Glycation in food and metabolic transit of dietary AGEs (advanced glycation end-products): studies on the urinary excretion of pyrraline

A. Foerster and T. Henle1
Institute of Food Chemistry, Technical University of Dresden, D-01062 Dresden, Germany

Abstract
Pyrraline \( [\varepsilon-(2\prime\text{-formyl}-5\prime\text{-hydroxymethyl-pyrrolyl})\text{-L-norleucin}] \) belongs to the group of AGEs (advanced glycation end-products) formed in the final stage of the Maillard reaction in foods and in vivo. As it is generally accepted that AGEs are pathophysiological relevant in aging and in diseases such as diabetes and uraemia, physiological consequences resulting from the ingestion of dietary AGEs are discussed, but balance studies for well defined AGEs are still lacking. The aim of our study was to investigate the influence of nutrition on the urinary excretion of pyrraline. After the first day without dietary restrictions, seven healthy volunteers were asked, starting on the morning of day 2, to ingest a diet virtually free of Maillard compounds (i.e. no cooked or roasted foods, no bakery products, no coffee, etc.). Dietary control was stopped on the morning of day 5. We collected 24 h urine samples for these 5 days, which were analysed for free pyrraline by reverse-phase HPLC with UV detection at 297 nm. We found that urinary excretion of free pyrraline was directly affected by the composition of the diet, decreasing from 4.8 ± 1.1 mg/day on day 1 to levels of 1.6, 0.4 and 0.3 mg/day on days 2, 3 and 4 respectively, followed by a significant increase to 3.2 ± 1.4 mg/day on the 5th day. The results of this work prove, for the first time, that urinary excretion of pyrraline is strongly dependent on its dietary intake. Thus the influence of nutrition should be taken into consideration in studies directed to the physiological role of glycation compounds.

Introduction
During industrial processes or home cooking, as well as during long-term storage of foods, side-chain modifications by carbohydrates in the course of the Maillard reaction, also referred to as non-enzymatic browning or glycation, are of particular importance for the nutritional and functional quality of food proteins. Individual protein-bound Maillard compounds can serve as markers for the extent of glycation reactions in foods and thus as parameters of food quality (for reviews, see [1,2]). Besides the quantitatively predominant Amadori products fructoselysine and lactuloselysine, which are formed during the ‘early stage’ of the complex reaction, up to now only a few amino acid derivatives of the advanced Maillard reaction, so-called AGEs (advanced glycation end-products), have been quantified in foods [3]. As it is generally accepted that the Maillard reaction in vivo contributes to the pathogenesis of diabetes, uraemia and aging, questions arise concerning the intake of AGEs via daily food and their possible pathophysiological role [4,5]. In this context, however, only limited information concerning digestion, resorption and elimination of dietary glycation compounds can be found in the literature (for review, see [6]). Metabolic transit data for early Maillard compounds suggest that only around 5–10% of ingested peptide-bound Amadori products from foods are excreted unmetabolized in urine and faeces, whereas the major part is degraded by currently unknown mechanisms, among which the intestinal microbial activity might be of central importance [7]. Balance studies for advanced Maillard compounds are still lacking. It has been proposed that serum AGE levels, analysed as immuno-reactivity by an ELISA technique, may be influenced by a diet containing AGEs [8–11]. Based on the observation that renal excretion of this AGE immunoresponse was slower in patients with renal failure compared with healthy subjects, it was speculated that dietary AGEs, or glycotoxins, may represent a risk factor in diabetic and uraemic patients [8]. Biotransformation of individual AGEs, however, has not been studied up to now.

Protein-bound pyrraline \( [\varepsilon-(2\prime\text{-formyl}-5\prime\text{-hydroxymethyl-pyrrolyl})\text{-L-norleucin}] \) is formed from the \( \varepsilon \)-amino group of lysine and 3-deoxy-D-erythrohexos-2-ulose (3-deoxyglucosulose), which is a degradation product of reducing sugars and Amadori products (Scheme 1) [12–14]. Pyrraline is one of the few AGEs that has been quantified in foods. Using chromatographic techniques after enzymatic hydrolysis, this acid-labile lysine derivative was found in milk products such as sterilized or evaporated milk in amounts of up to 150 mg/kg of protein, as well as in bakery products, where it accounts in bread crust, with levels of up to 3700 mg/kg of protein, for 15% of lysine derivatization [15,16]. Several studies have investigated the pyrraline

Key words: 3-deoxyglucosulose, diet, glycation, Maillard reaction, pyrraline, urinary excretion.

Abbreviation used: AGE, advanced glycation end-product.

1To whom correspondence should be addressed (e-mail Thomas.Henle@chemie.tu-dresden.de).
Proposed pathway for the formation of pyrraline (compound 3) from peptide-bound lysine (compound 1) and 3-deoxyglucosulose (compound 2)

R₁ and R₂ denote the peptide chain.

content of human urine. Portero-Otin et al. [17] were the first to report higher levels of urinary pyrraline for patients with diabetes compared with healthy controls, as already observed for N-ε-carboxymethyl-lysine [18] and pentosidine [19]. This finding was recently confirmed by others, resulting in the conclusion that circulating levels of pyrraline might be directly influenced by glycaemic status, thus supporting a role for AGEs in the pathogenesis of diabetes [20–22]. The role of dietary pyrraline, however, has not been taken into account by the authors. As no information about the metabolic transit of pyrraline is currently available, the aim of our present study was to investigate whether dietary pyrraline is biologically available during the digestion process and whether dietary restriction influences the urinary excretion of this lysine derivative.

Materials and methods

Seven healthy volunteers (four male, three female; age 28–64 years) took part in the dietary study. Following day 1 of the study, on which there were no dietary restrictions, the intake of foods containing Maillard products (i.e., cooked or roasted foods, bakery products, beer, coffee, etc.) had to be avoided for 3 days. On the morning of the fifth day, dietary control was stopped. Volunteers had to record their nutrition and to collect 24 h urine samples. Urine samples were stored at −18°C until analysis.

The content of free pyrraline was determined by reverse-phase HPLC with UV detection at 297 nm, using a modified method according to [17]. The HPLC was an Åkta Basic system (Amersham Biosciences, Freiburg, Germany), equipped with an analytical column (4.6 mm × 125 mm) filled with Eurospher 100 C18 material (Knauer, Berlin, Germany). The column temperature was 37°C. Gradient elution was performed. Solvent A was 0.02 M ammonia acetate, and solvent B was a mixture of water and acetonitrile (1:1, v/v) containing 0.1% trifluoroacetic acid. Flow rate was 1 ml/min. The gradient started with 0% solvent B for 5 min, followed by a linear increase to 10% solvent B within 13 min and then a linear increase to 100% B within 7 min. After 5 min at 100% B, the gradient was decreased linearly to 0% solvent B within 5 min, after which equilibration for 11 min was achieved. A 50 µl sample of the membrane-filtered urine, diluted 1:1 (v/v) with solvent A, was injected. Quantification was achieved using external calibration with reference material of pyrraline synthesized according to [23].

Results and discussion

Using reverse-phase HPLC with UV detection, free pyrraline could be detected and quantified in urine samples from seven healthy volunteers without need for derivatization (Figure 1). Complete separation of the peak designated as pyrraline from other compounds was possible. Identification was achieved by comparing the retention time of the peak detected in urine samples with that of a reference sample of pure pyrraline, as well as by co-injection of sample and standard. Furthermore, ratios of peak areas recorded at various wavelengths for the pyrraline peak in urine and standard samples were identical.

As can be seen from Figure 1(A), a significant decrease in the area of the pyrraline peak was found in the chromatograms, in parallel with the dietary restriction. Quantitative measurement showed that the pyrraline content of human urine is directly affected by the composition of diet (Figure 2). On the first day, without dietary restrictions, mean pyrraline excretion was 4.8 ± 1.1 mg/day. This is in good agreement with values published by Portero-Otin et al. [17], who measured a daily pyrraline excretion of between 1.1 and 3.4 mg/day for healthy and diabetic subjects. Equivalent...
quantitative data were reported by Daimon et al. [21]. On the subsequent days of our study, when no Maillard-product-containing food was allowed, the mean level of pyrraline in urine decreased to 1.6 ± 0.6 and 0.4 ± 0.2 mg/day on days 2 and 3 respectively, and to 0.3 ± 0.1 mg/day on the 4th day. After the cessation of dietary control on the 5th day, the pyrraline level increased again to 3.2 ± 0.2 mg/day on days 2 and 3 respectively, and to 0.3 ± 0.1 mg/day on the 4th day. After the cessation of dietary control on the 5th day, the pyrraline level increased again to 3.2 ± 1.4 mg/day, reaching approximately the level as on the 1st day. Based on the diet protocols and available data for the pyrraline content of foods, a total supply of 3.1–6.0 mg pyrraline per day was calculated for days 1 and 5. Given the data for pyrraline excretion, it can be concluded that dietary pyrraline is nearly completely released and resorbed during digestion, followed by rapid elimination via the kidneys, thus leading to nearly complete recovery of dietary pyrraline in the urine. This indicates that, in contrast with Amadori products, of which only up to 5% are recovered in the urine [24,25], pyrraline obviously is not metabolized within the body.

In contrast with previously published reports, we therefore conclude that urinary pyrraline is almost exclusively of dietary origin and is affected to only a minor extent by in vivo catabolic pathways for AGEs. Recently published findings, in which close correlations between urinary pyrraline as well as other endogenously formed AGEs and oxidative stress [22] or glycaemic control [21] were postulated without taking dietary influence into consideration, are therefore questionable.

Our results indicate that the healthy kidney is capable of clearing dietary AGEs efficiently from the circulation. Nevertheless, further studies are necessary in order to clarify whether accumulating dietary AGEs might contribute significantly to the AGE load of the human body, especially in uraemia, and therefore may have to be taken into account for certain physiological consequences. Above all, however, it has to be realized that the term ‘AGE’ comprises a large number of individual amino acid derivatives, of which only a minority have been identified and quantified either in foods or in vivo.

References


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