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AGEs in foods: Do they play a role in uremia?

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AGEs in foods: Do they play a role in uremia? The so-called Maillard reaction, or nonenzymatic glycation between proteins and carbohydrates, is of particular importance for the flavor, color, and shelf life of food. Despite the great variety of possible AGEs, which can be formed during heating processes, only a few have unequivocally been identified and quantified in foods. From the quantitative point of view, the amount of AGEs ingested with a conventional diet is much higher than the total amount of AGEs in the plasma and tissue. To date, however, only preliminary studies concerning digestion, resorption, and elimination of AGE-modified food proteins can be found in the literature, indicating that for patients with impaired kidney function, dietary AGEs might contribute significantly to the total AGE load of the body. To date, however, no conclusive answers or recommendations can be given regarding a possible role of AGEs as uremic toxins in general, and of dietary AGEs in particular.

BACKGROUND AND GENERAL CONSIDERATIONS

The abbreviation "AGEs" stands for "advanced glycation end products," a great variety of individual compounds that are formed during nonenzymic reactions between side chains of proteins, in particular lysine and arginine residues, and glucose or glucose degradation products. The chemistry of this complex reaction, which was named by its first investigator, the french biochemist Louis-Camille Maillard, has been reviewed extensively [1, 2, 3, 4]. Today, it is generally accepted that AGEs, the peptide-bound reaction products of the advanced stages of protein-carbohydrate reactions, represent a class of uremic toxins, accumulating in the body of uremic patients with direct links to the complications of the uremic syndrome, such as beta-2-microglobulin-derived amyloidosis, endothelial dysfunction, and accelerated atherogenesis [5, 6, 7]. As referenced in Medline, about 300 papers dealing with "glycation" are presently published per year. Despite all of the effort that has been made throughout the last years, essential questions still remain unsolved. It is undisputed from the chemical standpoint that the AGEs we know today surely represent only a small proportion of all possible reaction products. Surprisingly, up until now *no* individual AGE has been directly linked to certain biologic effects on a molecular or cellular basis. Model studies generally are based on proteins like bovine or human serum albumin, which is incubated with glucose for a certain time in order to become a glycated "AGE-BSA." Such samples, however, do not seem to be good models for studying real structure-function relationships on a cellular level, as incubation of BSA in the presence of glucose predominantly forms *early* reaction products, the so-called Amadori products. The amino ketose fructoselysine accounts for more than 90% of the observable lysine derivatization. Advanced reaction products like N-ε-carboxymethyllysine, pyrraline, or pentosidine are formed only in minor amounts. Whether observed biologic effects thus really are due to AGEs, the predominating Amadori compounds or any other intrinsic protein modification remains open.

AGES IN FOOD: QUANTITATIVE CONSIDERATIONS

In addition to the "structure-function" question, an analytical chemist must also ask how much of each individual AGE compound is formed, either in vitro or in vivo? Literature reports dealing with this question are rare, indicating a lack of suitable and unambiguous methods for the quantification of individual compounds in complex matrices, such as plasma and urine [8]. In this context, uremia research might benefit from analytical food chemistry. In food science, one major problem for years was the quantitative determination of the Amadori products of lysine 1 (Fig. 1), and thus, the assessment of the extent of blockage of the essential amino acid lysine occurring during heating or storage of food. During technological treatment of milk, for example, a lysine modification of 10% to 20%, and in certain cases up to 70%, can be observed [9, 10]. Quantification can be achieved by measuring furosine 2 (Fig. 1), a reaction product which is formed from the Amadori products of lysine during acid hydrolysis [8, 9]. During further progress of the Maillard reaction in foods, an oxidative degradation of the Amadori compounds leads to the formation of N-ε-carboxymethyllysin 3 (CML) [11]. CML was found in milk

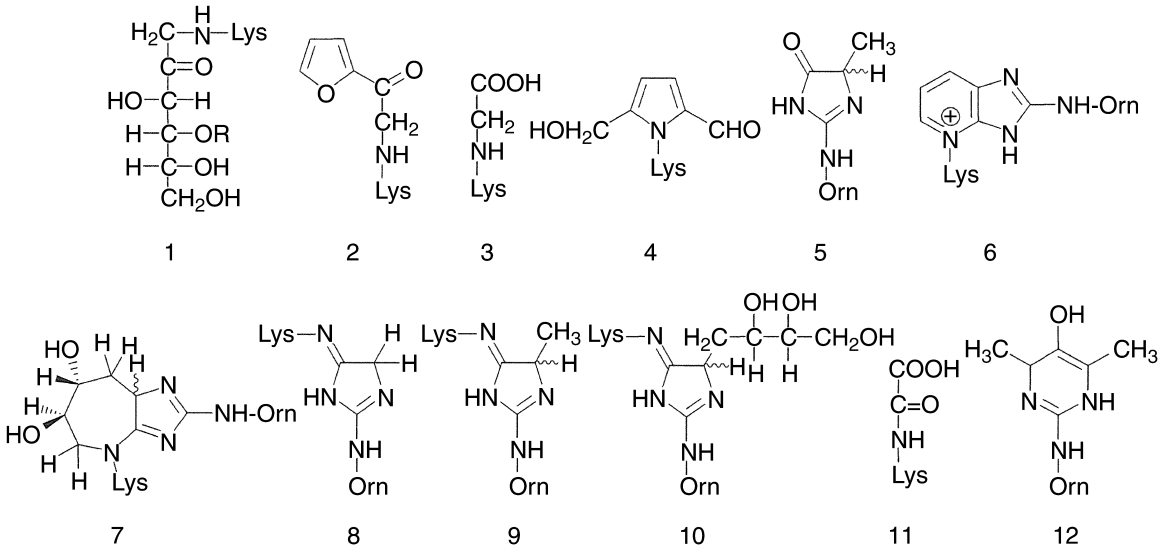

Fig. 1. Amino acid derivatives of the Maillard reaction which have been quantified in heated foods (see text for details).

and other foods, accounting for 3% to 10% of the Amadori compounds [12]. Concerning the formation of further "advanced" reaction products resulting from the reaction between proteins and α-dicarbonyls, only limited quantitative information is available. Few AGEs have unequivocally been quantified in foods. Milk products like sterilized or evaporated milk contain up to 150 mg/kg protein of pyrraline 4, an acid-labile pyrrolaldehyde of lysine. For bread crust, up to 3700 mg/kg protein were found, accounting for 15% of the lysine derivatization [13]. With identification and quantification of δ-N-(5-methyl-4-oxo-5-hydroimidazol-2-yl)-L-ornithine 5, imidazolinone, the first arginine reaction product of the advanced Maillard reaction, was found in foods [14]. Imidazolinone is the dominating form of arginine derivatization in bakery products and roasted coffee. Up to 30% of arginine is modified to imidazolinone during coffee roasting. Small amounts of another arginine derivative, the crosslink amino acid pentosidine 6, were measured in foods. With values ranging between 5 and 10 mg/kg protein, pentosidine does not play a major role within the Maillard reaction in foods [15]. Slightly higher values were found recently for the crosslink amino acids glucosepan 7, GODIC 8, MODIC 9, and DODIC 10 [16]. Using LC/MS after enzymic hydrolysis, up to 150 mg MODIC per kg protein were determined in bakery products. The amounts of compounds glucosepan, GODIC, and DODIC were between 10 and 50 mg/kg protein, indicating that, like pentosidine, these amino acid derivatives may not contribute significantly to crosslinking of food proteins. Using a polyclonal antibody, Hasenkopf et al were able to detect oxalic acid monolysylamide (OMA) 11, in milk products, but no quantitative data were given [17]. Very recently, argpyrimidine 12, an arginine derivative formed between arginine and two molecules of methylglyoxal, was found as free amino acid in beer [18].

Table 1. Estimated supply as calculated from quantitative data published in literature of selected glycation compounds with daily food

Glycation compound	Milk products	Bakery products	Coffee brew
Amadori compounds 1	500 to 2000	up to 1500	–
CML 3	up to 50	up to 160	–
Pyrraline 4	2 to 17	up to 80	–
Ornithinoimidazolinone 5	–	up to 700	20 to 70

Assuming a consumption of one litre of milk, 500 g of bakery products, or 400 ml of coffee, respectively. Data are given in μmole of AGE compound per day and depend largely on heat treatment of the corresponding food item.

Abbreviations are: CML, N-ε-carboxymethyllysine; AGE, advanced glycation end products.

FOOD AGES AS UREMIC TOXINS?

Based on this quantitative data it becomes clear that food is a main source of AGEs. With a "conventional" diet consisting of processed foods like heated milk, bakery products, and coffee, the amount of ingested protein-bound amino acid derivatives of the Maillard reaction per day is somewhere between 1500 and 4000 μmol (500 to 1200 mg, calculated as fructoselysine) for Amadori compounds, and 100 to 300 μmol (25 to 75 mg) for AGEs, mainly pyrraline and CML (Table 1). This means that ten to fifty times more AGEs are supplied compared with the total amount of AGEs in the plasma and tissue of an uremic patient. How does the body deal with this enormous load of nonphysiological amino acids? To date, only preliminary studies concerning digestion, resorption, and elimination of AGE-modified food pro-

teins can be found in the literature. It has been proposed that AGEs derived from food items, measured with an unspecific ELISA, can be found in the circulation, cumulating in patients with renal failure [19]. Using a chromatographic method and well characterized reference material for Amadori products, we were unable to show increased plasma levels for healthy volunteers after ingestion of milk with defined content of Amadori products [20]. Urinary excretion of Amadori products (measured via furosine) and of pyrraline was significantly affected by food consumption and could be decreased by diets free of AGEs (no baked or cooked foods, no coffee, etc.). Based on this, we can say that the major part of AGEs measured in urine is of dietary origin. Similar results were observed for PD-effluates. This gives the preliminary indication that dietary AGEs might significantly contribute to the total AGE load of the human body. The kidney, as well as the peritoneal membrane, has to deal with a "continuous" exposure to dietary AGEs. Therefore, biologic effects of these exogenously formed compounds have to be considered, in addition to AGEs formed endogenously. Corresponding studies, as well as nutrition studies with uremic patients are currently underway in our laboratories.

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