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Role of Sugar Fragmentation in an Early Stage Browning of Amino-carbonyl Reaction of Sugar with Amino Acid

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Glycolaldehyde and methylglyoxal, both model compounds structurally related to potential C_2 and C_3 sugar fragments, showed extremely high reaction rates in browning with β-alanine compared to the usual reducing sugars and even to such active intermediate products of amino-carbonyl reaction as the Amadori product and osones. Production of C_2 and C_3 sugar fragments in a glucose–β-alanine system was negligible in acidic conditions, but increased with pH in a manner parallel to the increase in browning and also to the N/C ratio of the melanoidins. These results indicated that the proposed new pathway of browning, involving sugar fragmentation, is very important in the initial stages of browning in the Maillard reaction of neutral or alkaline solutions.

The mechanism proposed by Hodge for the amino-carbonyl reaction of sugar with amines or amino acids has been accepted as the most appropriate description to give a browning polymer, melanoidin.[1,2] The main process of this pathway is initiated by the condensation of the sugar with the amino compound to yield a glycosylamine, followed by Amadori rearrangement as a key step, and deamination of the 1,2-enaminol form of this product to give 3-deoxy- or 1-deoxy-hexosones, considered to be important intermediates in the formation of melanoidin. In this mechanism, no consideration has been given to sugar fragmentation as a source of reactive intermediates at an early stage, and three-carbon carbonyl products have been assumed only as minor active products from 1-deoxyhexosones in the middle stage of the reaction.

As previously reported,[3～7] we have found that a free radical product was formed at an early stage in the browning reaction of sugar-amino compounds, and ESR analyses led to the assignment of an *N,N'*-dialkylpyrazine cation radical structure. This induced the assumption of formation of a C_2 sugar fragment as a precursor of the radical product, demonstrated by isolation and identification of glyoxal dialkylimine (C_2 imine). Thus we have proposed a new pathway for browning that involves cleavage of the sugar moiety of the Schiff base at the initial stage of the amino-carbonyl reaction, before the Amadori rearrangement, followed by formation of a C_2 fragmentation. Methylglyoxal dialkylimine (C_3 imine) was also identified as a C_3 sugar fragment at an early stage of the browning reaction and was considered to be produced directly from the Amadori product by another new pathway.[8] This paper deals with studies on the role of C_2 and C_3 carbonyl products in the early stages of browning of sugars and amino acids.

EXPERIMENTAL

D-Glucosyl-β-alanine (glucosylamine), 1-β-alanino-1-deoxy-D-fructose (Amadori product), 3-deoxy-D-glucosone and D-glucosone were prepared according to the literature.[9～12] Other reagents were of guaranteed grade. Mixtures of sugar or a carbonyl compound with an amino acid in water were heated at a constant temperature for a given time. When necessary, the pH of the mixed solution was adjusted at the starting point by sodium hydroxide or hydrochloric acid.

Amounts of C_2 and C_3 compounds were measured by a modification of the *o*-phenylenediamine method,[13] using capillary GC, and the details have been shown.[8] The

Amadori product was analyzed by HPLC using a JEOL TWINCLE pump with a Shodex RI SE-II detector and a column (30 cm × 0.4 cm i.d.) of LiChrosorb NH_2 with acetonitrile–water (70:30) as a solvent (2 ml/min). Browning was measured as the O.D. at 420 nm.

To prepare melanoidin, a mixture of glucose and β-alanine in water reacted at a definite pH was adjusted to neutral pH with sodium hydroxide or hydrochloric acid, put on a Sephadex G-15 column (2.9 i.d. × 42 cm), and separated with distilled water. The melanoidin fraction was observed by its O.D. at 440 nm using a Hitachi 034 UV-VIS effluent monitor. After removal of the first part of the elution (about 90 ml), a brown fraction (30 ~ 110 ml) was collected, lyophilized, and used for elemental analysis. By TLC this fraction of melanoidin contained no low molecular weight products or materials.

RESULTS AND DISCUSSION

1. *Effects of pH on the formation of sugar fragmentation products*

It was shown previously[3] that, with increases in pH of the reaction mixture of glucose and β-alanine, the amount of free radicals increased parallel to browning. Therefore, it is considered that occurrence of the fragmentation depends greatly on the pH of the reaction mixture. Formation of C_2 and C_3 products at different pHs was investigated with the glucose-β-alanine system and compared with browning and the production of the Amadori compound. As shown in Fig. 1A, production of both C_2 and C_3 products was greatly influenced by the pH of the reaction mixture, large in alkaline but negligible in acidic mixtures. Development of browning (Fig. 1B), as already known, intensified greatly in higher pH solutions, but the effects of pH on the formation of the Amadori product (Fig. 1C) seemed not nearly so large.

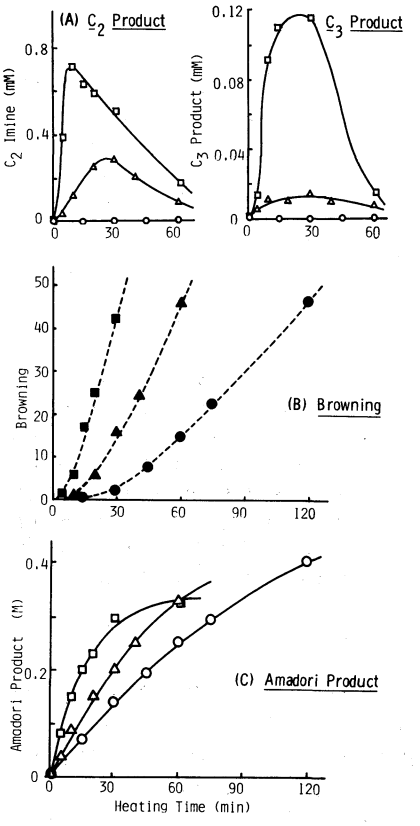

FIG. 1. Formation of C_2 and C_3 Imines (A), Browning (B) and Amadori Product (C) during the Reaction of Glucose with β-Alanine (2 M each), Heated in a Boiling Water Bath.

○ ●, acidic conditions (pH 3.51); △ ▲, neutral conditions (pH 6.42); □ ■, alkaline conditions (pH 9.29).

2. *Browning ability of fragmentation products*

The C_2 and C_3 fragmentation products are assumed to be as glycolaldehyde, glyoxal, methylglyoxal, glyceraldehyde *etc.*, or their imine derivatives. The browning abilities of these carbonyl compounds were measured and compared with those of xylose and glucose in sugar–β-alanine systems by monitoring the O.D. at 420 nm. Rates of browning of glycolaldehyde and methylglyoxal were so high that they could not be compared directly with that of glucose under the same reaction temperature, so first, they were compared with xylose at 80°C, and then xylose and glucose were compared at 95°C, as shown in Fig. 2. In the cases of C_2 and C_3 compounds, the browning reaction had hardly any induction time, while the sugars had definite induction periods. As a result of this, the rates of early browning could not be strictly compared, and a tentative comparison between glucose and small molecular weight carbonyl compounds was made by using the value of the reciprocal of the time taken to reach O.D. 5.0.(Table I), where sugar fragmentation was assumed to occur rapidly in most glucose–amine sys-

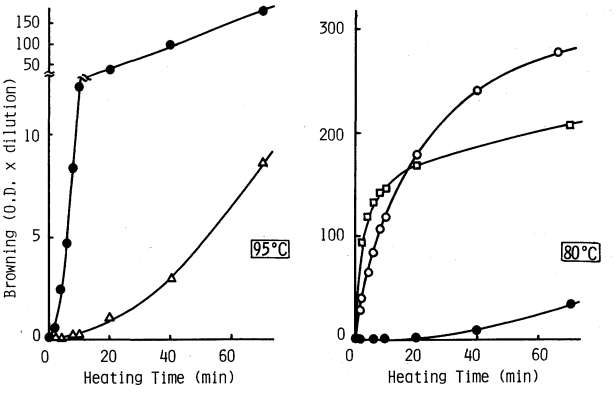

FIG. 2. Browning by the Reaction of Sugar or Carbonyl Compound with β-Alanine (1 M each in Water).
●, xylose; △, glucose; ○, methylglyoxal; □, glycolaldehyde.

TABLE I. BROWNING BY THE REACTION OF β-ALANINE WITH SUGAR OR CARBONYL COMPOUNDS

Sugar or carbonyl compds.	Reaction temp. (°C)	Browning activity (1/min)	Relative value
Glucose	95	0.019	1
Fructose	95	0.014	0.74
Xylose	95	0.166	8.74
Xylose	80	0.037	
Methylglyoxal	80	2.77	654.3
Glyceraldehyde	80	8.33	1967
Glyoxal	80	0.515	121.6
Glycolaldehyde	80	8.93	2109

Mixtures (1 M each) were heated for 20 min.

TABLE II. BROWNING BY THE REACTION OF INTERMEDIATE PRODUCTS FROM β-ALANINE SYSTEMS

Materials	Browning activity (1/min)	Relative value
Glucose+β-Ala	0.028	1
Glucosylamine	0.138	4.93
Amadori product	0.023	0.821
Amadori product+β-Ala	0.072	2.57
Glucosone+β-Ala	0.666	23.7
3-Deoxyglucosone+β-Ala	3.85	137.5

Materials (1 M each) were heated for 20 min in a boiling water bath.

tems.[3,5,6] In the table, the browning abilities of various sugar-related compounds are also indicated relative to that of glucose. As shown in Table I, small molecular weight aldehyde compounds had extremely high browning abilities compared with the sugars, *e.g.*, the relative values for glycolaldehyde and glyceraldehyde were about 2,000 times and that of methylglyoxal was about 650 times that of glucose.

The browning abilities of the well-known intermediate products of the browning reaction were measured in a similar way and are shown in Table II. 3-Deoxyglucosone and glucosone had higher abilities than glucosylamine and its Amadori product. However, the values for glycolaldehyde and glyceraldehyde were far higher than those of these intermediates. This means that, even if only a small amount of sugar fragmentation occurs, browning by sugar fragmentation will make a major contribution to browning in the early stage.

3. *Contribution of sugar fragmentation to early browning*

In attempts to evaluate the contribution of sugar fragmentation to browning at early stages of the Maillard reaction, pH effects on the fragmentation, browning, and Amadori compound production shown in Fig. 1 were analyzed using their approximate rates. Table III showed a comparison of their rates between

TABLE III. INITIAL RATE OF BROWNING AND PRODUCT FORMATION ON THE BASIS OF THE DATA IN FIG. 1.

Condition	Browning (O.D./min)	Amadori product (mM/min)	C_2 Imine (mM/min)	C_3 Imine (mM/min)
Neutral	0.243*	9.8	0.017	0.001
Alkaline	0.989**	14.8	0.079	0.015
Alkaline/Neutral	4.0	1.5	4.6	15

Asterisks mean average values with heating for 30 (*) and 15 min (**).

TABLE IV. N/C RATIO OF MELANOIDINS PRODUCED BY THE REACTION OF β-ALANINE WITH GLUCOSE OR GLYCOLALDEHYDE

Materials	Initial pH	Heating time (min)	Browning	N/C
Glucose (2 M each)	2.3	190	21	0.083 ± 0.006
Glucose (2 M each)	6.5	53	21	0.111 ± 0.009
Glucose (2 M each)	9.2	15～25	31～37	0.132 ± 0.008
Glycolaldehyde (1 M each)	5.9	30	183	0.15

Mixtures were heated in a boiling water bath.

neutral and alkaline conditions. The increase in the rate of browning caused by alkalinity was about 4.0 times while the increases in the cases of C_2 and C_3 formations were 4.6 and 15 times, respectively, and that in Amadori product formation was only about 1.5 times. These differences suggest that the increase in browning rate depends only to a limited extent on browning caused by the Amadori product and its breakdown products.

Here, the rates of formation of C_2 and C_3 products were known to be very small compared to that of the Amadori product even under alkaline conditions. However, the contribution of these fragmentation products to browning at an early stage could not be evaluated as negligible due to their low yields, because, as shown in Tables I and II, the browning abilities of these fragmentation product are estimated to be at least several hundred times as high as that of the Amadori product. Thus in increase in formation rates of the fragmentation products with increase in pH might contribute very significantly to the observed increase in browning.

Based on these results, it seems that the fragmentation of its sugar moiety may contribute significantly to browning at an early stage of the Maillard reaction, especially at neutral pH or above. In other words, the browning observed at early stages of the Maillard reaction at pHs above neutral might be caused mainly by browning through the fragmentation pathways.

4. *Supplementary evidence on the role of sugar fragmentation*

The above mentioned proposal, that sugar fragmentation may contribute greatly to early stages of the browning reaction, especially in neutral or alkaline solutions, led to the assumption that browning products, melanoidins, produced at alkaline pHs might be somewhat different in their components to those formed at acidic pHs, due to differences in their main precursor compounds.

Gel chromatographic separation of browning products from the glucose–β-alanine mixtures of different pHs and almost the same value in O.D. at 420 nm was done by using Sephadex G-15. The nitrogen to carbon ratio (N/C) of each isolated browning product, melanoidin, from its elementary analysis is shown in Table IV. This ratio was different depending on the initial pH value of the reaction, that is, the ratio increased with in-

creases in pH value and reached that of the melanoidin from glycolaldehyde–β-alanine system.

The chemical structure of so-called melanoidin is not yet clear but probably it does not have a definite one and there exists a range of various types of melanoidin, depending on such preparative conditions as materials, pH, and temperature. If we suppose the melanoidins obtained at alkaline and acidic conditions were those formed with or without an important contribution from sugar fragmentation products, the difference in N/C ratio obtained is reasonable.

In the case of the well-known pathway, melanoidin is said to be produced mainly without cleavage of the sugar part, and is basically composed of equimolar amounts of sugar and amino compound.[14] If melanoidin is induced with equimolar sugar fragment and the amino compound in the same manner as the known pathway, a higher N/C ratio than that from the known pathway is expected, though we have no information on the ratio. This assumption was supported by the results in Table IV, where melanoidin from the glycolaldehyde system, as a sugar fragment model, had a higher N/C ratio than that from glucose system. Thus, the increased part of the N/C ratio in glucose system with increase of pH is assumed to be due to the sugar fragmentation pathway.

CONCLUSION

Some evidences for the role of sugar fragmentation on the browning reaction are presented here. These indicate that the sugar fragmentation pathway is related to browning at early stages of the reaction at neutral or alkaline pHs.

Figure 3 is a summary of the mechanisms for the early browning reaction. In acidic conditions, the main pathway inducing melanoidin formation is naturally considered to be the route of osone formation through Amadori rearrangement. However, there was no reasonable explanation for the increase of browning by increasing pH until our presentation, which proposes that an appreciable amount of this might be due to the pathway containing the sugar fragmentation caused mostly by amino-carbonyl reactions.

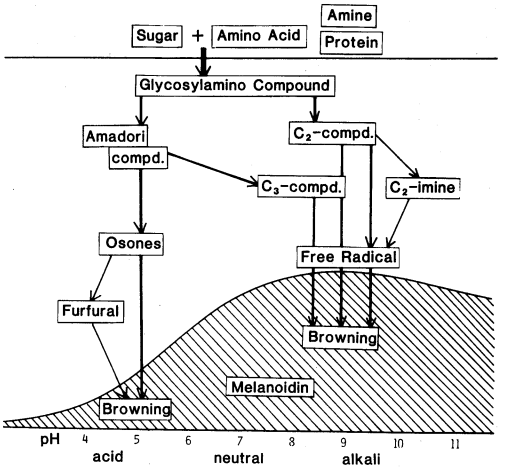

FIG. 3. A Type Figure on Different Pathways for Melanoidin Formation Depending on Reaction pH.

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