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Electrospray ionization mass spectrometric investigations of α-dicarbonyl compounds—Probing intermediates formed in the course of the nonenzymatic browning reaction of L-ascorbic acid

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Abstract

A new method is presented which allows the simultaneous detection of various α-dicarbonyl compounds generated in the course of the nonenzymatic browning reaction initiated by thermal treatment of L-ascorbic acid, namely: glyoxal, methylglyoxal, diacetyl, 3-deoxy-L-pentosone, and L-threosone. 3-Deoxy-L-threosone was successfully identified as a new C_4-α-dicarbonyl structure for the first time in the degradation of Vitamin C by application of this non-chromatographic mass spectrometric approach. Moreover, a more detailed elucidation of the mechanistic scenario with respect to the oxidative and nonoxidative pathways is presented by using dehydro-L-ascorbic acid and 2,3-diketo-L-gulonic acid instead of L-ascorbic acid as a starting material. Furthermore, the postulated pathways are corroborated with the aid of ^{13}C-isotopic labeling studies. The investigations were extended to baby food, and the successful detection of α-dicarbonyl compounds characteristic for Vitamin C degradation proved the matrix tolerance of the introduced method.

Keywords: L-Ascorbic acid; Nonenzymatic browning; α-Dicarbonyl compounds; 3-Deoxy-L-threosone; Electrospray ionization mass spectrometry

1. Introduction

Chemically, L-ascorbic acid belongs to the family of carbohydrates, functionally it is classified as an organic acid and a reducing reagent, and physiologically the compound is attributed to the class of essential vitamins. The so-called Vitamin C is a natural component in a wide range of esculent goods. Furthermore, artificial addition of L-ascorbic acid is successfully utilized to protect food against oxidation. Hence, it is vital to have knowledge and control over the Vitamin C content in foods. Unfortunately, L-ascorbic acid is relatively unstable under common storage and processing conditions such as heat, oxygen, and exposure to transition metals. Thus, it is of essential importance to understand the nonenzymatic degradation of Vitamin C. However, this process is complex and hence it is not completely understood yet. It is widely accepted to classify the decomposition into two types of reactions, namely the oxidative and a nonoxidative pathway. Hitherto, more than 100 different intermediates and degradation products are known to play a role in the course of the nonenzymatic browning reaction [1]. In this context, fragments with α-dicarbonyl structure represent a category, which has not yet been in the center of attention. Therefore, we focussed on the formation of the highly reactive intermediates, which need to be derivatized prior to detection. The trapping reagent fulfills two important roles: on the one hand it is necessary to stabilize the α-dicarbonyl compounds to avoid fast subsequent reactions and on the other hand it is compulsory to enhance the sensitivity in electrospray ionization (ESI) at the same time. Here, the derivatizing agent of choice is *o*-phenylenediamine (OPD) as it is capable of fulfilling both tasks. In a two-fold condensation reaction the α-dicarbonyl structures are converted to stable quinoxalines. The latter are probed by a high electrospray ionization response owing to the relatively high basicity of nitrogen centers associated with more facile protonation, which has been verified by test calculations.

Furthermore, it would be desirable to develop a method which allows the direct detection of highly reactive α-dicarbonyl intermediates generated in course of the browning reaction of

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L-ascorbic acid in the presence of complex matrices as encountered in food systems. If chromatographic or laboratory work-up could be omitted, this method would be even more attractive for time and cost efficiency reasons. In order to allow a simultaneous analysis of the various α-dicarbonyl compounds generated via the oxidative and nonoxidative Vitamin C degradation pathway, we describe here, is the successful development and application of such an approach.

2. Experimental

2.1. Materials

L-Ascorbic acid was generously provided from Pascoe pharmazeutische Präperate GmbH. 2,3-Diketo-L-gulonic acid was synthesized according to established preparative procedures [2]. In brief, L-ascorbic acid was oxidized to dehydro-L-ascorbic acid by addition of *p*-benzochinone, oxalic acid, and formic acid to a dimethylacetamide solution of L-ascorbic acid. The reaction mixture was heated to 50 °C and the newly generated dehydro-L-ascorbic acid dimer was crystallized. The intermediate dimer was converted to the barium salt of 2,3-diketo-L-gulonic acid by formation of the monomer in an alkaline sodium hydroxide solution, and addition of hydriodic acid induced spontaneous lactone cleavage followed by crystallization with the aid of bariumiodide. In a final step, the 2,3-diketo-L-gulonic acid was liberated by ion exchange.

The remaining compounds were all commercially purchased and employed without further purification unless stated otherwise: L-[1-^{13}C] ascorbic acid (Eur iso-top), dehydro-L-ascorbic acid (Aldrich), methanol (Merck), hydrochloric acid (Merck), *p*-benzochinone (Fluka), oxalic acid (Fluka), formic acid (Merck), dimethylacetamide (Merck), sodium hydroxide solution (Merck), hydriodic acid (Merck), bariumiodide (Riedel-de-Haën), sodium hydrogen sulfite (Merck), activated charcoal (Merck) and *o*-phenylenediamine (Aldrich). The latter compound was dissolved in water, augmented with sodium hydrogen sulfite and activated charcoal, refluxed, hot filtrated and recrystallized prior to use.

The quinoxaline standards glyoxal and methylglyoxal were purchased from Serva, diacetyl from Lancaster and 3-deoxy-L-pentosone was synthesized and kindly provided by Hollnagel [3].

The baby food samples, HIPP Baby-C-juice and HIPP Vitamin C enriched fruit tea, were publicly obtained from a supermarket.

2.2. Sample preparation

For preparation of the reaction mixtures L-ascorbic acid, L-[1-^{13}C] ascorbic acid, dehydro-L-ascorbic acid, or 2,3-diketo-L-gulonic acid are dissolved in distilled water and pH adjusted to 3.5 with hydrochloric acid to yield 0.5, 0.5, 0.025, and 0.025 M solutions, respectively. In each case, 0.5 ml aliquots are pipetted into 10 ml ampules and sealed. The thermolysis is initiated by placing the sample ampules in a thermo block (Behrotherm) at 120 °C for 120 min.

The derivatization is brought about by preparing a 1:1 mixture (v/v) of a 0.5 M *o*-phenylenediamine aqueous solution with the thermally treated samples and allowing them to react for 30 min at room temperature, dilution with methanol, and introduction to the ESI-MS without further chromatographic treatment.

The baby food samples were handled analogously by pipetting 0.5 ml of either the unaltered HIPP Baby-C-juice or preparing the HIPP Vitamin C enriched fruit tea according to instructions of the manufacturer (1 g dissolved in 2.5 ml water) into 10 ml ampules and the treated accordingly (see above).

2.3. Electrospray ionization mass spectrometry

The mass spectrometric experiments were carried out on a commercial VG BIO-Q mass spectrometer, which has been described in detail previously [4]. In brief, the VG BIO-Q consists of an ESI source combined with a tandem mass spectrometer of QHQ configuration (Q: quadrupole; H: hexapole). In the present experiments, methanolic mmolar solutions of OPD-derivatized solutions of thermally treated L-ascorbic acid, dehydro-L-ascorbic acid, and 2,3-diketo-L-gulonic acid or the respective food sample were introduced via a syringe pump (flow rate 10 μl min^{-1}) to the fused-silica capillary of the ESI source. Nitrogen was used as drying and nebulizer gas. The source temperature was kept at 80 °C and the cone voltages applied in the desolvation zone of the differentially pumped ESI source were systematically varied for the ions of interest which were then selected at unit mass resolution by means of Q1.

The isotope patterns of all ions described below agreed with expectation on the basis of natural isotope abundances [5]. The cone voltage U_C determines the amount of collisional activation of the ions evolving from solution in the differential pumping system of the ESI source. Collision-induced dissociation (CID) experiments were performed with argon at various collision energies (E_{lab} = 0–30 eV) and a pressure of ca. 3×10^{-4} mbar, which approximately correspond to single-collision conditions [4]. The collision energies were converted to the center-of-mass frame, $E_{CM} = [m/(M+m)]E_{lab}$, where m and M are the masses of the collision gas and the ionic species, respectively. The product ions formed in the hexapole were then analyzed by scanning Q2.

3. Results and discussion

In general, the nonenzymatic browning reaction of L-ascorbic acid is discussed in terms of two major decomposition pathways, which are referred to as the oxidative and nonoxidative pathway, respectively. In this work, the terms oxidative and nonoxidative are used as follows in order to avoid misunderstanding: The oxidative pathway describes the reaction branch which involves the oxidation of L-ascorbic acid to dehydro-L-ascorbic acid as an initial step. Likewise, the nonoxidative pathway relates to the direct decomposition of L-ascorbic acid with exclusion of dehydro-L-ascorbic acid as an intermediate structure. It is noted that the expression nonoxidative refers solely to the nature of the initial step, subsequent transformations may indeed involve various oxidation steps.