Carbohydrate Research, 256 (1994) 41–47
Elsevier Science B.V., Amsterdam

The degradation of L-threose at Maillard reaction conditions †

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(Received May 9th, 1993; accepted September 25th, 1993)

ABSTRACT

L-Threose, a comparatively unstable aldose, is produced from L-ascorbic acid in the presence of oxygen and participates vigorously in Maillard reactions, even at comparatively mild conditions. In the present study, the degradation of L-threose at pH 7.0 alone, in the presence of *N*-α-acetyl-L-lysine, and at pH 2.0 alone at 37°C was investigated by identification of some of the products produced in the reactions by means of GLC and GLC–MS. Among the compounds identified were 3-deoxy-tetros-2-ulose (1), the predicted alkaline rearrangement product derived from 1 (2,4-dihydroxybutyrate, the 4-carbon metasaccharinic acid), as well as glyceraldehyde. Isotopic tracer studies clearly show that the glyceraldehyde is produced by loss of C-1 from the starting L-threose molecule. The presence of *N*-acetyl lysine in incubation solutions appears to accelerate the production of 1, but the formation of glyceraldehyde appears to be independent of the lysine derivative.

INTRODUCTION

3-Deoxy-glycos-2-uloses have been proposed as intermediates in the Maillard reaction[1,2], as well as in acid- and base-catalyzed degradation reactions undergone by reducing sugars in solution[3,4]. They serve as precursors for 2-furaldehydes (acid-catalyzed degradation reactions) as well as for "metasaccharinic acids" (base-catalyzed degradation reactions). Such compounds are thought to be formed by pathways shown in Fig. 1, and represent a partially dehydrated sugar that exists in the same oxidation state as the original sugars from which they are formed. The formation of osuloses and their subsequent degradation constitute a portion of the complex of reactions known as the Maillard reaction. In addition to functioning as intermediates in degradation reactions at elevated temperatures, there is some evidence to show that they may well be formed in vivo and may play a role in in vivo Maillard reactions[5,6], participating in protein crosslinking reactions and related protein modifications that occur as a function of aging in an organism.

† Journal Paper No. 11916 Of the Missouri Agricultural Experiment Station.
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SSDI 0008-6215(93)E0348-5

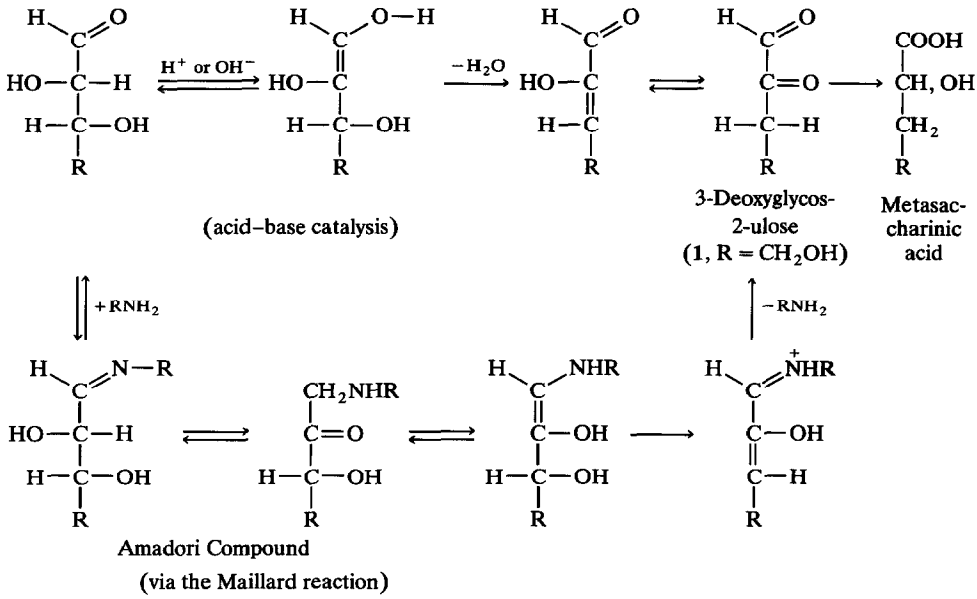

Fig. 1. Pathways for the formation of **1** from L-threose via acid-base catalysis, and via the formation of an intermediate Amadori compound, as would occur during the Maillard reaction.

3-Deoxy-D-*erythro*-hexos-2-ulose (the 6 carbon analogue) has been detected in human serum and urine[7], and, in a series of papers[8,9], Kato and his group have shown that this compound is highly reactive in terms of protein crosslinking at in vivo conditions. Thus, 3-deoxyosuloses may well play a role in biological aging.

Although threose, a highly reactive but not a commonly occurring sugar, has not been the subject of serious study in terms of its degradation, it may well be a significant participant in Maillard reactions (both in vitro and in vivo) involving L-ascorbic acid. A recent study[10] of the latter has shown that high levels of threose (up to 22% yields) are produced from ascorbate when it undergoes degradation at pH 7.0 and 37°C in the presence of oxygen. Thus, although ascorbate solutions undergo Maillard-type nonenzymatic browning readily, it is probable that the reactive component in the mixture is not ascorbate itself, but threose that is produced from it in the presence of oxygen.

Baynes and his group recently published a study of ascorbate and its role in in vivo Maillard reactions[11] and have suggested that threose, produced from ascorbic acid, interacts with protein amino groups to form an Amadori compound and then undergoes further degradation. Although they did not identify the osulose intermediate, they reported preliminary evidence for the formation of an Amadori compound, produced by the reaction of threose with the epsilon amino group of lysine. They further postulate that this compound serves as the precursor of N^{ϵ}-carboxymethyl-L-lysine (CML) by oxidative cleavage between C-2 and C-3 of

the Amadori compound produced in the reaction. CML represents a substituted amino acid that is thought to be produced as a result of in vivo Maillard reactions, and appears to be formed as a result of the oxidative degradation of Amadori compounds that are produced as a result of in vivo glycation reactions.

In this paper, we report the identification of some of the degradation products that arise when L-threose is incubated in solution, alone, and in the presence of *N*-α-acetyl-L-lysine, a model amino-containing compound that is useful in studies of the Maillard reaction.

RESULTS AND DISCUSSION

The experimental protocol used both for the identification of and the quantitative estimation of glyceraldehyde and **1** involved the reduction of an aliquot of an incubation solution with borohydride, followed by acetylation and examination of this derivative by GLC and by GLC–MS. Using this approach, glycerol triacetate and 1,2,4-butanetriol triacetate were readily detected in the incubation reactions so treated, having retention times and mass spectra identical to authentic standards. For both samples, the base peak (100% relative abundance) was *m/z* 43. For the glycerol derivative, a major peak is observed at *m/z* 145 (M $-CH_2OC-OCH_3$) corresponding to a cleavage between the primary and secondary carbons. In addition, a peak at *m/z* 103 (145 $-CH_2CO$) was observed. For the butanetriol derivative, a peak was observed at *m/z* 159 (M $-CH_2OCOCH_3$), corresponding to a cleavage between C-1 and C-2 of the butanetriol. The latter three peaks (*m/z* 145, 103, and 159) were used to obtain information on the origin of both the glyceraldehyde and **1** derived from threose. That the compounds were derived from glyceraldehyde and **1** was confirmed by repeating the procedure as described above but using borodeuteride as the reducing agent, as well as L-(1-^{13}C)- and -(2-^{13}C)threose as starting compounds in conjunction with borohydride and borodeuteride reduction. Using these reagents, and observing shifts for the peaks at 145 and 103 (for ordinary glycerol triacetate), and, the peak at 159 (for ordinary butanetriol triacetate), the positions of the carbonyl groups, as well as the carbon atom lost in the production of glyceraldehyde from threose could be easily verified. For an incubation starting with ordinary L-threose followed by borodeuteride reduction, the MS showed peaks at both *m/z* 145 (60%) and 146 (88%) for the glycerol derivative and at *m/z* 160 (53%) for the butanetriol derivative. Data for the other experiments are as follows: for glycerol derived from L-(1-^{13}C)threose and reduced with borohydride: *m/z* 145 (74%); For glycerol derived from L-(1-^{13}C)threose and reduced with borodeuteride: *m/z* 145 (57%) and 146 (79%). For glycerol derived from L-(2-^{13}C)threose and reduced with borohydride: *m/z* 145 (75%) and 146 (60%). For glycerol derived from L-(2-^{13}C)threose and reduced with borodeuteride: *m/z* 145 (70%), 146 (70%), and 147 (82%). In addition, the peak at *m/z* 103 (145 $-CH_2CO$) also showed an analogous series of shifts for isotope

content. For an incubation starting with ordinary L-threose and reduced with borodeuteride, the butanetriol showed a peak at m/z 160 (53%). For butanetriol derived from L-(1-^{13}C)threose and reduced with borohydride: m/z 159 (58%). For the butanetriol produced from L-(1-^{13}C)threose and reduced with borodeuteride: m/z 160 (58%). For butanetriol produced from L-(2-^{13}C)threose and reduced with borohydride: m/z 160 (53%). For butanetriol produced from L-(2-^{13}C)threose and reduced with borodeuteride: m/z 161 (57%).

These data confirm that the glyceraldehyde produced in the reaction is derived from carbons 2, 3, and 4 of L-threose and that compound **1** is produced as would be expected. That is, the carbonyl groups of **1** are derived from C-1 and C-2 of the original L-threose.

Both glyceraldehyde and **1** were found to be present in commercially available preparations of L-threose as well as in preparations made in our laboratories and are probably produced during the work up of the threose, which involves hydrolysis of the isopropylidene group, followed by treatment with hot, dilute acetic acid solution.

Measurement of the compounds over a time course (Figs. 2 and 3) gives an estimation of their rates of formation at the incubation conditions studied and suggests that they are constantly being produced from threose during its degradation. At pH 7.0 and 37°C, L-threose has a measured half life of ca. 3.5 days[10]. The more or less constant levels of both compounds at pH 2.0 is consistent with the well-known fact that sugars are most stable in slightly acidic solution. At this pH, minimal degradation of threose would be expected. The high amounts of **1** in the presence of the lysine derivative is also not surprising, since the osulose would be

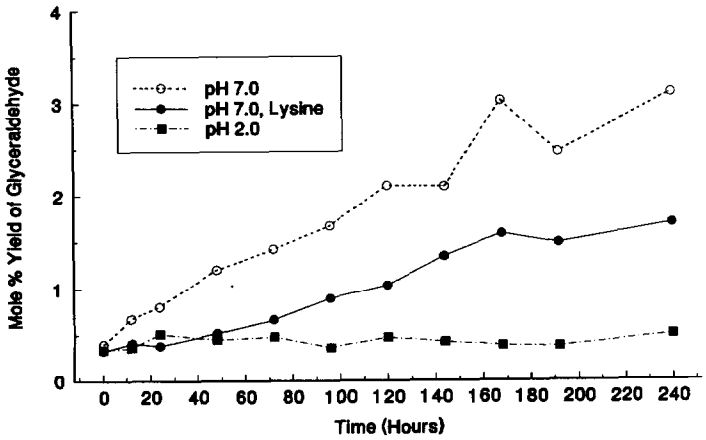

Fig. 2. Levels of glyceraldehyde in incubation solutions at several different conditions. GLC parameters for this separation were: initial temperature, 140°C (2 min hold) followed by a ramp of 5°C per min to a final temperature of 250°C.

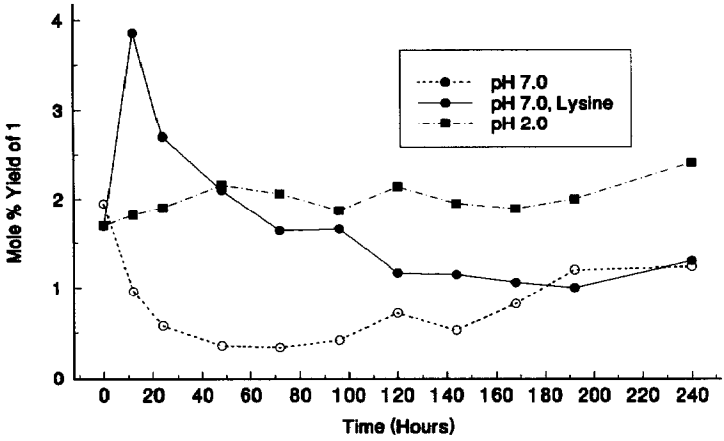

Fig. 3. Levels of 1 in incubation solutions at several different conditions. GLC parameters for this separation were: initial temperature, 140°C (2 min hold) followed by a ramp of 5°C per min to a final temperature of 250°C.

expected to be formed via the intermediate formation of an Amadori compound. The latter are known to be less stable than the aldoses from which they are derived and, therefore, would be expected to give higher initial yields of the osulose.

It should be noted that the levels of glyceraldehyde and 1 in the incubation solution are quite low, as might be expected for degradation intermediates, and it is probable they react further during the degradation reaction, particularly in the presence of lysine. Glyceraldehyde levels range from 0.25 mol% (initial) to 2.0 mol% at its highest level, while for 1, the corresponding values range from 1.1% (initial) to 2.5% (maximum).

The fact that the highest levels of glyceraldehyde are detected at pH 7.0 in the absence of an amino group, suggests that this may well be a simple base-catalyzed cleavage reaction, involving the 1,2-enediol of D-threose. That comparatively high levels of glyceraldehyde are present may well be due to the fact that a comparatively large proportion of threose exists in the open chain form in solution. Serianni and co-workers[12] reported that, over the temperature range 17–81°C, ca. 11% of the population of threose exists in the open chain form (a combination of aldehyde and hydrated forms). This would allow a much larger population of enediol precursor (the open chain form) to be present at any time, compared to other higher carbon aldoses.

Compound 1, in alkaline solution, would be predicted to rearrange, via a benzylic acid type rearrangement, to the appropriate metasaccharinic acids, a mixture of 3-deoxy-threonic and -erythronic acids (the C-2 racemic 2,4-dihydroxybutyric acids). This compound was, in fact, found in trace quantities in the mixture (after ca. 3 months incubation) and was identified by conversion to the Me_3Si derivative and comparison of its GLC retention time and its MS with an authentic standard. The MS of the Me_3Si derivatives (both authentic and isolated) showed

peaks at m/z 321 (M -15) and 219 (M $-COOMe_3Si$). Other peaks appear at m/z 147, 103, and 73. These data suggest that at least a portion of **1** disappears from the reaction solution by rearrangement via typical and expected alkaline degradation mechanisms.

EXPERIMENTAL

General methods.—GLC measurements were made using a Varian model 3400 chromatograph equipped with an onboard computer. All separations were performed using a Quadrex 007 0V-17 0.25 mm × 25 m capillary column. Separation parameters are given in the figure legends. GLC–MS data were collected using a Hewlett–Packard 5890 series chromatograph coupled to a Hewlett–Packard 5970 mass selective detector. L-Threose was prepared from 1,3-*O*-benzylidene-L-arabinitol as described by Perlin. DL-2,4-Dihydroxybutyric acid was prepared by treatment of commercially available DL-2,4-diaminobytyric acid with nitrous acid.

Analytical protocols.—In typical experiments, the incubation of solutions was carried out in constant temperature baths in cap sealed tubes. Solutions of D-threose (0.067 M) were dissolved in phosphate-buffered solutions, which were 0.023 M in perseitol, which was used as an internal standard. For the experiments in the presence of *N*-α-acetyl-L-lysine, the L-threose was present in a 6 molar excess relative to the amino acid. For sampling, a 1.0-mL aliquot was removed, placed in a 3.0 mL reactivial and immediately reduced with 1.0 mL of 0.26 M $NaBH_4$ (or deuteride). This solution was allowed to stand at room temperature for 60 min, dried, acidified with methanolic HCl (3 N), and evaporated to dryness under a stream of air. This process was repeated twice. The residue was dissolved in 0.5 mL of pyridine and then 0.5 mL of Ac_2O was added. This solution was heated at 100°C for 1.0 h, followed by evaporation to dryness under a stream of air. The residue was dissolved in 1.0 mL of CH_2Cl_2 and a 1-μL aliquot injected into the GLC. For Me_3Si derivatives, samples were additionally dried by vacuum desiccation overnight and then derivatized by treatment with bis(trimethylsilyl)trifluoroacetamide in MeCN at 70°C for 30 min. A 1-μL aliquot of this mixture was injected. Yields of glyceraldehyde and **1** were calculated by comparason with the perseitol peak (internal standard).

ACKNOWLEDGMENT

This work was supported, in part, by NIH grant EY 07070.

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