ATP-Induced Inhibition of Potato Browning. Effect of Ascorbic Acid Oxidase and of Reducing Substances

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When potato tubers and some other plants are sliced, polyphenol oxidase catalyzes the oxidation of phenols to form dark polymers. Reducing substances, such as ascorbic acid, prevent this browning by reducing the quinones formed in diphenol oxidation (6, 8). Browning of potato slices begins only after all ascorbic acid has been oxidized.

Previous studies have shown that plant slices treated with solutions of ATP remain substantially lighter than untreated controls (5). The interference of ATP with enzymic color formation appears to depend on metabolically active particulate systems. With purified polyphenol oxidase enzyme or particle-free extracts, ATP has no effect on the course or the products of the polyphenol oxidase reaction (4). Although ATP itself is not a reducing substance, its effect on plant slices resembles the effect of ascorbic acid or other reducing substances.

A possible explanation of the effect of ATP on potato slices is the activation of a mechanism for reduction of dehydroascorbic acid, or reduction of oxidized glutathione, or both. These reductions are closely interrelated inasmuch as the enzymic reduction of dehydroascorbic acid requires glutathione (6, 7).

If a reductive formation of ascorbic acid or glutathione were responsible for inhibition of browning by ATP, then this inhibition should be enhanced by adding the precursors, oxidized glutathione or dehydroascorbic acid, to slices treated with ATP. Furthermore, the prevention of browning by ATP should be nullified on addition of ascorbic acid oxidase. It will be shown that ascorbic acid does not appear to be the reducing substance formed upon addition of ATP. The relative effectiveness of several reducing compounds on enzymic browning of potato slices will also be given.

Materials and Methods

Russet and White Rose potatoes (Solanum tuberosum, L.) were stored at 5° before use. Methods and experimental conditions were similar to those used earlier (5). Substrate 3,4-dihydroxyphenylalanine was added to all samples including controls. Essentially the same results were obtained with and without added substrate but the rate of browning was slower without it. All materials and solutions were kept at 1 to 2°. Polyphenol oxidase was purchased from Mann Biochemicals.2 Crude preparations of ascorbic acid oxidase were made from cucumber and from summer squash (1); a highly purified preparation was a gift from C. R. Dawson. Dehydroascorbic acid was from Nutritional Biochemicals Company and was further purified by precipitation at 0° with absolute ethanol (3). Crystalline disodium salt of ATP was purchased from Pabst Laboratories.

Color changes were measured by reflectance directly on 6 mm thick potato slices and read on the Rd scale of a Gardner Automatic Color Difference Meter. Two slices with matched opposing surfaces from a single cut served as control and treatment

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1 Received Sept. 20, 1963.

2 Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others.
pair. Coded markings insured the same orientation for each measurement. The slices were held in a moist chamber at 20°C.

Reflectance measurements were expressed as decrease in per cent reflectance with time, \(-\Delta \text{Rd}\). Initial readings were subtracted from subsequent measurements. The data were plotted and values for \(-\Delta \text{Rd}\) for selected time intervals were read from the graphs.

Results and Discussion

Effect of Precursors and of Ascorbic Acid Oxidase on Browning of Potato Slices. Precursors of reducing substances, oxidized glutathione, cystine, and dehydroascorbic acid had little, if any, effect on color formation without ATP; none were reduced directly, nor did any one of them interfere with the normal browning reaction. Figure 1 shows that adding oxidized glutathione with ATP resulted in slightly less browning than adding ATP alone, but the difference is too small to be significant. Similar results were obtained with dehydroascorbic acid and with cystine.

Results shown in Figure 2 with purified ascorbic acid oxidase are representative of all three ascorbic acid oxidase preparations used. Figure 2 also includes results of experiments to determine the individual effects of components of the system. Slices treated with the oxidase alone developed as much color as the untreated controls. The addition of ascorbic acid alone completely prevented browning. The curve labeled “AA-oxidase + AA” shows the high efficiency of the ascorbic acid oxidase catalysis in the presence of the polyphenol oxidase system. Since the concentration of added ascorbic acid was high (0.05 M), the rate of its oxidation catalyzed by the oxidase must have been greater than the rate of ascorbic acid oxidation by quinones formed in the simultaneous oxidation of phenols. The development of color on addition of the oxidase with ATP was no different from the response to ATP alone. All three preparations of ascorbic acid oxidase failed to nullify the effect of ATP. Direct measurement in potato homogenates treated with ATP also failed to show the presence of ascorbic acid. (Makower, unpublished). These results suggest that the ATP-induced

Fig. 1. Effect of 0.05 M ATP, 0.05 M oxidized glutathione (GSGG), and both (ATP + GSSG) on color development in White Rose potato slices in the presence of 0.0005 M 3,4-DL-dihydroxyphenylalanine. Average of 8 determinations. Reagents were at pH 6.4. Measured as decrease in per cent reflectance, \(-\Delta \text{Rd}\), with time.

Fig. 2. The effect of ascorbic acid oxidase (AA-oxidase) alone, with 0.05 M ascorbic acid (AA), and with 0.05 M ATP on color development in Russet potato slices. The change in control (no treatment) and the effect of AA alone and ATP alone are included for comparison. 3,4-DL-dihydroxyphenylalanine (0.0005 M) was added to all slices. Average of 8 determinations. Reagents were at pH 6.2. Measured as decrease in per cent reflectance, \(-\Delta \text{Rd}\), with time.

Fig. 3. Inhibition of polyphenol oxidase activity by 0.0016 M and 0.0083 M dihydroxyfumaric acid. Measured spectrophotometrically at 420 m\(\mu\) in aerated 0.1 M citrate, pH 5.8, with 3,4-M-dihydroxyphenylalanine as substrate. Control contained no dihydroxyfumaric acid.
reduction of quinones in slices is not caused by ascorbic acid or glutathione, but by some other substance. The nature of this substance is being investigated.

Effect of Several Reducing Compounds on Browning of Potato Slices. Effectiveness in preventing enzymic color development by several thiols, some carboxyls, and dihydroxyfumaric acid have been tested. Thiols prevented browning as well as, or better than, ascorbic acid; carbonyl compounds were far less effective; the effect of dihydroxyfumaric acid was intermediate between those of thiols and of thiols (table I). Glucose, fructose, and glyceraldehyde (all at 0.05 M) had no effect on enzymic browning.

The fairly strong inhibition of browning by dihydroxyfumaric acid was also demonstrated spectrophotometrically with purified soluble polyphenol oxidase (#fig 3).

The chemical mechanisms of reduction, or other means of color prevention, may differ for the various compounds listed in table I. The reduction of quinones by ascorbic acid is well known (8). The reactions of cysteine and glutathione, which form complexes with quinones, have also been studied (2, 9). Little is known about the chemistry of color inhibition by carbonyl compounds. More chemical information is necessary to explain effectiveness of dihydroxyfumaric acid (10).

Summary

Addition of adenosine triphosphate (ATP) to potato slices inhibits the browning produced by enzymic oxidation of phenols. This inhibition is due to formation of reducing compound(s) from naturally occurring potato constituents. Formation of ascorbic acid as the active reducing agent is unlikely because adding oxidized glutathione or dihydroxyascorbic acid to slices does not enhance the effect of ATP, and adding both ascorbic acid oxidase and ATP does not counteract the decrease in browning produced by ATP alone. Comparative data are presented on the inhibition of enzymic browning in potato slices by ascorbic acid, several thiols, dihydroxyfumaric acid, and several carbonyl compounds.

Acknowledgment

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Literature Cited


Table I. Inhibition of Color Development in Potato Slices by Ascorbic Acid and Other Reducing Substances

<table>
<thead>
<tr>
<th>Compounds used for treatment</th>
<th>Difference from control</th>
<th>% inhibition**</th>
<th>Inhibition as % of ascorbic acid inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- △ Rd*</td>
<td>- △ Rd</td>
<td>- △ Rd</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>18.7</td>
<td>97</td>
<td>100</td>
</tr>
<tr>
<td>Cysteine</td>
<td>19.4</td>
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<td>104</td>
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<tr>
<td>Ascorbic acid</td>
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<tr>
<td>Dihydroxyacetone</td>
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<tr>
<td>Dihydroxyfumaric acid</td>
<td>11.0</td>
<td>62</td>
<td>68</td>
</tr>
</tbody>
</table>

- △ Rd*: decrease in percent reflectance with time.
- ** Some values for percent inhibition of color are above 100 because the inhibitors decolorized slices, making them lighter than the controls.

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