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Electrochemical Study of the Maillard Reaction

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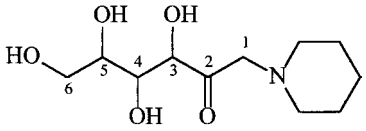
Electrochemical properties of β-alanine/carbohydrate Maillard reaction products were measured using a combination platinum/Ag−AgCl (Cl^-) redox electrode. Changes toward more negative voltages were observed, which were consistent with reductone formation during the course of the Maillard reaction. Using voltage change as a guide, the propensity for reductone formation among various sugars was ribose > xylose ∼ arabinose > glucose ∼ rhamnose ∼ mannose ∼ lactose > fructose. Similar electrochemical behavior indicative of reductone formation was observed in the decomposition products of a model Amadori compound, *N*-(1-deoxyfructos-1-yl)piperidine (**1**).

KEYWORDS: Maillard; redox potential; electrochemistry; Amadori; reductone; browning

INTRODUCTION

The Maillard reaction initiated by amine−carbonyl reactions of proteins or amino acids and reducing sugars has received much attention because of its relevance in both food science and medicine (*1*). In view of the widespread interest in the Maillard reaction it seemed worthwhile to consider rapid, noninvasive analytical techniques to follow the progress of the reaction in terms of its reactive intermediates.

During the early stage of the Maillard reaction highly reactive intermediates known as reductones are formed, which are known to have enhanced chemical reducing properties. Reductones, that is, hydroxymethylglyoxal and related compounds, have been detected in foodstuffs and recognized as important indicators of a Maillard reaction in progress (*2*). Reductone intermediates of Maillard reactions have been analyzed, but usually they are first trapped as stable chemical derivatives such as quinoxalines (*3*), pyrazoles (*4*), or oximes (*2*) prior to analysis by gas chromatography or polarography (*5*). Conceivably, faster, more direct analysis can take advantage of the well-known redox property of the reductones. For example, it has been shown that reducing substances formed in the Maillard reaction between D-glucose and γ-aminobutyric acid can readily be quantified by ferricyanide titration (*6*), and more recently the redox behavior of a tetrazolium salt was used to spectroscopically quantify the amount of an aminoreductone in heat-treated milk (*7*). The Maillard reaction is by the nature of its reaction intermediates a viable electrochemical system. Although simple reductones such as ascorbic acid, reductic acid, and triose reductone are known to be electrochemically active (*8*), apparently no attempt has been made to measure electrochemical evidence of reductone activity during the course of the Maillard reaction. Typical reductones to be expected among Maillard products are the well-known decomposition products of the



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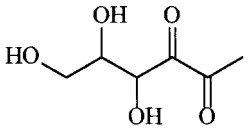

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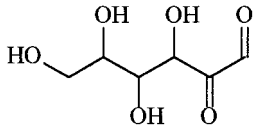

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Figure 1. Amadori compound **1** and related reductones **2** and **3**.

Amadori compound *N*-(1-deoxyfructos-1-yl)piperidine (**1**) such as 1-deoxy-2,3-diketoses (**2**), diacetylformoin, aminoreductones (*1*), and 1,2-diketoses (**3**) (**Figure 1**) (*9*). A Maillard product structurally similar to a reductone, 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one, has previously been analyzed by liquid chromatography coupled to an oxidative electrolytic detector (*10*). The objective of the present work was to investigate the redox potentials of Maillard reactions and those of a related Amadori compound as noninvasive measurements for tracking the formation of reductones and related substances during the course of the reactions.

EXPERIMENTAL PROCEDURES

Materials. Purchased chemicals were all high-purity, reagent grade materials obtained from the following commercial suppliers: D-glucose, D-arabinose, D-sorbitol, β-alanine, 2-deoxy-D-glucose, 2,2-bis(hy-

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droxymethyl)-2,2′,2″-nitrilotriethanol (commonly called "bis-tris"), Aldrich Chemical Co.; D-(+)-xylose, D-ribose, α-L-rhamnose; D-(+)-mannose, Sigma Chemical; D-xylitol (Fluka); l-(+)-ascorbic acid (J. T. Baker); D-(−)-fructose (MCB Manufacturing Chemists); and anhydrous lactose (Spectrum Labs). Low-conductivity water obtained by ion exchange filtration was distilled before use. The buffer used in all experiments was prepared by adding 6 N HCl to an aqueous solution of bis-tris.

***N*-(1-Deoxy-D-fructos-1-yl)piperidine (1).** The Amadori compound (**1**) was prepared by a two-step synthesis described in detail previously (*11*). Briefly, D-glucose and piperidine were reacted to form *N*-D-glucosylpiperidine, which was isolated and purified by recrystallization from MeOH/acetone (55% yield). Rearrangement of the *N*-glycoside catalyzed by diethyl malonate gave **1** as colorless crystals after two recrystallizations from MeOH/acetone (27% yield): mp 126−128 °C (dec) (corr); lit. mp 126−128 °C (dec) (corr) (*11*); ^{13}C NMR (D_2O) 44.94 (C-1), 63.47 (C-6), 69.39 (C-5), 69.98 (C-4), 70.32 (C-3), and 97.7 (C-2) ppm. In solution **1** appears to be mostly the β-pyranohexose isomer on the basis of close similarity of our spectral data with those published for the β-pyranohexose isomer of *N*-(1-deoxy-D-fructos-1-yl)glycine (*12*).

Methods. Redox potentials were measured with a platinum redox electrode (Orion model 977800) containing a built-in Ag/AgCl (saturated KCl) reference electrode and coupled to a Corning model 240 pH meter (millivolt mode). Time-dependent voltage changes were followed with a Kipp and Zonen type BD 41 strip chart recorder. Accuracy of potential measurements within 5 mV was confirmed by comparative measurement with a laboratory grade digital voltmeter. Correct electrode operation was verified by ferricyanide/ferrocyanide calibration tests described in Orion electrode literature. In our setup the Pt electrode was connected as the cathode of an electrochemical cell (+) with $Ag + Cl^- \rightarrow AgCl + e^-$ serving as its anodic reference (−). Thus, test substances capable of reducing silver ion should produce a negative voltage at the cell's terminals.

Maillard reactions were generally performed by adding 30.0 mL of 0.10 M buffer to solid mixtures of β-alanine and carbohydrate components to obtain 0.067 and 0.20 M solutions, respectively, at 23 °C. Within 2 min of obtaining a clear solution the electrode was introduced and an initial potential, E_i, was recorded exactly 1 min after immersion. Following removal of the electrode, the solutions were boiled under reflux for various times. After each refluxing period, ice cooling was used to rapidly cool the mixtures to 23 °C (2−3 min). A final potential, E_f, was recorded immediately and exactly 1 min after electrode immersion. The change in redox potential during an experiment, $E_f - E_i$, is interpreted to represent a change in the oxidative state of the reaction mixture caused by the Maillard reaction.

An ascorbic acid control experiment was performed by adding 1.00 mL of a freshly prepared solution containing 3.77 mg of L-(+)-ascorbic acid in buffer to 20.0 mL of similar 0.1 M, pH 6.86, buffer at 22 °C. After 1 min, the 0.001 M ascorbic acid solution developed an $E_f - E_i$ of −102 mV.

Microwave heating experiments were done in a standard, household unit (Panasonic model NN-S5488A) equipped with multiple power settings and a rotating glass tray. To obtain the necessary heating profile in small samples, a 500 mL glass beaker containing 250 mL of water (initially at 23 °C) served as a ballast and was placed at the center of the tray. The sample cell, a 20 mL glass scintillation vial containing 10.0 mL of reactant solution, was located 7.5 cm from the center of the tray. At the lowest power setting (P10) the following heating profile was obtained (heating time/temperature): 5 min/61 °C, 8 min/72 °C, 10 min/77 °C, 12 min /77 °C, and 15 min/76 °C.

Proton-decoupled ^{13}C NMR data were obtained in D_2O solution on a Varian Unity Inova 300 unit operating at 75.429 MHz. The external reference standard was the center line of $CDCl_3$ at 77.0 ppm.

RESULTS AND DISCUSSION

Theoretical Aspects. The reductone redox system (**Figure 2**) consists of a reductone in its ene−diol tautomeric form (RH_2) and its oxidized triketo counterpart (R). The system is formally similar to the well-known *o/p*-dihydroxybenzene/quinone redox

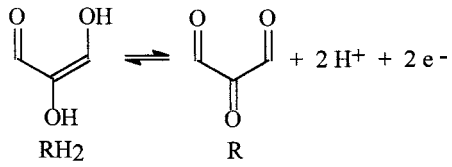

Figure 2. Reductone half-cell reaction.

pair, and an expression for its half-cell electromotive force (EMF) is given by eq 1, where E represents the half-cell

$$E = E_o - \mathscr{R}T/2F \ln(R)/(RH_2) - \mathscr{R}T/2F \ln [(H^+)^2 + k_1(H^+) + k_1k_2] \quad (1)$$

oxidation potential (in volts) for solutions containing specific concentrations of R, RH_2, and hydrogen ions, T is the temperature in K, and $\mathscr{R}/F$ is a universal constant. E_o is the standard oxidation potential produced by R and RH_2, each being at unit activity, and $k_1$and k_2 are the first and second acidic dissociation constants for the weakly acidic ene−diol RH_2 (*13*).

For practical purposes because k_1 and k_2 are very small for weak acids, this equation can be simplified to

$$E = E_o - \mathscr{R}T/2F \ln(R)/(RH_2) - \mathscr{R}T/F \ln(H^+) \quad (2)$$

According to eq 2 the half-cell oxidation potential (E) should vary as the concentration ratio R/RH_2 at constant temperature and pH.

In our setup with the Pt electrode connected to the positive terminal of the voltmeter, that is, as the cathode, a decrease in R/RH_2 is predicted to produce a more negative cell voltage, and because RH_2 is a primary Maillard product, an increasingly negative cell voltage should correlate directly with an increased presence of RH_2 in Maillard reactions.

Reactions of β-Alanine and Carbohydrates. The Maillard reaction was studied in a model system consisting of β-alanine and a variety of carbohydrate molecules initially at 0.067 and 0.20 M, respectively, in aqueous 0.10 M buffer at near neutral pH. β-Alanine was chosen to minimize side effects of Strecker degradations, and an organic tertiary amine buffer avoided the long-known interaction of polyvalent inorganic anions, that is, phosphate with carbohydrates (*14*). Reactions performed with reducing sugars at reflux temperature (∼100 °C) gradually changed color during 0−6 h from colorless to deep orange, indicating the smooth continuous progress of a Maillard reaction. No attempt was made to exclude atmospheric oxygen from reactions, but gas solubilities were expected to be negligible at reflux temperatures.

Measurement of EMF values was done with a combination redox electrode containing a platinum working electrode [cathode (+)] and an Ag/AgCl (saturated KCl) reference electrode [anode (−)]. Preliminary experiments with D-xylose/β-alanine were promising in that increasingly negative cell potentials were observed with time, indicating the formation of chemically reducing species as browning progressed. The increasingly negative cell potential indicated a growing negative value for the reverse of the oxidative half-cell reaction (**Figure 2**) because E_{ref}, that is, for $Ag + Cl^- \rightarrow AgCl + e^-$, was constant at − 0.23 V. Thus, reducing species in the D-xylose reaction mixture were at least capable of spontaneously reducing silver ion.

Measurement of cell EMF values proved to be problematic at first because voltages measured in cooled reaction aliquots tended to drift toward still more negative values with time. In retrospect, this effect is reasonable because the continued

Table 1. Changes in Redox Potential during β-Alanine/Carbohydrate Maillard Reactions[a]

carbohydrate compd	$E_f - E_i$, Δ in mV	±σ	replicates	buffer pH
D-glucose	−102	1	3	6.94–7.22
D-mannose	−100		1	6.94
L-rhamnose	−105	3	2	6.94
D-fructose	−83		1	6.94
2-deoxy-D-glucose	−22	8	2	6.85
D-sorbitol	−3.7	27	3	6.94–7.22
D-xylose	−155	27	3	7.22
D-arabinose	−157	7.5	2	6.94
D-ribose	−192	4.5	2	6.94
D-xylitol	−7.7	27	3	6.94–7.22
lactose	−99		1	6.94

[a] Initial concentrations: carbohydrates, 0.20 M; β-alanine, 0.067 M. Refluxed for 1.0 h in 0.10 M bis-tris buffer.

reactivity of R and RH_2 in Maillard mixtures probably precludes a true reversible measurement of EMF as defined by eq 2. To circumvent this problem, we chose not to interpret absolute values of EMF but instead to consider changes in EMF produced under a set of standardized measurement conditions. Potentials were recorded exactly 1 min after the electrode had been inserted into reaction mixtures previously cooled to 23 °C. The changes in EMF, $\Delta = E_f - E_i$, where E_f and E_i represent potentials measured before (i) and after (f) treatments, were noted for a series of carbohydrate materials, for example, before and after 1.0 h of reaction with β-alanine (**Table 1**).

D-Glucose exhibited a significantly negative Δ of −102 ± 1 mV indicative of the formation of reductones or other highly reducing substances. Similar behavior was observed for other hexoses, that is, D-(+)-mannose (−100 mV), α-L-rhamnose (−105 ± 3 mV), and D-(−)-fructose (−83 mV), and also for a single disaccharide example, lactose (−99 mV).

Control experiments were done with carbohydrates that do not participate in the Maillard reaction, that is, compounds that are structurally incapable of forming Amadori compounds. As expected, D-sorbitol (−3.7 ± 27 mV) and 2-deoxy-D-glucose (−22 ± 8 mV) led to relatively small changes in electrode potential. In addition, the latter compounds produced no color change during the reaction periods. In addition, L-(+)-ascorbic acid in 0.1 M, pH 6.85, buffer served as a positive control. A freshly prepared 0.001 M solution of ascorbic acid in buffer developed a Δ of −102 mV within 1 min of preparation at 22 °C.

A careful comparison showed that pentose sugars produced more electrochemical activity than hexoses under comparable conditions. Thus, D-xylose with Δ = −155 ± 27 mV exceeded its higher homologue, D-glucose (−101 ± 1 mV). Predictably, the nonreducing sugar alcohol D-xylitol gave no browning and negligible Δ (−7.7 ± 27 mV). Other pentose sugars, D-arabinose (−157 ± 7.5 mV) and D-ribose (−192 ± 4.5 mV), also displayed larger changes in potential relative to D-glucose. Therefore, for reducing saccharides the electrochemical data suggest an order of reactivity of ribose > xylose ∼ arabinose > glucose ∼ rhamnose ∼ mannose ∼ lactose > fructose in the Maillard reaction.

For the D-glucose/β-alanine reaction longer reaction times at reflux temperature led to greater Δ values at pH 6.85 that paralleled visual color development (**Figure 3**), with Δ ranging from −101 to − 204 mV in 1−6 h. However, long reaction times failed to produce a significant Δ effect in reactions run under physiological conditions. A 0.1 M buffer solution (pH 7.04) containing D-xylose (0.25 M) and glycine (0.05 M) stored for 28 days at 37 °C developed a pale yellow color, but its Δ value (−20 mV) hardly differed from that of a colorless 0.25 M D-xylitol control (−35 mV). In contrast to our results, reductones have been detected previously in Maillard systems under physiological conditions using chemical derivatization (*15*).

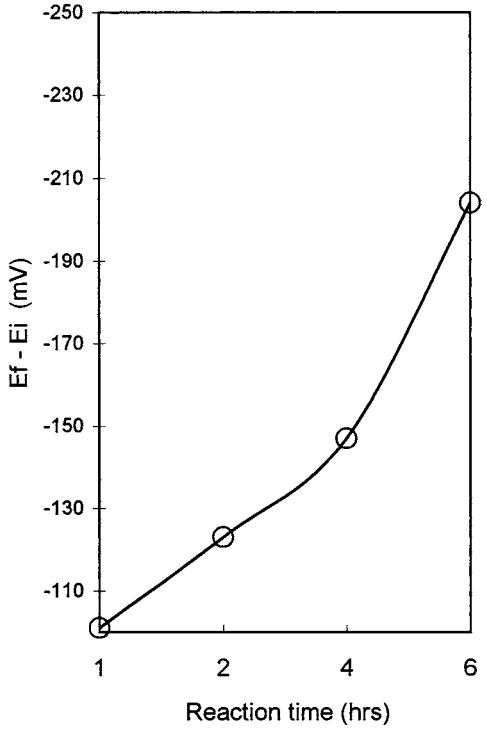

Figure 3. Changes in redox potential for the D-glucose/β-alanine Maillard reaction at 100 °C.

The effect of buffer alone on reducing sugars appeared to be minimal. Control experiments with D-xylose and D-glucose (without β-alanine) run under **Table 1** conditions gave Δ values of −42 ± 28 and −25 mV, respectively. In addition, no visual browning was observed in the absence of amino acids.

Electrochemical Effects Caused by Amadori Compound Decomposition. Because Amadori compounds are believed to be the penultimate precursors of reductones, it was also of interest to examine their behavior under our standardized Maillard reaction conditions. For study, we selected as a model compound *N*-(1-deoxy-D-fructos-1-yl)piperidine (**1**) because it was readily synthesized and its thermal decomposition products had already been well characterized (*16*).

Exposure of Amadori compound **1**, initially at 0.01 M, to 1 h of refluxing in 0.10 M buffer at pH 6.85 led to an expected deep yellow coloration and a Δ value of −153 mV. In view of the apparent rapid reaction of **1** at ∼100 °C, we decided to study the effects of milder conditions obtainable under controlled microwave heating (**Table 2**). In these experiments small vials of reactant solutions in 0.10 M buffer at pH 6.85 were heated for various times at ∼77 °C. After 15 min of microwave heating, 0.01 M **1** developed a small Δ of −26 mV, whereas under the same conditions a solution containing 0.21 M D-xylose and 0.061 M β-alanine was virtually unchanged with a Δ of +4 mV. When the experiment was begun instead with 0.025 M **1**, a more pronounced potential change, −124 mV, was observed in the same time of heating. At 0.05 M **1** a steady decline in Δ from −55 to −223 mV was observed between 5 and 15 min, and in a single experiment starting with 0.101 M **1**, a Δ of − 362 mV was obtained in 15 min. These data suggest that at 77 °C under these reaction conditions the rate of Amadori compound formation from D-xylose and β-alanine is very slow

Table 2. Heat-Induced Changes in Redox Potential of *N*-(1-Deoxy-D-fructos-1-yl)piperidine (**1**) in 0.10 M Bis-tris Buffer at pH 6.85

microwave heating conditions[a]	initial reactant(s) concn	$E_f - E_i$, Δ in mV
~77 °C, 15 min	0.01 M compd **1**	−26
control: 22 °C, 1 h	0.01 M compd **1**	−16
control: 22 °C, 5 h	0.01 M compd **1**	−15
~77 °C, 15 min	0.025 M compd **1**	−124
~77 °C, 5 min	0.05 M compd **1**	−55
~77 °C, 10 min	0.05 M compd **1**	−132
~77 °C, 15 min	0.05 M compd **1**	−223
~77 °C, 15 min	0.21 M D-xylose + 0.061 M β-alanine	+4

[a] ~77 °C = lowest power microwave setting (see Experimental Procedures).

relative to its decomposition rate. Had a concentration of 0.01−0.025 M Amadori compound been rapidly formed, its presence would have been evidenced by a Δ value more negative than 4 mV for xylose/β-alanine. The value of −124 mV from 0.025 M **1** indicated a buildup of reducing substances in 15 min at 77 °C comparable to that obtained in Maillard reactions of pentose sugars after 1 h at ~100 °C (**Table 1**). Evidently at 100 °C both formation and decomposition of Amadori compounds take place rapidly as shown by the accumulation of reducing species with time. Presumably, at some point beyond our longest experiment (6 h at 100 °C) the level of reducing substances will reach a maximum value before beginning to decline. If true, this would be consistent with the well-accepted notion of polymerization of reactive intermediates to form high molecular weight melanoidins. Although quite reactive at 77 °C, the Amadori compound **1** seemed to be fairly stable in buffer at 22 °C and pH 6.85. A 0.01 M solution of **1** developed Δ values of only −16 and −15 mV and failed to develop any coloration after 1 and 5 h, respectively.

Electrochemical redox behavior was observed in Maillard reactions that was consistent with theoretical predictions based on the presence of reductones. An increasingly negative cell potential paralleled the course of the reactions, indicating the buildup of chemically reducing species. Electrochemical measurement of Maillard reactions is a viable analytical tool for following the course of the reaction in terms of reactive intermediates. Further research is required to determine whether this technique can be applied to more complex systems such as food matrices.

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