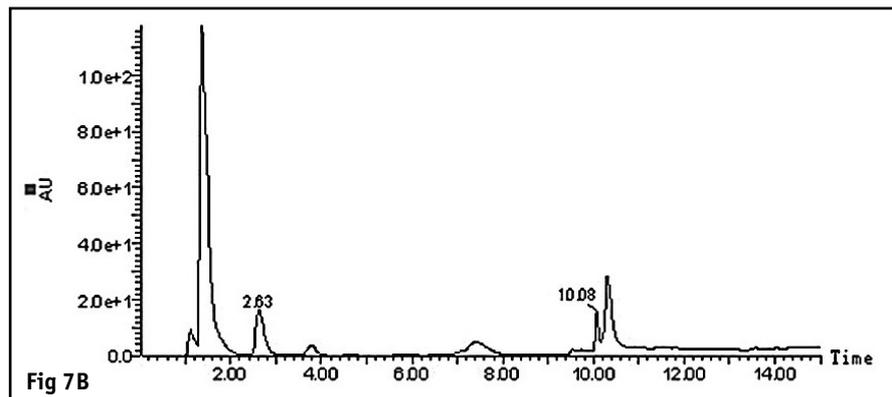
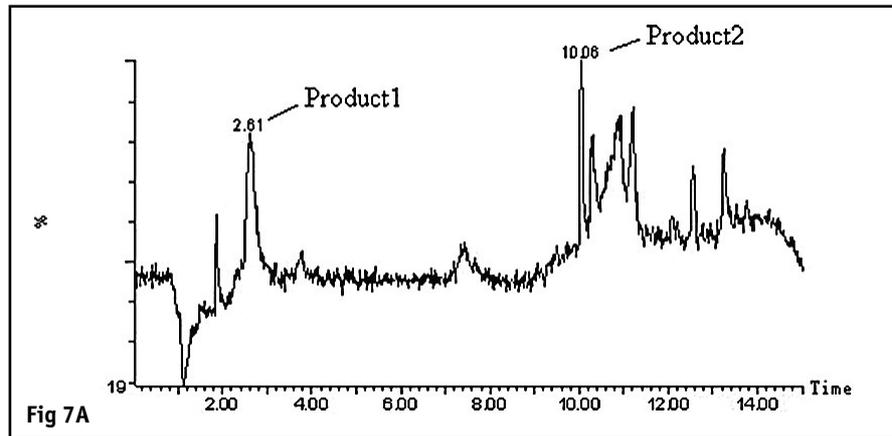
Considering the four major kinds of neurotransmitters in human brain, i.e. serotonin, norepinephrine, dopamine and histamine, all of which possess the carbonyl-reactable amino groups, it is important to study the MDA toxification on these neuroamines in a clear-cut model system [1,6]. Some of these neurotransmitters have been reported to be carbonylated by malondialdehyde in diseases and diminished in aged brain [19–21]. Such decrease of neuroamines is therefore proposed to be rel-

evant to the carbonyl stress at pathological conditions [21].

Our results clearly demonstrated for the first time that HA can also react with MDA in supra-physiological conditions, and form stable products. Isolated by HPLC and assayed by different techniques, two products were identified in this reaction: the nonfluorescent compound, product 1, and the fluorescent compound, product 2. According to the ion mass measured by LC/MS-ESI, the products 1 and 2 were proposed to be an enamine derivative and an 1, 4-dihydropyridine adduct respectively (Fig. 8). The proposed structure of products suggested that the

Figure 7 (A, B, C and D).
LC/MS analysis of reaction mixture after 2d incubation.

- (A) The total ion chromatogram.
(B) The diode array detector chromatogram.
(C) The mass spectra corresponding to the total ion chromatogram at retention time 2.61min.
(D) The mass spectra corresponding to the total ion chromatogram at retention time 10.06min.



molecular proportion of HA: MDA was 1:1 in the product 1, and 1:3 in the product 2. As high concentrations of MDA may form acetaldehyde under certain conditions [22,23], two molecules of MDA and one molecule of acetaldehyde were proposed to react with one molecule of HA to form dihydropyridine adducts as reported similarly in the literature [24,25]. Although our data showed that the reaction mechanism of MDA with HA was similar to that of with other neuroamines, like dopamine, the dominative products of 1.0 mM HA with 1.0 mM MDA was the non-fluorescent enamine [24,25].

The effect of HA and MDA concentrations on HA/MDA reactions showed that when reacting with 1.0 mM MDA, excessive HA failed to enhance the fluorescence yield (Fig. 5). In contrast, when reacting with 1.0 mM HA, high MDA concentration enhanced the fluorescence intensity progressively, which was most possibly owing to the crosslinking potential of MDA (Fig. 4). The produced fluorophores showed the typical ceroid/lipofus-

cin-like fluorescence (310–395nm/395–460nm), which implicated that HA could reduce toxic carbonyl compounds under certain conditions [6]. Such de-carbonylation reaction may also explain the increase of HA level in stresses accompanying with the formation of multifarious toxic unsaturated carbonyls. On the other hand, the results may also imply a possible mechanism of neuronal degeneration by carbonyl stress and ceroid/lipofuscin formation in brain-disordered patients.

The pH effects on fluorescence intensity have often been observed in relevant studies [6]. In neutral and alkaline conditions the predominant form of MDA is the enolate anion, whereas at acidic pH, MDA mainly exists in the tautomeric undissociated enol form as β -hydroxy acrolein, which may hinder the reactivity of this molecule [1]. In our study, the highest fluorescence intensity of the reaction products was found at pH 4.8, while in basic condition, the reaction mixture of HA and MDA hardly showed any fluorescence.

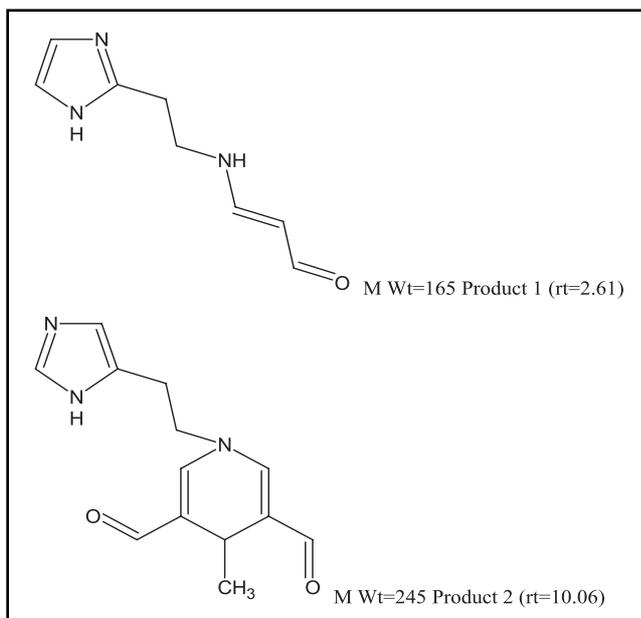


Figure 8. Proposed product structures of HA+MDA reaction.

We have proposed before that de-carbonylation may be a possible 'anti-aging' process during sleep [26,27]. Here, we further propose that carbonylation may be part of fatigue biochemistry in CNS. In regard to a possible reversion of carbonylation during rest and sleep (cleaning of Schiff's base formed during oxidative/glycation stresses), we suggest that the enhanced histamine level and activities should thus be detected and explained following the process of wake-up. Such biochemistry may have a general validity in explaining the fluctuations of performance of neuroamines in the CNS fatigue and sleep biochemistry.

In summary, our study demonstrated that HA reacted with MDA to form stable conjugated products, which can be either non-fluorescent or fluorescent. The reaction of HA with MDA suggested a toxic effect of carbonyl stress on neuroamines in the brain and it may, on the other hand, reflect a novel de-carbonylation function of histamine in patho-physiological situations of inflammatory tissues.

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