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On the Effect of Tetraborate Ions in the Generation of Colored Products in Thermally Processed Glycine–Carbohydrate Solutions

GEORGE P. RIZZI*

Procter & Gamble Company, Winton Hill Business Center, 6300 Center Hill Road, Cincinnati, Ohio 45224

The effect of tetraborate ions on Maillard browning was investigated in a series of monosaccharide−glycine reactions in aqueous bis-tris buffer at pH 7.2. Addition of borax (sodium tetraborate) in catalytic amounts led to enhanced browning measured by absorbance at 420 nm in the order xylose > arabinose > galactose ∼ fructose > ribose > mannose > rhamnose, and the degree of browning with borax was uniformly greater than that produced by phosphate on an equimolar basis. A mechanism is proposed for borax catalysis in which monosaccharide−borate complexation shifts carbohydrate equilibria to favor open-chain (carbonyl) forms, thereby enhancing the rate of the Maillard reaction.

KEYWORDS: Maillard; browning; tetraborate; complexation; catalysis

INTRODUCTION

In Maillard reactions, amino groups of protein (or amino acids) react with reducing sugars to initiate a complex series of reactions whose major product is eventually a brown polymer. In these reactions, the free (or hydrated) carbonyl form of sugars is believed to be a key reactive species. However, from mechanistic considerations, the concentration of said carbonyl components required for these reactions is limited by unfavorable equilibria involving more stable, cyclic (hemi-acetal) forms of sugars. In fact, the amount of free sugar carbonyl in aqueous solution is reported to vary from zero to only a fraction of one percent (*1*). In a previous study, we observed that the degree of Maillard browning in a series of aldose sugars increased significantly with a higher free aldehyde content reported in sugar equilibria (*2*) (**Table 1**). If the overall browning rate truly depends on the free carbonyl concentration, then factors that control the position of sugar equilibria should be involved in the kinetics of Maillard color development. We began our study by considering factors favoring enhanced formation of free carbonyls (i.e., factors that could promote the Maillard reaction). In this connection, it was significant that sodium tetraborate had recently been shown to enhance the amounts of the aldehydo form of sugars present in aqueous solution (*3*). Borates (i.e., various salts of boric acid) are commonly used per se as alkaline buffers, but apparently none have been considered as catalysts or reactants in the Maillard reaction. Borates and boric acid have been employed to a limited extent as preservative agents in foods (*4*). Sodium tetraborate decahydrate (borax) is used in French and Iranian caviar and is assigned the designation E285 in the international numbering system for food additives. In their role as preservatives, borates are added to foods at levels up to 4 g/kg to inhibit the growth of insects, bacteria, and fungi. The borates appear to have preservative properties in foods similar to those of common salt (NaCl). At present, boric acid and its salts are not permitted as food additives in the U.S. In view of the reported effect of borates on carbohydrate equilibria, it became the objective of this work to investigate the effect of sodium tetraborate decahydrate (borax) as an additive on the rate of browning in a series of glycine−carbohydrate reactions. Also, it was of interest to compare the effects of the tetraborate ion to that of another polyatomic anion (i.e., phosphate) that had been investigated previously (*2*).

Table 1. Maillard Browning as a Function of Sugar Carbonyl Form Present in Solution

sugar	browning degree (A_{420})[a]	free carbonyl (%)[b]
ribose	1.17	0.05
xylose	0.84	
arabinose	0.64	0.03
glucose	0.30	0.002
galactose	0.79	
mannose	0.021	0.005
rhamnose	0.094	
fructose	0.37	
lactose	0.21	
allose		0.01
idose		0.2

[a] Absorbance of sugar–β-ala reactions after 80 min in pH 7.3 bis-tris buffer at 100 °C (*2*). [b] Data from ref *1*.

* To whom correspondence should be addressed. Tel.: (513) 761-0816. E-mail: georgerizzi@yahoo.com.

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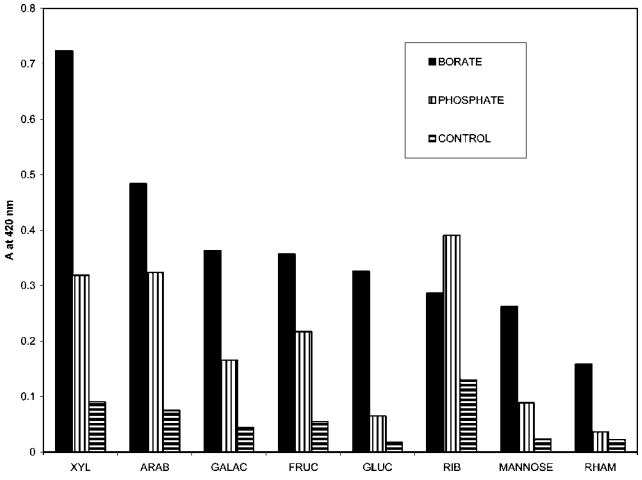


Figure 1. Degree of browning in monosaccharide–glycine reactions in pH 7.2 bis-tris buffer after 80 min at 100 °C.

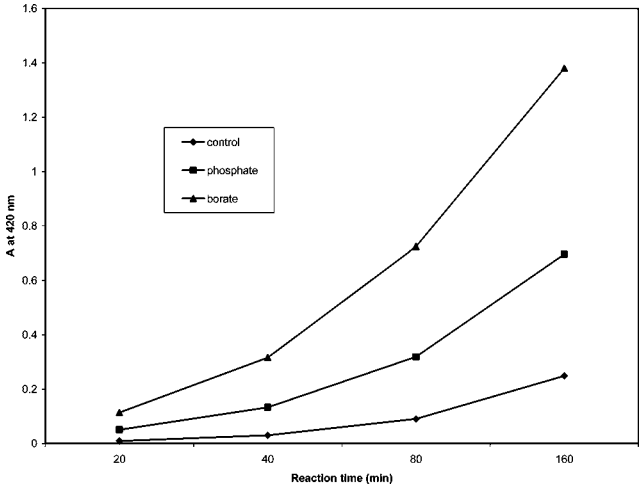


Figure 2. Browning produced in xylose–glycine reactions in pH 7.2 bis-tris buffer at 100 °C.

MATERIALS AND METHODS

Materials. All chemical reagents were high quality commercially available materials of at least 98% purity. Sugars were D-isomers except for l-rhamnose. Phosphate buffer (0.10 M, pH 7.0) was prepared by combining aqueous solutions of 0.10 M Na_2HPO_4 and 0.10 M NaH_2PO_4. Bis-Tris buffer consisted of 0.10 M aqueous 2,2-bis(hydroxymethyl)-2,2′,2″-nitrilotriethanol adjusted to pH 7.2 with 6 N HCl. A neutral 0.10 M borax buffer was prepared by adding concd. HCl to 0.10 M aqueous $Na_2B_4O_7 \cdot 10\ H_2O$ to reach pH 7.1.

Reaction Procedures. Maillard browning reactions were usually performed by refluxing 0.10 M aqueous bis-tris buffer solutions (initially at pH 7.2) containing various carbohydrates (initially 0.10 M) and glycine (initially 0.036 M) with or without Na_2HPO_4 (initially 0.0092 M) or $Na_2B_4O_7 \cdot 10\ H_2O$ (initially 0.0092 M) in round-bottomed flasks arranged for reflux and fitted with a rubber septa and steel hypodermic needles for withdrawal of samples. Reactions were maintained at approximately neutral pH under atmospheric pressure reflux at ca. 100−105 °C for periods ranging up to 160 min. The pH measured after each reaction did not vary more than ±0.3 pH units from the initial buffer pH. For rate measurements, small samples of hot reaction mixtures were withdrawn at increasing time intervals with pre-chilled syringes and immediately were diluted 1:4 with 23 °C water prior to absorbance measurement (vs pure water) in 1 cm cuvettes at 420 nm. Diluted reaction mixtures exhibited nearly constant absorbance (*A* values) within ca. ±0.002 *A* during measurements. Experimental precision for absorbance values was ca. ±20% for replicate experiments. For the measurement of blue pigment formation, ca. 2.5 mL of undiluted samples was removed at timed intervals and spectrophotometrically scanned from 400 to 700 nm. Absorbance (A_{620}) due to blue pigment formation was estimated by subtracting the absorbance of the sloping baseline under the well-defined peak at 620 nm.

Instrumental Analyses. UV−vis data were obtained with a computer interfaced Hitachi U-3010 spectrophotometer using a slit width of 2 nm and a scan speed of 250 nm/min.

RESULTS

Effects of Borax and Phosphate on Browning. Maillard browning was investigated in a model system consisting of an aqueous 0.1 M bis-tris buffer initially at pH 7.2, generally containing a monosaccharide initially at 0.10 M and glycine initially at 0.036 M with or without added borax ($Na_2B_4O_7 \cdot 10\ H_2O$) or disodium phosphate (Na_2HPO_4) at 0.0092 M. Reaction mixtures were heated at reflux (ca. 100 °C) for up to 160 min during which time samples were withdrawn to assess the degree of browning by spectrophotometric analysis at 420 nm. The reaction pH did not vary more than ±0.3 pH units during the course of the experiments. The addition of borax led to enhanced browning versus control in all glycine−monosaccharide reactions examined (**Figure 1**) with the degree of browning decreasing in the order xylose > arabinose > galactose ∼ fructose > ribose > mannose > rhamnose. The catalytic effect of borax persisted at levels added below 0.0092 M. For example, in 80 min ribose−glycine reactions, borax added at 0.0030 and 0.0015 M produced A_{420} values of 0.220 and 0.184, respectively, versus a control value of 0.130. With the exception of ribose, the addition of borax to monosaccharide−glycine reactions produced more browning than phosphate on an equimolar basis. The progression of browning versus time exemplified in the xylose−glycine reaction (**Figure 2**) suggested that a similar but retarded chemistry was taking place in the case of added phosphate. In phosphate catalyzed reactions, aldopentoses were again the most active browning agents with browning degrees decreasing in the order ribose > arabinose ∼ xylose > fructose > galactose > mannose > rhamnose.

Several attempts were made to observe the catalysis of Maillard browning in boric acid solutions adjusted to near neutral pH. Aqueous solutions of boric acid at 0.10 and 0.20 M adjusted to pH 7.3 with sodium hydroxide served as reaction media for ribose−glycine reactions with initial sugar−amino acid concentrations of 0.10 and 0.036 M, respectively. Little browning was observed in these systems after 80 min at 100 °C [absorbance (*A*) of 1:4 diluted reaction mixture < 0.05] probably as a result of ineffectual pH control by the boric acid−borate buffer system near neutral pH (boric acid pK_a is ca. 9.0). In 0.20 M boric acid−borate initially at pH 7.3, the final reaction pH fell to 5.45, a pH level where protonation of glycine itself would deter browning.

The intrinsic value of boric acid as a Maillard browning catalyst was examined by adding it on an equimolar basis to borax to a xylose−glycine reaction in bis-tris buffer. The effect of boric acid on browning was minimal as compared to borax. In an 80 min reaction, 0.009 M (added) boric acid led to A_{420} of 0.152 versus 0.091 for a control, whereas 0.009 M borax produced A_{420} of 0.724 under the same conditions. In all of these reactions, the pH was maintained at 7.2 ± 0.2 in the bis-tris buffer.

Color Changes and Observation of a Blue Pigment. Visual color changes were observed during the course of monosaccharide−glycine reactions that were indicative of progressive chemical changes taking place. Monosaccharide reactions with

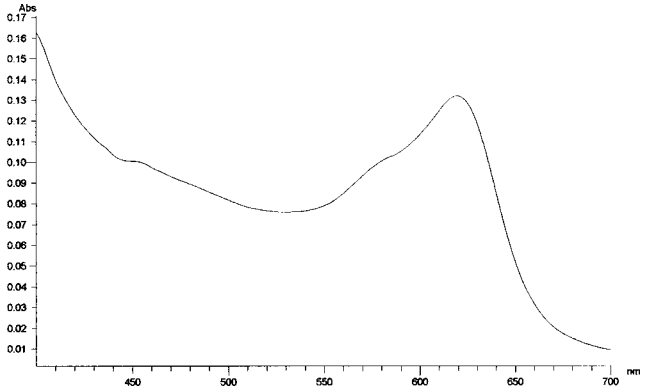
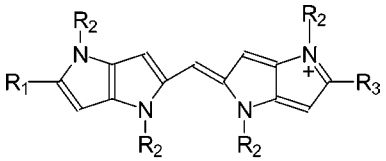


Figure 3. UV–vis spectrum of a xylose–glycine reaction in pH 7.2 bis-tris buffer after 30 min at 100 °C.

glycine in bis-tris buffer at pH 7.2 at 100 °C (with the exception of xylose and ribose) gradually changed color during 160 min from colorless through pale yellow to darker yellow and yellow−orange and finally to brown. For these sugars, similar qualitative changes in color were observed whether or not borax or phosphate had been added. The UV−vis spectra of these reaction mixtures exhibited no definite maxima in the range of 400−700 nm, only smooth, asymptotic decreases in absorbance extending toward visible wavelengths, behavior commonly associated with Maillard reactions (*5*).

However, the reactions of xylose and ribose with glycine in bis-tris buffer were unique with regard to visible color formation. In these reactions at 100 °C, colors ranged during 160 min from colorless through light blue, dark blue, blue−green, yellow−green, and finally to greenish black. Visually, it appeared that co-formation of blue and yellow products led to the appearance of the green hue and finally to the dark, almost black color. UV−vis spectra of xylose and ribose reaction mixtures at short reaction times revealed an absorption maximum at 618−620 nm indicative of a discrete blue chromophore (**Figure 3**). The compound responsible for the blue color was not identified, but it was presumed to be the same or similar to a blue pigment recently identified in xylose−glycine reactions by Japanese workers (*6*), which they designated Blue M1. Chemically, Blue M1 was shown to be 5-{[1,4-(dicarboxymethyl)-5-(2,3-dihydroxypropyl)-2-pyrrolo[3,2-*b*]pyrrolylmethine}-1,4-(dicarboxymethyl)-2-(1,2,3-trihydroxypropyl)-pyrrolo[3,2-*b*]pyrrolylium (**1**). The UV−vis spectrum of Blue M1 was reported to exhibit a maximum at 625 nm ($\epsilon = 8.24 \times 10^4$ mol^{-1} L cm^{-1}) with a shoulder peak at 580 nm similar to what we have observed.



1

$R_1 = CH_2CHOHCH_2OH$
$R_2 = CH_2CO_2H$
$R_3 = (CHOH)_2CH_2OH$

In our system, the blue substance appeared to be a transient intermediate, gradually disappearing with time as yellow products became more dominant (**Table 2**). The proportion of blue pigment assessed by the ratio A_{620}/A_{420} was larger in xylose−glycine reactions versus ribose reactions and decreased rapidly with time in both reactions. In xylose or ribose reactions containing added borax or phosphate, less blue pigment was formed, but similar decreases in amounts occurred with time. Some differences were observed on the effects of borax versus phosphate on the formation of the blue pigment in various sugars, but this effect was not examined in detail. When xylose−glycine or ribose−glycine reactions were performed in 0.10 M phosphate buffer (initially pH 7.0), rapid browning occurred at 100 °C, but no trace of discrete chromophores could be detected in the range of 400−700 nm in reaction mixtures at various reaction times.

Table 2. Effect of Added Borate or Phosphate on the Degree of Blue Pigment Formation in Aldose–Glycine Reactions in pH 7.2 Bis-Tris Buffer at 100 °C[a]

	A_{620}/A_{420}		
reaction time (min)	control	with borate	with phosphate
	[ribose]		
20	0.55	0.21	0.22
40	0.55	0.20	0.16
80	0.33	0.12	0.079
160	0.12	0.034	0.0021
	[xylose]		
20	0.80	0.049	0.41
40	0.71	0.036	0.29
80	0.39		0.16
160	0.14		

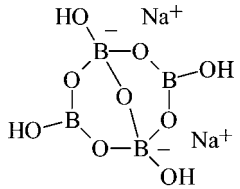
[a] Initial reactant concentrations: sugars, 0.10 M; glycine, 0.036 M; and borate or phosphate 0.009 M. Reaction time 80 min.

DISCUSSION

The chemistry of boric acid salts (i.e., borates) suggested that they might be unique catalysts for the Maillard reaction. Boric acid itself is a weak acid (pK_a ca. 9.0) that ionizes to a slight extent by reaction with water at neutral pH to form the tetrahydroxyborate ion (eq 1).

$$B(OH)_3 + H_2O \rightleftharpoons [B(OH)_4]^{-1} + H^+ \qquad (1)$$

At higher pH, polymeric borates are formed, notably the stable compound, sodium tetraborate decahydrate (borax), $Na_2B_4O_7 \cdot 10H_2O$. Structurally, borax is known to be an octahydrate of the bicyclic tetrameric compound $Na_2B_4O_5(OH)_4$ **2** (*7*).



2

The relevance of borate chemistry to Maillard catalysis was suggested by a recent study in which the addition of borax was shown to increase the amount of sugar carbonyl (open-chain form) present in aqueous carbohydrate equilibria (*3*). Since sugar carbonyls are reactive partners in the Maillard reaction, it follows that borates might serve as Maillard catalysts by raising a reactant concentration. Mechanistically, the enhancement of sugar carbonyl concentration can be explained in terms of carbohydrate complex formation with tetrahydroxyborate ions. At near neutral pH, tetrahydroxyborate ions can be formed by hydrolysis of borax (eq 2). Borate−polyol complexation is a well studied phenomenon, and borate-enhanced electrophoretic mobility of sugars has been explained by invoking negatively

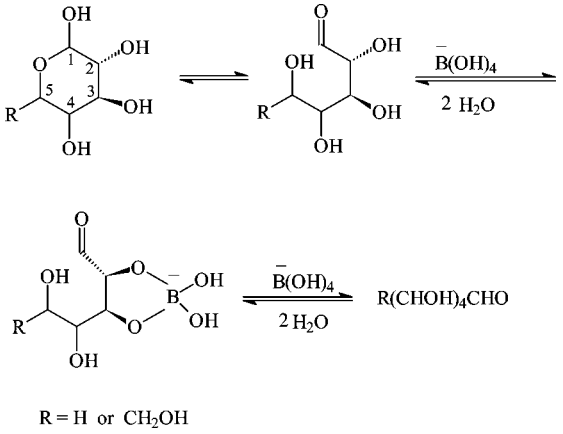
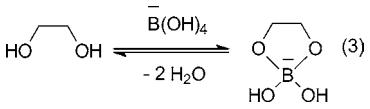


Figure 4. Mechanism for enhancement of sugar aldehyde forms via borate complexation.

charged complexes derived from tetrahydroxyborate ions and adjacent hydroxyl groups on the sugars (*3*).

$$[B_4O_5(OH)_4]^{-2} + 5H_2O \rightleftarrows 3\,B(OH)_3 + [B(OH)_4]^{-1} + OH^{-1} \quad (2)$$

In addition, this complexation has been shown to be stereoselective in that borate appears to react exclusively with *cis*-vicinal glycols to form cyclic cis-complexes (*8*) (eq 3).



The stereochemical (cis) preference for borate complexation also extends to acyclic systems in which 1,2-*syn*-glycols are selectively complexed versus 1,2-*anti*-glycols (*9*).

On the basis of the knowledge of borate−polyol complexation behavior, it is predicted that carbohydrate structures with rigid 1,2-*cis*-glycol configurations (i.e., cyclic hemiacetals with *cis*-vicinal hydroxyls or open-chain structures capable of adopting a 1,2-syn-glycol configurations) would be favored to form borate complexes. Moreover, under equilibrating conditions, borate complexation was envisioned to encourage the formation of open-chain forms of trans-configured sugars by reaction with pairs of conformationally mobile hydroxyl groups (**Figure 4**). Thus, borax was predicted to accelerate Maillard browning indirectly for aldoses and ketoses containing trans-configured vicinal hydroxyl groups. Sugars with cis-configurations could react directly in their hemiacetal forms and thereby not be a source of increased free carbonyls.

The effects of borax on the rate of browning (**Figure 1**) are mostly explained by our aforementioned hypothesis provided that complexation takes place mainly involving sugar hydroxyls at C-2 and C-3. Sugars exhibiting the most browning with borax (i.e., xylose, arabinose, glucose, galactose, and fructose) all have trans-configured hydroxyls in the C-2/C-3 positions of their hemiacetal forms. Therefore, in these sugars, complexation occurs in their open-chain form following ring-chain tautomerization (**Figure 4**). The net effect is a higher concentration of carbonyl moieties and thereby enhanced Maillard browning. Conversely, sugars with cis-configured C-2/C-3 hydroxyls in their hemiacetal forms (i.e., ribose and mannose complexation) do not require ring-chain tautomerization explaining why for these sugars less browning occurred with borax addition. Rhamnose, an all-*trans*-polyol-like glucose, showed less browning than expected as predicted by our model. Possibly, deoxysaccharides like rhamnose (5-deoxyglucose) are less prone to borate complexation via ring-chain tautomerization.

The addition of phosphate to monosaccharide−glycine reactions led to relatively less browning than equimolar amounts of borax (**Figure 1**). With phosphate, the greatest browning occurred in aldopentose−glycine reactions in the order ribose > xylose > arabinose, similar to that previously observed in monosaccharide−β-alanine reactions (*2*) (**Table 1**). It is reasonable to assume that browning catalysis by phosphate and borax operates by completely different mechanisms acting during the early stage of the Maillard reaction. If this assumption is correct, then a combined phosphate−borax catalyst is predicted to have a synergistic effect on the Maillard reaction. This and other aspects of Maillard reaction catalysis will be the subject of a further investigation.

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