Monitoring of methylglyoxal/indole interaction by ATR-FTIR spectroscopy and qTOF/MS/MS analysis

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ABSTRACT

Sugar derived reactive 1,2-dicarbonyl intermediates are considered important precursors for the formation of Maillard reaction products. Efficient strategies are needed to modulate their formation in food. Indole, a major thermal degradation product of tryptophan, has been shown to scavenge such 1,2-dicarbonyls at high temperatures. In this study, the trapping of methylglyoxal by indole was monitored at various temperatures either by (a) ATR-FTIR spectroscopy or (b) in-solution using qTOF/MS/MS analysis. Information obtained through these studies have indicated that even at room temperature indole can quickly react with methylglyoxal forming an adduct as confirmed by the emergence of a new peak at 1729 cm\(^{-1}\) and by qTOF/MS/MS analysis. On the open surface of the ATR crystal this adduct underwent a fast oxidization into carboxylic acid as evidenced by the disappearance of the band at 1729 cm\(^{-1}\) and the formation of a new band at 1712 cm\(^{-1}\) and its subsequent conversion into a carboxylate band under basic conditions.

1. Introduction

The reactive 1,2-dicarbonyl species such as methylglyoxal (MG) are formed from the degradation of reducing sugars or through the Maillard reaction during food processing or storage or in vivo. These intermediates are considered as major precursors in the generation of Maillard reaction products such as colors, aromas and toxicants. In patients suffering from type-2 diabetes the elevated levels of MG causes the formation of various adducts with amino acid residues on proteins such as cysteine, lysine, and arginine (Lo et al., 1994). Scavenging such reactive 1,2-dicarbonyl species is considered an important aspect of controlling the formation of various Maillard Reaction Products (MRPs) in food and Advanced Glycation End products (AGEs) in vivo. Several strategies have been proposed to prevent MG related complications in vivo such as pharmacological intervention using thiamine (Rabbani et al., 2009), aminoguanidine (Thornalley et al., 2000; Solis-Calero, 2014) metformin (Kinsky et al., 2016) and creatine (Löbner et al., 2015). These reagents react with MG and form stable adducts. On the other hand, several flavonoids such as quercetin and (−)-epigallocatechin gallate (EGCG) have been shown to scavenge MG in food through electrophilic aromatic substitution reaction at ring A (Shin et al., 2018; Shao et al., 2014). Recently, we have demonstrated (Ghassem Zadeh and Yaylayan, 2019) the concept of in situ generation of carbonyl trapping agents from the degradation of amino acids during thermal treatment of foods, using tryptophan as an example. Indole, a major thermal degradation product of tryptophan has been shown to scavenge 1,2-dicarbonyl compounds, similar to that of flavonoids. These preliminary studies have been conducted at high temperatures (150–250 °C) and under this conditions reaction generated various adducts trapping up to 3 mol of MG per mole of indole. This diversity of products was possible due to the ability of indole to undergo electrophilic aromatic substitutions at carbon atoms 2 and 3 and on the ring nitrogen. Furthermore, the initial carbonyl containing adducts were able to undergo similar substitution reactions with a second mole of indole and generate for example 1,2-di(1H-indol-3-yl)propan-1-one (Ghassem Zadeh and Yaylayan, 2019). To verify the reactivity of indole with 1,2-dicarbonyl compounds at room temperature, the reaction was conducted under mild conditions and followed using qTOF/MS/MS and FTIR spectroscopy which is well suited to monitor changes in the carbonyl region of MG and its molecular transformations in the presence of indole.

2. Materials & methods

2.1. Materials, reagents, and equipment

Methylglyoxal (40% MG solution in water), indole (98%), ethanol (98%), methanol (99%) and KOH were purchased from Sigma-Aldrich.

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In order to monitor the initial phase of the interaction of indole with methylglyoxal, indole was mixed with methylglyoxal hydrate (MG) in ethanol as described under Materials and Methods. A two-ul of the reaction solution was immediately applied onto the ATR crystal and after the evaporation of the solvent the spectra were acquired every minute at 30 °C over 30 min (see Fig. 1). Fig. 2 shows the same reaction where the spectra were acquired every 5 min for 30 min at 30 °C. After 10 min when the water and ethanol have evaporated completely and a thin film has been formed, a wide absorption band centered at 1726 cm⁻¹ (not shown in the figure for clarity) appeared and slowly started decreasing giving rise to a new absorption band at 1712 cm⁻¹ which continuously increased and reached its maximum intensity after 30 min of the reaction (see Fig. 2). The second derivative spectrum of the initial wide band centered at 1726 cm⁻¹ indicated the presence two distinct bands one centered at 1724 cm⁻¹ (identical to the absorption band of the starting MG shown in Figure S1) and the second band at 1729 cm⁻¹. The latter band was assigned to the initial adduct A shown in Fig. 3 based on the analysis of the reaction mixture by qTOF-MS/MS (see section 3.1). Furthermore, the new band at 1712 cm⁻¹ that formed and increased at the expense of the band at 1729 cm⁻¹ was assigned to its oxidation product the carboxylic acid shown in Fig. 3 (see also Figure S2). This band at 1712 cm⁻¹ was completely shifted to 1561 cm⁻¹ (carboxylate form) when KOH was added to the ATR crystal at the end of the 30 min run (Figure S3). Apparently, the oxidation of the aldehyde A is very fast on the exposed surface of the ATR crystal, as can be seen in Fig. 2 within 30 min at 30 °C there was a complete conversion of the peak centered at 1729 cm⁻¹ to 1712 cm⁻¹. When the reaction was performed at 100 °C on the ATR crystal this oxidation was completed within 6 min (see Fig. 4). Interestingly, when the reaction was carried out in solution in a reaction vessel and the spectrum was acquired immediately after sample application onto the ATR cell, even after 3 h of heating in the reactor tube at 37 °C no band was observed at 1712 cm⁻¹ in the IR spectrum, or a mass spectral peak at the expected carboxylic acid peak value when analyzed by qTOF/MS, indicating the need of efficient exposure to air for this oxidation to occur. However, when the sample was left on the ATR cell and monitored, within 15 min the band at 1712 cm⁻¹ appeared and increased over time. Furthermore, when the solvent-phase reaction was performed under forced aeration at 60 °C for 10 min and the sample was applied onto the ATR cell it immediately showed an intense peak at 1712 cm⁻¹. On the other hand, when the reaction mixture heated at 37 °C for 3 h was analyzed by qTOF/MS/MS two major ions were observed at [M+Na]⁺ = 212.0688 and [M+Na]⁺ = 311.1154 the former had an elemental composition consistent with that of aldehyde A shown in Fig. 3 and the second ion consistent with the structure of 1,2-di(indol-3-yl)propan-1-one reported earlier (Ghassem Zadeh and Yaylayan, 2019) and confirmed in this study by comparison of their MS/MS data (see Fig. 5 and Table 1). The ion at [M+Na]⁺ = 311 is proposed to form by the interaction of a second mole of indole with adduct A as shown in Fig. 3. In an effort to separate some of the indole adducts from the solution, water was added to the reaction mixture that was heated at 60 °C for 3 h and the resulting solution was kept at RT until a brown oil separated after 2 days. Q-TOF/MS/MS analysis of this oil showed mainly the presence of ion at [M+Na]⁻ 311 and the FTIR spectrum (Figure S4) of this oil was consistent with the proposed structure.

3.1. Proposed mechanism of interaction of indole with methylglyoxal

Neglecting the stereoisomers, the reaction of indole with MG theoretically can generate six isomers resulting from the interaction of C-5, C-2 atoms and the ring nitrogen of indole with each of the two carbonyl carbons of MG. Two of the isomers are shown in Fig. 3 as isomers A and B. The C-3 carbon of indole being the most reactive atom (Ziarani et al., 2018) in nucleophilic substitution reactions of indole, we propose the formation of adduct A as the major product. Furthermore, due to the fast rate of this interaction and due to the presence of mainly hydrated methylglyoxal at the beginning of the reaction - which effectively blocks the aldehyde carbonyl from reacting at low temperatures - it appears that indole reacts rapidly with the keto carbonyl and form an adduct

2.2. Sample preparation

Indole/methylglyoxal were either (a) reacted at specific temperatures (30, 60 and 100 °C) on the surface of ATR cell of an FTIR spectrometer equipped with a temperature controlled ATR crystal or (b) heated in ethanol at 37 °C or 60 °C in a temperature controlled reactor (J-KEM Scientific, Inc. St Louis, MO, USA) for 3 h and samples were analyzed by FTIR and qTOF-MS/MS. In an effort to separate some of the indole adducts from the solution which was analyzed by FTIR and qTOF-MS/MS. The samples were analyzed on an Bruker Maxis Impact quadrupole time-of-flight mass spectrometer (Bruker Daltonics, Bremen, Germany) operating in positive ion mode. Calibration of the instrument was carried out by using sodium formate. The diluted samples were infused continuously into the detector. The acquisition parameters for electrospray interface were the following: nebulizer pressure, 0.6 Bar; drying gas, 4.0 L/min, 180 °C; capillary voltage, 4500 V. Scan range was done from m/z 90 to 1000. The data were analyzed by Bruker Compass Data Analysis software version 4.2. Temdam mass spectrometry (MS/MS) was carried out in multiple reaction monitoring (MRM) mode using 35 eV and 18eV collision energies for the ion at [M+Na]⁻ = 212.0688 and 35eV for the ion at [M+Na]⁺ = 311.1154.

2.3. Electrospary ionization/quadrupole time of flight/mass spectrometric analysis (ESI/qTOF/MS)

The samples were diluted in methanol (90% v/v) before analyzing by ESI/qTOF/MS. The system used was a Bruker Maxis Impact quadrupole time-of-flight mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with a deuterated triglycine sulfate (DTGS) detector, a temperature-controlled single-bounce diamond attenuated total reflectance (ATR) crystal, and a pressure application device for solid samples. A total of 24 scans at 4-cm⁻¹ resolution were co-added. Background spectra were routinely acquired under the same conditions without the sample and automatically subtracted from the sample spectra. Processing of the FTIR data was performed using Bruker OPUS software. Second-order derivatization of the spectra was performed using the Savitsky-Golay function (second-order polynomial, 12 points).

2.5. Trapping of MG by indole

Indole (0.1 g) was mixed with ethanol (5 mL) at room temperature in a test tube, subsequently, 0.1 g of 40% MG solution (Sigma-Aldrich) was added to the indole solution and the reaction mixture was stirred using a magnetic stirrer at 37 °C or 60 °C for 3 h and monitored by FTIR by sampling the mixture at specific time intervals (see section 2.3). At time zero a 2 μL of the reaction solution was removed from the test tube and applied onto the ATR crystal and after the evaporation of the solvent the spectra were acquired every 5 min for 30 min at 30 °C. Similarly, in a subsequent experiment, a reaction sample (2 μL) obtained at time zero was applied onto the ATR crystal and the spectra were acquired at 100 °C every minute for a total of 6 min. Samples from the reaction mixture heated in ethanol at 37 °C were analyzed by qTOF/MS/MS at the end of the 3 h period. Finally, distilled water (1 mL) was added to the reaction mixture that was heated at 60 °C for 3 h in the test tube and the resulting solution was kept at room temperature until a brown oil separated from the solution which was analyzed by FTIR and qTOF/MS/MS.

3. Results and discussion

In order to monitor the initial phase of the interaction of indole with methylglyoxal, indole was mixed with methylglyoxal hydrate (MG) in a chemical company (Milwaukee, WI) and used without further purification.
Fig. 1. Time-dependent FTIR spectra in the 1790-1630 cm$^{-1}$ region of freshly prepared indole methylglyoxal solution acquired on an open ATR crystal at 30 °C over 30 min. Spectra are acquired every 1 min.

Fig. 2. Time-dependent FTIR spectra in the 1780-1650 cm$^{-1}$ region of freshly prepared indole methylglyoxal solution acquired on an open ATR crystal at 30 °C over 30 min. Spectra are acquired every 5 min.
The expected ions at [M+H]$^+$ 206 and [M+Na]$^+$ 228 were not observed in the absence of air.

Fig. 3. Proposed mechanism of methylglyoxal reaction with indole (shown here reacting at C-3 of indole) (ppm values in parenthesis represent errors associated in calculating elemental formulas).

Fig. 4. Time-dependent FTIR spectra in the 1780-1650 cm$^{-1}$ region of freshly prepared indole methylglyoxal solution acquired on an open ATR crystal at 100 °C over 6 min.
Fig. 5. MS/MS fragmentations of [M+Na]+ 211.068 and [M+Na]+ 311.1154. (ppm values in parenthesis represent errors associated in calculating elemental formulas).

Table 1

| Carbonyl Absorption band | Structure | Proposed structure | MS/MS |
|--------------------------|-----------|--------------------|-------|
| No bands (see Figure S5) |           |                    |       |
| 1724 cm⁻¹                |           |                    |       |
| 1729 cm⁻¹                |           |                    |       |
| 1702 cm⁻¹                | 2-hydroxy-2-(1H-indol-3-yl)propanoic acid | C₁₁H₁₀NO⁺ (100%) | m/z 172.0755 |
| Shifted completely to 1561 cm⁻¹ when KOH was added | 2-(1H-indol-3-yl)prop-2-enal | | |
| 1712 cm⁻¹                | 1,2-di(1H-indol-3-yl)propan-1-one | C₁₉H₁₆N₂NaO⁺ (100%) | m/z 311.1154 |
|                          | 2-hydroxy-2-(1H-indol-3-yl)propanal | C₁₁H₉N⁺ (2.8 ppm) | m/z 130.0644 (25.7%) |
|                          | 1720.0771 (18%) | C₁₁H₉N⁺ (9.7 ppm) | m/z 212.0688 |
|                          | m/z 144.0437 (7.6%) | C₁₁H₉N⁺ (7.1 ppm) | m/z 144.0804 (100%) |
|                          | m/z 311.1154 | C₁₉H₁₆N₂NaO⁺ (2.034 ppm) | |
| FTIR                     | 3401 cm⁻¹ (NH), 1702 cm⁻¹ (CO), 2920, 1455 & 1352 cm⁻¹ (-CH₃), 739 cm⁻¹ (aromatic CH) | |
|                          | Observed only on the open ATR crystal | |
|                          | 3401 cm⁻¹ (NH), 3348 cm⁻¹ (COOH), 1712 cm⁻¹ (COOH), 1097, 1080 cm⁻¹ (OH), 744 cm⁻¹ (aromatic CH) cm⁻¹, 1458 & 1355 cm⁻¹ (-CH₃) | |

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tentatively assigned structure A. Reaction at the keto carbonyl of MG will generate an aldehyde after loss of hydration water as shown in Fig. 3 and it appears that this aldehyde undergoes facile oxidation into its corresponding carboxylic acid when exposed to air, as it was observed when the sample was analyzed on the surface of the ATR crystal (see Figs. 1 and 2). When the same reaction was carried out in solution in a test tube and the mixture was analyzed by qTOF/MS/MS, the expected molecular ion of the carboxylic acid was not observed, only ions at m/z 212 and m/z 311 was detected as shown in Fig. 3. However, when the same solution was applied on the surface of the ATR crystal and monitored at 30 °C, within 15 min carboxylic acid band emerged and increased. This band was shifted completely to 1561 cm⁻¹ in the presence of KOH, indicating the presence of carboxylic acid (Figure S3). The initial aldehyde can undergo similar interaction with another mole of indole to generate 1,2-di(1H-indol-3-yl)propan-1-one at m/z 311. To provide further evidence for the formation of isomer A rather than isomer B, the ion at m/z 212 was further studied by collision induced fragmentations (Fig. 5) at various energy levels. The collision induced decomposition under 18eV energy generated a fragment ion from the aliphatic portion of the molecule at m/z 97 (C₃H₆NaO₂) (see Fig. 5) and fragmentation at 35eV generated two indole containing moieties through loss of water and CO 1 at m/z 144.0804 (C₇H₇NaO₃) and the other at m/z 130.0644 (C₆H₇N₃) as shown in Fig. 5, confirming the assigned structure A considering structure B is not capable of generating such fragment ions. Furthermore, the reaction mixture also showed a lower intensity ion at m/z 172 which can arise from dehydration of m/z 212. It is difficult for isomer B to undergo such dehydration to generate m/z 172, which now requires lower energy (18 eV) to generate the same ions at m/z 144 and m/z 130 generated from m/z 212 under 35eV energy. On the other hand, both isomers A and B can undergo a second reaction with indole to generate ion at m/z 311 which was assigned 1,2-di(1H-indol-3-yl)propan-1-one structure based on its MS/MS dissociation into m/z 172 and 144 as shown in Fig. 5. Table 1 summarizes the FTIR and MS/MS data of the observed intermediates.

4. Conclusion

Indole can scavenge methylglyoxal under mild conditions at room temperature and the initial adduct formed can either undergo oxidation to carboxylic acid or react with another mole of indole to form a more stable di-indolyl adduct.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.