Mechanism for the suppressive effect of salts on ascorbate Oxidase activity of vegetables

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Summary

Ascorbate oxidase (EC 1.10.3.3, AAO) is widely distributed in plants, and responsible for the oxidation of ascorbate (vitamin C) during storage, processing, and cooking of vegetables. It is well known that the level of ascorbic acid in Momijioroshi, a grated radish and carrot mixture, is decreased rapidly after preparation. We determined that 99% of ascorbic acid of mung bean (*Vigna mungo*) sprouts was oxidized after homogenization with water, but the oxidation was prevented by the addition of NaCl. To elucidate the mechanism for suppressive effect of salts on AAO activity of vegetables, we tried to purification of AAO from mung bean sprouts. Two isozymes (I and II) of AAO were separated by a DE-52 cellulose column chromatography after ammonium sulfate fractionation. The partial purified two isozymes were used for further studies. The optimum pHs of I and II were 5.0 and 4.5~5.2, respectively. The isozymes I and II showed the apparent Km values of 12.5×10^{-5}M and 4.4×10^{-5}M for ascorbic acid, respectively. The both isozymes were inhibited strongly by several salts and the effective order was Na-citrate> NaCl> KCl> Na-malate> NH_4Cl> $(NH_4)_2SO_4$. Lineweaver-Burk plots of isozyme I in the absence and presence of Na-citrate and NaCl showed that inhibitions by Na-citrate and NaCl were non-competitive with the substrate ascorbic acid. Ki values for Na-citrate and NaCl were 50 mM and 130 mM, respectively. On the other hand, Isozyme II was inhibited competitively by the two salts, and the Ki values for Na-citrate and NaCl were 25 mM and 28 mM, respectively. These results suggest that the concentration of NaCl used in cooking of fresh vegetables and lemon juice effectively inhibit AAO activity, and results in preventing of oxidation of ascorbic acid in fresh vegetables after cutting and grating.