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Ascorbic acid browning: the incorporation of C_1 from ascorbic acid into melanoidins

C. G. A. Davies & B. L. Wedzicha

Procter Department of Food Science, University of Leeds, Leeds, UK, LS2 9JT.

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Labelled $^{14}C_1$-ascorbic acid and ^{14}C(U)-glycine were used to measure the incorporation of glycine and *non-decarboxylated* ascorbic acid, C_1-ASA, into melanoidins (molecular weight > 12 kdaltons) formed in the browning reaction between ascorbic acid, ASA, and glycine. The C_1-ASA : glycine stoichiometry is 1 : 1. With account taken of microanalysis data, the results suggest that the melanoidins are made up from two residues of decarboxylated ASA, i.e. perhaps 3-deoxypentosulose, one C_1-ASA molecule and one glycine molecule. Possible methods of the binding of ASA to intermediates in browning are considered.

INTRODUCTION

Under anaerobic conditions, ascorbic acid, ASA, undergoes a spontaneous decarboxylation and dehydration to form 3-deoxypentosulose, DP, and furfural, by way of the α,β-unsaturated dicarbonyl intermediate, 3,4-dideoxypentosulos-3-ene, DDP (Kurata & Sakurai, 1967). These products are the same as the intermediates in the Maillard browning of pentoses, and it is reasonable to suggest that the mechanisms of colour formation are similar in both cases (Wedzicha, 1984). The idea is further supported by the fact that the inhibition of these browning reactions by sulphite species leads to the formation of 3,4-dideoxy-4-sulphopentosulose as a result of the reaction of DDP with HSO_3^- (Wedzicha & McWeeny, 1974). Thus DP and DDP are regarded as key intermediates in both forms of browning, and they are analogous to 3-deoxyhexosulose and 3,4-dideoxyhexosulos-3-ene formed in the Maillard browning of hexoses (Wedzicha, 1984).

Despite these similarities, the coloured products (melanoidins) with molecular weight >12 kdaltons formed in arabinose-glycine browning have a somewhat different composition from those formed in ASA-glycine browning as shown in Table 1 (Davies & Wedzicha, 1992)*. The compositions of these melanoidins were calculated from microanalysis data assuming the reaction of a mol ASA with one mole glycine to give the melanoidin ($C_{6a+2q}H_{8a+5-2p}NO_{6a+2-p-2q}$) + p mol H_2O + q mol CO_2, and the reaction of b mol arabinose with one mole glycine to give the melanoidin ($C_{5b+2-y}H_{10b+5-2x}NO_{5b+2-x-2y}$) + x mol H_2O + y mol CO_2.

The ratio (1 : 1) of arabinose- to glycine-derived residues incorporated into melanoidins with molecular weights >12 kdaltons is similar to that found (1·25 : 1) for glucose- to glycine-derived residues (Wedzicha & Kaputo, 1987) in melanoidins from glucose + glycine. In contrast, the melanoidins obtained from ASA + glycine contain 3·1 times more ASA-derived residues per glycine incorporated into the polymer than pentose units in the Maillard reaction. The mechanism for the incorporation of ASA-derived residues in melanoidins is clearly different from that in the Maillard reaction of pentoses. This suggests that the colour-forming reaction in ASA-glycine mixtures may not pass exclusively through DP or it may also involve the addition of other, nitrogen-free, components of the reaction mixture to the growing polymer.

In the early stages of browning ($A_{450} < 1$), the extent of decarboxylation of ASA is *c.* 0·3 mol CO_2 per mol ASA incorporated (q = 1·1), which increases to 0·7 mol CO_2 per mol ASA (q = 2·44) in the later stages of reaction ($A_{450} > 5$), whereas the number of ASA-to-glycine-derived residues is constant and in the range (3·1–3·5) : 1 (Davies & Wedzicha, 1992). If DP were the only intermediate in colour formation, each ascorbic-acid-derived residue incorporated into the melanoidin would be accompanied by the liberation of one mole of CO_2 (i.e. q = 3·1–3·5). Hence the previous work suggests that non-decarboxylated ascorbic acid

* The 'sugar' : glycine ratios for melanoidins from arabinose and ASA were given in Davies & Wedzicha (1992) as 1·4 : 1 and 3·5 : 1, respectively. These were based on incorrect empirical formulae, and the correct values are shown in the present paper. The substance of the discussion in the previous paper is, however, not altered by this error.

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Table 1. Composition of non-dialysable (molecular weight >12 kdaltons) melanoidins obtained by heating mixtures of arabinose or ascorbic acid, ASA, with glycine*

Model system	Melanoidin	'Sugar' : glycine
Arabinose+glycine	$C_{7\cdot3}H_{11\cdot1}O_{5\cdot1}N$	1 : 1
ASA-glycine	$C_{20\cdot6}H_{30\cdot6}O_{20\cdot6}N$	3·1 : 1

* The 'sugar' : glycine ratio is the number of arabinose- or ASA-derived residues per glycine-derived residue in the melanoidin.

molecules are being incorporated into the melanoidin to a large extent, either as a result of a direct reaction with glycine, or by adding to a growing polymer, which may also involve DP residues.

The aim of the present work is to investigate rigorously the incorporation of C_1 from ASA into melanoidins from the ASA-glycine reaction by means of specifically labelled $^{14}C_1$-ASA and ^{14}C-glycine.

MATERIALS AND METHODS

Reaction mixtures were prepared by mixing aqueous solutions (10 ml) of ASA (2M) and glycine (1M) at pH 4 with $^{14}C_1$-ASA or $^{14}C(U)$-glycine (Amersham International plc) (10 ml) to give specific activities of 6·35 and 27·1 MBq mmol^{-1} for ASA and glycine, respectively. Each reaction mixture was dispensed as 1-ml aliquots into 2-ml sample vials, which were placed individually into 10-ml sample bottles containing aqueous NaOH (2 ml, 1M) to trap evolved CO_2. Reaction mixtures were held at 40°C and analysed at timed intervals (13–41 days).

Analysis was for total ^{14}C-activity and ^{14}C in melanoidins with molecular weight >12 kdaltons. The latter procedure was carried out by placing an aliquot of reaction mixture (1 ml) in visking tubing, dialysing against water (3 × 500 ml) until the ^{14}C-activity in the dialysate equalled the background, and counting the retentate after appropriate dilution. Quench correction was by the external-standard-channels-ratio method.

RESULTS AND DISCUSSION

Figure 1 shows the amount of ASA and glycine found to be associated with the high-molecular-weight melanoidins that remained after ASA-glycine reaction mixtures were dialysed. These amounts were calculated from the ^{14}C-activity in the retentate; in the case of glycine, the conversion of activity to number of moles assumes that both carbon atoms of each glycine molecule used to make up the melanoidin are present in the polymer, i.e. no decarboxylation of the amino acid has taken place. On the other hand, since the ASA is specifically labelled at position 1, the results refer only to the amount of non-decarboxylated ASA incorporated. The slope of the best line through the data in

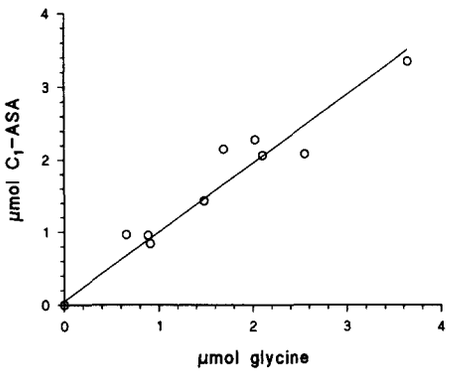
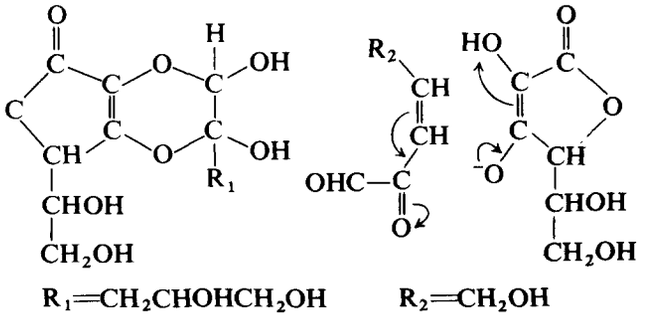


Fig 1. Amount of non-decarboxylated ascorbic acid (C_1-ASA) and glycine converted to melanoidin with molecular weight >12 kdaltons when 1 mmol ASA is heated with 0·5 mmol glycine at 40°C, pH 4·0 for varying lengths of time.

Fig. 1 is 1·0, suggesting that the ratio of non-decarboxylated ASA- to glycine-derived residues in the polymer is 1 : 1. This stoichiometry is maintained as the amount of melanoidin increases, though the maximum conversion of glycine in the set of experiments reported is only some 0·7% (mol/mol).

Microanalysis of these melanoidins (Davies & Wedzicha, 1992) gives the ratio of ASA- to glycine-derived residues (irrespective of decarboxylation) as (3·1–3·5) : 1. If decarboxylation of ASA occurs prior to the formation of melanoidins, the intermediate so formed is DP, and it is reasonable to suggest that DP or DP-derived residues form part of the structure of the melanoidin, as they would do in the Maillard browning of pentoses. If this is the case, the radiochemical data indicate that the repeating unit of the polymer could arise by the combination of two DP molecules with one molecule of each of ASA and glycine. The fact that inhibition of ascorbic acid browning by S(IV) gives rise to a high yield of DSP (Wedzicha & McWeeny, 1974) is evidence that DP is, indeed, formed prior to the formation of melanoidins.

The data shown in Fig. 1 were obtained with melanoidins whose A_{450} value was >1·0, rising to values in excess of 5·0. Thus the suggested degree of decarboxylation of ASA is in agreement with the higher over-all value for CO_2 production (2·44 mol CO_2 per mol glycine used to make up the melanoidin) obtained from microanalysis data. These data also show that the extent of incorporation of decarboxylated glycine into the melanoidin from arabinose + glycine is negligible.



There is no reason to expect the decarboxylation of glycine in the ASA-glycine reaction to be significant for a similar extent of browning; decarboxylation of amino acids is the result of the Strecker degradation reaction, which is caused by dicarbonyl compounds such as DP.

Possible reactions for the incorporation of non-decarboxylated ASA into melanoidins include the formation of an acetal, I, between the ene-diol and an α-dicarbonyl compound or the Michael addition, II, of the anion of ASA to an α,β-unsaturated carbonyl compound (Fodor *et al.*, 1983).

The reaction could involve DP (acetal formation) or DDP (both acetal and Michael-addition product) prior to polymerisation, or the reaction of ASA with these structures in a melanoidin molecule. Since the melanoidin formed in the Maillard reaction of arabinose consists of DP- and glycine-derived residues with 1:1 stoichiometry, a 1:1 reaction of ASA with the DP-derived subunit of such a melanoidin would give an over-all 2:1 stoichiometry of ASA-derived residues:glycine-derived residues. The observed stoichiometry is (3·1–3·5):1 (Davies & Wedzicha, 1992). An attractive idea is that ASA may react with DP or DDP (1:1) to form an intermediate. One molecule of this product could either add to a subunit of melanoidin formed from DP and glycine (1:1) or is involved in a distinct polymerisation reaction with a molecule of DP and glycine. In either case, the resulting stoichiometry would be 3:1 (ASA:glycine) in agreement with the microanalysis data.

It would now be of interest to examine the possible reactivity of DP–glycine mixtures, and melanoidins from the Maillard reaction of pentoses, towards ASA.

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