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Reactivity of nitrogen atoms in adenine and $(Ade)_2Cu$ complexes towards ribose and 2-furanmethanol: Formation of adenosine and kinetin

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To explore the interaction of nucleosides and nucleobases in the context of the Maillard reaction and to identify the selectivity of purine nitrogen atoms towards various electrophiles, model systems composed of adenine or adenosine, glycine, ribose and/or 2-furanmethanol (with and without copper) were studied in aqueous solutions heated at 110 °C for 2 h and subsequently analyzed by ESI/qTOF/MS/MS in addition to isotope labelling techniques. The results indicated that ribose selectively formed mono-ribosylated N^6 adenine, but in the presence of $(Ade)_2Cu$ complex the reaction mixture generated mono-, di- and tri-substituted sugar complexes and their hydrolysis products of mono-ribosylated N^6 and N^9 adenine adducts and di-ribosylated $N^{6,9}$ adenine. Furthermore, the reaction of 2-furanmethanol with adenine in the presence of ribose generated kinetin and its isomer, while its reaction with adenosine generated kinetin riboside, as confirmed by comparing the MS/MS profiles of these adducts to those of commercial standards.

1. Introduction

Investigation of the chemistry of interaction of reducing sugars with nucleobases may contribute to our understanding of the role of the Maillard reaction in transforming purine and pyrimidine bases into various Maillard modified products. Due to the spontaneous nature of the Maillard reaction such studies may also help elucidate how small molecules in the prebiotic world could assemble into complex structures forming nucleosides, nucleotides and DNA. Nucleosides for example can be viewed as N^9-Schiff bases of ribose and under relatively mild temperatures (37–100 °C) various groups have indeed reported the formation of Amadori/Schiff base adducts in sugar/nucleobase mixtures (Dutta, Cohenford, Guha, & Dain, 2006; Dutta, Cohenford, & Dain, 2005; Knerr, Ochs, & Severin, 1994; Nissl, Ochs, & Severin, 1996). When ribose was reacted with adenine at 100 °C in the dry state, it generated two products, an N^6-ribosyladenine (Schiff base or Amadori adduct) as the major product and around 2% adenosine as a minor product (Fuller, Sanchez, & Orgel, 1972) indicating the preference of sugars to undergo carbonyl amine reaction with adenine at a nitrogen atom other than N^9. Unlike the behaviour of amino acids in the Maillard reaction, which has been extensively studied over the past century, such reactions with nucleobases have been relatively less explored in detail. Many foods contain nucleic acid material that can generate free purine and pyrimidine bases upon thermal hydrolysis (Cambero, Pereira-Lima, Ordoñez, & García de Fernando, 2000; Wondrak, Tressl, & Rewicki, 1997), and their addition or substitution with conventional Maillard reactants can consequently lead to the generation of a variety of new Maillard reaction intermediates or products. In food mimicking model systems, the thermal hydrolysis of ATP and nucleic acids was reported to contribute to the Maillard reaction by enhancing the flavour perception of certain foods such as meats (Cambero et al., 2000; Chen, Chin, & Ho, 2004). However, the exact chemistry of this interaction was not reported, and the behaviour of these components under cooking conditions is currently unknown. In 1997, Wondrak et al. (1997) reported that heating herring sperm DNA at 160 °C in aqueous solution and/or incubating it (free acid) without any sugars resulted in the hydrolysis of DNA and the formation of several furanone derivatives, including 5-methyl-3(2*H*)-furanone, a known Maillard reaction product (Cerny & Davidek, 2003) and N^6-furfuryladenine, a furan derivative of adenine commonly known as kinetin. Kinetin is considered to be a highly bioactive plant hormone (Amasino, 2005) that is known to possess several desirable properties for human applications, including

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anti-carcinogenic activity (Voller et al., 2010). Although the presence of kinetin has not been reported to the best of our knowledge in the Maillard reaction mixtures containing free adenine and ribose, the conditions needed for its formation are available in foods. Therefore, in this study we explore the possible interactions of adenine with sugars (ribose, glucose and fructose) and 2-furanmethanol in the presence and absence of copper and glycine in aqueous model systems heated at 110 °C for 2 h.

2. Materials and methods

2.1. Materials

Glucose, fructose, ribose, glycine (98%), copper (II) chloride ($CuCl_2$) (99.9%), potassium hydroxide, kinetin (99%), Kinetin riboside, adenine ($\geqslant$99%), adenosine ($\geqslant$99%), 2′-deoxyadenosine-5′-monophosphate (98–100%), and 2-furanmethanol (98%) were purchased from Sigma-Aldrich Chemical Co. (Oakville, ON, Canada). Labelled [U_5-^{13}C]ribose (99%) was purchased from Cambridge Isotope Laboratories (Andover, MI).

2.2. Preparation of the adenine containing model systems

Model systems (10 mg) comprising of 2′-deoxyadenosine-5′-monophosphate, adenine, or adenosine alone or with sugars (glucose or fructose or ribose and/or 2-furanmethanol), and/or glycine were dissolved in water (2 mL) and heated on a sand bath in an open vial (5 mL capacity) at 110 °C for 2 h until dry. The detailed composition of model systems and their relative ratios are presented in Supplementary Table S1. All the samples were analyzed in duplicates. Although ribose, glucose and fructose were used in all the experiments, the results were mainly discussed using ribose as a representative sugar due to its intrinsic presence in nucleotides.

2.3. Synthesis of bis(adeninato)copper(II) complex

The *bis*(adeninato)copper(II) or $(Ade)_2Cu$ complexes were prepared by dissolving adenine (1.35 g) in methanol (10 mL) in the presence of KOH (0.05 g) followed by the addition of half a mole of $CuCl_2$ (0.67 g). The precipitate was washed with methanol, filtered and dried. $(Ade)_2Cu$ complex was confirmed by obtaining its elemental composition from its accurate mass determined by ESI/qTOF/MS analysis at $[M+H]^+ = 332.0102$ (calculated for $C_{10}H_9CuN_{10}$ with an error of 1.9 ppm).

2.4. Electrospray ionization/quadrupole time of flight/mass spectrometry (ESI/qTOF/MS) analysis

The dry reaction mixtures were dissolved in LC grade water to a concentration of 1 mg/mL. The samples were then diluted 10-fold in 10% methanol prior to analysis by ESI/qTOF/MS. The ESI/qTOF/MS system was comprised of a Bruker Maxis Impact quadrupole-time of flight mass spectrometer (Bruker Daltonics, Bremen, Germany) operated in positive ion mode. Samples (1 μL) were injected directly into the ESI/qTOF/MS. Instrument calibration was performed using sodium formate clusters. The electrospray interface settings were the following: nebulizer pressure 0.6 bar, drying gas 4 L/min, 180 °C, capillary voltage 4500 V. Scan range was from m/z 100–1000. The data were analyzed using Bruker Compass Data Analysis software version 4.1. Tandem mass spectrometry (MS/MS) was carried out in MRM mode using 20.0 eV collision energy for selected ions.

2.5. Liquid chromatography-diode array detector-mass spectrometry (LC-DAD-MS) analysis

Selected samples in Supplementary Table S1 were analyzed by LC-DAD-MS using a Dionex Ultimate 3000 UHPLC with a DAD detector (200–600 nm) coupled to a Bruker Maxis Impact QTOF in positive ESI mode. Samples were separated on a Phenomenex (Torrance, CA) Luna C18(2) column (5 μM, 100 Å, 2.2 × 50 mm) using a gradient of 98% mobile phase **A** (0.1% formic acid in H_2O) and 2% mobile phase **B** (0.1% formic acid in acetonitrile) to 100% mobile phase **B** in 8 min. The data was processed using Bruker data analysis software version 4.1.

2.6. Structural identification

Evidence for the proposed structures of non-volatile reaction intermediates were provided through ESI/qTOF/MS analysis of their elemental composition, and by the use of commercial or synthetic intermediates and by isotope labelling studies. Furthermore, retention times, UV spectrum and MS/MS spectra of selected product ions were compared to those of commercial standards. The incorporation of copper in the identified complexes was confirmed by the isotopic signature of copper through detection of M+2 peaks at 30% relative intensity.

3. Results and discussion

Although the reaction of nucleobases with sugars has been studied in the past; however, no information is available on the selectivity of the purine base nitrogen atoms towards various electrophiles. The glycation of adenine with sugars such as ribose has been shown to generate mainly N^6-substituted ribosyladenine in addition to negligible amounts of adenosine, (Fuller et al., 1972; Maurel & Convert, 1990). Additionally, the generation of adenosine has been shown to be enhanced in the presence of metal ions, which in turn, are known to coordinate with adenine at various locations generating stable complexes (Harkins & Freiser, 1958; Ilavarasi, Rao, & Udupa, 1997; Lippert, 2000). Currently, the pathways of generation of Maillard-type reaction products from nucleobases such as adenine under cooking conditions are not known. To understand the nature of such interactions under Maillard reaction conditions, adenine/sugar model systems were chosen to investigate the role of added amino acids and metal salts or metal-bound adenine such as *bis*(adeninato)copper on the selectivity and the profile of their interaction in aqueous systems heated at 110 °C for 2 h and subsequently analyzed by electrospray ionization tandem mass spectrometry (ESI/qTOF/MS/MS). Although ribose, glucose and fructose were used in all the experiments, the results were mainly discussed using ribose as a representative sugar due to its intrinsic presence in nucleotides.

3.1. Interaction of adenine with ribose or glucose and formation of monoglycated adducts

To confirm the literature information (Dutta et al., 2006; Maurel & Convert, 1990) that adenine sugar mixtures generate only N^6 monoglycated products, adenine/ribose or glucose aqueous model systems were heated at 110 °C for 2 h. The ESI/qTOF/MS analysis indicated that the reaction indeed generated only mono-ribosylated or mono-glucosylated adenine adducts at $[M+H] = 268$ and $[M+H] = 298$, respectively, which were detected as major ions in the ESI/qTOF/MS spectrum of these mixtures (Table 1 and Fig. 1). These adducts can be formed through the known carbonyl-amine reaction between the sugars and adenine or nucleosides such as guanosine (Knerr, Ochs, & Severin, 1994; Nissl et al.,