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The multiple Maillard reactions of ribose and deoxyribose sugars and sugar phosphates

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Abstract—Ribose 5-phosphate (R5P) undergoes the Maillard reaction with amines at significantly higher rates than most other sugars and sugar phosphates. The presence of an intramolecular phosphate group, which catalyzes the early stages of the Maillard reaction, provides the opportunity for the R5P molecule to undergo novel reaction paths creating unique Maillard products. The initial set of reactions leading to an Amadori product (phosphorylated) and to an α-dicarbonyl phosphate compound follows a typical Maillard reaction sequence, but an observed phosphate hydrolysis accompanying the reaction adds to the complexity of the products formed. The reaction rate for the loss of R5P is partially dependent on the pK_a of the amine but also is correlated to the protonation of an early intermediate of the reaction sequence. In the presence of oxygen, a carboxymethyl group conjugated to the amine is a major product of the reaction of R5P with *N*-acetyllysine while little of this product is generated in the absence of oxygen. Despite lacking a critical hydroxyl group necessary for the Maillard reaction, 2-deoxyribose 5-phosphate (dR5P) still generates an Amadori-like product (with a carbonyl on the C-3 carbon) and undergoes phosphate cleavage. Two highly UV-absorbing products of dR5P were amine derivatives of 5-methylene-2-pyrrolone and 2-formylpyrrole. The reaction of dR5P with certain amines generates a set of products that exhibit an interesting absorbance at 340 nm and a high fluorescence.

Keywords: Ribose 5-phosphate; Deoxyribose 5-phosphate; Maillard; Glycation

1. Introduction

A member of the pentose phosphate pathway, ribose 5-phosphate (R5P) exists at higher levels (5–20 μM) in those cells requiring reduced nicotinamide adenine dinucleotide phosphate (NADPH) for reductive biosynthesis or in dividing cells that require the molecule as a precursor for purine and pyrimidine biosynthesis. As a sugar, R5P is subject to spontaneous Maillard reactions with cellular amines, including the N-terminal, lysine (Lys) and arginine (Arg) groups of proteins. In fact, the molecule has been shown to react at much higher rates than most other common sugars (including glucose, ribose, and fructose) and sugar phosphates (including glucose 6-phosphate and fructose 1,6-bisphosphate).[1–3] Based on UV absorbance and browning results, R5P reacts at rates that exceed 100-fold that of glucose.[1] A reaction with glycine at pH 8 and 37 °C monitored by ^{1}H NMR spectroscopy gave a bimolecular rate constant for the disappearance of R5P from solution of 0.22 $M^{-1}\,h^{-1}$.[1] Considering this first step of the Maillard reaction involves the attack of the deprotonated form of the amine, a pH-independent rate equation for this system can be rewritten as

$$\text{Rate} = k[\text{sugar}][\text{R–NH}_2]$$

where [R–NH_2] reflects the pH-dependent concentration of the deprotonated amine. For the reported glycine system,[1] the rate constant would be recalculated as approximately 7 $M^{-1}\,h^{-1}$. Given an amine of moderately low pK_a (e.g., 8.0) and 100 μM concentration, this translates into an in vivo R5P glycation rate equaling

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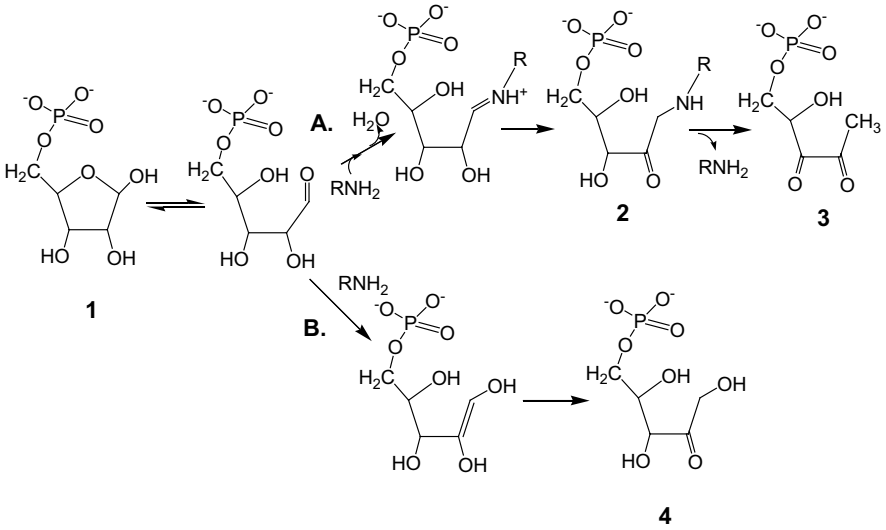
approximately 0.05% of available amine per day at physiologic R5P levels. Longer lived proteins would thus experience 1% glycation in a 3-week period.

The relatively rapid glycation rate of R5P is clearly related in some way to its attached phosphate group.[1] Phosphates have been long employed as catalysts of the Maillard browning reaction via a mechanism speculated to be either general base catalysis[4] or covalent catalysis.[5] In the case of R5P, a proximity effect of the phosphate group perched over the reactive anomeric center apparently promotes a greater reactivity in comparison to that observed by intermolecular phosphate. (Sugar phosphates such as glucose 6-phosphate contain intramolecular phosphate, but the location of the phosphate in an equatorial position is stereochemically distant from the C-1 carbon of the glucopyranoside.) The proximity/orientation rate enhancements could also be supplemented by steric effects of the phosphate on the furanose ring structure leading to a higher level of the acyclic structure, a form generally acknowledged as the true initial reactant of the Maillard sequence. While large discrepancies have been reported in the acyclic levels of sugars as a result of the technique employed, the acyclic level of R5P appears to be at least an order of magnitude in excess of D-ribose and 2–3 orders of magnitude greater than D-glucose.[6–8]

The early steps of the reaction of R5P with amines (Scheme 1) appear to be similar to the Maillard reaction of other sugars. The initial attack of the nitrogen to produce an N-glycoside is followed by a rearrangement to generate an Amadori compound.[2,9] The formation of a typical α-dicarbonyl compound has then been suggested,[1] which subsequently proceeds to a variety of highly UV- and visible-absorbing advanced glycation end-products (AGEs). The reaction of R5P in this sequence appears, however, to be more complex than other sugars. It is possible that the presence of the amine encourages isomerization of R5P to ribulose 5-phosphate (Ru5P) (see Scheme 1), and this reaction may or may not be connected to an observed loss of phosphate from the molecule.[1,10] As in other Maillard systems, the availability of O_2 allows a different series of subsequent reactions (compared to anaerobic conditions) and the formation of reactive oxygen species superoxide and hydrogen peroxide.[11]

Like R5P, 2-deoxyribose 5-phosphate (dR5P) has a phosphate moiety perched over a reactive hemiacetal group of a furanose ring. The sugar, however, lacks the hydroxyl group at C-2 necessary for the enaminol tautomerization step that occurs during the Amadori rearrangement process. Thus, while it is likely that dR5P undergoes some initial N-glycoside reaction with amines in solution, it is impossible for dR5P to proceed to melanoidin production and browning via a traditional Maillard series of reactions. However, as previously reported by Wondrak et al.[12] and as we report here, dR5P overcomes this 'deoxy' barrier to generate a variety of Maillard-like compounds.

We report here the kinetics and major reaction products of R5P and dR5P (along with their corresponding non-phosphorylated counterparts ribose and deoxyribose) with simple amines. The molecules are capable of producing a diverse set of products in a relatively rapid timeframe in simulated physiological conditions and, thus, need to be considered as potentially significant glycation agents in cells.



Scheme 1. Proposed early reactions of R5P (**1**) with amines stemming from the acyclic form in equilibrium with the furanose. A traditional series of Maillard reactions (path A) leads through an Amadori product (**2**) to an α-dicarbonyl product (**3**), while an isomerization series (path B) leading to ribulose 5-phosphate (**4**) may be encouraged by a general base catalysis (i.e., removal of the C-2 hydrogen) of the amine.